

DANIEL C. BAUMGART
EDITOR

Crohn's Disease and Ulcerative Colitis

From Epidemiology and
Immunobiology to a
Rational Diagnostic and
Therapeutic Approach

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Daniel C. Baumgart

Division of Gastroenterology and Hepatology,

Department of Medicine, Charité Medical Center – Virchow Hospital

Medical School of the Humboldt–University of Berlin

Berlin, Germany

daniel.baumgart@charite.de

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Foreword

The clinical features of the major forms of Inflammatory Bowel Disease, Crohn's disease and ulcerative colitis, have been generally well-known for decades. After a long period in which advances in our understanding of the causation and pathogenesis of Inflammatory Bowel Diseases remained painfully slow and, in parallel, improvements in management were at best incremental, the momentum of progress has accelerated over the past several years, making a textbook that draws together the full continuum of these timely advances.

The recent pace of progress in understanding underlying pathogenesis has been especially remarkable. This has been possible because of more powerful methodological approaches as well as a growing community of investigators focused on these disorders and the basic processes associated with them. Progress in recent years has been rapid along a number of fronts and a general paradigm has emerged to suggest that these disorders result from alterations in the host response to the microflora present within the GI tract. These host responses comprise the collective functional integrity of the mucosal epithelium and the complex set of innate and adaptive immune responses. While many details remain to be fleshed out, molecular pathways intrinsic to the interactions and functional regulation of these responses have been identified and it is clear that for many the "set point" is determined by the genotypic variations at dozens of susceptibility genes. Undoubtedly surprises remain. History suggests the skeptic of any entrenched dogma has a good likelihood of eventually being proven right. However, this paradigm or model has proven a powerful context to ask better questions that should eventuate in more complete answers about the causation and pathogenesis of Inflammatory Bowel Diseases. Progress in understanding of the basic processes underlying the development of Inflammatory Bowel Diseases should be an engine for still more effective therapies as well as diagnostic tools to facilitate management.

On the clinical front, the global epidemiology of Inflammatory Bowel Diseases continues to evolve. While incidence and prevalence plateaued after a long period of steady rise in those regions that remain highest, Europe and North America, other areas of the world are seeing a characteristic pattern of increases in the frequency of ulcerative colitis followed *pari passu* by increases in Crohn's disease. Clinicians caring for these patients cannot be complacent. There is more information that needs to be incorporated into management decisions and, most importantly, much more to offer patients. Improvements in management include evolving surgical approaches and, in some instances, alternative interventions via endoscopy offering efficacy with less morbidity. Advances in non-surgical medical therapy have had an even greater impact on treatment of IBD patients. These include categorically new agents, which have been developed on the basis of advances in our understanding of mechanisms relevant to the pathogenesis of these disorders. As exemplified by anti-TNF agents, the age of biologics has arrived. Given that understanding of pathogenesis is still progressing rapidly, one can anticipate that more and more effective agents will yet be forthcoming.

These advances have resulted in greater complexity in good decision-making. Those caring for these patients should welcome this complexity in so far as it reflects the possibility of finding management strategies better tailored to the specific needs of an individual patient. The opportunities to make more confident management decisions, if not initial diagnosis as well, have also become both more complicated and more promising with the advent of new imaging modalities (both “radiologic” and “endoscopic”), as well as various biomarkers. While it is not yet clear how specific susceptibility genotypes might be best used clinically there is, in concept, the possibility of more definitive diagnosis after decades in which diagnosis has remained, in the final analysis, an empiric process.

In these general reflections on recent progress are clear indications of the timeliness for a textbook that endeavors to bring these new advances into better focus. The editor has embraced this ambitious goal and assembled an outstanding group of authors who have been at the forefront of much of this progress. This volume provides both the clinician and the scientist with an understanding of the most recent advances as well as the context for each of them to be pursuing their mission of caring for patients and advancing our knowledge, respectively. However, these general reflections also come with an embedded caution. Clinicians will recognize that even with this progress, unmet needs persist and there remain many patients for whom current approaches are simply not good enough. The scientist will recognize how still incomplete our understanding of these diseases remains. So, this textbook provides a powerful tool to ensure that clinicians can provide today’s best care and scientists can pose today’s best questions. One can only hope that within a few years the next volume will be ready to be written.

Dallas, TX, USA

Daniel K. Podolsky

Preface

Crohn's disease and ulcerative colitis – two chronic inflammatory diseases (IBD) on the rise – result from an inappropriate immune response, in genetically susceptible individuals, to microbial antigens of commensal microorganisms. This inappropriate response is promoted by certain environmental factors including Western life style, explaining their globally increasing incidence. As a systemic disorder of the immune system IBD manifest itself primarily in the gastrointestinal tract but can affect all of the organ systems of the human body. Thus, not only gastroenterologist, but many other clinicians are confronted with IBD.

On the other hand, IBD is an excellent example of how the exponential growth of knowledge in biomedical science can make a remarkable impact on clinical practice and patient's quality of life. It has led to the development of a number of novel targeted and tailored treatments. These are continuously being refined to treat not only the two original conditions in the gut, but also the variety of associated immune disorders. New therapies are sometime complex and associated with important risks requiring a deeper understanding of their molecular principles from clinicians.

This book is intended to serve as a unique combined resource for physicians and scientists addressing the needs of both groups. It is meant to help stimulate exchange and collaboration and shorten the path between discovery and application of new knowledge and also help clinicians understand new therapeutic concepts from their origins.

I am grateful to my colleagues from all over the world who contributed chapters in their respective fields of expertise and made accomplishing the goals of this book possible.

Berlin, Germany

Daniel C. Baumgart

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Contributors

C. Agnè, MD Departments of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Caroline P. Allen, MA, MBBS, MRCP Department of Dermatology, The Churchill Hospital, University of Oxford, Headington, Oxford, UK

Koji Atarashi, PhD Department of Immunology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Daniel C. Baumgart, MD, PhD Division of Gastroenterology and Hepatology, Department of Medicine, Charité Medical Center–Virchow Hospital, Medical School of the Humboldt–University of Berlin, Berlin, Germany

Daniel Benítez-Ribas, PhD Department of Gastroenterology, CIBERehd Villarroel 170, Barcelona, Spain

Charles N. Bernstein, MD Department of Internal Medicine, University of Manitoba IBD Clinical and Research Centre, Bingham Chair in Gastroenterology, Health Sciences Centre, University of Manitoba, Winnipeg, MB, Canada

Charles L. Bevens, MD, PhD Department of Microbiology and Immunology, University of California, Davis School of Medicine, Davis, CA, USA

Atul K. Bhan, MBBS, MD Department of Pathology, Harvard University, Massachusetts General Hospital, Boston, MA, USA

Wojciech Blonski, MD, PhD Division of Gastroenterology, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA, USA

Emily M. Bradford, PhD Department of Pathology, University of Chicago, Chicago, IL, USA

Per Brandtzaeg, MS, DSS, PhD Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Centre for Immune Regulation (CIR), University of Oslo, Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Alan L. Buchman, MD, MSPH Department of Gastroenterology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Susan M. Burge, BSc, DM, FRCP Department of Dermatology, The Churchill Hospital, University of Oxford, Headington, Oxford OX3 7LJ, UK

Eduard Cabré, MD, PhD Department of Gastroenterology, Hospital Universitari Germans Trias I Pujol, Badalona, Spain

Roger W. Chapman, MD, FRCP Department of Hepatology/Translational Gastroenterology, John Radcliffe Hospital, University of Oxford, Oxford, Oxfordshire, UK

Tanya P. Chawla, MBBS, MRCP, FRCP, FRCPC Abdominal Imaging Division, Joint Department of Medical Imaging, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada

Judy H. Cho, MD Department of Internal Medicine: Digestive Diseases, Genetics, Yale University School of Medicine, New Haven, CT, USA

Alvin Chung, MBBS Department of Gastroenterology, Box Hill Hospital, Box Hill, VIC, Australia

Robert R. Cima, MD, MA Division of Colon and Rectal Surgery, Department of Surgery, Mayo Clinic College of Medicine, MN, USA

Jacques Cosnes, MD Department of Gastroenterology and Nutrition, Saint-Antoine, Paris, France

Nigel Crawford, MBBS, MPH, FRACP Royal Childrens Hospital, Parkville, VIC, Australia

Stephanie Dahan, PhD Immunology Institute, Mount Sinai School of Medicine, New York, NY, USA

Vera K. Denmark, MD Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA

Iris Dotan, MD Department of Gastroenterology and Liver Disease, IBD Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Laurence Egan, MB, BCh, BAO Department of Pharmacology, Clinical Science Institute, University Hospital Galway, Galway, Ireland

Francis A. Farraye, MD, MSc Section of Gastroenterology, Boston Medical Center, Boston University School of Medicine, Boston, MA, USA

Richard A. Flavell, PhD Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA

Joel G. Fletcher, MD Department of Radiology, Mayo Clinic, Rochester, MN, USA

Miquel A. Gassull, MD, PhD Health Sciences Research Institute, Germans Trias I Pujol Foundation, Badalona, Spain

Grace N. Gathungu, MA, MD Division of Pediatric Gastroenterology, Stonybrook University Hospital, Stonybrook, NY, USA

Michael J. Gebel, MD, PhD Departments of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

Peter R. Gibson, MD, FRACP Department of Gastroenterology and Hepatology, Eastern Health Clinical School, Eastern Health and Monash University, Box Hill Hospital, Box Hill, VIC, Australia

Paolo Gionchetti, MD Department of Internal Clinical Medicine and Gastroenterology, University of Bologna, Policlinico S. Orsola, Bologna, Italy

D. Neil Granger, PhD Department of Molecular and Cellular Physiology,
Louisiana State University Health Sciences Center, Shreveport, LA, USA

Marco Greco, JD, PhD EFCCA – European Federation of ulcerative Colitis and Crohn’s
Association, Brussels, Belgium

Matthew B. Grisham, PhD Immunology and Inflammation Research Group,
Department of Molecular and Cellular Physiology, Louisiana State University
Health Sciences Center, Shreveport, LA, USA

John Halliday, MBBS (Hons.), FRACP Department of Hepatology/Translational
Gastroenterology, John Radcliffe Hospital, University of Oxford, Oxford, Oxfordshire, UK

Stephen Hanauer, MD Section of Gastroenterology, Hepatology and Nutrition,
Pritzker School of Medicine, University of Chicago, Chicago, IL, USA

Norman R. Harris, PhD Department of Molecular and Cellular Physiology,
Louisiana State University Health Sciences Center, Shreveport, LA, USA

Toshifumi Hibi, MD, PhD Department of Internal Medicine, Keio University,
School of Medicine, Tokyo, Japan

Frank Hoentjen, MD, PhD Hepatology and Nutrition, Pritzker School of Medicine,
University of Chicago, Chicago, IL, USA

Department of Gastroenterology, Free University Medical Center, Amsterdam, The Netherlands

Daniel W. Hommes, MD, PhD Department of Gastroenterology and Hepatology,
Leiden University Medical Center, Leiden, The Netherlands

Kenya Honda, MD, PhD Department of Immunology, Graduate School of Medicine,
The University of Tokyo, Tokyo, Japan

Jeffrey S. Hyams, MD Division of Digestive Diseases, Hepatology, and Nutrition,
Departments of Digestive Diseases, Hepatology, and Nutrition, Connecticut Children’s
Medical Center, University of Connecticut School of Medicine, Hartford, CT, USA
Department of Pediatrics, University of Connecticut School of Medicine, Farmington, CT, USA

Nora E. Joseph, MD Department of Pathology, University of Chicago, Chicago, IL, USA

Aarti Kakkar, MD Section of Gastroenterology, Boston Medical Center,
Boston University School of Medicine, Boston, MA, USA

Terumi Kamisawa, MD, PhD Department of Internal Medicine, Tokyo Metropolitan
Komagome Hospital, Tokyo, Japan

Sunanda Kane, MD, MSPH Department of Medicine, Mayo Clinic, Rochester, MN, USA

Gil Kaplan, MD, MPH Department of Medicine, Foothills Medical Center,
Calgary, AB, Canada

Fridrik Karlsson, PhD Immunology and Inflammation Research Group,
Department of Molecular and Cellular Physiology, Louisiana State University
Health Sciences Center, Shreveport, LA, USA

Reena Khanna, MD, FRCPC Division of Gastroenterology, McMaster University
Medical Centre, Hamilton, ON, Canada

Ralf Kiesslich, MD, PhD Endoscopy Department, I. Med. Klinik und Poliklinik, Universitätsmedizin Mainz, Johannes Gutenberg Universität Mainz, Mainz, Deutschland, Germany

Iurii Kobozev, BS Immunology and Inflammation Research Group, Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, LA, USA

Koichi Koizumi, MD Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Go Kuwata, MD Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Barrett G. Levesque, MD, MS Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Gary R. Lichtenstein, MD Division of Gastroenterology, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA, USA

Edward V. Loftus, MD Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Edouard Louis, MD, PhD Department of Gastroenterology, CHU Liège and GIGA Research, University of Liège, service de gastroentérologie, CHU de Liège, Domaine du Sart Tilman, Liège, Belgium

Kristine Macartney, BMedSci, MBBS (Hons), DipPaeds, MD, FRACP National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead, Westmead, Australia

Peter Mannon, MD, MPH Division of Gastroenterology and Hepatology, University of Alabama at Birmingham, Birmingham, AL, USA

Michael P. Manns, MD Departments of Gastroenterology, Hepatology, and Endocrinology, Medical School of Hannover, Hannover, Germany

John K. Marshall, MD, MSc, FRCPC, ACAF Division of Gastroenterology, McMaster University, Hamilton, ON, Canada

Lloyd Mayer, MD Immunology Institute, Mount Sinai School of Medicine, New York, NY, USA

Division of Clinical Immunology, Mount Sinai Medical Center, New York, NY, USA

Marjorie Merrick, MD Department of Research and Scientific Programs, Crohn's and Colitis Foundation of America, New York, NY, USA

Atsushi Mizoguchi, MD, PhD Department of Pathology, Harvard University, Massachusetts General Hospital, Boston, MA, USA

Pia Munkholm, MD, DMSci Department of Gastroenterology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark

Yasuhiro Nemoto, MD, PhD Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

Deanna D. Nguyen, MD Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

Jagtar Singh Nijjar, MBChB (Hons.), BSc (Med.Sci.) Centre for Rheumatic Diseases, Glasgow Royal Infirmary, University of Glasgow, Glasgow, UK

Junko Nishio, MD, PhD Department of Immunology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Haruhiko Ogata, MD, PhD Center for Diagnostic and Therapeutic Endoscopy, Keio University School of Medicine, Tokyo, Japan

Remo Panaccione, MD, FRCPC Department of Medicine, Foothills Medical Centre, Calgary, AB, Canada

Julián Panés, MD, PhD Department of Gastroenterology, Hospital Clínic Barcelona, CIBERehd Instituto Salud Carlos III, Barcelona, Spain

Natalia Pedersen, MD, PhD Herlev University Hospital, Copenhagen, Denmark

John H. Pemberton, MD Division of Colon and Rectal Surgery, Department of Surgery, Mayo Clinic College of Medicine, MN, USA

Catherine Reenaers, MD, PhD Department of Gastroenterology, CHU Liège and GIGA Research, University of Liège, service de gastroentérologie, CHU de Liège, Domaine du Sart Tilman, Liege, Belgium

Walter Reinisch, MD Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

Fernando Rizzello, MD Department of Internal Clinical Medicine and Gastroenterology, University of Bologna, Policlinico S. Orsola, Bologna, Italy

Patrik Rogalla, MD Department of Medical Imaging, Toronto General Hospital, University of Toronto, Toronto, ON, Canada

Atsushi Sakuraba, MD Section of Gastroenterology, Hepatology and Nutrition, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA

Azucena Salas, PhD Department of Gastroenterology, Hospital Clínic de Barcelona, Barcelona, Spain,

Institut Investigacions Biomètiques August Pi Sunyer (IDIBAPS), Barcelona, Spain

Nora Schweitzer, MD Departments of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany

Philippe Seksik, MD, PhD Department of Gastroenterology and Nutrition, Saint-Antoine, Paris, France

Scott B. Snapper, MD, PhD Gastroenterology, Children's Hospital and Brigham and Women's Hospital, Boston, MA, USA

Harry Sokol, MD, PhD Department of Gastroenterology and Nutrition, Saint-Antoine, Paris, France

A. Hillary Steinhart, MD, MSc, FRCP(C) Department of Medicine, University of Toronto, IBD Centre, Mount Sinai Hospital, Toronto, ON, Canada

Eoin R. Storan, MB, BCh, BAO Department of Gastroenterology, Clinical Science Institute, University Hospital Galway, Galway, Ireland

Till Strowig, PhD Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA

Roger Sturrock, BD, MD, FRCP Division of Immunology, Infection and Inflammation,
University of Glasgow, Glasgow, UK
Centre for Rheumatic Diseases, Glasgow Royal Infirmary, University of Glasgow, Glasgow, UK

Lloyd R. Sutherland, MDCM, FRCPC, FACP Division of Gastroenterology, Department of
Medicine, University of Calgary, Foothills Hospital, Calgary, AB, Canada

Taku Tabata, MD Department of Internal Medicine, Tokyo Metropolitan
Komagome Hospital, Tokyo, Japan

Emily S. Turner Department of Pathology, University of Chicago, Chicago, IL, USA

Jerrold R. Turner, MD, PhD Department of Pathology, University of Chicago,
Chicago, IL, USA

Gert Van Assche, MD, PhD Department of Gastroenterology, Leuven University Hospitals,
Leuven, Belgium

Catherine van Kemseke, MD, PhD Department of Gastroenterology, CHU Liège and GIGA
Research, University of Liège, service de gastroentérologie, CHU de Liège,
Domaine du Sart Tilman, Liège, Belgium

Morten H. Vatn, MD, PhD Section of Gastroenterology, Oslo University Hospital,
Oslo, Norway

University of Oslo, Institute of Clinical Medicine, Akershus University Hospital,
Akershus , Norway

Anne Christine W. Vos, MSc Department of Gastroenterology and Hepatology,
Leiden University Medical Center, Leiden, The Netherlands

Alan W. Walker, BSc (Hons), PhD Pathogen Genomics Group, Wellcome Trust
Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK

Sharheel K. Wasan, MD Department of Medicine, Section of Gastroenterology,
Boston Medical Center, Boston, MA, USA

Mamoru Watanabe, MD, PhD Department of Gastroenterology and Hepatology,
Tokyo Medical and Dental University, Tokyo, Japan

Christopher R. Weber, MD, PhD Department of Pathology, The University of Chicago,
Chicago, IL, USA

Ben Wilson, BA(Hons), MSc EFCCA – European Federation of ulcerative Colitis
and Crohn’s Association, Brussels, Belgium

Kevin J. Woollard, BSc, PhD Division of Immunology, Infection and Inflammatory
Diseases, Centre for Molecular and Cellular Biology of Inflammation, Kings College London,
London, UK

Part I

Epidemiology

Chapter 1

The Genetics of Inflammatory Bowel Disease

Grace N. Gathungu and Judy H. Cho

Keywords Crohn's disease • Ulcerative colitis • Interleukin 23 • Autophagy • Innate immunity • Epithelial defense • Intestinal microbiome

Introduction

The inflammatory bowel disorders (IBD) are comprised of Crohn's disease (CD) and ulcerative colitis (UC). The hallmark of IBD is a loss of tolerance to commensal organisms that results in a chronic, uncontrolled inflammation of the intestinal mucosa. The peak age of onset is typically during young adulthood between 25 and 35 years of age, but about 20–25% of patients have onset of symptoms during childhood or adolescence [1]. The pathogenesis of IBD is not fully defined, but is clearly multifactorial, resulting from multiple genes, developmental and environmental factors, which together result in a dysregulated innate and adaptive mucosal immune response.

The intestinal immune system maintains a delicate balance between the identification and elimination of pathogens while co-existing with commensal bacteria and food antigens. A unique property of the intestine is the close apposition of microorganisms to the single cell intestinal epithelial barrier that serves as the first line of defense. The factors that disrupt intestinal homeostasis and likely play a role in IBD pathogenesis include alterations in epithelial barrier function, innate immune cells [including macrophages and dendritic cells (DCs)], lymphocyte function and stromal-cell-derived factors [such as transforming growth factor- β (beta) (TGF β)] that can have tolerizing effects on intestinal immune responses [2]. The interleukin (IL) 23 pathway was identified through genome-wide association studies (GWAS). This led to the discovery of the role of the IL23 pathway in IBD. Th17 cells are involved in clearance of pathogens not dealt with by Th1 or Th2 cells and are potent inducers of tissue inflammation [3]. Presently, approximately 100 genomic loci have been associated with either CD or UC and implicate a broad array of genes and pathophysiologic mechanisms.

G.N. Gathungu, MA, MD
Division of Pediatric Gastroenterology, Stonybrook University Hospital, Stonybrook, NY, USA

J.H. Cho, MD (✉)
Department of Internal Medicine, Digestive Diseases, Genetics, Yale University School of Medicine,
333 Cedar Street/1080 LMP, P.O. Box 208019, New Haven, CT 06520-8019, USA
e-mail: judy.cho@yale.edu

Epidemiology

The epidemiology of IBD is under constant change. Emerging nations such as China, Eastern Europe, and India have a predominance of UC, whereas developed nations have a predominance of CD. Current estimates are that the prevalence of IBD in the USA ranges between 1 and 1.5 million people [4, 5]. The incidence of pediatric CD in particular is on the rise but the causes for this are still unclear [6]. The majority of IBD patients in most US studies are European ancestry; but other studies report an increasing burden of IBD among non-European ancestry populations outside the USA. One study examined racial disparities in disease burden and treatment within a US healthcare system [7]. Overall, African-American patients had higher proportion of CD than Hispanics and Asians. Hispanic and Asian IBD patients had a later age of diagnosis, shorter disease duration, and less frequent family history of IBD. Disease behavior, choice of medical therapy and rates of surgery were the same for all population cohorts examined. This study highlights the need to examine non-European ancestry individuals in greater numbers.

Epidemiologists have proposed various factors contributing to disease etiology [8]. The hygiene hypothesis argues that as a consequence of the industrial revolution, the rates of communicable infectious diseases have decreased, but resulted in a rise in the incidence of chronic immune diseases [9]. A study by Fumagalli et al. [10] surveyed the abundance of gene polymorphisms and gut microbial pathogens from several geographical regions around the world and determined the existence of a selective pressure for genetic polymorphisms within immune-response genes, especially the interleukin genes.

The diversity of gut microbial organisms has also been examined. Under normal conditions, the human gut microbiota is highly diverse and abundant and has co-evolved to maintain an ongoing, commensal relationship to the host [11–15]. For example, differences in IBD intestinal microbiota could result from both genetic variants resulting in different host–microbe responses, as well as secondary effects of the inflammatory process. In general, studies have shown an imbalance or *dysbiosis* in microbiota that comprise the *Firmicutes* phylum, *Bacteroidetes*, and facultative anaerobes such as *Enterobacteriaceae* [16]. It is unclear if infectious agents have shaped the genetic variation at interleukin loci or human beings exposed to the microbial pathogens have evolved in response to the infections. A paucity of the organism *Fecalobacterium prausnitzii* was associated with active CD in one study [17]. Another study showed that the expression of an adhesion molecule carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), correlated with the colonization by adherent-invasive *Escherichia coli* is increased in patients with ileal CD [18].

Other factors that have been examined include the effects of smoking. It is well established that CD patients are more likely to be smokers than their matched controls, while in contrast to this, patients with UC are less likely to be smokers than controls or patients with CD [19–21]. In addition, smoking cessation after diagnosis is detrimental for UC patients but beneficial for CD patients [21–24]. Breastfeeding has many advantages and is the preferred mode of nutrition for growing infants. A meta-analysis reported a protective effect of breastfeeding on IBD development (OR 0.69; 95%CI, 0.51–0.94) [25]. Additional associations include an increased risk of antibiotics use in children diagnosed with IBD [26]. Finally, an increased dietary consumption of animal protein is associated with increased risk of an IBD diagnosis [27].

Heritability

Heritability studies of multiple relatives and twins affected with IBD have revealed a prominent genetic component. For example, Orholm et al., observed that the risk of CD to first-degree relatives of CD patients was tenfold greater while the risk of UC to relatives of UC patients was eightfold

greater than the risk observed in first-degree relatives of healthy controls [28]. An even greater risk was conferred to monozygotic twins when compared to dizygotic twins [29–34]. Furthermore, disease concordance is high among relatives with 75–80% of families having the same disease type of either CD or UC [35]. The heritability of IBD has been postulated to arise from polymorphisms in multiple genes, each contributing in an additive fashion to the overall disease risk. The first IBD-associated gene, the nucleotide-binding oligomerization domain 2 (NOD2), was described in 2001 using genetic linkage studies done by two independent groups [36, 37]. Subsequently, we have had tremendous progress in high-throughput genotyping technology. Through the International HapMap Project and the Human Genome Project single-nucleotide polymorphisms (SNPs) were described. The variation introduced by a SNP can result in functional alterations of genes. GWAS test a large number of SNPs throughout the genome in multiple individuals using genotyping platforms. Current genotyping platforms screen for variations in hundreds of thousands of common SNPs (minor allele frequency greater than 5%) across large cohorts. Such studies have led to the discovery of 99 confirmed IBD loci [38, 39] in genes that belong to different molecular pathways. Some loci are specific to CD or UC, whereas over 25 polymorphisms or SNPs have significant association signals in both UC and CD.

Scope of Genetic Associations Identified Through GWAS

Through the study of large, well-powered case–control cohorts, GWAS has identified approximately 100 loci association to CD and/or UC. At some loci, the definitive causal allele/gene has been identified, but for most of the loci, only general association regions have been implicated. Candidate genes within these loci (Table 1.1) implicate a broad array of genes potentially contributing to IBD pathogenesis. Perhaps not surprisingly, the largest class of candidate genes involved cytokine and cytokine receptors. Multiple innate immune genes mediating microbial recognition have been implicated, especially for CD. Other classes of candidate genes include genes mediating lymphocyte activation, epithelial defense, endoplasmic reticulum stress, intracellular trafficking, and a broad array of transcription factors. Taken together, the multiplicity of these associations highlight the pathophysiologic complexity underlying IBD.

Genetic Variants Predominantly Associated with Crohn's Disease

GWAS have led to the identification of over 71 CD-associated genes or loci [39, 40]. The determination of the functional significance for many of the putative loci or genes is still ongoing. However, some of the genes implicated have highlighted key pathways including bacterial recognition (NOD2), autophagy [autophagy-related 16-like 1 (ATG16L1), immunity-related GTPase family, M (IRGM)].

NOD2

NOD2 polymorphisms are associated with an increased susceptibility to CD but not UC. NOD2 is a pattern-recognition receptor protein found in many cells including monocytes, macrophages, DCs, intestinal epithelial cells, B and T lymphocytes. It senses the intracellular bacterial cell wall component

Table 1.1 Candidate genes within IBD-associated regions

Gene symbol	Gene name	Chromosomal region	IBD association
<i>Cytokines and cytokine receptors</i>			
IL23R	Interleukin 23 receptor	1p31	CD-UC [38, 39]
IL10, IL19	Interleukin 10; interleukin 19	1q32	CD-UC [38, 39]
IL1R2	Interleukin 1 receptor, type II	2q11	CD-UC [38, 39]
IL1RL1; IL18R1; IL18RAP	Interleukin 1 receptor; interleukin 18 receptor 1; Interleukin 18 receptor accessory protein	2q12	CD [39]
IL8RA; IL8RB	Interleukin 8 receptor, alpha; interleukin 8 receptor, beta	2q35	UC [38]
IL2; IL21	Interleukin 2; interleukin 21	4q27	UC [38]
IL7R	Interleukin 7 receptor	5p13	UC [38]
IL12B (p40)	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	5q33	CD-UC [38, 39]
CCR6	Chemokine (C-C motif) receptor 6	6q27	CD [39]
IL2RA	Interleukin 2 receptor, alpha	10p15	CD [39]
IL26	Interleukin 26	12q14	UC [38]
IL27	Interleukin 27	16p11	CD [39]
CCL2; CCL7	Chemokine (C-C motif) ligand 2; chemokine (C-C motif) ligand 7	17q12	CD [39]
TNFSF11	tumor necrosis factor (ligand) superfamily, member 11	13q14	CD [39]
TNFRSF6B	Tumor necrosis factor receptor superfamily, member 6b, decoy	20q13	CD-UC [38, 39]
TNFSF4; TNFSF18	tumor necrosis factor (ligand) superfamily, member 4; tumor necrosis factor (ligand) superfamily, member 18	1q24	CD [39]
TNFSF8; TNFSF15	Tumor necrosis factor (ligand) superfamily, member 8; tumor necrosis factor (ligand) superfamily, member 15	9q32	CD-UC [38, 39]
TNFRSF9; TNFRSF14	Tumor necrosis factor (ligand) superfamily, member 9; tumor necrosis factor (ligand) superfamily, member 14	1p36	UC [38]
IL17REL	Interleukin 17 receptor E-like	22q13	UC [38]
<i>Microbial recognition</i>			
ATG16I1	ATG16 autophagy-related 16-like 1	2q37	CD [39]
IRGM	Immunity-related GTPase family, M	5q33	CD [39]
CARD9	CASPASE recruitment domain family, member 9	9q34	CD-UC [38, 39]
NOD2	Nucleotide-binding oligomerization domain containing 2	16q12	CD [39]
<i>Leukocyte activation</i>			
MHC class II	Major Histocompatibility Complex, class II	6p21	CD-UC [38, 39]
TAGAP	T-cell activation RhoGTPase activating protein	6q25	CD [39]
LSP1	Lymphocyte-specific protein 1	11p15	UC [38]
ICOSLG	Inducible T-cell co-stimulator ligand	21q22	CD-UC [38, 39]

(continued)

Table 1.1 (continued)

Gene symbol	Gene name	Chromosomal region	IBD association
<i>Epithelial defense</i>			
MUC19	Mucin 19, oligomeric	12q12	CD [39]
MUC1	Mucin 1, cell surface associated	1q22	CD [39]
LAMB1	Aminin, beta 1	7q31	
<i>ER stress</i>			
ORMDL3	ORM1-like 3	17q12	CD-UC [38, 39]
XBP1	X-box-binding protein 1	22q12	CD-UC [38, 39]
<i>Intracellular trafficking</i>			
VAMP3	Vesicle-associated membrane protein 3 (cellubrevin)	1p36	CD [39]
SCAMP3	Secretory carrier membrane protein 3	1q22	CD [39]
DENND1B	DENN/MADD domain containing 1B	1q31	CD [39]
GALC	Galactosylceramidase	14q35	CD [39]
<i>Transcription factors</i>			
NKX2-3	NK2 transcription factor related, locus 3	10q24	CD-UC [38, 39]
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	17q21	CD [39]
SMAD3	SMAD family member 3	15q22	CD-UC [38]
ZMIZ1	Zinc finger, MIZ-type containing 1	10q22	CD [39]
BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2 BTB and CNC homolog 2	6q15	CD [39]
REL	C-Rel proto-oncogene protein	2p16	CD-UC [38, 39]
HNF4A	hepatocyte nuclear factor 4, alpha	20q13	
<i>Enzymes</i>			
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	2p23	CD [39]
GCKR	GLUCOKINASE (hexokinase 4) regulator	2p23	CD [39]
FADS2	Fatty acid desaturase 2	11q12	
TYK2	Tyrosine kinase 2	19p13	CD-UC [38, 39]
ERAP2	Endoplasmic reticulum aminopeptidase 2	5q15	CD [39]
FUT2	Fucosyltransferase 2	19q13	CD [39]
<i>Intracellular signaling</i>			
PTPN22	Protein tyrosine phosphatase, nonreceptor type 22 (lymphoid)	1p13	CD [39]
JAK2	Janus kinase 2	9p24	CD-UC [38, 39]
PTPN2	Protein tyrosine phosphatase, nonreceptor type 2	18p11	CD [39]
<i>Other membrane proteins</i>			
PTGER4	prostaglandin E receptor 4 (subtype EP4)	5p13	CD-UC [38, 39]
SLC22A4; SLC22A5	Solute carrier family 22 (organic cation/ergothioneine transporter), member 4; solute carrier family 22 (organic cation/carnitine transporter), member 5	5q31	CD [39]
PLCL1	Phospholipase C-like 1	2q33	CD [39]
FCGR2A; FCGR2B	Fc fragment of IgG, low affinity IIa, receptor (CD32); Fc fragment of IgG, low affinity IIb, receptor (CD32)	1q23	UC [38]
CD244	CD244 molecule, natural killer cell receptor 2B4	1q23	CD [39]
ICAM3	Intercellular adhesion molecule 3	19p13	CD [39]

muramyl dipeptide (MDP) [41, 42] and activation of NOD2 leads to the downstream activation of nuclear factor- κ B (NF- κ B) and mitogen-activated protein (MAP) kinase signaling pathways [43]. The NOD2 gene is located on chromosome 16 and three rare polymorphisms, Arg702Trp, Gly908Arg, and Leu1007fsinsC are independently associated with CD [44]. Each of these variants are located within or near the leucine-rich repeat region of NOD2, the bacteria-sensing domain, and each results in decreased NF- κ B (beta) activation after peptidoglycan or MDP stimulation [42, 43, 45]. Interestingly, NOD2 mutations are highly associated with European ancestry CD. Approximately 30% of patients of European ancestry have at least one of the three polymorphisms yet these variants are rare in Africans and absent in those of Asian ancestry [46–49]. A meta-analysis of 39 studies in Caucasians showed an odds ratio for simple heterozygotes of 2.4 (confidence interval, CI 2.0–2.9), and for homozygous or compound heterozygous carriers of 17.1 (CI 10.7–27.2) [44]. NOD2 carriers have more ileal disease involvement, they are more likely to develop strictures and fistulae as a complication leading to resectional surgery and NOD2 carriage has also been associated with earlier age of onset [50].

Recently, it has been established that certain classes of bacteria (Actinomyces and mycobacteria) contain a hydroxylase enzyme that covalently modifies MDP to form glycolyl MDP [51]. This modified form more potently activates the NOD2 pathway and opens up the intriguing possibility that CD is associated with a differential sensing or clearance of certain classes of bacteria. A GWAS of leprosy patients from China established an intriguing overlap with Crohn's disease loci, including polymorphisms in the NOD2 region, as well as autophagy genes (LRRK2) and TNFSF15 (implicated in a Japanese GWAS study) [52, 53].

ATG16L1

In a German cohort of 735 individuals with CD and 368 controls, Hampe et al. genotyped 19,779 SNPs and identified a significant association of the rs2241880 variant in the ATG16L1 on chromosome 2q37.1 [54]. Subsequently, many studies including pediatric cohorts have confirmed this association with CD [55–57]. The rs2241880 SNP encodes a threonine to alanine substitution (T300A) at amino acid position 300 located at the N-terminus of the WD-repeat domain in ATG16L1 [54]. It is expressed by intestinal epithelial cells, antigen-presenting cells, B cells, CD4+, and CD8+ T cells [54, 57]. ATG16L1 forms a complex with ATG5 and ATG7 that is necessary for autophagosome formation [57]. A working hypothesis is that mutations affecting components of autophagy would result in reduced pathogen clearance. In one study, the expression of ATG16L1 by intestinal epithelial cells was necessary for effective autophagy of *Salmonella typhimurium* [57]. More recently, Cooney et al. determined that activation of NOD2 by MDP induces autophagy in DCs [58]. This process required receptor-interacting serine-threonine kinase-2 (RIPK-2), ATG5, ATG7, and ATG16L1. They showed that NOD2-mediated autophagy is required for both bacterial handling and generation of major histocompatibility complex (MHC) class II antigen-specific CD4+ T-cell responses in DCs. Individuals with CD and NOD2 or ATG16L1 risk variants were defective in autophagy induction, bacterial trafficking and antigen presentation [58]. Similarly, Homer et al. demonstrated that MDP stimulation of epithelial cells, macrophages, and dendritic cells activated autophagy, NF- κ B (beta) and MAP kinase signaling while it increased killing of *Salmonella*. These processes were impaired among individuals with CD and NOD2 or ATG16L1 risk variants [59]. Importantly, a functional overlap between ATG16L1 and the intestinal pathogen norovirus was reported to modulate the gene expression program in intestinal Paneth cells, further highlighting the importance between host innate immune genes and the intestinal microbiota [60].

Additional Autophagy Genes

A second autophagy gene that has been linked to CD susceptibility is the IRGM gene on chromosome 5q33.1 [61, 62]. IRGM is an atypical member of the IRG family of p47 immunity-related GTPase genes. It encodes a 181 amino acid protein and induces autophagy against intracellular pathogens [63, 64]. Irgm1-deficient mice have increased severity of infection when challenged by intracellular pathogens including *Toxoplasma gondii*, *Mycobacterium tuberculosis*, and *Salmonella typhimurium* [65]. In a Wellcome Trust Case Control Consortium (WTCCC) GWAS, two flanking IRGM polymorphisms (rs13361189 and rs4958847) were significantly associated with CD, with replication in an independent panel ($p=2.1 \times 10^{-10}$) [61, 62]. Extensive re-sequencing of the IRGM coding region did not reveal any obvious causal variants associated with CD, suggesting that the causal variants are *cis*-regulating and in linkage disequilibrium with the associated variants. McCarroll et al. showed that the deletion allele of a 20-kb copy number variant (CNV) 1.6 kb upstream of IRGM was significantly associated with CD ($P<0.01$) and is completely correlated ($r^2=1.0$) with the CD risk allele at the SNP rs13361189 [66]. They subsequently compared gene expression using quantitative allele-specific methods and discovered a strong difference in the extent to which the risk and protective variants are expressed in different cell types [66]. Recently, Prescott et al. [67], found an additional component to this IRGM picture. They describe a small insertion/deletion polymorphism in the promoter and 5'-untranslated region of IRGM that is strongly associated with CD and serves as an independent risk factor from the previously described CNV. The CD risk haplotype carrying the indel is associated with a significant decrease in IRGM expression ($P<10^{-12}$) in untransformed lymphocytes from CD patients. Interestingly, analysis of these variants in a Japanese CD case-control sample and of IRGM expression in HapMap populations revealed that the IRGM insertion/deletion polymorphisms and the CNV were not associated with CD or with altered IRGM expression in the Asian population. They propose that the involvement of the IRGM risk haplotype in pathogenesis of CD requires other genetic factors or environment interactions which may be absent in Asian populations. Alternatively, none of the variants described are causal, and that the true risk variants are more recent in evolution and thus absent in Asian ancestry individuals [67].

Alterations in bacterial sensing or antigen-processing point to innate immune defects. This is a central feature of CD as evidenced by the associations of SNPs in NOD2, ATG16L1, and IRGM with CD but not UC. Further studies examining the altered function of CD-associated polymorphisms will be important in establishing the extent to which Crohn's disease represents a primary innate immune defect in bacterial processing.

Genetic Variants Associated with CD and UC

Polymorphisms in cytokine genes and cytokine pathway genes are associated with both CD and UC. Duerr and colleagues completed the first large-scale GWAS in IBD of 308,332 SNPs [68]. A non-synonymous SNP (rs11209026 Arg381Gln) in the interleukin 23R gene conferred significant protection against IBD. Heterozygous carriage of the glutamine allele confers an approximately threefold increase in protection against developing CD, with a more modest protective effect observed in UC. Several other SNPs within IL23R were also shown to be associated with ileal CD. Further replication studies for IL23R have been published in several adult and pediatric IBD cohorts but IL23R is also associated with autoimmune diseases including psoriasis [69] and ankylosing spondylitis [70].

IL23 is a regulatory cytokine expressed by natural killer (NK) cells, NKT cells, CD4+ T cells, CD8+ T cells, activated macrophages and DCs [71]. This heterodimeric cytokine discovered in 2000 [72] is comprised of IL12p40 and an IL23-specific p19 subunit. Studies have shown that IL23 has a distinct role from IL12 in end-organ inflammation. Cua et al. [73], elegantly demonstrated this in knock-out mice of the IL23 gene. They showed that in experimental autoimmune encephalomyelitis (EAE), only mice that lacked the IL23 p19 subunit (p19^{-/-}) or the p40 subunit (common to both IL12 and IL23) were resistant to EAE [73]. Subsequent to this other studies have shown that IL23 is the most likely culprit in other chronic inflammatory disorders including joint inflammation [74], psoriasis [75] and intestinal inflammation [76]. In further support of this, the use of an anti-p19 monoclonal antibody to block IL23 expression in *Helicobacter hepaticus* infected and Rag ^{-/-} mice significantly decreased intestinal inflammation and decreased proinflammatory cytokines (TNF- α (alpha), IFN γ (gamma), IL6, IL1 β (beta), and IL17) in the intestine [77]. Interestingly, Ustekinumab (p40-specific antibodies) induced a clinical response in patients with moderate-to-severe CD highlighting a promising approach for the treatment of CD [78]. Future studies will examine the relative safety and efficacy of blockade of both IL23 and IL12 (p40-specific antibodies) compared with blockade of IL23 alone (p19-specific antibodies) in clinical trials.

IL23 expression is enriched within the intestine and mediates T-cell-dependent and independent pathways of intestinal inflammation through effects on Th1 and Th17-producing T cells. Furthermore, IL23 suppresses regulatory T-cell responses in the gut, favoring inflammation. Increased levels of IL-17 expression have been demonstrated in the intestinal lamina propria of patients with UC [79–81]. Functional studies of the effects of the IL23R variants on IL17 expression may shed further light on the role of IL17 in intestinal inflammation.

The IL23 Signaling Pathway

Gene polymorphisms within IL23 receptor (IL23R), IL12B, and STAT3 are significantly associated with CD and UC risk [40, 82]. The mechanism by which these SNPs alter gene function and drive IBD pathogenesis is not clear. However, these genes form part of the IL23 signaling pathway that culminates in the differentiation of effector CD4+ T cells, key components of tissue inflammation. IL23R is an alternatively spliced gene with more than four described mRNA transcripts [83]. It is composed of two heterodimeric subunits. The IL12Rb1 subunit binds to IL12p40 and is shared with the IL12 receptor. The other subunit is IL23R and is specific to IL23. The p40 subunit of IL12 and IL23 is encoded by the IL12B gene located on chromosome 5. The SNP rs3212227 found in the 3' untranslated region of this gene confers increased risk to many chronic inflammatory diseases including IBD [40, 62, 69, 84]. Further, lamina propria mononuclear cells extracted from patients with active CD had increased expression of both IL12p70 and IL23 [85].

Once the IL23 cytokine engages its receptor, it causes activation and dimerization. This leads to the transphosphorylation and activation of Janus kinase 2 (JAK2), a tyrosine kinase. Subsequently, the intracellular domain of JAK2 is phosphorylated resulting in the recruitment of the signal transducer and activator of transcription 3 (STAT3) [71, 86].

The STAT3 proteins form homodimers but can also form heterodimers with STAT1. The dimers translocate to the nucleus and bind to promoter regions of target genes [71, 87]. STAT3 and the transcription factor ROR γ T [88, 89] are important to the differentiation of Th17-producing CD4+ T cells (Th17 cells) [89–92]. In parallel studies STAT4 activation by IL12 or IL23 cytokines culminates in differentiation of Th1 and Th17 producing T cells, respectively [93, 94]. Also, STAT5 activation by IL2 results in a reduction of TH17 cell differentiation [89]. These findings demonstrate the complex interplay of these genes. Understanding this pathway and the distinct role it plays in driving the differentiation of effector CD4+ T cells into Th1 or Th17 cells is paramount to unraveling IBD pathogenesis.

Genetic Variants Predominantly Associated with UC

While much progress has been made in deciphering the genetic architecture of CD, we have only recently started to unravel the genetic susceptibility loci in UC. Although both disorders belong to a spectrum of IBD, monozygotic and dizygotic twin studies reported a more modest contribution of genetics to the predisposition to ulcerative colitis. In addition, IBD genes like NOD2 which were clearly associated with CD did not predispose to UC. Recently, GWAS with large cohorts have dramatically increased our knowledge of genetics in UC [95]. A meta-analysis of six UC GWAS datasets, comprising 6,687 cases and 19,718 controls, identified 29 new risk loci ($P < 5 \times 10^{-8}$), so that a total of 47 UC-associated loci have now been identified [38]. These 47 loci are estimated to explain at least 16% of the heritability in UC. In a study by Franke et al., 50 loci previously shown to be associated with CD were tested for their effect on the predisposition to UC. Polymorphisms in PTPN2, HERC2 and STAT3 were associated only with UC [96]. In a subsequent study of non-synonymous SNPs the strongest UC susceptibility loci mapped to the ECM1 gene [97]. Although the causative variants have not been identified, ECM1 itself is an attractive candidate gene for UC, as it is implicated in the interaction between epithelium and basal membrane and it is involved in NF- κ B activation [98]. GWAS have also reported strong associations for variants within the HLA complex, especially HLADRB1 [62]. Interestingly, HLA variants that are risk markers in UC also correlate with an increased risk for pure colonic CD [99]. However, they are protective against ileal CD, highlighting the major genetic differences between ileal and colonic IBDs. Additionally, an important finding from the most recent GWAS is with SNPs near the 3'-UTR of the IL10 gene at 1q32 [96, 100]. There is some evidence to suggest that UC-predominant associations include genes modulating epithelial barrier function. Specifically, gene associations including CDH [38] HNF4A, LAMB1, and ECM1 [97] have been reported in UC and all play a role in modulating epithelial barrier function.

IL10

It is well-known that IL10 is a very important anti-inflammatory cytokine in intestinal immune homeostasis, and there is growing knowledge that it is potentially involved in the pathogenesis of IBD. The first convincing evidence implicating IL10 in intestinal immune regulation came from the IL10 mouse model in which IL10-deficient mice spontaneously develop enterocolitis [101]. In this model, the inflammation mainly affects the mucosa along the gastrointestinal tract and is characterized by enlarged and branched crypts in the colon, reduced number of goblet cells, degeneration of superficial epithelial cells and increased expression of major MHC class II molecules. An important protective role of IL10 was demonstrated in other models of colitis. For example, the transfer of CD4⁺ CD45RB^{high} T cells to immunodeficient mice did not result in inflammation in the presence of IL10-producing regulatory T cells [102]. Production of IL10 by these cells was shown to be essential for the prevention of colitis. During the healing process, IL10-producing regulatory T cells selectively accumulate within the colonic lamina propria, suggesting compartmentalization of these regulatory T cells at sites of inflammation. Of note, IL10-deficient mice do not develop spontaneous colitis in germ-free environments, pointing to the necessity of intestinal bacteria in the induction of chronic inflammation [103]. The proper function and sufficient production of this cytokine could thus regulate the mucosal immune response to certain enteric flora, preventing the development of chronic inflammation. IL10 was shown to downregulate IL1 β and TNF- α secretion including the messenger RNA levels when added to peripheral blood monocytes and lamina propria mononuclear cells obtained from IBD patients [104]. Physiologically important defects in IL10 signaling in lamina propria mononuclear cells from UC patients were described in the same study.

Early clinical trials of subcutaneous recombinant IL10 showed some benefit in CD patients although not in Phase II trials [105, 106]. Further exploration of IL10 as a therapeutic option in UC should be considered. An additionally important finding is that IL10 appeared to play a key role in preventing tumor development. Studies show that bacteria-induced chronic inflammation is associated with the development of colonic cancer, proposing an underlying mechanism for cancer development in UC [107]. Other agents that trigger inflammation in IBD include nonsteroidal anti-inflammatory drugs (NSAIDs) and stress in predisposed subjects.

Importantly, autosomal recessive mutations in either subunit of the interleukin 10 receptor [108], or cytokine [109] can result in an early onset, severe IBD phenotype. Taken together with the fact that a major phenotype of interleukin 10-deficient mice is the presence of enterocolitis [101] interleukin 10 deficiency contributes to Mendelian form of IBD in humans.

The Future of Genetic Studies of Inflammatory Bowel Disease

In the past four decades there has been a rapid advance in genomic biotechnology. As a result genetic studies, particularly GWAS, have identified almost 100 susceptibility genes which confer an increased risk of developing IBD. Some estimate that the currently identified susceptibility genes account for less than 30% of the genetic contribution to CD susceptibility [39, 40]. The limitations of such an estimation is that it does not take into consideration key factors such as gene–gene interactions or other important cofactors such as changes in the environment, and the intestinal microbiota. We must also address gaps in our knowledge on the role of rare alleles. In addition, many loci are surrogate markers for the true disease causative mutations while others confer risk as part of a haplotype effect.

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Chapter 2

Environmental Factors in the Epidemiology of Inflammatory Bowel Disease

Morten H. Vatn

Keywords Environment • Geography • Socioeconomy • Nutrition • Microbiology • Pharmacology • Smoking • Risk factors

Introduction

The occurrence of IBD has been increasing in Western Europe and North America [1, 2] over several decades after the Second World War. It has been assumed that socioeconomic factors represent the most important explanation for this increase [3]. A part of this increase may have been related to more awareness and recognition of the diseases, as well as generally better registration in all countries.

It is important to realize that the general acceptance of endoscopy as the main internationally accepted diagnostic procedure, is quite young, and that we may divide the history into, a pre-endoscopic area before 1970, an early endoscopic period between 1970 and 1990 characterized by a relatively large heterogeneity among studies, and a post-endoscopic period from around 1990, where after a widespread distribution of equipment and skills of endoscopy enabled all countries to perform uniform diagnostic procedures (Table 2.1). Moreover, after this point in time, most international studies have been performed according to generally accepted definitions and criteria of diagnosis [4].

However, even if we generally include only endoscopy-based studies, the heterogeneity of even the Western materials is striking and difficult to compare, regarding incidence, prevalence, and subtypes. One important reason for this is the selection of cohorts in the different countries. In most centers, the registration of IBD has been hospital based, by which the type of recorded patients were depending on the level of each hospital in the health-care system of each country, including access to health care. Additionally, great variations exist in the recording systems, both between hospitals and between countries, in how well the patients were characterized on the basis of first or later admissions [4, 5]. The centers which have achieved most experience in IBD are second or third line hospitals with large databases, including patients with relatively more complicated disease [6–8].

M.H. Vatn, MD, PhD (✉)

Section of Gastroenterology, Oslo University Hospital, P.O. Box 4959, Nydalen, Oslo 0424, Norway

University of Oslo, Institute of Clinical Medicine, Akershus University Hospital,

1474 Nordbyhagen, Akershus, Norway

e-mail: m.h.vatn@medisin.uio.no

Table 2.1 Registration of IBD

Possible causes for change over time

- Before 1970
 - Retrospective data
 - Hospital based
 - Cross sectional studies
 - Unclear definitions
 - Pre-endoscopic period
- After 1970
 - Early endoscopic period
 - Defined populations
 - Prospective registration
 - GP/hospital based
- After 1990
 - Endoscopy-based diagnosis
 - International criteria
 - Subgroups: proctitis/indeterminate
 - Controlled on specialist level
 - Follow-up controls
 - Possibility for “Case control” studies

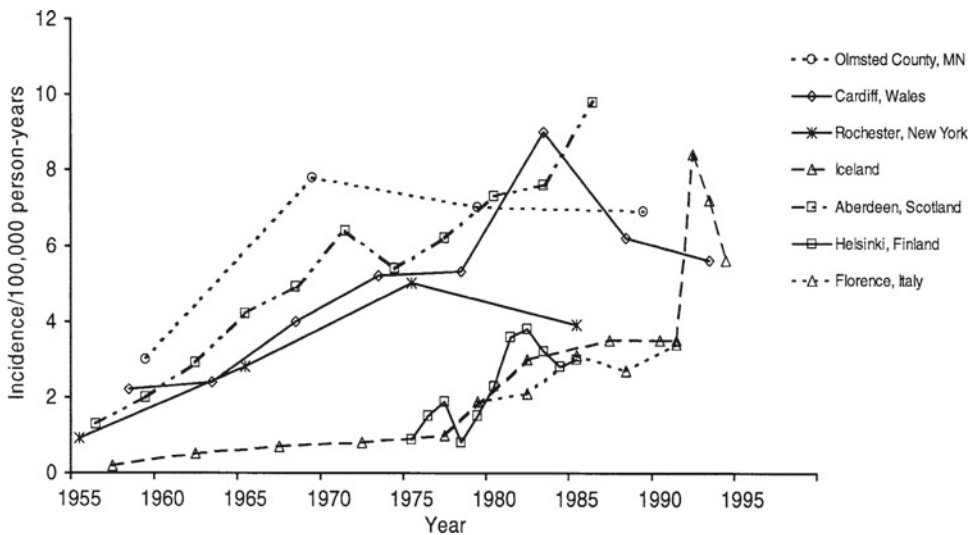


Fig. 2.1 Temporal trends in incidence rates (cases per 100,000 person-years) of Crohn's disease in selected areas (Olmsted County, Minnesota; Cardiff, Wales, UK; Rochester, New York; Iceland; Aberdeen, Scotland, UK; Helsinki, Finland; and Florence, Italy). [Reprinted from *Gastroenterology*; 126(6). Loftus E. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences:1504–17.©2004. with permission from Elsevier]

In population-based studies, in which the cohorts better represent the total number of patients with IBD in an area, increased number of patients are recorded with light to moderate disease and less complications, and with a relatively higher age at diagnosis [8–14].

In spite of the variation in incidence and prevalence of IBD between the Western countries, the recognition of increased occurrence has been a common feature (Figs. 2.1 and 2.2). Although

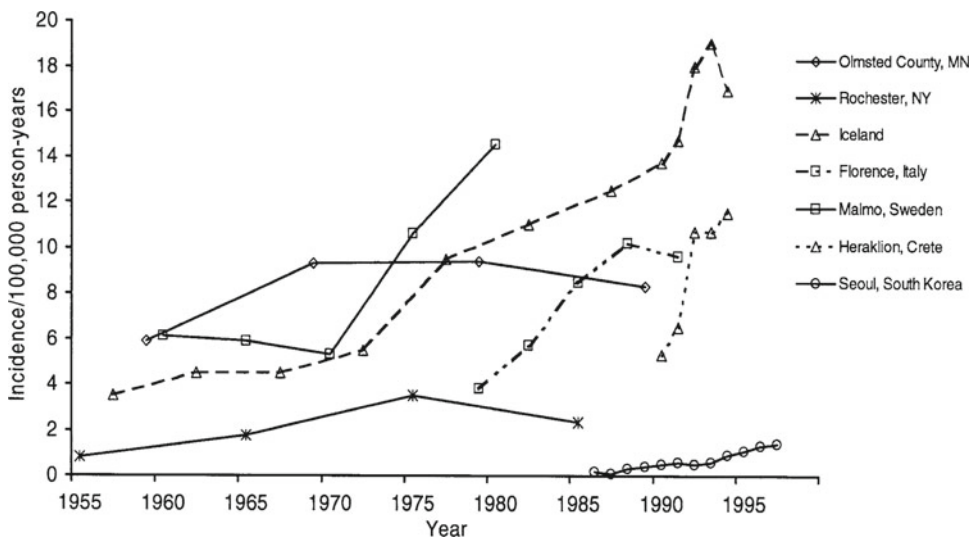


Fig. 2.2 Temporal trends in incidence rates (cases per 100,000 person-years) of ulcerative colitis in selected geographic regions (Olmsted County, Minnesota; Rochester, New York; Iceland; Florence, Italy; Malmö, Sweden; Heraklion, Crete, Greece; and Seoul, South Korea). [Reprinted from *Gastroenterology*; 126(6). Loftus E. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences:1504–17.©2004. with permission from Elsevier]

follow-up studies have given increased knowledge of outcome of disease, repeated prospective studies on incidence have only recently been performed [9], and mostly in children (Figs. 2.3 and 2.4; Tables 2.2 and 2.3) [15, 16]. These studies tend to suggest that the incidence of CD may still be increasing in the Western world, despite signs of a stable frequency of UC in the same cohorts [15–17]. In spite of the reported higher incidence rates of CD than of UC from Canada and the middle of Europe, UC is the predominant phenotype of IBD in the rest of Europe. Moreover, studies from certain areas of Northern [18, 19] and Eastern Europe [20], as well as New Zealand [21], may suggest that UC is still increasing among adults.

When looking for a cause relationship behind CD and UC, the environmental factors of importance mainly seem to be related to the Western life style. Nevertheless, the variation in life style, between countries and areas within countries, is great. Additionally, the emerging increase in prevalence reported from outside the Western countries, makes the focus on environmental factors even more important. A burning question is therefore, whether certain specific risk factors for the development of IBD are related to increased socioeconomic status, regardless of geography, and in addition to public awareness and access to health care? Additionally, we have to bear in mind that in diseases like IBD, with a multifactorial etiology, different risk factors may cause imbalance of the environmental–host relationship in different parts of the world. Suspected consequences of industrialization might not necessarily be relevant for disease development in different geographic regions, although our traditional reductionism of logic thinking tends to look for a simplified explanation for cause relationships.

In the following, it seems necessary to relate environmental factors to the reported occurrence of IBD in the different geographic areas, and thereafter discuss the degree of potential risk factors of disease present in each specific region, to the best of our present knowledge.



Fig. 2.4 Incidence of CD in Children (Table 2.3)

Table 2.2 Incidence of pediatric IBD in the Nordic countries

Country	Year	Incidence of CD	Incidence of UC	N
Norway (IBSEN)	1990–1993	2.7	2.0	29 <16 years
Norway (AHUS)	1993–2004	2.8	3.9	49 <16 years
Norway (IBSEN II)	2005–2007	6.7	3.9	48 <16 years
West Norway	1984–1985	2.5	4.3	27 <16 years
Denmark	1998–2006	3.1	1.6	50 <15 years
Sweden	1990–2001	4.9	2.5	152 <16 years
Finland	1987–2003	1.9	3.9	604 <18 years

Table 2.3 Incidence of pediatric IBD in Europe

Country	Year	Incidence of CD	Incidence of UC	N
Scotland	1981–1995	2.5	3.8	665 <16 years
	1980–1990	2.2		107 <16 years
	1990–1999	4.4		107 <16 years
Iceland	1990–1994	8.5	2.6	38 <16 years
Wales	1996–1997	1.36		
	1996–2003	3.6		39 <16 years
England	1998–1999	3.1	5.2	739 <16 years
Checkoslovakia	1990–1999	1.25		470 <15 years
	1999–2001			
North France	1988–1999	2.3	3.1	509 <17 years
Netherlands	1999–2001	2.1		
			7.3	220 <18 years

Geography

Variation Between Countries

The fact that IBD occurs with the highest frequency in the Western world is undisputable, and the experience is based on hospital materials from the large centers in Europe and the USA. These areas also have in common that remarkable increasing prevalence rates have been recorded during the second half of the twentieth century [1, 2] (Figs. 2.1 and 2.2).

In addition to that, differences between regions of Europe and North America have been recorded. In Europe, a North–South gradient for incidence rate, phenotype, and recurrence, has been demonstrated [10, 22, 23] based on modern diagnostic procedures and prospective follow up.

Interestingly, the highest incidence rates of both the North and South of Europe have been demonstrated in the islands of Iceland and Faroe Islands [10], and the islands of Crete and Sicily [10], respectively, (Fig. 2.5). This might raise interesting questions regarding both genetic and environmental explanations. The recently reported high incidence rates of IBD in New Zealand may also contribute to this discussion. In Japan, most of the experience in IBD is based on the reports from large hospital-based centers, all reporting on increased prevalence rates, although definitively much lower than in the Western world [24]. Some reports have also come from South America [25].

Racial differences of IBD prevalence rates have been reported from North America [26], showing much lower rates among Hispanic and Asian people compared to whites and African Americans. High prevalence rates for Crohn’s disease and ulcerative colitis have also been shown for North American Ashkenazi and Israeli Jews [27, 28]. The suggested effect of ethnicity on disease location, complications, and anticipation may be partly explained by genetic and environmental factors [25–27].

Developing regions have traditionally reported lower prevalence of IBD, which seems to increase, probably as a consequence of a rising incidence of IBD in many of these nations, such as India and China, as they have become industrialized [29, 30].

Furthermore, migrant studies have demonstrated that individuals immigrating from regions with low prevalence to countries with higher prevalence rates are at an increased risk for developing IBD, particularly among first-generation children [30, 31].

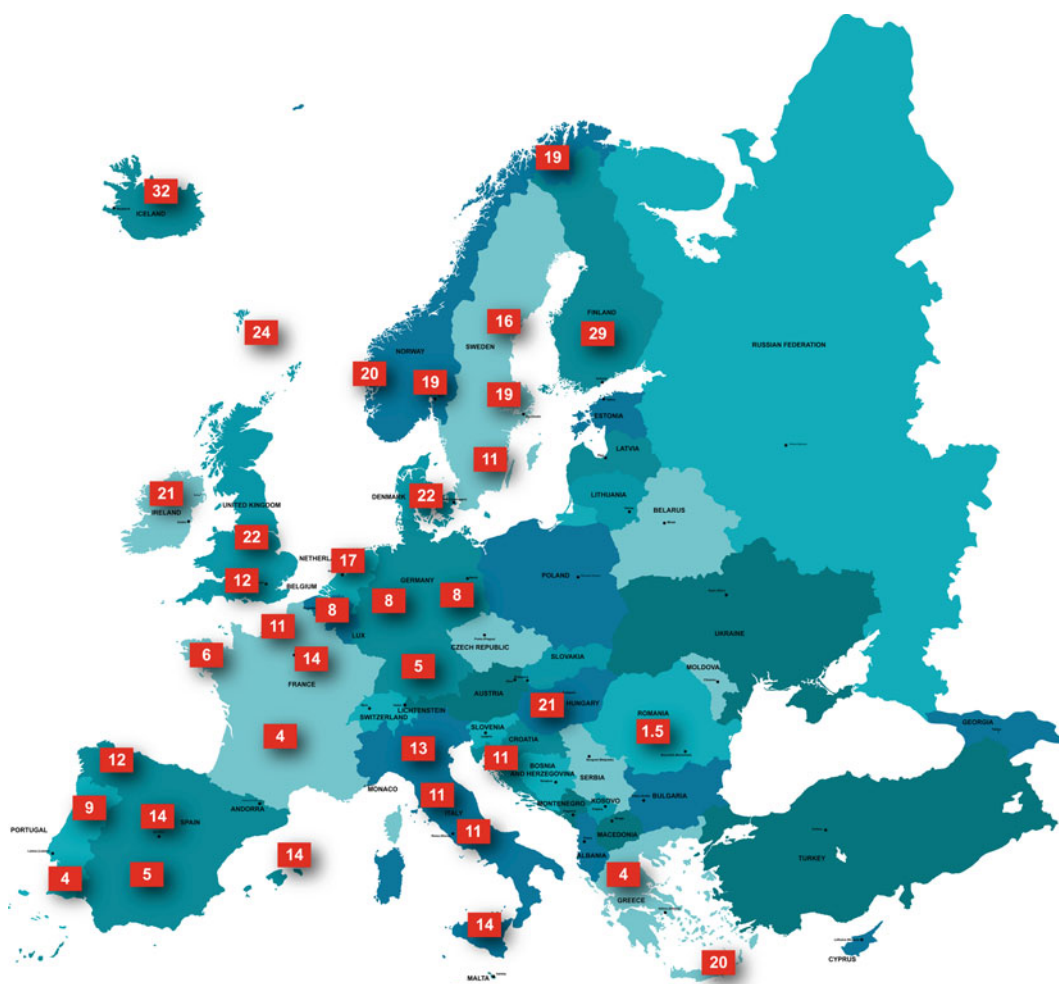
In the USA, also, a North–South gradient has been shown by hospital-based registrations [6, 32, 33], whereas in Canada, an East–West gradient has been demonstrated in a nationwide comparison [13] (Fig. 2.6). Moreover, the population-based registry of Manitoba, Canada [13] has demonstrated some of the highest incidence rates of IBD in the world.

Other data on intracontinental differences of IBD in general are few, and mostly based on results from single countries. Standardized population-based data of comparisons between the East and West of Europe are underway.

Variations Within European Countries: Population-Based Studies (Fig. 2.7)

In Europe, great differences have been reported regarding variation in frequency of IBD between centers within countries. In Greece, the island of Crete showed a markedly higher incidence of IBD compared to Joannina in the North [10] similar to a higher incidence in Sicily compared to the North of Italy [10]. Based on comparisons on the Italian continent, also a North–South gradient is indicated, similar to a North–South gradient in Portugal [10].

In France, a higher incidence and prevalence in the North compared to the South has been reported, based on a partly hospital-based Nationwide registry [34].



Within Germany, Spain, and the UK, National variations based on direct comparisons have not been reported, although variations between single center studies are obvious within countries. High incidence rates have been reported from Ireland, Scotland, and the Netherlands compared to the UK and Western Germany [10]. Many of these differences, however, might be explained by variation in type of cohorts and organization of health care.

From Denmark and Norway, generally high incidence rates have been reported in population-based studies [1, 10–12]. In Norway, similar incidence rates have been shown between the Northern [35], Western [36], and the South-eastern part [12], whereas great differences were shown between counties for the two latter areas compared to a more even distribution within the former [35]. This could be explained by a generally mixed urban rural population in the North and a better separation between urban and rural areas in the two others. Both the Western and South-Eastern part showed a generally higher incidence rate in the scattered rural populations, opposite to previous international experience, in which urban areas have been considered to be areas of increased risk of IBD.

An explanation for this discrepancy within the literature might be that different risk factors are acting concomitantly within an area in addition to the existence of different risk factors between areas. One should not, however rule out the possibility of variations in efficiency and quality of registration between areas.

In Norway, the counties with most scattered and rural populations were also the areas with only one hospital, in contrast to the many recording hospitals and multidisciplinary doctors in the cities. This gave a variation in incidence rate between 17/100,000 in Oslo and 28/100,000 in the scattered populated area of Aust Agder, with one hospital in the only city of the county.

These data may provide evidence for the importance of access to health care and awareness of the population under examination. To increase the understanding of the complexity of this problem, one might add, that the area with the highest incidence of IBD, had the highest increase in socioeconomic status during the decade prior to the incidence study, as measured by the number of individuals obtaining higher education. Although a much higher level of education was seen for Oslo, this level had been stable during the previous decades. With this respect, a factor that might be of importance in the Western societies is the increasing rate of immigration. Recent reports from the UK, reported on an unexpected high prevalence rate of IBD among immigrants from South-eastern Asia [37].

Relationship Between UC and CD

Although a change in socioeconomic level seems to be a common risk factor for UC and CD, it is important to note that these diseases may react quite differently, not only genetically, but also to environmental risk factors. A solid background of geographic examinations is therefore important.

In addition to variations between countries and regions regarding incidence and prevalence of IBD in general, the ratio between UC and CD has also shown geographic variations. A generally higher incidence rate for UC than for CD has been shown both in the North and the South of Europe, but with a smaller difference in the South [9]. Both in Canada and the USA, CD seems to occur with a higher frequency than UC [13], similar to Northern France [10]. Since this now also seems to be the case for Southern Germany [38] and parts of Eastern Europe [20], we may no longer describe this as a French enigma, but rather as a tendency for middle Europe. The variation in ratios of UC/CD between countries and areas might reflect differences in environmental risk factors, although genetic predispositions may occur. It might be interesting to note that in Europe, the incidence of NOD2-mutations seems to be highest in the middle part, corresponding to the region with an increased CD to UC ratio.

Although unsolved questions exist regarding patterns of IBD in Europe, the risk factors and frequency of IBD in Eastern Europe must principally be regarded as related to the same socioeconomic trends as the rest of the continent and North America, in contrast to the developing countries. It is, therefore, relevant to discuss the environmental risk factors of IBD generally for the whole Europe as one area.

The question, if the incidence and prevalence rates of IBD still are increasing, is generally an unsolved question in most parts of the world. The reasons for this are different in the Western world compared to the developing countries. In the USA and Europe, few data exist based on comparable prospective studies performed during different time periods within the same area. A study from Copenhagen may suggest that the incidence of CD in adults is still increasing during the last decade [9]. In children, however, studies from several countries have suggested that the incidence still is increasing for CD but not for UC [15, 16]. The relationship between this increase and immigration is unclear, but studies on the risk of acquiring IBD among first-generation immigrants are underway. In the recent study from Oslo [17], the incident cases of CD representing first-degree immigrants from developing countries, were partly responsible for the 100% increase in incidence of CD over the last decade. To what extent the shift of environment has an impact on the development of IBD, will have to be focused in the future.

The geographic difference between the North and South of Europe was the same for UC as for CD [10, 22, 23], also with regard to outcome and complications. For the above-mentioned reasons, it is not quite clear if the incidence of UC has stabilized or in some areas still is changing. A recent study from Finland indicates a dramatic increase in UC, based on partly population-based data from a regional registry. For all registry-derived data, some uncertainty exists, regarding reliability of the recording system over time. On the other hand, since recently also an increase of UC has been suggested in Hungary, a combined causality of environmental factors and ethnicity could explain a parallel increase in Finland and Hungary.

Reports from developing countries on the incidence and prevalence of IBD are still missing as regards population-based studies. Regional studies from India [24], may, however, suggest an increase over time in well-defined regions. Documented increase of incidence or prevalence in Japan would, however, be of particular interest, since Japan may be the only country in Asia with stable socioeconomic conditions over several decades. An eventual increase in frequency of IBD would then have to be related to other than direct socioeconomic factors, and rather to other changes in the environment or life style, such as dietary habits.

Environmental Factors

Relationship to Microbiology

Today, the most important environmental factor, with a cause relationship to the development of IBD, is considered to be related to an imbalance in the microbial–host relationship with mucosal barrier dysfunction and reduced microbial diversity [39]. The hygiene hypothesis is an attempt to explain why improvement of hygienic conditions may result in intestinal dysbiosis as a primary event, resulting in IBD among genetically predisposed individuals. This hypothesis implies that the rising frequency of immunologic disorders can be attributed to lack of childhood exposure to enteric pathogens. This dysbiosis may on one hand be characterized by an imbalance between commensal bacteria, and on the other hand, by secondary development of pathogens, which by omitting the immunocompromized cells of the different barrier systems, may lead to chronic inflammation. The suggested “Cold chain hypothesis” represents a more direct explanation of a cause relationship between specific bacteria and the immunocompromized host, from a molecular perspective,

postulating that CD is a result of a defect in the host recognition of pathogenic bacterial components that usually escape the immune response (e.g., Yop molecules), leading to an excessive host response to bacteria, such as *Yersinia spp.* and *Listeria spp.*, which can survive refrigerator temperature [40]. The definition relies on the introduction of refrigeration in society, which was related to the time of increased prevalence of CD. A support for this hypothesis has been reported in case-control studies, partly in combination with other socioeconomic risk factors [41, 42].

In support of the hygiene hypothesis are the generally negative association to the epidemiology of *Helicobacter pylori* [43] and the inverse association to the prevalence of helminthic colonization [44, 45].

It is still an issue if primary pathogens like *Mycobacterium Avium Paratuberculosis spp* (MAP), Jones disease [46], may be an etiologic factor, but problems related to the biologic methodology has been a major concern, and further studies are expected in the years to come. Clinical studies up to now have been inconclusive with regard to the impact of MAP in IBD, and a study of seropositivity showed a high prevalence for IBD, but failed to demonstrate a difference between CD, UC, and controls [47].

Several studies, however, have detected a high prevalence rate of MAP in CD patients, and a meta-analysis of 28 case-control studies showed a positive association, both for enzyme-linked immunosorbent assay (ELISA) and PCR [48].

A recent examination [49], however, performed with highly sensitive methods in intestinal mucosa, could not detect the presence of MAP in newly diagnosed, treatment naïve cases, in contrast to many affected cases among hospitalized CD patients on treatment, in the same catchment area. MAP was not found among patients with long-standing UC. According to these results, MAP is probably not an etiologic factor, but a bystander appearing during the course of disease and appearing in patients on treatment. Another interpretation could be that MAP remains elusive to detection during the early phase of disease, and that a longer duration of immune decompensation is needed for diagnosis by the present methods.

The high prevalence of adherent-invasive *Escherichia coli spp.* associated to ileal CD may represent another primary pathogenic strain of bacteria, which is able to adhere to intestinal epithelial cells, to invade epithelial cells via a mechanism involving actin polymerization and microtubules, and to survive and replicate within macrophages [50].

Other hospital-based studies have demonstrated a geographic covariation related to hospitalization and mortality for IBD and *Clostridium difficile spp.* [51].

Since IBD is most common in the Northern hemisphere, most studies with regard to microbial risk factors have been performed in this region. As, on one hand, one might speculate that improvement in sanitary conditions is responsible for reduced microbial diversity, industrial pollution in society might serve as another explanation for changed environment. It is probably unlikely that the exogenous predisposition for IBD can be explained by one single environmental factor. At the moment, our knowledge regarding possible risk factors derived from industrialization must be divided mainly into primary direct effects of endogenous dysbiosis and secondary effects on this microbial imbalance. The latter explanation will include all the risk factors that will either increase the microbial instability or increase the vulnerability of the host organism.

Relationship Between Environment and Geography

Of factors that may act on the intestinal microbial composition, geography may represent a risk in addition to socioeconomic development. Living on the Northern hemisphere may therefore explain the increased incidence of IBD, only based on this single factor, which might be explained by increasing intestinal dysbiosis. It has been suggested that this risk increases with increasing latitude from the

Equator to the North Pole [52]. This explanation needs support from more comparable studies, which need to be performed by standardized examinations on preferentially unselected materials.

Although latitude alone may represent a risk for development of IBD, the reports from Canada, showing a marked East–West gradient, which at least up to now, might have been the case also for Europe, seems to indicate that contemporary differences within a society or within a region over time, represent the most important risk factors. These two examples of East–West gradients may therefore strengthen the argumentation for industrialization as a main causative factor for IBD. The North–South gradient in Europe does not necessarily depend on the same differences, because industrialization and socioeconomic growth patterns have in part ran more parallel in the North and South of Europe.

Other environmental factors, such as water supply, may act in addition to, or increase, the instability, primarily caused by the dysbiotic intestine. In a recent study, a strong association between iron concentration in the sources of drinking water and the community incidence of IBD, both CD and UC, was found [53]. Other metals showed no association to IBD, opposed to the proposed focus on aluminum as a risk factor [54]. One explanation why inorganic iron might be a risk factor is its known ability to cause oxidative stress, whereas another might be its effect on bacterial growth. The results might generally agree with a role for oxygen radicals in animals and humans [55].

Relationship to latitude might also be explained by changes in sun exposure and vitamin D [56].

Socioeconomic Factors

One might speculate that the role of latitude is part of the North–South gradient in Europe, although other environmental factors, such as diet or socioeconomics, may be responsible for the variation in the occurrence of IBD.

Several studies have reported on increased incidence of both UC and CD in more densely populated areas [57–61]. Both family size and number of older siblings, as well as birth order, have been related to increased risk of UC, and with smaller families and few older siblings related to CD [62], which might be a sign that UC is more directly affected by environmental factors than CD. This explanation was also supported by a shorter interval between first-degree relatives acquiring UC compared to CD [63]. The relationships between these diseases and other household-related conditions, such as pets, are unclear [64–67].

It has previously been reported that both UC and CD are affecting white collar more than blue collar employees [68]. Further studies among German employees suggested that work in the open air and physical exercise were protective, while being exposed to air conditioned, artificial working conditions or extending and irregular shift working increased the risk of IBD [69]. In population-based studies in Norway, the incidence of IBD was higher in rural areas with a recent increase in socioeconomic status, based on years of education, compared to urban areas with a stable high socioeconomic level [12, 36].

Other factors which might be related to socioeconomics are sanitary conditions, which actually formed the basis for the hygiene hypothesis. In an epidemiological study in the UK, the availability of a fixed hot water supply in childhood before the age of 11 was associated with Crohn's disease [3].

Smoking

Smoking has generally been accepted as a risk factor in Crohn's disease, for worsening of the disease course, such as reduced response to treatment, increased relapse rate, and complications [70], whereas in UC, smoking has a protective effect against the same outcomes of disease [23].

Regarding onset of disease, the connection to smoking is less clear, but a meta-analysis showed an OR of 0.58 for UC and 1.76 for CD among smokers [71] in the general population which could implicate that smoking is a part of a primary event, and not only as a secondary factor influencing the course of disease. Passive exposure to smoking during childhood has also been shown to influence the risk of IBD [72]. A possible relationship between age at diagnosis and smoking has also been suggested [73].

No single explanation for the mechanism behind smoking and the onset of IBD has been postulated, but among siblings discordant for smoking, smokers tended to develop CD, whereas non-smokers tended to develop UC [74]. This may suggest an interaction between smoking and genetic susceptibility. In UC, the significantly reduced frequency of pANCA positivity among smokers, and a tendency of increased frequency of ASCA positivity, may either be supportive of such a mechanism or may be explained by other mechanisms, such as direct reaction to yeast, a result of disease activity or exposure to treatment [23].

The meta-analysis of 245 articles [71], reported on evidence for an association between current smoking and CD (OR, 1.76) and former smoking and UC (OR). Current smoking had a protective effect on the development of UC when compared with controls (OR, 0.58). These results confirmed that smoking is an important environmental factor in IBD with differing effects in UC and CD.

Nutrition and Diet

Considerable efforts have been made in the search for nutritional factors related to, and maybe even responsible for the development of IBD. The methodology of this research has been hampered by the problems of confounding factors and the fact that many patients will change their nutritional habits as a consequence of the disease. There are many studies in small cohorts of patients, who claim that intake of certain diet constituents like fat, refined sugar, fruits, vegetables, and fiber affect the expression of IBD. These studies do not provide unequivocal evidence to incriminate any particular dietary factor. A recent survey of Medline and the Cochrane database concluded that, based on the current levels of knowledge concerning dietary risk factors for IBD and the therapeutic efficacy of dietary and nutritional interventions, the results need to be supported by well-designed trials in large cohorts of patients [75].

A multitude of factors, including drug–nutrient interactions, disease location, symptoms, and dietary restrictions can lead to protein energy malnutrition and specific nutritional deficiencies.

Studies have revealed that nutritional deficiencies are relatively common in IBD, both regarding reduced intake of food and vitamin and mineral deficiencies. It is estimated that up to 85% of hospitalized IBD patients have protein energy malnutrition, based on abnormal anthropometric and biochemical parameters [76, 77].

It is clear that nutrition plays an important role in the management of patients with IBD. The need and advice for nutritional therapy is, due to the heterogeneity of the diseases, quite variable and based on the individual subtype of disease, disease stage, and the patients' total situation. Consequently, there are no specific nutritional therapies that may be recommended to all patients.

Attention to weight changes, to eating habits, and to GI symptoms are the best guides for the clinician. Any abnormality, regarding general health, clinical, or biochemical measurements, must be considered as risk factors regarding disease outcome. Nutritional factors of importance for the outcome of IBD represent the basis for prophylaxis against complicated disease and malnutrition. Specific dietary therapy to avoid symptoms and supplements to meet nutritional depletion are active measures to avoid complications to disease. Metabolic dysfunction and secondary osteoporosis and osteomalacia are serious complications related to malabsorption in CD.

Regarding primary risk factors for development of disease, studies have focused on preventive measures and potential risk factors.

In a relatively large surveillance of patients with UC and CD in Italy [78], in addition to the previously documented relationships between these diseases and smoking, the study reported that lack of breast feeding was associated with increased risk of both UC (OR 1.5) and CD (OR 1.9). A meta-analysis of 14 case-control studies reported on a protective role for breastfeeding in both CD and UC [79].

In a French case-control study of incident cases with CD and UC occurring before 17 years of age, performed between January 1988 and December 1997, 140 variables covering familial history of IBD, events during the perinatal period, infant and child diet, vaccinations, childhood diseases, household amenities, and family socioeconomic status were recorded. Among nutritional factors, regular drinking of tap water was protective against IBD, whereas breast feeding was a risk factor [80], opposite to the previously mentioned report [79]. The preponderance of evidence suggests that breastfeeding is a protective factor for IBD, with a greater effect for CD than UC, based on a recent meta-analysis [81].

The role of dietary macronutrients in the etiology of IBD was recently examined in a large prospective cohort of women living in France, aged 40–65 years. Based on questionnaires on disease occurrence and lifestyle factors that were completed every 24 months, high total protein intake, specifically animal protein, was associated with a significantly increased risk of IBD [82]. This could fit well with the previously reported association with consumption of fast food for both UC (OR 3.4, 1.3–3.9) and CD (OR 3.9, 1.4–10.6) in a population-based incidence study in Sweden. The study, performed by a questionnaire, covered retrospectively the 5 years prior to diagnosis of IBD [83].

Another study showed that total fat and intake of mono-unsaturated and polyunsaturated fats, as well as intake of vitamin B6, were related to UC, whereas a negative association was found for carbohydrates [84]. A relationship to fat consumption was also found in another study, for UC [75], whereas intake of dietary fiber, fruit, and vegetables were reported to be protective for both [85, 86].

In another prospective controlled study of pre-illness changes in IBD, approximately one-third of patients changed their diet prior to the diagnosis of IBD due to nonspecific symptoms. Of the patients not changing their diet, moderate and high consumption of margarine (OR=11.8 and OR=21.37) was associated with ulcerative colitis, while high consumption of red meat (OR=7.8) and high intake of cheese were associated with Crohn's disease [87].

In a retrospective study performed within 3 years after diagnosis, the results also showed different, but significant associations for both UC and CD with regard to food consumption [86].

Especially, the French study [82], being the first large-scale prospectively performed study of diet recorded before the onset of disease, lends strong support to fat and meat consumptions as risk factors of IBD. Especially, the animal proteins from meat and fish represented a risk factor, whereas dairy and vegetable proteins did not. Again the risks were increased for both UC and CD; however, the study was limited to middle aged females. Nevertheless, the study partly supported the previous findings from Japan, where the reported increase of CD during the period 1966–1985 was strongly associated to increased intake of animal protein and somewhat less to increased $n-6/n-3$ polyunsaturated fatty acid ratio [88], by multivariate analysis.

A nested case-control study of a prospective cohort study within seven regions in Europe, identified linoleic acid, in contrast to docosahexaenoic acid, as a significant risk factor for the development of UC [89], however, failed to find a significant association between micro- or macronutrients and disease, based on data from partly the same regions [90]. The evidence for these dietary risk factors in IBD, therefore, has to await further documentation from different populations and subgroups of patients in the future.

Based on epidemiological data, case-control studies, and search in Medline, it has been speculated that the reported relationships between changes in food consumption would fit, in a timely manner, with a change of intestinal microbes associated to IBD [91]. A time relationship between IBD and change in dietary consumption has also been reported for intake of carbohydrates.

Case–control studies from Germany [92] and the UK [93] demonstrated an association between intake of sugar and CD. Another case–control study from the UK showed that intake of sugar and smoking were separate but interactive risk factors [94]. The association between both monosaccharide's and disaccharides and CD was also shown in Israel [95], Japan [96], and Italy [97]. The general question regarding the carbohydrate hypothesis is, to what extent reporting of increased consumption is related to early change of diet due to onset of disease, or if it represents an etiologic factor. This question may also be raised regarding the increased frequency of bran eaters among patients with CD [98].

Another factor of increasing interest is the host response to yeast. Several studies have shown increased IgG and IgA antibodies to baker's yeast (*Saccharomyces cerevisiae* spp.) in patients with CD but not UC [99, 100] as a consequence of intake of wheat. A recent report from studies in twins, however, suggested ASCA to be a marker of shared environment but with a genetic susceptibility, other than NOD2/Card15, as regards the titer level [101].

Recent reports have focused on the possibility of a nutrient–gene interaction, which might be a part of an individualized immunogenic therapy in the future [102].

Mechanisms by way of food consumption might also be further elucidated by studies on the role of epigenetic factors for the development of IBD in the future [103, 104].

Micronutrients and Microparticles

Both in food and water supply, metals and minerals, as well as other microparticles, are abundant, and as such more common as part of pollution in industrialized areas. These particles may act in different ways with the immune system, causing primary or secondary effects. It has been suggested that exposure to xenobiotic-like metals may induce immune responses in autoimmune diseases. Such reactions have been related to effects of mercury [105], cobalt, zirconium, beryllium, silver, and aluminum [54]. Especially aluminum is ubiquitous in the Western culture and represents the most widely used trace element in food, water, soil, and pharmaceutical agents. Moreover, food additives and processed foods, such as cheese, baked goods, grain products, cake, and pancake mixes, vending machine powdery, milk, cream powder substitute, and soy-based milk formulae, sugar and frozen dough, add substantial amount to Al intake. Additionally, different substances, when added to water and even water purification procedures, may increase the bioavailability and toxicity in aqueous organisms resulting in facilitating Al entry into the food chain. On these grounds, a hypothesis of a bacterial–metal interaction was put forward as a factor in CD induction [106].

In line with this, the recently reported strong association between iron concentration in the sources of drinking water and the community incidence of IBD, both CD and UC, may support a bacterial–metal interaction [53]. In this study, however, other metals showed no association to IBD, opposed to the proposed focus on aluminum as a risk factor in IBD. Interactions between microparticles and the immune system, possibly by accumulation in macrophages, has also been postulated as a basis for the use of low microparticle diets in the treatment of IBD [107].

Nonsteroidal Anti-inflammatory Drugs

Nonsteroid anti-inflammatory drugs have been considered as potential risk factors for outbreak of inflammation, relapse, and increasing activity of established IBD.

Several hypotheses have been put forward on the pathophysiology of intestinal damage by NSAIDs [108], such as, enhanced intestinal permeability, inhibition of cyclooxygenase (COX), enterohepatic recirculation, and formation of adducts. The effects of COX-2 selective inhibitors, which appear to have better gastric tolerability when compared to nonselective NSAIDs, on normal and inflamed intestinal mucosa, as in Crohn's disease or ulcerative colitis, are still largely unexplored. If COX-2 inhibition plays a key role in suppressing the inflammatory process, recent evidence suggests that COX-2 products are involved in maintaining the integrity of intestinal mucosa, in the healing of gastrointestinal ulcers and in the modulation of inflammatory bowel disease (IBD). Animal models of intestinal inflammation have so far yielded conflicting results on the effects of COX-2 selective inhibitors on the intestinal mucosa. It is now clear that NSAIDs do not act through cyclooxygenase inhibition alone, but also have different effects on targets, such as nuclear factor-kappaB and/or on peroxisome proliferator-activated receptors (PPAR). The peculiar pharmacological profile of each compound may help to explain the different impact of each NSAID on the inflammatory process and on IBD. Notably, the salicylic acid derivative 5-ASA is widely used in the treatment of IBD and is believed to act through nuclear factor-kappaB interaction. Although the use of COX-2 selective inhibitors remains contraindicated in patients with IBD, studying their effects on intestinal mucosa may offer new insights into their subcellular mechanisms of action and open new avenues for the development of novel therapies for IBD.

The general agreement that NSAIDs increase intestinal permeability, still makes these drugs potential risk factor for both exacerbation of disease, relapse rate, and increasing activity [109].

Recent studies of tolerability of selective Cox-2 inhibitors have demonstrated that these drugs are safe and beneficial in most patients with IBD and not associated with exacerbation of the underlying IBD- and GI-related complications [110].

Oral Contraceptives

Several studies have reported an increased risk of IBD following the use of oral contraception [111]. The association has been shown especially for Crohn's disease, in contrast to ulcerative colitis, and an interaction has been shown for current smoking [111]. This risk was also shown in a population-based case-control study from the USA. Women who reported oral contraceptive use within 6 months before disease onset were at increased risk for both diseases compared with never users. Women who had used oral contraceptives for more than 6 years had the highest risk of Crohn's disease (RR=5.1, 95% CI 1.8–14.3). In contrast, increasing duration of use was not associated with increased risk of ulcerative colitis. There has been a concern that females on contraceptives are at increased risk of disease relapse and other adverse events, such as thrombosis [112]. In this research of 207 articles in PubMed, results gave little evidence to suggest an increased risk of disease relapse among women with IBD who use oral contraceptives, and there seemed to be no differences in the absorption of higher-dose combined oral contraceptives, between women with mild ulcerative colitis or small ileal resections and healthy women.

A recent meta-analysis reported on a positive association for use of oral contraceptives and both UC and CD [113], with a reduced effect upon discontinuation. The study was not able to show an effect of dose reduction.

Although most of the literature recommend oral contraceptives in IBD, however, monitoring for thromboembolic events has been recommended [114].

Seasonal Variability

It has been speculated that the reported seasonal variations of relapse rate in Ulcerative colitis [115] or incidence in UC [116], may be explained by change in environmental risk factors throughout the year. In the Swedish cohort study [115], a significantly higher relapse rate was found during the summer, whereas a significantly increased incidence rate of IBD was found during the early winter months of December and January in the Norwegian population-based incidence study. A recent controlled cohort study based on the Hospital Episode statistics (HES) in the UK of all admitted IBD patients between 1997 and 2006 did not show a seasonal birth pattern [117]. By monthly comparison year by year, different fluctuations were found for CD and UC, with a weak but significant correlation (0.078, $p=0.018$). A slight trend for stronger correlation occurred during the later decades. Nevertheless, the author concluded that patterns of birth dates among IBD patients do not support the contention that seasonally or monthly varying environmental factors during early childhood shape the subsequent risk of developing IBD.

In a study from the USA, norovirus was associated to exacerbation of both UC and CD in pediatric patients, in all cases associated with bloody diarrhea, and with demand for hospitalization. This was in contrast to diarrhea without hematochezia when the infection occurs in the absence of IBD [118].

Based on these observations, the relationship between seasonal environmental risk factors may affect the outbreak of IBD differently according to the occurrence of risk factors around the world. One cannot rule out the possibility that also perinatal risk factors might be of importance at the presents of combination of risk factors, such as in selected areas or individual groups. Large nationwide studies might dilute important local variations, which might seem negligible when the results are not broken down into smaller regions, such as communities [119].

Appendectomy

The inverse relationship between previous appendectomy and ulcerative colitis has been confirmed in several studies. In a study including 213 patients with UC, 110 with CD and 337 controls, a highly significant association to appendectomy was found for UC (OR 0.20), and even higher when the operation was performed before the age of 20 (OR 0.14). No association was found for CD, and no association for tonsillectomy for either disease [120]. Moreover, the study from Spain also showed that appendectomy was less frequent, not only among UC and CD patients, but also among their relatives [120], compared to the general population. A case-control study from Iran confirmed the inverse relationship between appendectomy and UC in contrast to Crohn's disease [121]. A population-based study from New Zealand reported that not only appendectomy, but also tonsillectomy, infectious mononucleosis, and asthma were more common in CD patients than controls [122]. Another case-control study from Spain [123] showed that both appendectomy and current smoking were protective for UC. Additionally, this study showed that better living conditions during childhood were associated to increased risk for IBD. A case-control study from Greece [124] could neither confirm a significantly inverse relationship to UC (OR 0.6) nor a significant association to CD (OR 2.2), although well-known risk factors, such as family history and smoking were confirmed. A multivariate regression analysis, however, showed positive associations between appendectomy and tonsillectomy for CD, but no independent inverse association to UC.

With some variation in results, however, the literature shows strong relationships between previous appendectomy and the development of CD, and inversely to UC, which again might indirectly support the impact of the hygiene hypothesis as an explanation for the importance of socioeconomic conditions in the development of IBD.

General Remarks and Future Aspects Regarding Global Environmental Risk Factors in IBD

The evidence for increased frequency of IBD in the industrialized parts of the world is strong and is mainly explained by environmental risk factors. Of all the identified risk factors, not a single one alone may up to now, totally explain the increasing incidence in any part of the world. This might be explained by a multiplicity of potential routes for disturbances of the microbial–host interactions. It is also possible that the strength of influence by risk factors or lack of protective factors in a society is different, depending on geography or urbanization.

It is still possible that a common single explanation for the microbial–host imbalance will be found in the future, based on an explanation which up to now has been too difficult to grasp. If we hypothesize that change in human adaption to the socioeconomic burden is related to endogenous changes, caused by individually based stressors of psychoimmunological origin, a model for such a hypothesis is at the moment not at hand. If any one new single explanation for the microbial–host imbalance was found, it might leave all our present risk factors as confounders.

Nevertheless, the increased molecular understanding of disease development in IBD during the last decades must be expected to reveal new and important contributions, also to the evaluation of the different environmental risk factors, together with geographic and individual susceptibility of the person at risk.

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Part II

Immunobiology

Chapter 3

The Intestinal Immune System in Health

Per Brandtzaeg

Keywords Epithelial barrier • Oral tolerance • Pattern recognition receptors • Microbe-associated molecular patterns • Secretory IgA • Polymeric Ig receptor • IgG • IgE • Food allergy • Inflammatory bowel disease

Introduction

Understanding the pathogenesis of inflammatory bowel disease (IBD) requires detailed knowledge about the immunophysiology of gut mucosa and gut-associated lymphoid tissue (GALT). The gut epithelium is monolayered and vulnerable; in an adult human being it covers a surface area of perhaps 300 m² when villi, microvilli, crypts, and folds are taken into account. It is protected by numerous chemical and physical innate defense mechanisms which cooperate intimately with a local adaptive immune system [1]. The dominating component of the latter is an immunoglobulin A (IgA)-generating B-cell population which basically provides an anti-inflammatory first-line defense by mainly giving rise to secretory IgA (SIgA) antibodies performing “immune exclusion” [2, 3]. This term is coined for low- and high-affinity antibody functions at the mucosal surface, aiming to control both microbial colonization and penetration of noxious antigens through the epithelial barrier [4, 5].

The generation of SIgA depends on IgA-producing plasma cells (PCs) and their immediate precursors (plasmablasts) which accumulate in the mucosa by selective homing mechanisms after priming of B cells in GALT – including Peyer’s patches, isolated lymphoid follicles (ILFs) and the appendix [2, 6]. At least 80% of the body’s Ig-producing cells are located in gut mucosa, which therefore constitutes the body’s largest effector organ of humoral immunity [7]. In addition, hypersensitivity due to excessive penetration of exogenous antigens into the mucosa is normally counteracted by adaptive hyporesponsiveness to innocuous agents. This phenomenon is traditionally referred to as “oral tolerance” when induced via the gut; [8] it counteracts particularly overreaction to dietary proteins and components of the commensal microbiota [1, 2, 9].

P. Brandtzaeg, MS, DSS, PhD (✉)

Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Centre for Immune Regulation (CIR), University of Oslo, Department of Pathology, Oslo University Hospital, Rikshospitalet, P.O. Box 4950, Nydalen N-0424, Oslo, Norway
e-mail: per.brandtzaeg@medisin.uio.no

The mucosal induction of all these homeostatic mechanisms depends on immunological stimuli, and the neonatal period is critical in this respect. The mucosal barrier and its reinforcement by SIgA, as well as the immunoregulatory network, require both adaptive and innate induction by antigens and conserved microbe-associated molecular patterns (MAMPs) – the latter activating cellular pattern recognition receptors (PRRs) [1, 10–12]. This adaptation is remarkably successful in view of the fact that a ton of food may pass through the gut of an adult human being every year, usually without causing adverse reactions. In addition, the healthy gut harbors a number of “kind” bacteria that is some ten times the number of cells in the body, perhaps amounting to 10^{13} – 10^{14} microbial cells and a total weight of 1–2 kg [10]. Classical food allergy and IBD apparently reflect lack of this adaptive homeostasis, either due to retarded immunological development and immaturity of the mucosal barrier with its innate defense, abrogation of the epithelial barrier function, or a persistently imbalanced immunoregulatory network – probably on a polygenic susceptibility background.

The mainstream theory explaining the rise observed for allergies and other immune-mediated disorders, such as IBD in affluent societies [13], is based on the extended hygiene hypothesis, underscoring an important role of changes in the environmental microbial impact on the immune system [14]. In essence, the idea is that industrialized societies, by modern measures introduced over the last decades, have deprived the infants of adequate immunological stimuli [15]. The relations between a westernized life-style and the origins of human disease comprise hygienic, dietary, and medical practices that have altered the pattern of microbial exposure, including the composition of the gut microbiota. Therefore, research on microbial–host interactions aims to reverse hypersensitivity by promoting tolerance [12, 16, 17].

For effective strategies to prevent immune-mediated diseases it is essential to understand how exogenous variables influence the adaptive immunological programming and how the effector mechanisms are regulated. The scientific basis of such efforts will be discussed in this chapter.

Induction and Regulation of Intestinal Immunity

Mechanisms Promoting Homeostasis

Numerous genes regulate both the innate and adaptive arms of the immune system. Human immunogenetics has evolved to identify “danger” under the pressure of a “dirty environment,” even long beyond the hunting–gathering period. In this evolutionary process the intestinal immune system has generated its two adaptive anti-inflammatory strategies: immune exclusion performed by SIgA to control surface colonization of microorganisms and inhibit mucosal penetration of potentially dangerous agents; and oral tolerance to avoid local and peripheral hypersensitivity against innocuous antigens which have trespassed the epithelial barrier (Fig. 3.1). Together, the two strategies apparently explain why overt and persistent problems with gut immunopathology are relatively rare. Remarkably, the suppressive mechanisms usually operate successfully in the face of the enormous commensal microbiota separated from the internal body milieu only by the monolayered epithelium with its mucus coat [10, 11, 18, 19]. Notably, however, immunoregulatory differences apparently exist between humans and clean laboratory mice due to a more strict shielding of gut bacteria from the systemic immune system in the rodent species [10, 11, 20–22].

Oral tolerance is clearly a robust adaptive immune function because even healthy adult subjects absorb substantial amounts of intact food antigens, particularly after meals – corresponding to 10^{-5} of the intake and reaching a circulating level of 3–10 ng/ml [23] – as part of the total daily protein uptake of 130–190 g [24]. The epithelial tightness and the immunoregulatory network remain fragile for a variable period after birth [25, 26]. Importantly, animal experiments show that the postnatal

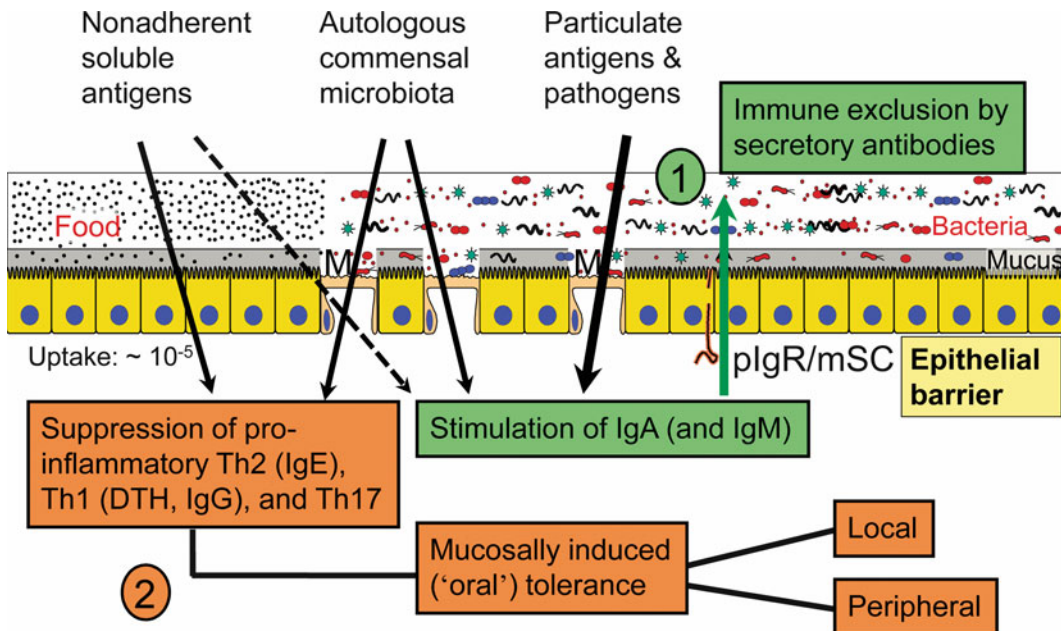


Fig. 3.1 Two-layers of anti-inflammatory mucosal immune defense preserving the integrity of the intestinal epithelial barrier. Depiction of the homeostatic mechanisms: (1) Productive immunity providing immune exclusion to limit epithelial colonization of pathogens and penetration of harmful exogenous antigens. This first line of defense is principally mediated by secretory antibodies of the IgA (and IgM) class in cooperation with various nonspecific innate protective factors (not shown). The secretory antibodies are actively exported by the epithelial polymeric Ig receptor (pIgR), also called membrane secretory component (mSC). Secretory immunity is preferentially stimulated by particulate antigens such as pathogens taken up through M cells (M) located in the dome epithelium covering gut-associated lymphoid tissue (see Fig. 3.2). (2) Innocuous soluble antigens (e.g., food proteins; magnitude of normal uptake indicated) and the commensal microbiota are also stimulatory for secretory immunity (graded arrows), but induce additionally suppression of pro-inflammatory Th2-dependent responses (IgE antibodies), Th1-dependent delayed-type hypersensitivity (DTH), IgG antibodies, and Th17-dependent neutrophilic reactions. This homeostatic Th-cell balance is regulated by a complex mucosally induced phenomenon called “oral tolerance,” in which regulatory T cells are important (not shown). The suppressive effects can be observed both locally and in the periphery

development of mucosal homeostasis depends on the establishment of a balanced commensal microbiota as well as on adequate timing and dosing of dietary antigens when first introduced [8, 10, 19, 26]. The effect of the indigenous bacteria on the postnatal development of GALT is strikingly revealed in experimental animals colonized with a conventional microbiota after being reared in a germ-free state [27, 28]. Also food proteins apparently contribute to the development of GALT and the intestinal immune effector compartments, as observed in mice reared on a diet containing casein compared with a balanced amino acid-based diet [29].

Inductive Gut-Associated Lymphoid Tissue Structures

Intestinal immune cells are located in three compartments: GALT, the mucosal lamina propria, and the surface epithelium (Fig. 3.2). GALT comprises Peyer’s patches, the appendix, and numerous ILFs [2, 6, 30]. These organized lymphoid structures represent inductive sites for intestinal immune responses, while the lamina propria and epithelial compartment principally constitute effector sites but may nevertheless contribute to retention, proliferation, and differentiation of immune cells [31].

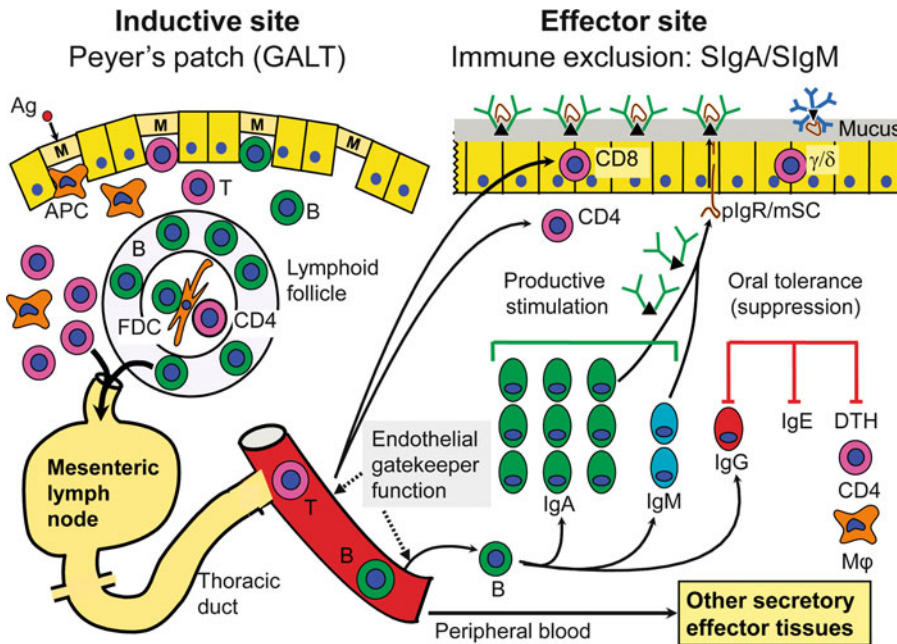


Fig. 3.2 Depiction of the intestinal mucosal immune system. Inductive sites for T and B cells are constituted by gut-associated lymphoid tissue (GALT) such as Peyer's patches with B-cell follicles and M cell (M)-containing follicle-associated epithelium through which exogenous antigens (Ag) are transported to reach antigen-presenting cells (APC), including dendritic cells, macrophages (Mφ), and follicular dendritic cells (FDC). After being primed, naïve T and B cells become memory/effector cells and migrate from GALT to mesenteric lymph nodes via lymph and then via the thoracic duct to peripheral blood for subsequent extravasation at mucosal effector sites. This process is directed by the profile of adhesion molecules and chemokines expressed on the local microvasculature – the endothelial cells thus exerting a “gatekeeper function” for mucosal immunity (see Figs. 3.3 and 3.4). The lamina propria (effector site) is illustrated with its various immune cells, including B cells (B), the approximate proportions of various Ig-producing plasma cells, and CD4⁺ T cells. The distribution of intraepithelial T lymphocytes (mainly CD8⁺ with α/β T-cell receptor; some γ/δ) is also depicted. Additional features are the generation of secretory IgA (SIgA) and secretory IgM (SIgM) via pIgR/membrane secretory component (mSC)-mediated epithelial export. The combined effect of oral tolerance mechanisms, mainly the action of regulatory T cells (not shown), provides a suppressive tone in the gut, normally keeping inflammation driven by IgG and IgE antibodies as well as cell-mediated (CD4⁺ T cells and Mφ) delayed-type hypersensitivity (DTH) under control

Although Peyer's patches are present at birth, it takes some time before they become activated as signified by germinal centers, and the induction of ILF organogenesis depends on postnatal exogenous stimuli, at least in mice [6, 32]. The B-cell follicles of GALT are covered by a specialized epithelium containing very thin “microfold” or membrane (M) cells which, together with intraepithelial dendritic cells (DCs), transport antigens from the gut lumen into the lymphoid tissue [33].

Peyer's patches resemble lymph nodes with interfollicular T-cell zones and a variety of antigen-presenting cells (APCs) such as DCs and macrophages, whereas ILFs have a more sparse T-cell zone. All GALT structures are devoid of encapsulation and contain no afferent lymphatics; [6] their supply of antigens depends exclusively on sampling directly from the mucosal surface through the specialized follicle-associated epithelium. Induction and regulation of mucosal immunity hence takes place primarily in GALT and draining mesenteric lymph nodes (MLNs), while terminal differentiation of B cells to PCs occurs in the lamina propria (Fig. 3.2) where secondary T-cell signals are generated when antigens are presented by local DCs and macrophages [31]. However, animal experiments have shown that oral tolerance can be induced in the absence of GALT, thus being dependent on antigen transport to MLNs from gut mucosa through lymph by specialized DCs, as discussed later [34, 35].

Activation and Homing of Intestinal B Cells

Antigens are presented to T cells in GALT and MLNs by APCs after intracellular processing [1]. The activated helper T (Th) cells release mediators (cytokines), and especially transforming growth factor (TGF)- β induces the switch of B cells from surface membrane expression of IgM to IgA in GALT follicles [2, 6, 31]. Memory/effector cells migrate rapidly via lymphatics to MLNs where B cells may be further differentiated to plasmablasts, which then reach peripheral blood via the thoracic duct and finally become seeded into secretory effector sites (Fig. 3.2). This homing targets particularly the intestinal lamina propria but to some extent also distant secretory mucosae and glandular sites—notably the lactating mammary glands [36].

The extravasation of plasmablasts at effector sites is facilitated by compartmentalized homing receptors interacting with ligands (addressins) on the microvascular endothelium, while additional fine-tuned navigation is conducted by chemokines [2, 6]. The extent of B-cell retention and terminal differentiation to PCs in the gut lamina propria (Fig. 3.2) depends on the local intensity of second signals, provided by chemokines and APC-processed antigens via activated CD4⁺ Th cells and their cytokines [31].

Retinoic acid (RA) derived from vitamin A in the diet exerts a positive impact both on intestinal differentiation of naïve B cells arriving in GALT and their subsequent migration as precursors for the mucosal IgA-producing PCs [37, 38]. Thus, the heavy chain switching to IgA both in humans and mice is enhanced by RA, and so is the expression of the gut-homing molecules integrin $\alpha 4\beta 7$ and the CC chemokine receptor CCR9 (Figs. 3.3 and 3.4). The phenotype of APCs in GALT, which seems to be imprinted by the action of gut bacteria on cellular PRRs, promotes RA generation by the APC expression of retinaldehyde dehydrogenase (RALDH); and the expression of inducible nitric oxide synthase (iNOS) enhances, via nitric oxide (NO), the release of innate switch factors which are cytokine members of the tumor necrosis factor (TNF) family, namely APRIL (A Proliferation Inducing Ligand) and BAFF/BlyS (B cell-Activating Factor of the TNF Family/B lymphocyte Stimulator) [38], as well as the release of the activated IgA switch factor TGF- β from Th cells [39]. Furthermore, in mice it has been shown that a fraction for these follicular T helper (T_{FH}) cells may be derived from regulatory T (T_{reg}) cells [40]. There may thus be a cellular link between intestinal IgA and mucosal tolerance induction, for which TGF- β is likewise important. Also, interleukin (IL)-10 that contributes to terminal differentiation of IgA⁺ PCs together with IL-6 (Fig. 3.3) is an important cytokine in oral tolerance, as discussed later.

The propensity of the mucosal immune system to generate low-affinity cross-reactive background antibodies is probably explained by the extensive innate drive imposed on it by MAMPs derived from the abundant commensal microbiota interacting with PRRs [6, 41]. Thus, experiments have revealed a role of the important PRRs designated toll-like receptors (TLRs) for B-cell differentiation in GALT [6, 41]. Interestingly, human GALT follicles contain the apparatus to support both T cell-dependent and T cell-independent (not involving CD40–CD40L interactions) class switch recombination (CSR) pathways to IgA (Fig. 3.3). This has recently been documented by showing GALT-restricted human expression of activation-induced cytidine deaminase (AID) – an essential enzyme for CSR to take place [42, 43]. Also notable, the T cell-independent switch factor APRIL, and its receptors TACI (Transmembrane Activator and CAMEL Interaction) and BCMA (B Cell Maturation Antigen) are expressed both in human GALT and human intestinal mucosa but, importantly, there is no co-expression of these receptors and AID beyond the GALT structures [42].

Thus, previous claims about B-cell switch to IgA in human lamina propria appear questionable, and the same is true for an extrafollicular switch from IgA1 to IgA2 which has also been proposed [38]. The possibility remains, however, that the unique T cell-independent B1 cell population generated in the mouse omentum [44] may provide a substantial fraction of lamina propria PCs and be subjected to IgA switch either in the peritoneal cavity or in the lamina propria of this species [45, 46]. However, recent evidence suggests that T cell-independent CSR in the mouse gut does depend on GALT

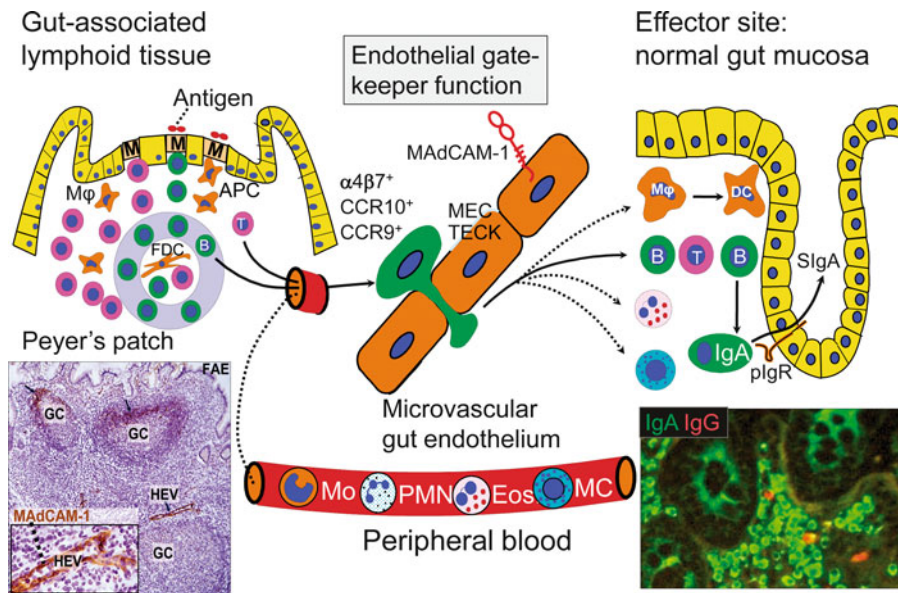


Fig. 3.4 Depiction of homing mechanisms that attract gut-associated lymphoid tissue (GALT)-derived B and T memory/effector cells to the small intestinal lamina propria (*to the right*; see Figs. 3.2 and 3.3). Interactions between the multidomain unmodified (containing no L-selectin-binding O-linked carbohydrates) mucosal addressin cell adhesion molecule (MAdCAM)-1 expressed on ordinary flat lamina propria venules is important as part of the endothelial “gatekeeper function” to direct mucosal $\alpha 4\beta 7$ -bearing memory/effector B and T cells to the normal gut mucosa (*solid arrows*). Selectively produced by the epithelium of the small intestine, the chemokine TECK (CCL25) attracts GALT-derived B and T cells expressing CCR9 to this segment of the gut, whereas MEC (CCL29) is a more generalized chemokine interacting with CCR10 on mucosal B cells. A GALT structure with its M cells (M), antigen-presenting cells (APC) such as macrophages (M ϕ) and follicular dendritic cells (FDC), is depicted on the left. The *bottom left panel* shows immunohistology of a Peyer’s patch with lymphoid follicles containing germinal centers (GC); the *insert* shows that MAdCAM-1 (with L-selectin-binding capacity) is expressed (*brown color*) on high endothelial venules (HEV) to attract naïve lymphocytes for priming. The access to the mucosal effector site is normally limited (*broken arrows*) for circulating proinflammatory cells such as monocyte (Mo)-derived M ϕ , polymorphonuclear neutrophils (PMN), eosinophils (Eos), and mast-cell (MC) precursors, whereas the favored GALT-derived B and T cells promote mucosal immunity including polymeric Ig receptor (pIgR)-dependent secretory IgA (SIgA) generation. Mo-derived mucosal dendritic cell (DC) may develop through a transitional M ϕ phenotype, as indicated. *Right bottom panel* shows paired immunofluorescence staining for IgA- and IgG-producing plasma cells (see color key) in normal colonic human mucosa and crypts with selective transport of IgA to the lumen. Note the negatively stained goblet cells

Intestinal Immunity in Infancy

Only few IgA⁺ plasmablasts circulate in the blood of newborns (<8 per million mononuclear cells), although this number is remarkably increased (~600 per million mononuclear cells) already after 1 month, mainly reflecting the progressive microbial stimulation of GALT [52]. An initial early elevation of circulating plasmablasts (mainly IgM⁺) occurs in preterm infants, especially those with intra-uterine infections [53]. Thus, mucosal immune cells are competent at least during the final trimester, but APCs need to be activated by microbial factors that enable them to provide appropriate co-stimulatory signals to naïve T cells [54]. The commensal microbiota is important to this end as shown by the fact that the number of intestinal IgA⁺ PCs is normalized 4 weeks after exposure of germ-free mice to a conventional gut microbiota [55, 56]. *Bacteroides* and *Escherichia coli* strains seem to be particularly immunostimulatory, but also lactic acid-producing bacteria contribute [57, 58].

The retarded postnatal activation of GALT parallels the functionally decreased systemic immunocompetence in the newborn period [25, 26, 54, 59]. Thus, peripheral CD4⁺ Th cells of infants show reduced capacity for cytokine production and B-cell help. One reason might be that there are relatively few circulating memory (CD45R0⁺) T cells in infancy, although the responsiveness of neonatal naïve (CD45RA⁺) T cells does not differ significantly from that of virgin counterparts in adults. Indeed, the chief explanation for the immunological immaturity in infancy appears to be a deficient APC function [60].

Secretory Immunity and the Epithelial Barrier Function

Formation and Properties of Secretory Antibodies

Most mucosal PCs produce dimers and larger polymers of IgA (collectively called pIgA), which contain a disulfide-linked 15-kD polypeptide called the “joining” or J chain (Fig. 3.5). The J chain is a prerequisite for active dimerization and the export of pIgA through secretory epithelia such as the intestinal crypts [61–63]. This transport is mediated by pIgR, which is also known as membrane secretory component (SC) [30]. J chain-containing pentameric IgM is externally transported by the same mechanism [2, 61, 64].

Apical proteolytic cleavage of the extracellular portion of pIgR enables release of SIgA and secretory IgM (SIgM) to the lumen. In this manner the ectodomain of pIgR is “sacrificed” to

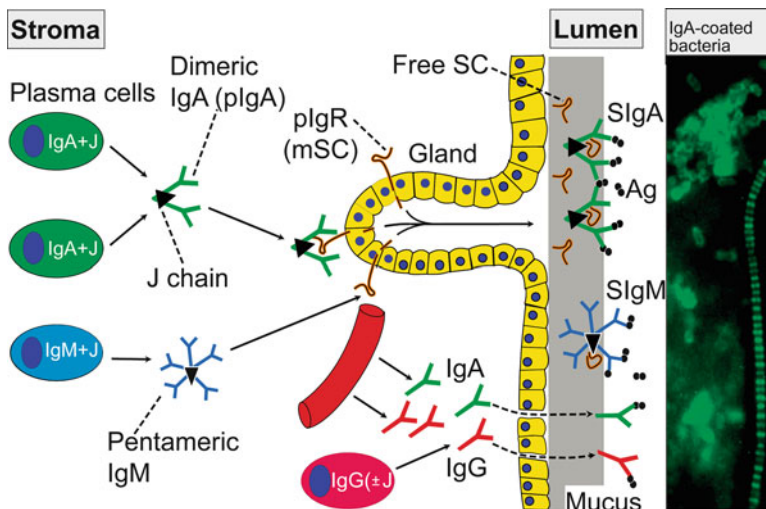


Fig. 3.5 Receptor-mediated export of dimeric IgA and pentameric IgM to provide secretory antibodies (SIgA and SIgM) functioning in immune exclusion of antigen (Ag) at the mucosal surface. Polymeric Ig receptor (pIgR) is expressed basolaterally as membrane secretory component (mSC) on mucosal epithelial cells and mediates transcytosis of dimeric IgA and pentameric IgM, which are produced locally with incorporated J chain ($\text{IgA} + \text{J}$ and $\text{IgM} + \text{J}$) by local plasma cells. Although J chain is often expressed by mucosal IgG plasma cells (70–90%), it does not combine with this isotype and is therefore degraded intracellularly as denoted ($\pm \text{J}$). Locally produced (and serum-derived) IgG is therefore not subject to pIgR-mediated transport, but can be transmitted paracellularly to the lumen together with monomeric IgA, as indicated. Free SC (depicted in mucus) is generated when pIgR in its unoccupied state (*top basolateral symbol*) is cleaved at the apical face of the epithelium like bound SC in SIgA and SIgM. Commensal bacteria in the *right-hand panel* are coated *in vivo* with SIgA, which aids their containment and thereby promotes microbial–host mutualism

become bound SC which stabilizes the quaternary structure of the secretory antibodies, particularly SIgA [2, 31]. Unoccupied pIgR is constitutively exported in the same manner; the cleaved ectodomain is called free SC [30] and exhibits certain innate immune properties such as affinity for *E. coli* and *Clostridium difficile* toxin [3]. Bound SC confers such properties to SIgA and contributes to its mucus-binding capacity and its trapping of bacteria in biofilms [65].

Immune exclusion performed by SIgA and SIgM thus depends on an intimate cooperation between the mucosal B-cell system and the pIgR-expressing epithelium (Fig. 3.5). Serum-derived and locally produced IgG antibodies may also to some extent contribute to immune exclusion when reaching the lumen by noninjurious paracellular diffusion [66] or after being transported by the neonatal Fc receptor, FcRn [67]. However, IgG is rapidly degraded in the gut lumen, although the hepatic superantigen (protein Fv) may form large complexes with degraded antibodies of different specificities, thereby reinforcing their immune exclusion function [68].

As mentioned above, only smaller amounts of pentameric IgM are normally exported by pIgR, and SIgM is not as stable as SIgA because bound SC is covalently stabilized only in the latter [64]. Immune exclusion is therefore normally performed mainly by SIgA (Fig. 3.2) in cooperation with innate defenses such as mucus, defensins, and peristalsis [1, 41]. In newborns and subjects with selective IgA deficiency, however, SIgM antibodies are of greater importance than in healthy adults [25, 31]. Thus, IgA is generally undetectable in the mucosa before 10 days of age, and IgM⁺ PCs often remain predominant up to 1 month. Thereafter a rapid expansion of IgA⁺ PCs takes place, and some increase may be seen up to 1 year of age. Accordingly, only traces of SIgA and SIgM occur in intestinal fluid during the first postnatal period, whereas some IgG is often present – mainly reflecting passive transmission from the lamina propria which after 34 weeks of gestation contains readily detectable maternal IgG [25].

A much faster establishment of SIgA immunity can be seen in developing countries with a heavy microbial load [69]. Some reports suggest that also probiotic treatment enhances the production of IgA, but this was not confirmed by measuring IgA in saliva [70]. Nevertheless, after a combination of prebiotic and probiotic treatment given perinatally and for the first 6 months of life, infants that showed an early elevation of fecal IgA had reduced risk of allergies before 2 years of age [71]. Also, in a recent study it was shown that early intestinal colonization with *Bifidobacterium* species was associated with significantly elevated levels of SIgA in saliva at 6 months of age [72].

The Epithelial Barrier Function

Although so-called “gut closure” normally occurs in humans mainly before birth, the mucosal barrier may be inadequate up to 2 years of age; the mechanisms involved remain poorly defined [73], but the development of secretory immunity is probably one decisive variable. Importantly in this context, pIgR-deficient knockout mice that lack SIgA and SIgM exhibit aberrant mucosal leakiness [74] and have increased uptake of food proteins and commensal bacteria [75]. A thick virtually microbe-devoid inner mucus layer has been revealed at the apical epithelial surface of the normal [76] rodent colon; this protected zone apparently limits direct bacterial contact with host cells, but it may nevertheless be permeable for MAMPs and antigens derived from the abundant microbiota present in the outer mucus layer – particularly when SIgA is absent.

Accordingly, the pIgR knockout mice show significantly elevated production of IgG antibodies to commensal bacterial antigens but, interestingly, not to food proteins [74, 75]. The undue influx of microbial products causes a generalized hyperreactive state with overactivation of the innate cellular NF- κ B transcription pathway, resulting in 50% liability of these mice to anaphylactic death after systemic antigen sensitization (ovalbumin, OVA) and low-dose intradermal challenge [77]. However, the pIgR-deficient mice exhibit enhanced capacity for induction of oral tolerance, which

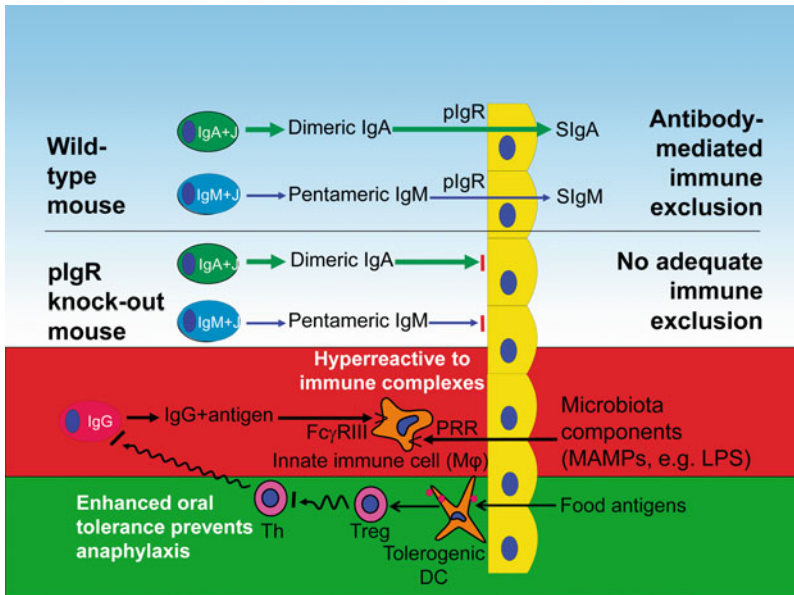


Fig. 3.6 Depiction of experimental model for hypersensitivity and oral tolerance. Lack of secretory antibodies (SIgA and SIgM) in pIgR knockout mice leads to inadequate immune exclusion of bacterial components from the gut microbiota. Such conserved microbe-associated molecular patterns (MAMPs), e.g., lipopolysaccharide (LPS), will interact with pattern recognition receptors (PRRs) on innate immune cells, including macrophages (Mφs), which become hyperreactive. These cells are therefore sensitive to IgG-containing immune complexes interacting with Fc receptors (FcγRIII), which renders the mice predisposed to hypersensitivity (anaphylaxis). The deficient epithelial barrier also allows increased uptake of food antigens from the gut lumen (e.g., fed ovalbumin) which enhances induction of oral tolerance, providing a net anti-inflammatory effect against undue penetration of the same antigen into the body by any route (e.g., dermal). Production of IgG antibodies against this sensitizing antigen will thus be down-regulated, and the animal is protected against anaphylaxis and delayed hypersensitivity (not shown) after antigen challenge. (Adapted from [77]. With permission from John Wiley & Sons, Inc.)

after OVA feeding was fully able to control IgG1- and T cell-dependent hypersensitivity against the same antigen (Fig. 3.6). This observation might imply that at the same time as an inadequate intestinal barrier in the infant represents a risk for hypersensitivity reactions, it will promote tolerance against cognate antigens when they are continuously present in the gut [12].

The postnatal balance between epithelial barrier function and oral tolerance thus appears to be critical for the induction of immunological homeostasis. Notably, although the incidence of food allergy (apparently non-IgE-mediated) is increased in children with IgA deficiency, it is not strikingly elevated [78] – perhaps because the induction of T_{reg} cells is enhanced in addition to compensatory SIgM, which in such individuals partially replaces the lacking SIgA in the gut [31, 79]. Also, bacterial overgrowth occurs in the jejunum of vagotomized patients only when IgA deficiency is combined with suboptimal function of innate defenses such as gastric acid and peristalsis [45, 80]. In this context, it is notable that the frequency of selective IgA deficiency among patients with Crohn's disease in Sweden is significantly increased, that is, one in 100 compared to one in 600 for the healthy population (Lennart Hammarström, personal communication).

AID knockout mice that have SIgA deficiency due to lack of Ig class switching, and also exhibit a defect IgM antibody response, show massive intestinal overgrowth of commensal anaerobic bacteria with a resulting striking hypertrophy of ILFs over time [81]. This development has some resemblance to the irregular lymphoid aggregates seen in long-standing IBD [82]. In contrast to AID and pIgR knockout mice, however, IgA-deficient mice (like IgA-deficient humans) have

compensatory SIgM antibodies in their gut lumen [83]. Interestingly, these mice show no increased susceptibility to various gut infections or to dextran sulfate sodium (DSS)-induced colitis, whereas pIgR-deficient mice do – probably because they lack both SIgA and SIgM [83, 84].

It has been suggested that postnatal hyperreactivity of the immune system may also result from intrauterine events, probably causing genetically or epigenetically determined poor T_{reg} -cell function and immunological immaturity [85, 86], as also suggested from cord blood studies [87]. In normal mice it has been shown that neonatal (but not adult) $CD4^+$ T cells are strikingly prone to differentiate into T_{reg} cells upon stimulation [88]. Moreover, the intestinal epithelium itself is equipped with a vast array of features to control immune barrier homeostasis [89]. These key intrinsic mechanisms have recently been reviewed and include variables such as secretion of mucins and defensins, inflammasome function, intercellular junctional complex regulation, and PRR signalling [90]. As discussed in a subsequent section, the latter is downregulated in the neonatal gut epithelium to preserve its integrity upon the encounter with the commensal microbiota [91, 92].

Additional IgA Antibody Functions

Antibody production by the numerous intestinal pIgA⁺ PCs may also be important for homeostasis within the lamina propria as a result of several anti-inflammatory mechanisms. IgA lacks ordinary complement-activating properties [93] and can therefore block nonspecific biological amplification triggered by locally produced or serum-derived IgG antibodies (Fig. 3.7), which may actually increase the penetration of exogenous bystander antigens through the surface epithelium [94]. This is important in view of the fact that immune complexes are probably formed even within the normal lamina propria due to some influx of soluble antigens, particularly following food intake [23]. Also, in vitro and in vivo experiments have suggested that soluble antigens – after pIgA-mediated noninflammatory trapping in immune complexes – may be cleared by the secretory epithelium via pIgR-mediated translocation to the lumen (Fig. 3.7) [3, 4, 95]. Similar experiments have suggested that pIgA antibodies can neutralize lipopolysaccharide (LPS) and viruses within secretory epithelial cells during pIgR-mediated export [3, 4], and thus return harmful microbial components to the gut lumen (Fig. 3.8). Mouse models have confirmed that the latter mechanism contributes to the intestinal defense against rotavirus infection [96], and IgA⁺ PCs with specificity for rotavirus occur in normal jejunal mucosa of adults with no signs of current infection [97].

The mucosal clearance function exhibited by pIgA (and probably pentameric IgM) antibodies reinforces the immune exclusion mediated by SIgA (and SIgM) with innate-like low-affinity antibody activity generated against commensal bacteria [6, 41, 98], and which to some extent may be cross-reactive (Fig. 3.8). The high-affinity antibodies induced by pathogens or properly adjuvanted oral vaccines may even more efficiently contribute to both immune exclusion and mucosal clearance, as reviewed elsewhere [4].

Locally produced pIgA may further influence homeostasis by interacting with the $Fc\alpha$ receptor ($Fc\alpha RI$, CD89) on leukocytes in the lamina propria. First, on the one hand it has been shown that pIgA-containing immune complexes are able to suppress attraction of neutrophils, eosinophils and monocytes, thereby reducing their proinflammatory activities [99]. On the other hand, when such complexes interact with CD89 on neutrophils, leukotriene B4 is released as a chemotactic factor attracting more of these cells [100]. Second, IgA can apparently downregulate the secretion of proinflammatory cytokines such as $TNF-\alpha$ from activated monocytes [101]. However, it is uncertain whether this mechanism operates in the normal gut (Fig. 3.7) because mucosal macrophages do not express detectable surface CD89 – at least not in the small intestine [102, 103]. Third, neutrophil and monocyte activation that results in generation of reactive oxygen metabolites (“respiratory burst”) is reportedly inhibited by IgA [104]. Conversely, pIgA may temporarily trigger monocytes

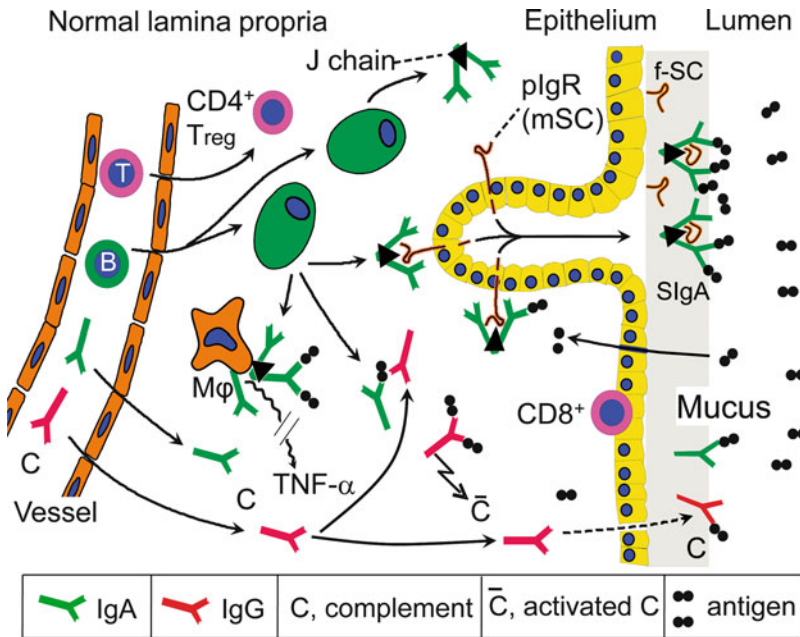


Fig. 3.7 Mucosal homeostasis in the normal gut. Contributing variables are presented as a balance between Ig classes (for simplicity, only IgA and IgG are indicated) and regulatory T (T_{reg}) cells. Secretory IgA (SIgA) is generated from dimeric IgA (with associated J chain) produced by local plasma cells and transported to the lumen by the polymeric Ig receptor (pIgR), also called membrane secretory component (mSC). After apical cleavage, unoccupied receptor is translocated in the same manner and called free SC (f-SC), in contrast to the bound SC in SIgA. The secretory antibodies act in first-line defense by performing antigen exclusion at the mucosal surface (*to the right*). Antigens penetrating the epithelial barrier may meet serum-derived IgG antibodies in the lamina propria. The formed immune complexes can activate complement, and the resulting inflammatory mediators may cause temporarily increased paracellular leakage of IgG antibodies (*broken arrow*). Sustained inflammation is normally inhibited by blocking antibody activities in the lamina propria exerted by serum-derived or locally produced IgA (competition for antigen depicted) and anti-inflammatory T_{reg} cells. Independent of antibody specificity, IgA-containing immune complexes may also inhibit pro-inflammatory mediator release (TNF- α depicted) from activated phagocytic cells such as macrophages (M ϕ). Moreover, antigens bound to dimeric IgA may be returned in a noninflammatory manner to the gut lumen by the pIgR-mediated transport mechanism, as indicated

to enhanced activity – including TNF- α secretion [105] – and IgA (particularly SIgA) appears to be a potent activator of eosinophils [106, 107]. Indeed, complexed SIgA induces respiratory burst and degranulation of these cells, while soluble SIgA enhances their survival *in vitro* [108].

Together, these *in vitro* results suggest that the participation of pIgA in mucosal homeostasis is quite fine-tuned [109] – perhaps being skewed toward a proinflammatory potential in IBD where there are numerous granulocytes [100] along with recently recruited monocyte-like macrophages which express the LPS co-receptor CD14 and PRRs such as TLR2 and TLR4 [110, 111].

Initially, the shift from the normal predominance of mucosal pIgA production to IgG and monomeric IgA in IBD lesions [112] may represent a powerful second line of defense because these antibodies may efficiently mediate immune elimination of penetrating bacteria via phagocytosis and antibody-dependent cell-mediated cytotoxicity (Fig. 3.8). Notably, by means of CD89 both neutrophils [100] and liver Kupffer cells [113] may phagocytose translocated gut bacteria opsonized even with serum-type monomeric IgA. Rodent studies suggest that the liver is designed to handle this antigen elimination in a silent manner because of its unique tolerogenic capacity, including induction of T_{reg} cells by plasmacytoid DCs [114]. Antigen elimination within the intestinal lamina

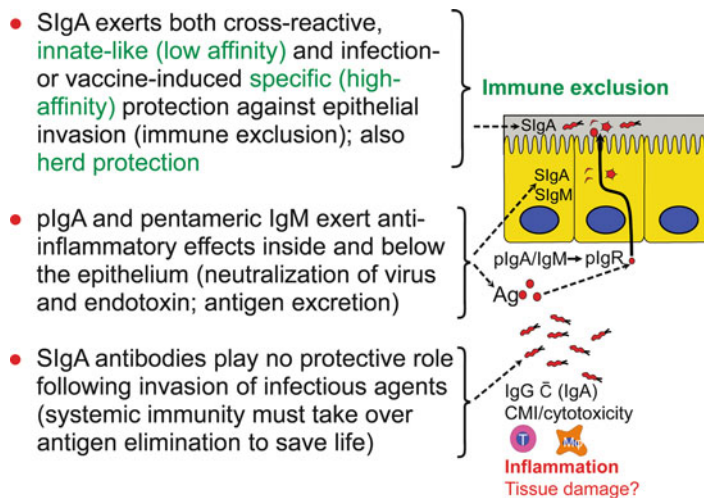


Fig. 3.8 Different principles for how secretory antibodies (SIgA and SIgM) may contribute to mucosal homeostasis. In addition to immune exclusion, the pIgR-mediated external transport of dimeric IgA and pentameric IgM (pIgA/IgM) may be exploited for intraepithelial virus and toxin neutralization, as well as non-inflammatory antigen (Ag) excretion from the lamina propria (see Fig. 3.7). However, when infection with invasion occurs, systemic immunity takes over; this involves proinflammatory mechanisms such as activation of complement ($\bar{C}$) by IgG antibodies, cell-mediated immunity (CMI), and cytotoxicity – all of which may cause tissue damage. Research forming the basis for the depicted mechanisms is reviewed in more detail elsewhere [4, 51]

propria, however, may more readily cause inflammation and tissue damage when systemic type of immunity is involved; this is a risk the immune system sometimes must take to hinder sepsis and save life (Fig. 3.8). The role of mucosal IgA and oral tolerance is to counteract overactivation of potentially harmful immune reactions.

Effect of Microbial–Host Interactions on Innate and Adaptive Immunity

Epithelial Permeability and Secretory Immunity

In fetal life, murine gut epithelial cells are sensitive to microbial factors such as LPS (endotoxin) because they express intracellularly a PRR for this MAMP, namely TLR4 [91]. Exposure to LPS in the vaginal tract during birth activates the neonatal gut epithelium via TLR4 and temporarily upregulates microRNA-146a, which leads to degradation of the TLR signaling molecule IL-1R-associated kinase 1 (IRAK1). Such translational repression of IRAK1 protects the epithelium from microbiota-induced damage during transition from a sterile environment [92, 115]. In remarkable contrast, epithelial tolerance to the commensal bacteria does not occur in mice delivered by cesarean section [91]. These experimental observations may be related to the fact that children delivered by cesarean section appear particularly prone to develop food allergy if they have a genetic predisposition for atopy [116, 117].

Mouse experiments have also demonstrated a crucial postnatal role of commensal gut colonization both in establishing and regulating the epithelial barrier, including upregulation of pIgR expression [118, 119]. Such beneficial effects of microbiota-derived MAMPs appear again to be mediated largely via PRRs, particularly TLRs and similar innate sensors on the epithelial plasma membrane

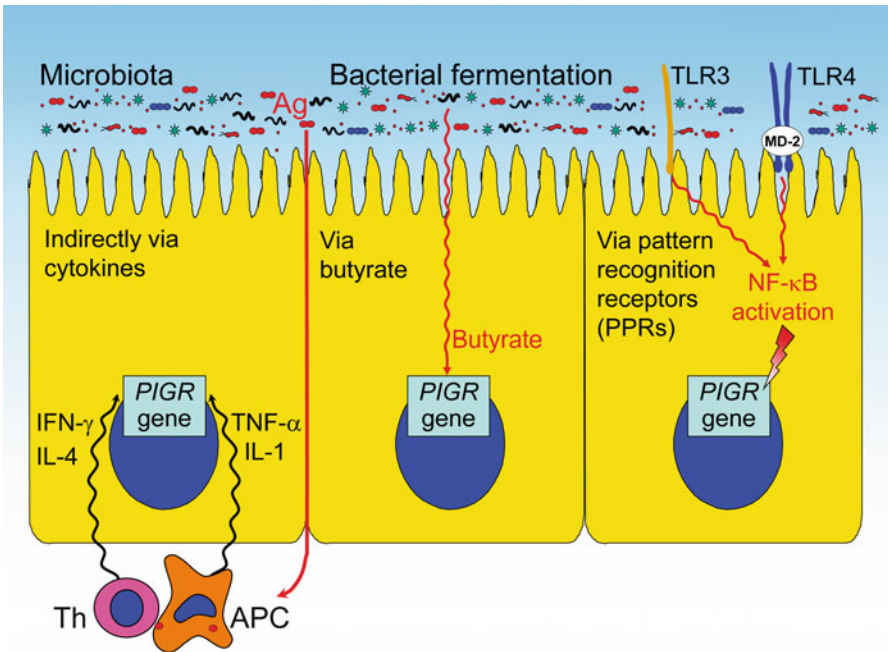


Fig. 3.9 Schematic illustrations of three possible manners in which pIgR expression can be upregulated by activation of its gene locus *PIGR*. The effect of the cytokines interferon (IFN)- γ , interleukin (IL)-4, tumor necrosis factor (TNF)- α , and IL-1 can be enhanced by the vitamin A derivative retinoic acid (not shown). Details are discussed in the text

(apically or basolaterally) or on endosomal membranes [89, 120]. Cell culture experiments with the polarized human colon carcinoma cell line HT-29 have shown that ligation of TLR3 and TLR4 with the respective MAMPs (double-stranded RNA and LPS) from the apical side upregulates pIgR expression (Fig. 3.9) [121], which may then increase the pIg export and thus enhance secretory immunity [41, 122]. Gut bacteria of the family *Enterobacteriaceae* can induce this effect [123].

Several cytokines derived from activated Th cells or APCs may likewise upregulate pIgR expression and thereby increase the export of secretory antibodies in response to microbial stimulation (Fig. 3.9). [41, 51, 122] A similar enhancing effect has been shown for butyrate in combination with various cytokines (Fig. 3.9) [41, 124]. Butyrate is an anaerobic microbial fermentation product of oligosaccharides and an important energy source of colonic epithelial cells. Notably, it can increase gene transcription levels through specific DNA sequences [125]. Vitamin A in the diet likewise supports secretory immunity, not only by enhancing IgA switch and homing of mucosal B cells (Fig. 3.3) but also by a positive effect of RA on cytokine-induced pIgR expression in intestinal epithelial cell at the transcriptional level [126, 127].

Altogether, there are many mechanisms by which exogenous variables may maintain the “tone” of intestinal pIgR expression above the constitutive level and thus reinforce immune exclusion. In addition, polarized gut epithelial cells seem to retain their ability to dampen the proinflammatory effect of PRR-mediated signals coming from the luminal side; [119, 120, 128] but after bacterial invasion, PRR signaling from the basolateral side results in NF- κ B activation with the release of epithelial defensins to join the combat against infection. [89, 129] Accumulating evidence suggests that barrier-related homeostasis depends on “cross-talk” between the epithelium (via cytokines and other factors) and lamina propria cells including macrophages, DCs, and T cells [130–133].

Thus, when immune regulation is operating in a healthy manner, the small amounts of MAMPs and exogenous antigen penetrating into the lamina propria seem to be handled in a homeostatic

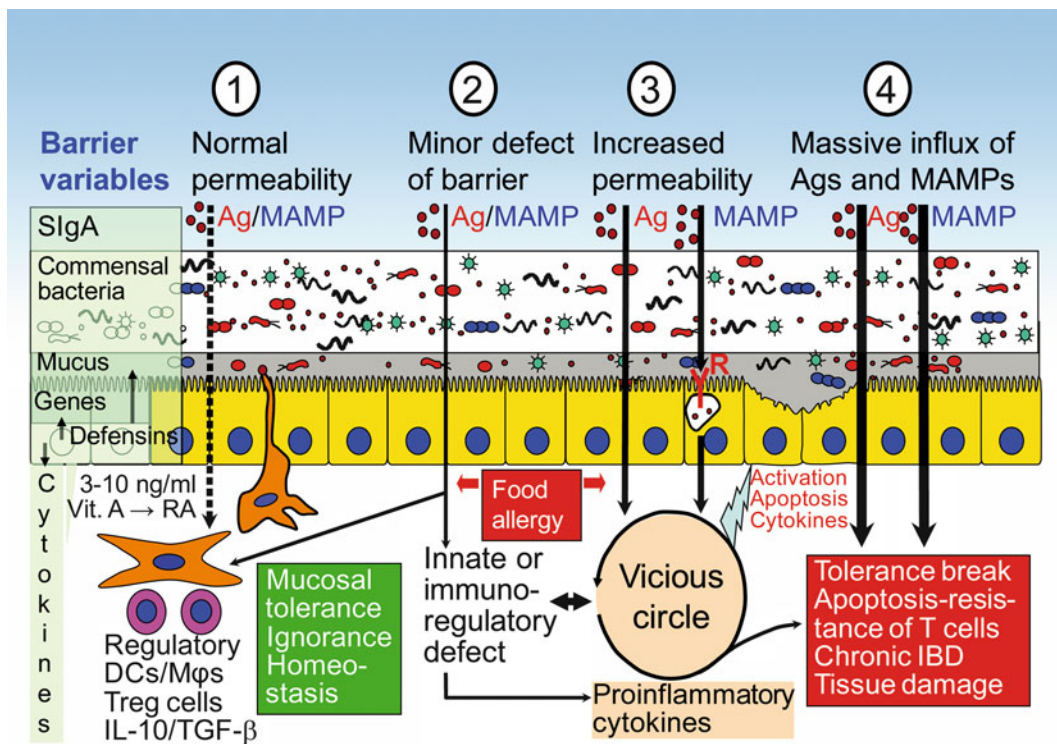


Fig. 3.10 Model for maintenance of mucosal homeostasis in the gut and abrogation of oral tolerance. Epithelial barrier variables, including secretory IgA (SIgA), mucus, defensins, genes (e.g., regulating tight junctions), and cytokines are indicated in light green panel to the left. (1) Normal permeability of gut epithelium allows some MAMP (microbe-associated molecular pattern) and exogenous antigen (Ag) uptake (amounts of dietary proteins detected in the circulation after a meal are indicated). (2) A minor barrier defect results in increased uptake, but mucosal homeostasis is maintained (green panel) when oral tolerance is adequately induced by quiescent dendritic cells (DCs) and macrophages (Mφs), which both can convert dietary vitamin A to retinoic acid (RA) as an aid in their induction of regulatory T (T_{reg}) cells (providing the suppressive cytokines IL-10 and TGF-β). If there is an innate or immunoregulatory defect, a vicious circle will develop which reciprocally acts also on the regulatory network. (3) With more skewing toward regulatory dysfunction, the vicious circle will activate the epithelium, and MAMP and Ag uptake is enhanced (graded arrows), both by increased permeability and aberrant receptor (R) expression apically on epithelial cells. Food allergy may occur in the dysfunctional, to some extent reversible, zone between 2 and 3, as indicated. (4) The adverse development may finally result in epithelial activation and apoptosis, increased secretion of proinflammatory cytokines, chronic inflammation, apoptosis-resistant effector T cells, chronic inflammatory bowel disease (IBD), and tissue damage (red panel)

manner by DCs and macrophages, with a balanced cytokine secretion and induction of T_{reg} cells (Fig. 3.10). However, if the influx is excessive or regulatory mechanisms are defect, immune reactions may be driven into hypersensitivity and enter a vicious circle with proinflammatory cytokines and epithelial apoptosis [12, 132–134]. In this manner a “point of no return” may be reached, as seen in IBD with apoptosis-resistant pathogenetic T cells and tissue damage (Fig. 3.10). The take-home lesson from most disease models in gene-manipulated animals is that a predilection exists for immunopathology to occur in the distal gut – where most commensals reside [10] – when adaptive immunity is dysregulated and innate immunity or the intestinal barrier function compromised [135, 136].

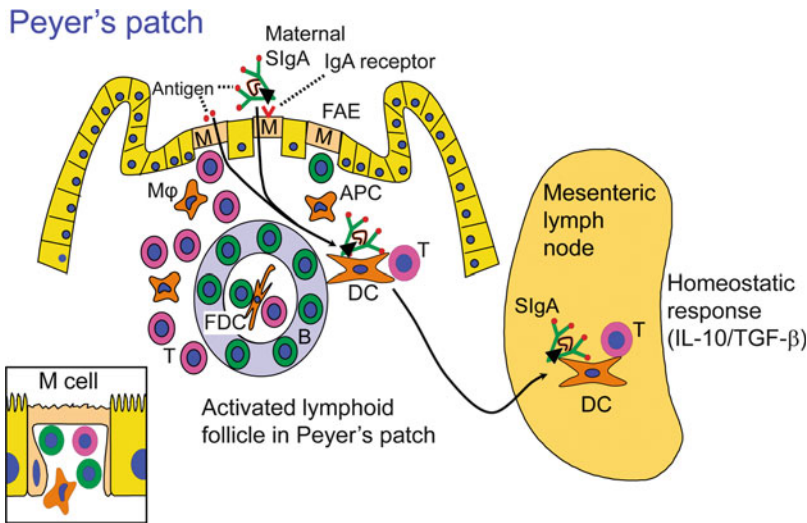


Fig. 3.11 Putative integration of the immune systems of mother and her breast-fed baby via M cell-mediated antigen uptake. Secretory IgA (SIgA) antibodies in breastmilk may guide induction of the infant's intestinal immune system because M cells of Peyer's patches express a receptor for IgA. This as yet uncharacterized receptor may facilitate uptake of antigens that have formed immune complexes with cognate maternal SIgA antibodies in the gut lumen of the infant. The complexes may be further target to dendritic cells (DCs) which carry them to mesenteric lymph nodes where a homeostatic immune response dominated by secretion of TGF- β and IL-10 is induced. Details of M-cell pocket with its cellular content is schematically shown in the panel to the left. FAE follicle-associated epithelium, M ϕ macrophage, APC antigen-presenting cell, FDC follicular DC, TGF transforming growth factor, IL interleukin. (Based on data from [3])

Interactions of SIgA with Commensal Bacteria

A great proportion of the commensal bacteria in the healthy human gut or at other mucosal surfaces are normally coated by IgA [137, 138], most likely representing low-affinity SIgA antibodies (Fig. 3.5). Such microbial–host interactions contain the commensal microbiota without eliminating it. Thus, it has been shown in a murine infection model with *Salmonella typhimurium* that bacteria coated with innate-like, cross-reactive SIgA antibodies showed reduced shedding and less liability to horizontal spreading by the fecal-oral route [83, 139]. Mouse experiments have further documented that a gut IgA response to a single commensal microbial epitope (capsular polysaccharide A, PSA) can be immunomodulatory and protect against pathogen-induced colitis [28]. Germ-free mice monocontaminated with the gut commensal *Bacteriodes thetaiotaomicron* demonstrated that specific SIgA antibodies directed against PSA in the gut inhibit activation of innate response markers such as oxidative burst and NF κ B, thereby inducing crucial modulation of immune homeostasis as well as antigenic drift [140]. SIgA antibodies can hence control the intestinal microbiota in a non-inflammatory, symbiotic relationship with the host [141].

Experimental studies have also revealed how intestinal homeostasis is mediated by microbial–host interactions in mice mono-colonized with the *Clostridia*-related segmented filamentous bacterium (SFB) which particularly grows in the distal ileum [142, 143]. SFB adheres to murine Peyer's patches and stimulate T-cell as well as IgA responses. Here a distinction is needed between B1 (T cell-independent) and B2 (T cell-dependent) responses when comparing Peyer's patches and lamina propria; this distinction is as yet not clear in mice [6, 46]. It is also possible that GALT may take up immune complexes containing SIgA bound to commensals via as yet uncharacterized receptors for IgA on M cells and then induce a homeostatic response (Fig. 3.11). Recently, this mechanism was suggested to direct the opportunistic bacterium *Alcaligenes* into murine GALT and thereby make these tissue structures more resistant to pathogen invasion [144].

Role of IgA and Breastfeeding in Immune Control

The level of SIgA appears to contribute to an individual's threshold for hypersensitivity reactions to exogenous antigens [12]. Thus, the risk of contracting allergy seems increased when the development of the IgA system is retarded and the SIgA-dependent barrier function insufficient [145]. Minor dysregulations of both innate and adaptive immunity (especially IgA) have been observed in children with multiple food allergy [146]. These clinical observations are in accordance with the hyperreactivity (Fig. 3.6) and sensitivity to DDS-induced colitis [84] seen in pIgR knockout mice.

It is therefore not surprising that exclusive breastfeeding up to the age of at least 4 months has an allergy-preventive effect, also in families without atopic heredity [147–149]. Moreover, having been breast-fed for at least 3 months protects against both Crohn's disease and ulcerative colitis in adulthood [150], and probably also against pediatric IBD [151]. Importantly, mothers with IBD receiving anti-inflammatory drugs can in most cases safely breastfeed their babies, and it may even provide a protective effect against disease flare [152–154].

In addition to the remarkable reinforcement of mucosal defense provided by maternal SIgA (and SIgM) antibodies as a natural immunological “substitution therapy,” it is important to emphasize the positive nutritional effect of breastfeeding on immune development [145]. Mother's milk also contains a number of immune cells, cytokines, and growth factors that may exert a significant biological effect in the breast-fed infant's gut, apparently enhancing in an indirect way even the subsequent health of the individual [36, 145, 155]. Moreover, its high content of oligosaccharides serves as a prebiotic promoting the growth of lactic acid-producing bacteria and reducing anaerobic bacteria in the gut of breast-fed infants [145]. Probiotic bacteria can reportedly also occur in breastmilk [156], perhaps after being transported to the mammary glands from the gut by DCs [157].

Numerous studies of the effect of breastfeeding on the development of secretory immunity have been performed with salivary IgA measurements as a read-out system. Discrepant observations have been made and the influence of contaminating the sample with milk SIgA, shielding of the suckling's mucosal immune system by maternal SIgA antibodies, and altered growth and composition of the infant's gut microbiota have been discussed as possible uncontrollable variables. Moreover, several prospective studies have reported that the postnatal increase of salivary IgA (and IgM) initially is more prominent in formula-fed than in solely breast-fed infants [25].

Nevertheless, evidence suggests that breastfeeding promotes the development of secretory immunity over time [26, 145], apparently even at extraintestinal sites such as the urinary tract [155]. Thus, although breastfeeding initially may reduce the induction of SIgA, it appears later on in infancy (up to 8 months) to boost secretory immunity. As mentioned above, one possibility is that SIgA antibodies in mother's milk guide the uptake of cognate luminal antigens via receptors for IgA on M cells (Fig. 3.11); as suggested by mouse experiments, the antigens may further be targeted to DCs which migrate to MLNs where they induce a homeostatic immune response [3].

Traces of exogenous antigen transferred into the milk of mothers may thus contribute to the induction of oral tolerance in the breast-fed infant; therefore, allergen avoidance during lactation is no longer recommended [12]. Various mouse models have indeed documented that antigen appears in the milk of exposed mothers and that it can induce T_{reg} cells in the suckling neonate, either together with TGF- β or after complexing with IgG antibody and transfer by FcRn into gut mucosa [158, 159]. In human milk, maternal SIgA-containing immune complexes may perform a similar function (Fig. 3.11). Interestingly in this context, a recent systematic review of published studies of the TGF- β levels in human milk suggested that this cytokine protects against allergy in the breast-fed infants and young children [160]. It was also recently reported that genes that are modulated during epithelial gut differentiation in the neonate are differentially expressed in breast-fed and formula-fed infants [161]. It is unknown whether this was a direct effect of milk components or caused by differences in the microbiota of the two feeding groups.

Mucosal Homeostasis vs. Hypersensitivity

Oral Tolerance in Humans

It is believed that oral tolerance is largely explained by different T-cell events such as anergy, clonal deletion, and induction of T_{reg} cells by conditioned APC, although other regulatory principles may be involved [12, 26, 145, 162–164]. For ethical reasons, the existence of mucosally induced tolerance in human beings is supported mainly by circumstantial evidence. Thus, the gut mucosa of healthy individuals contains virtually no hyperactivated T cells and hardly any proinflammatory IgG production, and their serum levels of IgG antibodies to food antigens are low [145]. Moreover, the systemic IgG response to dietary antigens tends to decrease with increasing age [165, 166], and a hyporesponsive state to bovine serum albumin has been demonstrated by intradermal testing in adults [167]. Interestingly, nasal application or feeding of a novel antigen (keyhole limpet hemocyanin) in healthy people induced peripheral downregulation of T-cell immunity and, less consistently, also suppressed systemic antibody responses to subsequent parenteral immunization [168, 169]. By contrast, oral tolerance could not be induced in patients with IBD where the epithelial barrier is severely deteriorated and the immunogenetics favors mucosal inflammation [170, 171].

Mucosal Tolerance Induction

In healthy human gut mucosa, resident APCs are quite inert in terms of immune-productive stimulatory properties [172], and they hardly express detectable surface levels of TLR2 or TLR4 [111]. Also, only negligible expression of the LPS co-receptor CD14 is normally observed on these cells, and their proinflammatory cytokine response is usually low after LPS stimulation [102, 173]. Nevertheless, the phagocytic and bacteriocidal activity of mucosal macrophages is maintained [174], which would be important for silent clearance of commensal bacteria normally penetrating into the mucosa in small numbers [11, 20, 175].

These observations support the notion that both macrophages and DCs play a central role in oral tolerance [130], and most human intestinal APCs come from a common myeloid progenitor (Fig. 3.4) and often show an intermediate phenotype [176, 177]. Heterogeneity of murine lamina propria APCs has also been highlighted [178, 179]. Recent in vivo observations in mice suggest that DC development from monocytes to the phenotype DC-SIGN/CD209⁺ is a predominant pathway when abundant LPS is available [180], such as in the gut. The subepithelial band of monocyte-derived CD103⁺CX3CR1⁺ DCs in the murine gut mucosa is indeed macrophage-like [177].

In a quiescent steady state, however, mucosal CD103⁺CCR7⁺ DCs (and possibly macrophages) are migratory and carry penetrating dietary and innocuous microbial antigens away from the gut mucosa to the MLNs [35, 181–183]. Here a portion of the same cells, in a maturation process, become further conditioned for tolerance induction and drive the expansion of T_{reg} cells [26, 184]. It has recently been shown in humans that the tolerogenic properties of these migrating mucosal DCs partly depends on expression of indoleamine 2,3-dioxygenase (IDO) [185] – an enzyme known to be involved in DC induction and function [186]. Hyperactivation of effector T cells in the intestinal mucosa with accompanying inflammation can thus be inhibited by this DC-dependent regulatory mechanism, both initially and subsequently. This is so because homeostatic control is also exerted when the generated T_{reg} cells home from MLNs to the lamina propria [12] – a homing that seems to be particularly active in infancy [187].

A dietary effect on the induction of T_{reg} cells is exerted through the conversion of vitamin A to RA by the enzyme RALDH, which is expressed both by intestinal DCs, macrophages, and epithelial

cells [37, 178], as well as by MLN stromal cells [188]. Together with IL-2, TGF- β , and IL-10, RA can drive induction of T_{reg} cells [189–192] – which in the human periphery are heterogenous and apparently may differentiate either by conversion from a naive (CD45RA⁺) phenotype or from rapidly proliferating T cells with a memory/effector (CD45RO⁺) phenotype with different migratory properties [193, 194]. It is the latter that expresses high levels of the transcription factor Foxp3 and seems to be actively suppressive [195].

Most information about T_{reg} cells is necessarily derived from mouse experiments. Human T_{reg} cells seem to be functionally and phenotypically more diverse than the murine counterparts, including the expression of the activation marker CD25 and the Foxp3 transcription factor [196]. There is also a need for better understanding for the functional stability and plasticity (e.g., acquisition of a Th17 phenotype) of T_{reg} cells [197].

Recognition of Microbial Components

Final cellular conditioning for oral tolerance in MLNs appears to depend on appropriate stimulation of the migrating mucosal APCs by certain MAMPs derived from commensal bacteria (Fig. 3.12), which induce the signaling molecules and transcription factors dictating the differentiation pathways and cytokine profiles of the activated T cells [11, 12, 198]. Components of intestinal parasites such as helminths can exert similar immune modulation [10, 14, 199–201] – apparently to some extent through induction of T_{reg} cells by mimicking the effect of TGF- β by ligation of its receptor [202]. Indeed, helminth-derived immunomodulators may be important in future novel medication for IBD [203]. Also of note, several studies suggest that LPS plays a central role in the early programming of the immune system; [204, 205] and there is an ongoing search to find out if allergy to food and aeroallergens is associated with hereditary single-nucleotide mutations (polymorphism) in PRRs recognizing this and other MAMPs, such as CD14, TLR2, TLR4, and NOD [206, 207]. The extended hygiene hypothesis implies that suboptimal PRR stimulation, with delayed maturation of the mucosal immune system and insufficient induction of T_{reg} cells, contributes significantly to the increasing incidence of not only allergy – commonly reflecting overactivation of Th2 cells – but also other immune-mediated inflammatory disorders such as IBD – reflecting overactivation of Th1 or Th17 cells, both of which are normally important for proper defense against infections [10, 14]. In the perspective of evolution, Th2 cells have had a crucial role in host defense against parasites [208].

This basis for the hygiene hypothesis has been tested in several clinical studies evaluating the beneficial effect on immune homeostasis exerted by probiotic bacterial preparations derived from the commensal intestinal microbiota and eggs of the porcine helminth (whipworm) *Trichuris suis* [14, 201]. In this context, viable strains of lactobacilli and bifidobacteria have been reported to enhance IgA, both in humans and experimental animals, but these responses have not translated convincingly into clinical effects [209, 210]. To select the right probiotic strains, or symbiotic combinations with prebiotics or breast milk, remains a difficult task – and there are safety issues [211, 212]. Experimental studies have indicated that certain probiotic bacterial strains in fact may be proinflammatory while others may induce DCs, directly or via epithelial mediators, to exert anti-inflammatory effects [213].

It is unknown whether probiotics and prebiotics might work mainly through SIgA-mediated reinforcement of the barrier function, expansion of T_{reg} cells, or the involvement of both these anti-inflammatory mechanism – perhaps combined with direct strengthening of epithelial integrity (Fig. 3.10). Notably, the most promising results have been reported for atopic eczema [209]. This skin disease is often seen in patients with IgE-mediated food allergy (20–40%) and is particularly associated with loss-of-function mutations in the filaggrin gene, which is involved in the epidermal barrier function [214]. Similar mutations appear to predispose for the combination of atopic eczema

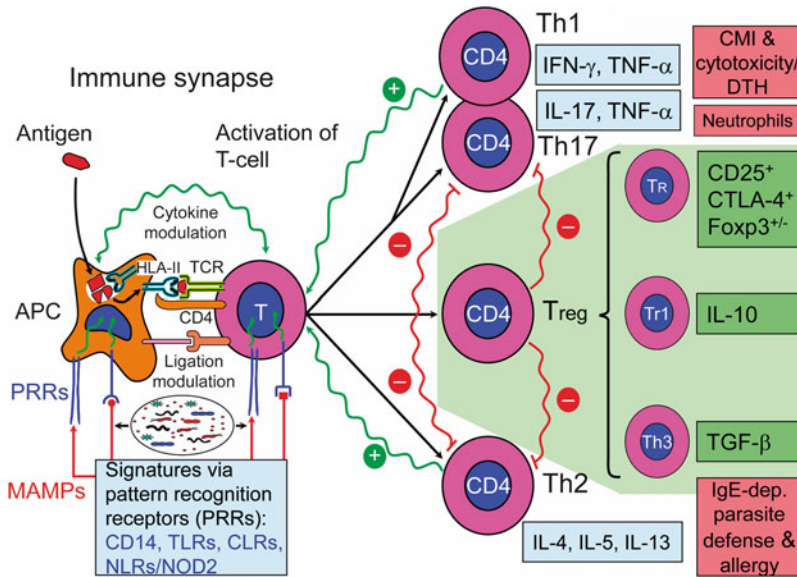


Fig. 3.12 Decision-making in the mucosal immune system is modulated by co-stimulatory signals (cytokines and ligands) operating in the synapse between antigen-presenting cell (APC) and T cell. Activation of CD4⁺ T cells occurs when APC takes up antigen and processes (degrades) it to immunogenic peptides for display to the T-cell receptor (TCR) in the polymorphic groove of HLA class II molecules (HLA-II). The level of co-stimulatory signals determines T-cell modulation (activation and conditioning). When CD4⁺ helper T (Th) cells are primed for productive immunity, they differentiate into Th1, Th17, or Th2 effector cells with polarized cytokine secretion (*blue panels to the right*). Such skewing of the adaptive immune response depends on the presence of microenvironmental factors, including cytokines, as well as signals from microbial components. Bacterial endotoxin (lipopolysaccharide), lipoproteins, unmethylated CpG DNA, and other conserved structural motifs are called microbe-associated molecular patterns (MAMPs); they are sensed by cellular pattern recognition receptors (PRRs) such as CD14, toll-like receptors (TLRs), C-lectin receptors (CLRs), and NOD-like receptors (NLRs), including NOD2. Signaling from PRRs to the nucleus of APCs and T cells stimulates various degrees of activation and functional maturation of APCs and will thereby dictate differential expression of various co-stimulatory signals directing either activation of Th1, Th17, or Th2 cells. Their cytokines induce various adaptive defense mechanisms or immunopathology (*red panels to the right*). The Th cytokine profiles are further promoted by the depicted positive and inhibitory feedback loops. Under certain conditions, rather immature but yet conditioned APCs may induce various subsets of regulatory T (T_{reg}) cells as indicated in the light green area; by their cytokines IL-10 and TGF- β , or by interactions depending on CTLA-4 and the transcription factor Foxp3, the T_{reg} cells can suppress Th1, Th17, and Th2 responses as well as innate immunity and inflammation. CTLA-4 cytotoxic T-lymphocyte antigen 4, IFN interferon, IL interleukin, TGF transforming growth factor, TNF tumor necrosis factor, CMI cell-mediated immunity, DTH delayed-type hypersensitivity

and asthma [215]. These findings apparently reflect that a leaky surface epithelium anywhere in the body may be a predisposing condition for allergen penetration, and that food allergy could be a consequence rather than a cause of atopic eczema [12]. Therefore, the use of this disorder as a clinical read-out of intervention effects on food allergy may not be scientifically acceptable.

Innate Signals Dictating Homeostasis

Microorganisms have inhabited Earth for at least 2.5 billion years, and the power of the immune system is a result of co-evolution in which especially commensal bacteria have shaped host defense in a state of mutualism [10, 14]. The prevailing mucosal homeostasis in the gut mucosa is indeed

remarkable because of the large surface area to be defended – continuously being exposed to at least 1,000 different bacterial species (Figs. 3.1 and 3.10). The gut microbiome is perhaps 100 times larger than the human genome [216]. In addition, the human gut harbors an unknown number of viruses [217]. Thus, while the intestine represents the largest exposure to microorganisms, it is also a major route of exposure to exogenous protein antigens (i.e., food) and micronutrients with immunomodulatory properties [12].

The original hygiene hypothesis postulated that the increasing incidence of allergy in westernized societies was explained by reduced or aberrant microbial exposure early in infancy, resulting in too little Th1-cell activity and therefore an insufficient IFN- γ level to downregulate optimally the prenatal Th2-cell responses which apparently can be ascribed to the cytokine thymic stromal lymphopoietin (TSLP) secreted by placental trophoblasts [218]. In this context, an appropriate postnatal encounter with a balanced commensal microbiota and exposure to foodborne and orofecal pathogens probably may exert an important homeostatic impact [219, 220], both by enhancing the SIgA-mediated barrier and promoting oral tolerance through a shift from a predominant Th2-cell activity in the newborn period [221]. The extended hygiene hypothesis postulates that induction of T_{reg} cells is an important part of such microbe-driven homeostasis to avoid both allergy and other immune-mediated inflammatory disorders such as IBD [14].

Naturally occurring T_{reg} cells with suppressive properties are present in large numbers in human fetal MLNs [222], probably as part of a peripheral tolerance to keep autoreactive effector T cells in check to avoid inflammation and tissue damage [192]. These T_{reg} cells are apparently induced in the thymus and expanded in the periphery [223, 224]. After birth, the decision for induction of hyporesponsiveness against innocuous exogenous antigen, vs. potentially harmful systemic-type productive immunity, may be largely instructed in mucosa-draining lymph nodes such as MLNs. As discussed above, the driving force in this homeostatic mechanism appears to be the microbial impact that conditions APCs and T cells for tolerance by balancing polarizing cytokines induced via PRRs (Fig. 3.12).

Thus, MAMPs do not only directly modulate the epithelial barrier function of neonates [91, 92] but also the activation profiles of innate and adaptive immune cells (Fig. 3.10). Appropriate balancing of the immune system appears to depend on a fine-tuned “cross-talk” between APCs/innate immunity and T cells/adaptive immunity during certain windows of opportunity, particularly early in the newborn period [12, 14, 26], and probably even late in fetal life [225]. Concepts such as epigenetic programming in utero and subsequent epigenetic regulation are being considered as critical pathways through which environmental changes could alter expression of genes which lead to immune homeostasis or dysregulation [86], apparently also involving T_{reg} cells and the Th1/Th17:Th2 balance [226, 227]. It is possible that opportunities for reprogramming may be a life-long process, thus explaining the late emergence of immune-mediated diseases in some individuals.

Effects of Genes vs. the Environment

The increasing incidence of immune-mediated diseases in affluent societies indicates that susceptibility genes for dysregulation are quite universal, such that they can be induced readily with environmental change. Epigenetics is an active research field providing novel understanding of how the environment can have heritable genomic effects and promote disease [86, 207, 227]. A number of early life exposures, including dietary nutrients and microbial exposure in utero, have been recently shown to have effects on gene expression with an impact on the clinical phenotype. For instance, mice born to dams exposed to bacteria during pregnancy experienced less allergy than those born to unexposed mothers, and maternal TLR signaling was needed for this transmission of protection [228]. Even the sensitivity of gut epithelial cells to LPS exposure via TLR4 may be subjected to epigenetic regulation [229].

However, the heredity of polygenic diseases is complex and the family history remains the best prediction of both allergy [230] and IBD [231]. Hopefully, the apparently inherent plasticity of the immune system may in the near future provide opportunities for reprogramming to facilitate more effective prevention and treatment of these disorders. Thus, even APCs of adults can be conditioned to induce T_{reg} cells by environmental factors such as LPS and cell wall lipids from parasites [232, 233]. Moreover, transient infestation with porcine helminths has been shown to have a beneficial effect on mucosal homeostasis in adult IBD patients [201], and the same has been shown in experimental models of allergy [200].

How Decisive Are Commensal Bacteria?

Indigenous gut bacteria play a central role in the extended hygiene hypothesis. Thus, the intestinal microbiota of young children in Sweden was found to contain a relatively large number of *Clostridium* spp., whereas high levels of *Lactobacillus* spp. and *Eubacterium* spp. were detected in [234] an age-matched population from Estonia; this difference might contribute to the lower incidence of allergy in the Baltic countries compared with Scandinavia [235]. A Finnish study likewise reported that allergic infants had more Clostridia and tended to have fewer bifidobacteria in their stools than non-allergic controls [236].

Absence of early postnatal gut colonization with a normal commensal microbiota dominated by lactic acid-producing bacteria might likewise contribute to the increased risk for food allergy generally noted in children delivered by cesarian section, particularly when genetically predisposed [116, 117]. Nevertheless, in clinical studies it has been difficult to reveal a convincing effect on oral tolerance by probiotic perinatal intervention, even in children at high risk for food allergy [209]. The same conclusion was reached with regard to IgE-mediated allergy at the age of 5 years (including food allergy) after extending the postnatal intervention (four probiotic strains combined with prebiotics) until the age of 6 months, as observed in a recent Finnish study [237]. However, for children delivered by cesarian section a modest but significant allergy reduction was noted when they were randomized to the same regime [237]. Thus, there is hope for clinical benefits by balancing the colonization of the gut microbiota and inducing homeostatic immune regulation [12].

The feeding and treatment conditions (e.g., antibiotics) to which the newborn is subjected, and also the general nutritional state, may have an impact on the indigenous microbiota and on epithelial integrity; such variables may hence modulate the programming of the mucosal immune system [238, 239]. Intestinal colonization of lactobacilli and bifidobacteria is promoted by breast milk because it acts as prebiotics through its large amounts of oligosaccharides [145, 239], and it may also contain probiotic bacteria [156, 240].

Cell culture studies have suggested that probiotics could be directly immunomodulatory by enhancing the Th1 profile via induction of IL-12, IL-18, and IFN- γ secretion [241, 242]. Also notably, *E. coli* is a strong inducer of IL-10 secretion, apparently derived both from APCs and T_{reg} cells [243, 244]. Importantly, T_{reg} cells bear PRRs for several MAMPs [245], and IL-10 is crucial for maintained expression of the Foxp3 transcription factor [246], which contributes significantly to the suppressive function of these cells [192]. IL-10 has been directly shown to be an important suppressive cytokine in the murine gut [247].

The above information collectively implies that the gut microbiota has an impact on mucosal homeostasis beyond that of enhancing the SIgA system, namely by promoting a balanced development of Th1, Th17, Th2, and T_{reg} cells [209, 211]. As mentioned previously, however, the selection of safe and effective probiotic strains remains difficult.

Loss of Mucosal Immune Homeostasis in IBD

Alterations of Mucosal IgA and the Epithelial Barrier

IBD lesions exhibit excessive numbers of IgA⁺ and IgG⁺ PCs with a remarkable skewing toward IgG production – depending on the severity of inflammation [112, 248, 249]. Initially this shift from the normal pIgA predominance may be beneficial as a powerful second line of defense because IgG antibodies can efficiently mediate immune elimination of bacteria via phagocytosis and antibody-dependent cell-mediated cytotoxicity (Fig. 3.8). However, the chronicity of IBD signifies that a defective epithelial barrier over time results in severely altered mucosal homeostasis and (Fig. 3.10). Thus, whereas fluorescent in situ hybridization on tissue sections from normal colon reveals no microorganisms, 83% of ulcerative colitis and 25% of colonic Crohn's disease specimens show mucosal invasion of commensal bacteria [250]. In ulcerative colitis a proinflammatory antimicrobial response is additionally promoted by a significant shift toward the highly complement-activating IgG1 subclass [251] – apparently reflecting a genetic impact as revealed by comparing identical twins, healthy or afflicted with this IBD [252].

In parallel with the disproportionately increased IgG⁺ PC subset, the J-chain expression is decreased in IBD lesions [248, 253] and there is a shift from the IgA2 to the less stable IgA1 subclass [248, 254]. Thus, more than 50% of the IgA1⁺ PCs are J chain-deficient, therefore producing monomers that cannot be exported by the pIgR [248, 255]. The same is true for a fraction (25–35%) of the expanded IgA2⁺ subset. These adverse local PC alterations supposedly reflect a less-restricted leukocyte extravasation due to a changed profile of adhesion molecules and chemokines on the mucosal microvascular endothelium (Fig. 3.4), allowing B cells expressing characteristics of systemic immunity to enter the lesion [112, 249].

A deficient epithelial barrier in IBD not only promotes bacterial invasion [250] but also increases food-antigen uptake and sensitization after rectal challenge, as shown in Crohn's disease [256]. This finding parallels with the increased mucosal leakiness of pIgR knockout mice [74, 75].

Antibodies to Commensals and Abrogated Tolerance

Locally produced IgG in IBD lesions has been reported to react with cytoplasmic antigens from a range of Gram-positive and Gram-negative fecal bacteria, with higher activity in Crohn's disease than in ulcerative colitis, and higher in ulcerative colitis than in other types of intestinal inflammation [257]. Thus, nonspecific mucosal damage and bacterial invasion alone do not seem to explain the intensified local IgG response to commensals. Studies in rodents have shown that indigenous gut bacteria normally are poorly stimulatory for the systemic B-cell system [258, 259]. One explanation might be that the indigenous microbiota, while permanently colonizing the gut, induces waves of SIgA responses performing immune exclusion and thus being self-limiting [260]. Such intermittent immune exclusion could contribute to the hyporesponsiveness or apparent oral tolerance against gut commensals [10, 11, 20]. This mechanism is clearly abrogated in IBD [18, 170, 171], which agrees with several experimental models of intestinal inflammation in rodents [135]. Also, it has been shown that dysfunction in either the adaptive or innate mucosal immune system leads to systemic antibody hyperreactivity to the gut microbiota in mice [74, 75, 77, 261].

In human IBD break of tolerance to the commensal microbiota is suggested by increased in vivo antibody coating of gut bacteria. In healthy controls, approximately 40% of fecal anaerobic bacteria are coated with IgA, 12% with IgG and 12% with IgM [138]. In IBD these figures are raised to 65, 45, and 50%, respectively [262]. This result parallels the markedly elevated mucosal Ig production

in IBD [99, 112, 248] – with the relative average increase being more prominent for IgG (x30) and IgM (x2.5) than for IgA (x1.7–2.0). In fact, adjacent to Crohn’s ulcers the number of PCs is increased 100- to 200-fold for the IgG class and 8- to 12-fold for the IgM class, compared with 1.2- to 6.7-fold for the IgA class.

Based on analysis of serum antibodies, however, there seems to be considerable heterogeneity in microbial specificities among IBD patients; rather than a global loss of tolerance against the intestinal microbiota, individual subsets of patients with varying immune responses to selected bacterial antigens has been identified [263]. Whether this is the cause or the effect of a more restricted gut microbiota [141] – with 25% fewer bacterial genes than normal – is currently unknown [216]. Interesting in this context, experimental colitis induced in a genetically manipulated mouse model can be transferred to healthy mice by a selected combination of commensal gut bacteria in the setting of a normal microbiota [264]. This result reflects the complexity of the microbial–microbial and the microbial–host interactions directing immune regulation in the gut [265].

Conclusions

A number of biological variables influence induction of oral tolerance and SIgA-dependent intestinal immunity. Increased epithelial permeability for exogenous antigens is clearly an important primary or secondary event in the pathogenesis of IBD. The postnatal mucosal barrier function is determined by the individual’s age (e.g., preterm vs. term infant), genetics, mucus composition, interactions between mast cells, nerves and neuropeptides, concurrent infection, and the mucosa-shielding effect of SIgA provided by breast milk or produced in the infant’s gut. The integrity of the epithelial barrier furthermore depends on homeostatic regulatory mechanisms, including mucosal induction of T_{reg} cells, where commensal microbial–host interactions apparently play decisive roles.

The incidence of both food allergy and Crohn’s disease is increased in IgA deficiency. In an experimental mouse model we found that a defect gut barrier due to SIgA/SIgM deficiency leads to systemic hyperreactivity of the host but may at the same time enhance oral tolerance induction by cognate antigen in a delicate balance (Fig. 3.6). Boirivant et al [266] likewise reported that a mild or transient breaching of zonula occludens in the intestinal epithelium of mice leads to a dominant anti-inflammatory T_{reg} cell response. Also notable, children who have grown out of their cow’s milk allergy, as revealed by oral challenge, show expansion of T_{reg} cells in peripheral blood [12, 267], perhaps reflecting that early infancy is the time when oral tolerance is best achieved. The relatively leaky gut epithelium probably promotes tolerance induced by continuous mucosal exposure to luminal antigens, and the homeostatic balance might be enhanced by cognate SIgA antibodies. Therefore, it is not surprising that epidemiological reports suggest that breastfeeding protects against food allergy and IBD. The remarkable output of SIgA during feeding represents an optimally targeted passive immunization of the breast-fed infant’s gut [36], and might serve as a positive homeostatic feedback loop (Fig. 3.11).

Altogether, the secretory immune system is critical for the mucosal barrier function because SIgA not only forms the first line of defense but also maintains mutualism with the indigenous microbiota. Notably, the epithelial barrier depends on exposure to microbial components (MAMPs) from the commensal microbiota and the environment, both by direct interaction with PRRs on the intestinal epithelium and induction of oral tolerance via mechanisms such as tolerogenic APCs and T_{reg} cells (Figs. 3.10 and 3.12). It has therefore been proposed that the hygiene hypothesis as an explanation of the increase of immune-mediated disease in affluent societies instead should be called the “microbial deprivation hypothesis” [15]. In mouse experiments it has indeed been shown that a single immunomodulatory molecule from a commensal gut bacterium can induce crucial modulation and homeostasis of the host’s immune system [140]. This gives hope for future therapeutic manipulation of the intestinal immune system of patients suffering from IBD.

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Chapter 4

Understanding the Epithelial Barrier in Inflammatory Bowel Disease

Emily M. Bradford, Emily S. Turner, and Jerrold R. Turner

Keywords MLCK • Claudin-2 • Tight junction • Occludin • ZO-1 • Intestine • Epithelial cell

Introduction

Mucosal surfaces are lined by epithelial cells, which form a barrier between the internal and external environment. The integrity of this barrier is critical, particularly within the intestine, where the luminal environment includes potentially pathogenic antigens and microorganisms. Although the term “barrier” may imply that the mucosa simply acts as a static impediment, preventing all trans-mucosal flux, this is not the case, as the epithelium must absorb and secrete the water, ions, and macromolecules necessary to maintain intestinal homeostasis and overall nutrition. Thus, the mucosa must integrate active and passive vectorial transport with a selectively permeable barrier that prevents entry of noxious luminal materials.

The mucosal barrier is formed primarily by the epithelial cells and the materials they secrete. The latter includes mucins, secreted by intestinal goblet cells, which form a hydrated gel over the epithelial surface. As discussed elsewhere (*see* Chaps. 1, 3, 5, and 6) defects in mucin synthesis and in the extracellular mucin layer have been associated with inflammatory bowel disease (IBD) in humans [1, 2] and spontaneous colitis in mice [3–5].

While mucin contributes to the development of the unstirred layer by limiting passage of large materials and trapping some bacteria [6], the epithelial cells are the primary determinant of mucosal barrier function. In erosions or ulcerations the epithelium is lost and, therefore, does not contribute to barrier function. In the presence of an intact epithelial layer, however, the intercellular junctions define the selective permeability of the mucosal barrier.

The Intestinal Barrier Is Compromised in IBD

Epithelial barrier function is compromised in Crohn’s disease (CD) and ulcerative colitis (UC). The association between decreased barrier function and intestinal disease, including IBD, was first reported in the early 1980s [7–9]. These studies, which made use of both *ex vivo* and *in vivo*

E.M. Bradford, PhD • E.S. Turner • J.R. Turner, MD, PhD (✉)
Department of Pathology, University of Chicago, 5841 S. Maryland Avenue,
MC 1089 AMB P-526, Chicago, IL 60637, USA
e-mail: jturner@bsd.uchicago.edu

approaches, demonstrated increased permeability both in IBD in the absence of ulcerations, and in intestinal diseases not typically associated with ulceration, e.g., celiac disease [10, 11].

In patients, measurement of intestinal permeability typically involves oral administration of small molecules, such as Cr-EDTA, polyethylene glycols, mannitol, and lactulose [12–15]. Intestinal absorption of these molecules occurs freely across erosions or, when the epithelium is intact, in a more restricted manner across intercellular junctions. These tracers are not metabolized in the circulation and are freely filtered at the glomerulus. Thus, fractional urinary recovery can be used as a noninvasive measure of intestinal permeability. Despite some advantages, Cr-EDTA and polyethylene glycols are used less often than lactulose and mannitol, as the latter combination allows assessment of barrier compromise per unit villous surface area when expressed as the lactulose mannitol recovery ratio [13]. However, lactulose and mannitol are not useful for analyzing colonic permeability, as these molecules are degraded by luminal bacteria [16, 17]. As a result, permeability has been studied to a far greater extent in patients with small intestinal Crohn's disease than in patients with Crohn's colitis or ulcerative colitis.

The contribution of the intestinal barrier to IBD was first suggested following the observation that increased permeability is present in a subset of healthy, first-degree relatives of CD patients [18, 19]. Further studies have linked barrier defects in these relatives to specific CD-associated mutations in *NOD2*, an immunoregulatory gene [20, 21]. Although the role of these barrier defects in disease pathogenesis is unclear, one case report describes the subsequent development of CD in a previously healthy relative with increased intestinal permeability [22]. In that case, risk of disease was increased in the patient on a genetic basis. Unfortunately, no studies have compared the long-term risk of developing CD in healthy relatives with or without increased permeability. However, increased small intestinal permeability is a known marker of impending relapse in CD patients with quiescent disease [23].

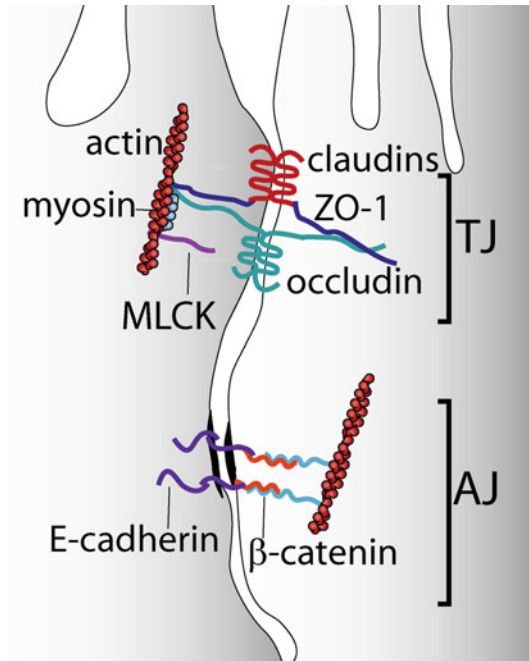
The Epithelial Monolayer

In a reductionist view, the intestinal barrier is formed by a single layer of epithelial cells linked together by intercellular junctions. In the absence of specific transporters, the epithelial plasma membrane serves as a barrier to most hydrophilic molecules; thus, the intercellular junctions are the limiting factor in transepithelial permeability. The most critical intercellular junctions are the tight junctions, adherens junctions, and desmosomes. The tight junction and adherens junction form the apical junctional complex, which associates with a dense perijunctional ring of actin and myosin II to encircle the apical aspect of each epithelial cell. These form a network that connects neighboring cells and acts as a selectively permeable paracellular barrier (Fig. 4.1).

Molecular Anatomy of the Tight Junction and IBD

The adherens junctions and desmosomes are required for the formation and maintenance of apical tight junctions. The adherens junction is formed by homotypic interactions between the epithelial cadherin isoform E-cadherin. The cytoplasmic domains of the cadherins interact with the catenins, which in turn bind to the actin cytoskeleton to regulate cell structure and function [24]. The critical role of the adherens junction in intestinal physiology was demonstrated by using chimeric mice in which some, but not all, villous enterocytes expressed a dominant-negative cytoplasmic tail of N-cadherin. Expression of the dominant-negative N-cadherin tail disrupted E-cadherin function and

Fig. 4.1 Structure of the epithelial barrier. Below the intestinal microvilli, the tight junction (TJ) and adherens junction (AJ) form the apical junctional complex. The tight junction is spanned by occludin and members of the claudin family, which dynamically regulate permeability between cells. The plaque protein zonula occludens 1 (ZO-1), myosin light chain kinase (MLCK), and the actin cytoskeleton also interact with these proteins to regulate barrier function. The adherens junction is formed by E-cadherin, several members of the catenin family, and the cytoskeleton



resulted in the loss of intercellular junctions, aberrant epithelial differentiation, Crohn's-like disease, and epithelial dysplasia [25]. E-cadherin may also be relevant to human disease as data from recent genome-wide association studies (GWAS) suggest that polymorphisms within *CDH1*, the gene encoding E-cadherin, are associated with ulcerative colitis [26].

Despite this critical role of the adherens junction, the tight junction is the primary determinant of paracellular permeability. It is, therefore, not surprising that alterations in tight junction structure and function are associated with increased permeability in IBD. For example, the normally complex anastomosing network of tight junction strands seen by freeze-fracture electron microscopy is simplified in IBD [27]. Moreover, the expression and distribution of individual tight junction proteins, several of which are not yet well-characterized, are altered in IBD [28]. Finally, the increased expression and enzymatic activity of myosin light chain kinase, a key regulator of the perijunctional actomyosin ring and tight junction permeability, have been associated with active IBD [29]. As discussed below, this appears to be the primary intracellular signaling pathway by which tumor necrosis factor (TNF) disrupts tight junction barrier function.

Discovery of the claudins, a large family of proteins now recognized to define tight junction ion selectivity and to be essential for the development of the paracellular barrier, was a major breakthrough in tight junction biology. The availability of reagents suitable for analyzing individual claudin proteins in human specimens revealed that expression of claudin-2 was upregulated in IBD [28, 30]. In parallel, *in vitro* studies demonstrated that claudin-2 expression increased the paracellular flux of cations, e.g., Na^+ , and small uncharged molecules [31, 32]. Furthermore, *in vitro* and *in vivo* studies have shown that claudin-2 expression can be induced by IL-13 (Fig. 4.2) [32]. Thus, increased flux through claudin-2 is a potential mechanism for the elevated paracellular permeability observed in IBD. While IL-13 may not be the only mediator of claudin-2 induction in IBD, it is interesting to note that claudin-2 expression is greater in UC than CD, and that there is a greater elevation of IL-13 production by lamina propria mononuclear cells from UC, relative to CD [28, 30]. However, as discussed below, increased claudin-2 expression cannot fully explain the barrier defects present in IBD, and it remains uncertain whether this represents a beneficial adaptive, or detrimental maladaptive, process.

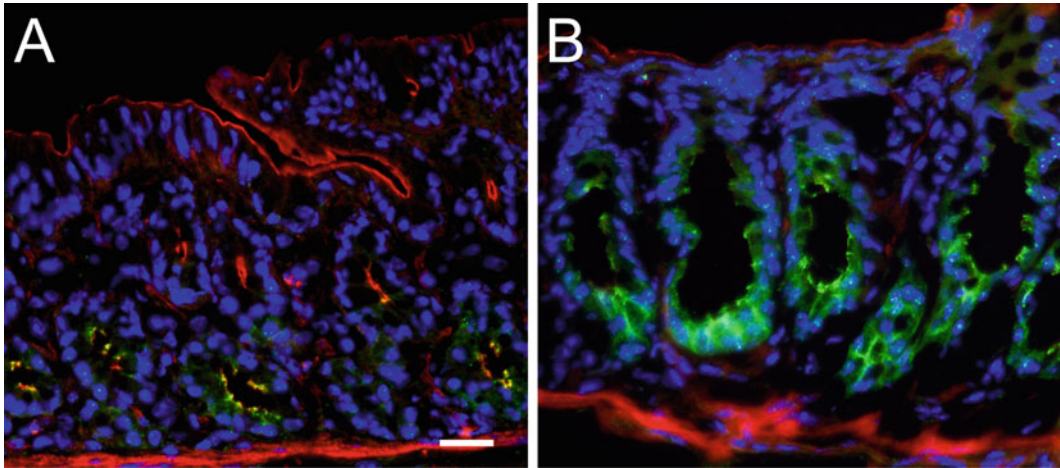


Fig. 4.2 IL-13 alters tight junction permeability via claudin-2 upregulation. Mice were injected with either vehicle (a) or 1 µg recombinant murine IL-13 (b), and colonic tissue was collected 12 h later. Immunofluorescent staining shows a marked increase in claudin-2 (green) expression after IL-13 treatment. Also note the dilated crypts in IL-13-treated mice, possibly caused by increased paracellular water and Na⁺ flux through claudin-2 pores. F-actin (red) and nuclei (blue) are shown for orientation. Bar, 20 µm. Images provided by Christopher R. Weber, M.D., Ph.D., The University of Chicago

While elevated levels of IL-13 are highly relevant to IBD, the impact of IL-13 neutralization on intestinal permeability and disease activity in human IBD is untested. In contrast, TNF neutralization reduces disease severity and also restores intestinal barrier function in IBD patients. While this is likely, in part, to reflect healing of mucosal ulcers and global immune downregulation, TNF plays a critical role in tight junction regulation. For example, studies in mice have shown that acute T-cell activation causes a TNF-dependent increase in intestinal permeability. This is associated with endocytosis of the tight junction protein occludin and redistribution of the cytoplasmic plaque protein zonula occludens-1 (ZO-1) [33]. The redistribution of occludin is directly related to TNF-induced barrier loss, as occludin overexpression in transgenic mice limits increases in permeability and prevents diarrhea following acute TNF exposure [34]. Although TNF administration is an acute model of disease, similar changes in occludin and ZO-1 have also been reported in chronic experimental colitis and human IBD [33–35].

TNF-induced barrier loss has also been shown, *in vitro* and *in vivo*, to require myosin light chain kinase- (MLCK-) dependent myosin II regulatory light chain (MLC) phosphorylation. TNF-induced MLCK activation precedes occludin internalization, as genetic or pharmacological MLCK inhibition prevents occludin redistribution, barrier loss, and diarrhea. In addition to enzymatic activation, TNF increases MLCK transcription and protein synthesis in cell culture and animal models. Moreover, transcriptional and enzymatic activation occur in human IBD, where they are associated with active disease [29]. MLCK activation is also required for barrier dysregulation by other cytokines relevant to IBD, including lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells (LIGHT), and interleukin 1β (IL-1β) [36–38]. As discussed below, these observations suggest that MLCK inhibition may have therapeutic efficacy.

The ability of proinflammatory cytokines to regulate tight junction barrier function may explain the association of increased permeability with human IBD. This would suggest that, for example, the permeability increases that precede CD reactivation may be secondary to limited immune activation. This idea is also consistent with the observation that barrier defects in first-degree relatives of CD patients are associated with mutations in *NOD2*, a known immunoregulatory gene. However, the fact that these relatives, as well as other individuals with reduced barrier function, do not have

disease demonstrates that increased intestinal permeability alone is insufficient to cause IBD. How is it possible to integrate these data and understand the relationship between intestinal barrier function and disease? One animal model that clarifies the relationship between immune signaling and barrier function in disease is the interleukin 10 knockout (IL-10 KO) mouse. This model is relevant to human disease, as polymorphisms of both IL-10 promoter and the IL-10 receptor have been linked to ulcerative colitis [39, 40].

IL-10 knockout mice develop spontaneous colitis when housed under “normal” specific pathogen-free conditions. However, like human IBD, development of disease in IL-10 KO mice is clearly multifactorial. Disease penetrance and presentation vary with genetic background and among different animal facilities, and disease does not develop at all in germ-free mice. Moreover, when disease does occur in IL-10 KO mice, clinical symptoms are not present at birth, but develop only after weeks or months [41]. In contrast, intestinal barrier defects are detectable in a large proportion of IL-10 KO mice within 4 weeks of birth, but are absent in antibiotic-treated or germ-free mice [42]. Thus, given that the primary defect in these mice is loss of the immunoregulatory cytokine IL-10, one can conclude that intestinal permeability defects arise secondary to an interaction between the immune system and luminal microbiota. This suggests that the barrier defects in IL-10 KO mice are a sensitive indicator of mucosal immune activation. However, these data do not address the question of whether the permeability defects contribute to disease pathogenesis.

One observation that suggests a contribution of barrier defects to disease evolution in IL-10 KO mice is the synchronization of disease onset by treatment with the non-steroidal anti-inflammatory drug (NSAID) piroxicam [43]. While the mechanisms by which NSAIDs exert these effects are unclear, NSAIDs are known to cause epithelial injury and barrier defects and to induce erosions and enhance mucosal bacterial invasion in IL-10 KO mice [44]. Thus, one might hypothesize that piroxicam triggers disease in IL-10 KO mice by enhancing intestinal permeability, although not by increasing tight junction permeability. This suggests that a transient elevation of intestinal permeability in a genetically susceptible host, e.g., IL-10 KO mice, is sufficient to initiate chronic disease. This model could also explain the observation, made in humans, that an acute episode of bacterial gastroenteritis is associated with increased risk of developing IBD [45]. Thus, the IL-10 KO mouse model emphasizes the interaction between genetic susceptibility, immune activation, intestinal microbiota, and epithelial barrier function in development of human IBD.

Barrier Defects and Mucosal Immune Regulation

The data discussed above suggest that barrier function can be a sensitive indicator of mucosal immune activation and that increased permeability may be able to amplify the effects of such immune activation. However, while the role of primary epithelial barrier dysfunction in IBD pathogenesis remains controversial, the associations are striking. For example, barrier defects precede clinical manifestations of disease in IL-10 KO mice, in which the primary defect is immunological [42]. Increased intestinal permeability has also been reported prior to clinical disease onset in the outbred SAMP1/YitFc mouse model of IBD [46]. Bone marrow chimera studies suggest that the primary defect in these mice is present within a radioresistant, non-bone marrow-derived cell population [46]. However, the precise abnormality that causes disease in SAMP1/YitFc mice has not been identified.

Studies of mice with targeted barrier defects provide further evidence that tight junction permeability may contribute to disease progression. One informative example comes from mice lacking junctional adhesion molecule A (JAM-A), an immunoglobulin superfamily member that facilitates tight junction assembly and leukocyte transmigration across endothelia and epithelia [47]. Loss of JAM-A expression within intestinal epithelia, both in vitro and in vivo, led to increased expression

of claudin-10 and claudin-15 and increased colonic epithelial apoptosis, proliferation, and migration *in vivo* [48]. This suggests that JAM-A deficiency results in chronic epithelial damage or compromised epithelial regenerative capacity. Consistent with this, colonic neutrophil accumulation was increased in JAM-A KO mice, and these mice were also hypersensitive to dextran sulfate sodium- (DSS)-induced epithelial injury and colitis [48]. Because JAM-A is normally expressed ubiquitously, defects in these mice could not be linked directly to loss of intestinal epithelial JAM-A. However, endothelial-specific JAM-A KO mice did not demonstrate increased DSS susceptibility, demonstrating that endothelial JAM-A loss was not responsible for the observed phenotype. In addition, the observation that intestinal epithelial, but not endothelial, JAM-A expression is reduced in human and experimental IBD supports the hypothesis that loss of intestinal epithelial JAM-A is responsible for the phenotype of JAM-A KO mice.

Although the phenotype of the JAM-A KO mice is informative, the cause of JAM-A loss in human and experimental IBD remains unclear. In contrast, the transcriptional and enzymatic activity of intestinal epithelial MLCK are well-documented in human and experimental IBD, and are, at least partly, regulated by proinflammatory cytokines such as TNF. Moreover, MLCK is required for *in vitro* and *in vivo* TNF-induced barrier loss. To model this form of barrier loss, transgenic mice that express constitutively active MLCK (CA-MLCK) from an intestinal epithelial-specific promoter were created. These mice have increased small intestinal and colonic paracellular permeability and exhibit mucosal immune activation, as evidenced by elevated mucosal IFN γ (gamma), TNF, IL-10 and IL-13 transcription and increased numbers of colonic lamina propria CD4⁺ cells. [49] However, despite mucosal immune activation, the mice thrive and do not develop spontaneous disease [49]. While this absence of disease may reflect the activity of immunoregulatory cells, such as those responsible for the increase in IL-10 transcription, expression of CA-MLCK in RAG1-deficient (RAG1^{-/-}) mice, which lack regulatory T cells, also fails to induce disease. However, when effector T lymphocytes are introduced into CA-MLCK/RAG1^{-/-} mice, using the CD4⁺CD45Rb^{hi} T lymphocyte adoptive transfer model of colitis, the onset of disease is accelerated and severity is increased relative to RAG1^{-/-} littermates that lack CA-MLCK [49]. Therefore, these CA-MLCK transgenic mice recapitulate the observation in humans that primary barrier dysfunction is insufficient to cause disease, e.g., in healthy relatives of CD patients, and provide evidence that barrier dysfunction can accelerate the onset of immune-mediated colitis.

Isolated Barrier Defects Are Insufficient to Cause Disease

Although the data above link increased intestinal permeability to disease severity and relapse, mild barrier defects may actually trigger regulatory processes that prevent inappropriate immune responses in immunocompetent hosts. The clearest example of this may be data indicating that, despite the presence of increased permeability in patients with infectious gastroenteritis, most of these individuals recover fully and do not develop IBD [45]. Recent studies in mice have provided some insight into the immunoregulatory mechanisms that prevent development of chronic disease in this situation. In wild-type mice, when mucosal damage was induced by intrarectal ethanol administration, the resulting transient increase in permeability induced a population of regulatory T cells characterized by surface expression of the TGF β propeptide latency-associated peptide (LAP) and increased IL-10 production by lamina propria mononuclear cells [50]. Moreover, this mild mucosal damage protected mice from colitis induced by subsequent intrarectal TNBS administration [50]. While the detailed mechanisms by which transient increases in permeability invoke immunoregulatory responses are incompletely characterized, it is notable that mucosal IL-10 production was also increased in CA-MLCK transgenic mice [49]. Thus, chronic barrier defects may induce immunoregulatory responses that prevent disease. On this basis, it can be inferred that in an

immunocompetent host limited barrier defects induce a robust immunoregulatory response that prevents disease. If correct, this hypothesis suggests that the difference between CD patients and their healthy relatives with increased permeability may be the quality of this regulatory response and the ability to manage inflammation induced by barrier loss. Overall, these studies emphasize the need for further clarification of the complex interactions between the intestinal epithelial barrier and mucosal immune system.

A Multifactorial Model of IBD

The data discussed above suggest a model of IBD in which paracellular defects, mucosal immune activation, and the luminal microbiota are interrelated and act cooperatively in the prevention, induction, and progression of disease (Fig. 4.3). The epithelial barrier balances the interaction between immunostimulatory luminal materials and the mucosal immune system. In immunocompetent subjects, mild barrier defects activate regulatory immune responses that compensate for and prevent excessive immune activation. In contrast, barrier defects may trigger inappropriate immune

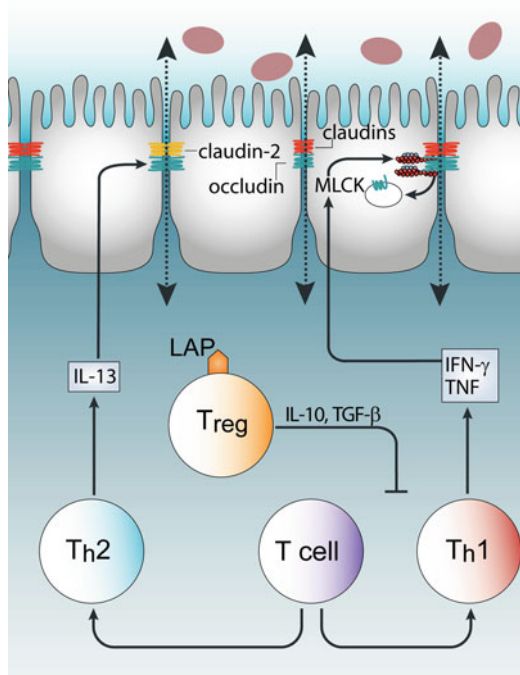


Fig. 4.3 The epithelial barrier regulates the balance between the luminal environment and mucosal immunity. Breaches in the epithelial barrier allow luminal material to cross the epithelium, where it may then activate mucosal immune responses. Under conditions of appropriate immune regulation, regulatory T cells (Tregs) produce anti-inflammatory signals to counter the proinflammatory immune response. A subset of these Tregs, those expressing latency-associated peptide (LAP), secrete IL-10 and TGF-β to attenuate disease. In contrast, under disease conditions, luminal material may activate Th1 or Th2 T cells. Th1 cells secrete TNF and IFNγ (gamma), which increase paracellular permeability via MLCK-dependent mechanisms. Alternatively, Th2 cells secrete IL-13, which increases epithelial permeability by enhancing claudin-2 expression. These increases in paracellular permeability may allow the passage of additional luminal material across the barrier, and ultimately, amplify the cycle of inflammation

activation and cytokine release in subjects that are unable to activate effective immunoregulatory responses. These cytokines may then signal to epithelial cells to cause further increases in paracellular permeability, which, in turn, allows further transmucosal passage of immunostimulatory materials and an even greater degree of immune activation. Thus defects in either barrier function or immune activation may initiate the cycle of barrier-immune dysregulation, but defects in both are necessary for perpetuation of this cycle and disease pathogenesis. This model also suggests that, in addition to the immunosuppressive and immunomodulatory agents that have been central to IBD therapy, it may be possible to break the cycle by restoring barrier function.

Barrier Restoration as a Future Therapeutic Intervention

Current immunosuppressive and immunomodulatory approaches are often effective in IBD management, but they treat the inflammation responsible for tissue damage rather than the underlying cause of inflammation. It has been recognized for some time that the epithelial barrier can be dysregulated by proinflammatory stimuli. The model described, which explains available data from *in vitro* and *in vivo* models as well as human studies, suggests that the barrier also regulates the immune response. Although increased paracellular permeability is a normal physiological response that is often beneficial and, in an immunocompetent host, triggers an immunoregulatory response, reduced barrier function may also be critical to disease pathogenesis. This is particularly true in individuals with immunoregulatory defects. For example, barrier loss precedes colitis in IL-10-deficient mice. Conversely, barrier restoration may blunt disease development [51]. Similarly, genetic deletion of intestinal epithelial long MLCK, which is necessary for TNF-induced barrier loss, partially protects immunodeficient mice from colitis induced by CD4+CD45Rbhi adoptive transfer. These data suggest that barrier restoration may be therapeutically useful, particularly in patients with IL-10 receptor mutations or single-nucleotide polymorphisms (SNPs) within the *IL10* promoter. However, as the effectors that regulate intestinal epithelial tight junctions are often shared with other epithelia and endothelia where they govern diverse processes, it will be critical to develop targeted approaches to restore intestinal barrier function without disrupting other tissues and functions. If this can be achieved, barrier restoration may have a promising future in IBD therapy.

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Chapter 5

Intestinal Microbiology and Ecology in Inflammatory Bowel Disease

Alan W. Walker

Keywords Microbiota • Microbiome • Ecology • Taxonomy • Inflammatory bowel disease • Dysbiosis

Introduction

Humans have evolved in the constant presence of microorganisms. It is unsurprising therefore that our physiology and anatomy have been shaped by interactions with microbes and that these interactions remain critical to our overall health and well-being [1]. This is perhaps nowhere more evident than in our intestinal tracts, where resident microbial communities (collectively termed the microbiota) have reached such incredible densities that the cumulative number of these cells exceeds the number of human cells that make up the body by a factor of ten [2].

While the role of the microbiota has been considered and studied for over a century [3], research was hampered until relatively recently by the fact that many intestinal microbes are recalcitrant to culture in the laboratory. Interest has intensified in recent years, however, driven by technological advancements in molecular biology and DNA sequencing that have largely bypassed the need to culture and so permit new insights into the relationship between ourselves and our microbial inhabitants.

The Human Gastrointestinal Tract Microbiota

The gastrointestinal tract contains a number of distinct physiological environments and therefore offers numerous niches for colonization by microorganisms [4] (Fig. 5.1). These microbial communities differ in composition along the length of the GI tract [2]. Bacteria present in the saliva may exceed 10^9 cells/ml but the stomach, with a local pH of around 2, acts as a barrier against entry into the lower intestinal tract [5]. This low pH, in combination with the short transit times of stomach contents, means that the number of microbes recoverable from the stomach is only approximately 10^3 cells/ml. The most abundant species found are acid tolerant streptococci and lactobacilli, which

A.W. Walker, BSc (Hons), PhD (✉)
Pathogen Genomics Group, Wellcome Trust Sanger Institute,
Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, UK
e-mail: aw6@sanger.ac.uk

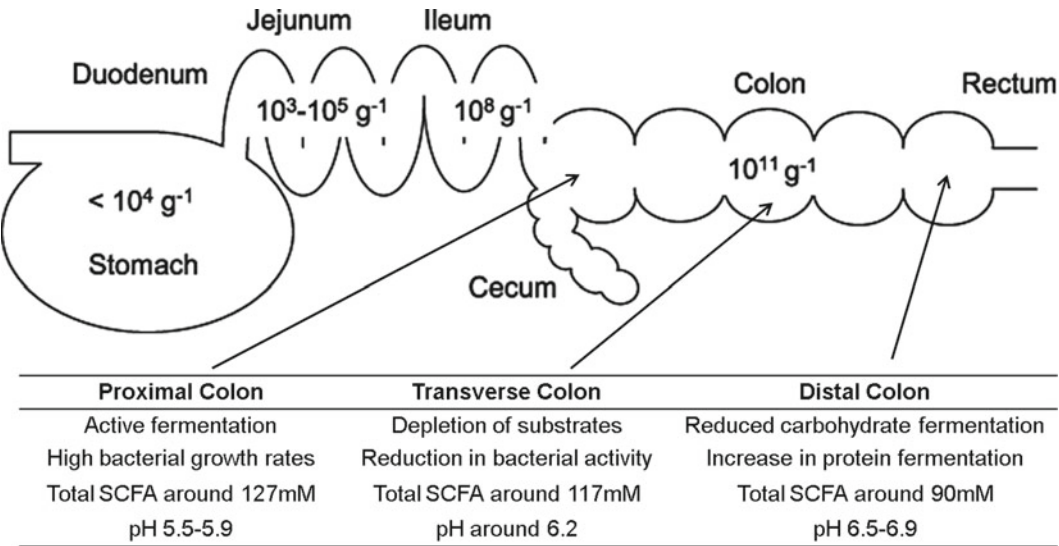


Fig. 5.1 Microbial colonization of the human gastrointestinal tract. Regional differences along the length of the gastrointestinal tract drive the development of distinct microbial communities. Figure illustrates the number of colonizing microbes in each region and shows the fermentative activities of bacteria within the colon. (Based on figures from Refs. 2 and 37)

can associate with the columnar epithelium of the gastric mucosa [4]. The duodenum is also subject to short transit times and this, in combination with biliary and pancreatic secretions, means it is also hostile to microbial survival and growth [6]. Therefore the upper regions of the small intestine contain only simple microbial communities of streptococci, lactobacilli, *Enterococcus*, and *Bacteroides* species [7]. It is not until the distal small intestine, with decreased peristalsis and acidity that both microbial abundance and species diversity increase significantly [2]. Beyond the ileum and into the colon, transit time slows and this allows the development of an abundance of microorganisms [8]. Around 10¹¹ bacterial cells/g of feces are reported [9] suggesting that the whole colon plays host to 10¹³⁻¹⁴ bacteria [10].

Colonization of the Human Large Intestine and Development of the Colonic Microbiota

The intestinal tract is sterile at birth and the composition of the resident microbiota undergoes dramatic changes during subsequent postnatal development [11, 12]. Colonization begins immediately after birth as the infant is exposed to bacterial contamination from the maternal intestinal and genital tract microbiota and from the surrounding environment [13]. Thereafter the composition of the colonic microbiota is directly influenced by the infant’s diet. In breast fed infants bifidobacteria usually become dominant, seemingly a result of their ability to utilize human milk oligosaccharides that escape digestion in the small intestine [14]. In contrast, formula fed infants tend to be colonized by a wider range of species [15]. Upon weaning, and subsequent exposure of the gut microbiota to more complex dietary carbohydrate sources, further compositional changes occur and by 12–24 months after birth a dense and complex microbiota resembling that of an adult becomes established [12, 16].

Table 5.1 Overview of the bacterial inhabitants of the human gastrointestinal tract

Phylum	Brief description	Commonly detected constituent genera
<i>Bacteroidetes</i>	Gram-negative bacilli, typically obligately anaerobic	<i>Bacteroides</i> , <i>Parabacteroides</i> , <i>Prevotella</i> , <i>Alistipes</i> , <i>Barnesiella</i>
<i>Firmicutes</i>	Low G+C content Gram positives, typically obligately anaerobic	<i>Roseburia</i> , <i>Dorea</i> , <i>Blautia</i> , <i>Anaerostipes</i> , <i>Coprococcus</i> , <i>Lachnospira</i> , <i>Butyrivibrio</i> , <i>Faecalibacterium</i> , <i>Ruminococcus</i> , <i>Subdoligranulum</i> , <i>Anaerotruncus</i> , <i>Coprobacillus</i> , <i>Holdemania</i> , <i>Veillonella</i> , <i>Phascolarctobacterium</i> , <i>Megasphaera</i> , <i>Dialister</i> , <i>Acidaminococcus</i> , <i>Eubacterium</i> , <i>Clostridium</i> , <i>Finegoldia</i> , <i>Lactobacillus</i> , <i>Streptococcus</i> , <i>Enterococcus</i>
<i>Actinobacteria</i>	High G+C content Gram positives, typically obligately anaerobic	<i>Bifidobacterium</i> , <i>Collinsella</i> , <i>Eggerthella</i> , <i>Atopobium</i>
<i>Proteobacteria</i>	Gram negative, mainly facultatively anaerobic species	<i>Escherichia</i> , <i>Enterobacter</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Hafnia</i> , <i>Sutterella</i> , <i>Parasutterella</i> , <i>Alcaligenes</i> , <i>Oxalobacter</i> , <i>Desulfovibrio</i> , <i>Bilophila</i> , <i>Campylobacter</i> , <i>Helicobacter</i>
Others	Typically less abundant members of intestinal bacterial communities	<i>Akkermansia</i> , <i>Victivallis</i> , <i>Fusobacterium</i>

The human intestinal microbiota is dominated by four bacterial phyla. The *Bacteroidetes* and *Firmicutes* are by far the most numerically abundant phyla and it appears that the *Firmicutes* phylum contains the greatest species diversity

The adult colonic microbiota is both extremely abundant and highly diverse. The vast majority of species are obligately anaerobic bacteria, which likely account for over 99% of the total microbiota [6]. Well over 1,000 bacterial species are known to be capable of colonizing the adult colon [17] and much of this diversity has only been appreciated in light of 16S rRNA gene sequence surveys. Indeed, it is estimated that 40–80% of the bacteria present in the gut have yet to be recovered by current culturing techniques, although these estimates vary between individuals and studies [18].

Despite this diversity a number of general themes are apparent. It appears that certain bacterial lineages are particularly well adapted to survival in the gut (Table 5.1). There are currently around fifty-five recognized divisions or phyla of bacteria and only nine of these are found in the human GI tract [17]. Indeed, the microbiota is typically dominated by just two bacterial phyla; the Gram-negative *Bacteroidetes* and the Gram-positive *Firmicutes* [19], with just two further phyla, the *Actinobacteria* and the *Proteobacteria*, commonly accounting for the remainder of the bacteria present [20]. Consistently identified genera from each of these phyla are shown in Table 5.1. It should be noted that taxonomy has not been finalized for many gut bacteria as many traditional designations, particularly within the *Firmicutes* phylum, have not been supported by subsequent DNA sequence data [21]. A number of species have since been reclassified based upon sequence information but this process is ongoing and incomplete [22]. It was previously thought that most *Firmicutes* were *Clostridium* and *Eubacterium* species but it is now well established that the predominant gut *Firmicutes* belong to two Families; the *Lachnospiraceae* (formerly known as clostridial cluster XIV or the *Clostridium coccoides* group) and the *Ruminococcaceae* (formerly known as clostridial cluster IV or the *Clostridium leptum* group), neither of which contain true clostridia or eubacteria [19, 21].

While bacteria have been the focus of most microbiota research eukaryotes, archaea and viruses can also colonize the human large intestine. Culture-based analyses of the eukaryotic microbiota suggest that *Candida* species are the most widespread group but these appear to be present at only 10^2 – 10^4 cfu/ml [17, 23]. In contrast to culture-based studies a recent molecular analysis identified

Blastocystis species as the predominant eukaryotes, although, again, these communities appear to be far simpler and less abundant than their bacterial counterparts [24].

Methanogenic archaea can also colonize the human large intestine, although they are not present in all individuals and colonization rates appear to be much lower in the developed world than in developing countries [25]. The vast majority of intestinal isolates appear to be accounted for by only a single species, *Methanobrevibacter smithii* [19], although *Methanosphaera stadtmanae* has also been isolated and a putative new order of archaea has recently been detected by sequence analysis [26].

In contrast to eukaryotes and archaea, viruses seem to be highly abundant in the human gut. The majority of DNA viruses appear to be phages (i.e., viruses that infect bacteria), while the majority of RNA viruses are plant viruses [27]. A recent metagenomic analysis has revealed interesting new insights into the interplay between viruses and bacteria in the human gut. In contrast to many other environments, where lytic phages are dominant, it appears that the human gut is instead dominated by temperate phages [28].

Variability Within the Microbiota

One of the most striking features of the intestinal microbiota is the large degree of variation in composition between different human hosts [29]. Indeed, while the predominant bacteria are usually members of the *Bacteroidetes* phylum and the *Lachnospiraceae* and *Ruminococcaceae* families of the *Firmicutes* phylum it appears that there is limited overlap at the species and strain level between individuals [29]. However, even though these results suggest that there is no core subset of species that are present in all individuals, it appears that there are dominant species which are widespread among the human population and are therefore assumed to be particularly well adapted to life in the human large intestine [20, 30, 31].

The final adult microbiota is influenced by factors such as host genotype and diet, historical microbial exposures during community assembly and temporal dynamics arising from interactions within the microbiota, and it is this set of influences that would appear to drive the establishment of microbial communities that are unique to the individual [1, 29, 32, 33].

It appears that, within individuals, species composition is relatively stable over time [34, 35]. However, it is probable that the relative abundance of at least some of these species is likely to vary in response to changes in diet [31, 36]. In addition, the physiology and prevailing environmental conditions vary considerably within each colonic region, offering distinct conditions for microbial growth. As a result there are likely to be spatial differences in species abundance depending on the location within the colon. The proximal colon is characterized by high substrate availability as undigested substrates enter the colon in this area and are rapidly fermented by the resident microbes. This leads to high production of short chain fatty acids (SCFAs), which in turn lower the luminal pH. As the digestive material moves distally along the gut carbohydrate sources become depleted. Consequently microbial growth and fermentation slow in the distal colon and pH rises toward neutrality [37] (Fig. 5.1).

Furthermore, microscopic examination of colonic material shows that the majority of bacteria are not freely dispersed but rather are arranged into large clumps and aggregates which are attached to plant cell wall structures, resistant starch granules, and the mucus layer covering the mucosa [38] (Fig. 5.2). These substrates promote the formation of differing attached populations and organisms found colonizing each of these substrates may be distinct from those in the gut lumen/feces [19, 39–41].

Perturbations in community structure due to insults such as antibiotic treatment and diarrheal disease are also likely to be important. While it appears that the individual microbiota is remarkably resilient to such insults recent evidence has shown that in some cases species recovery may be incomplete [42].

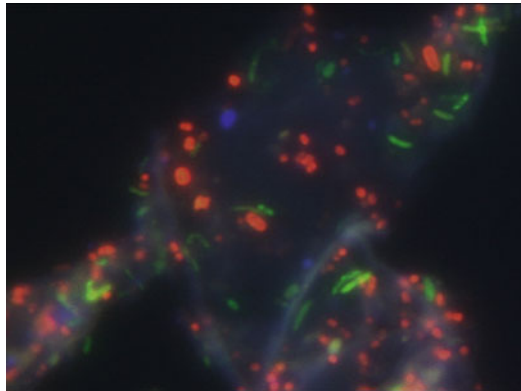


Fig. 5.2 Substrate-attached bacteria. Image shows FISH-labeled bacteria tightly adhered to a plant fiber recovered from a fecal sample. Distinct groups of *Firmicutes* species appear to be the predominant colonizers. *Red cells* represent *Ruminococcus* species, *green cells* represent *Lachnospiraceae*. The much rarer blue cells represent bacteria that have been stained with the universal DAPI label only and so belong to all other bacterial groups

This inherent variability has repercussions for investigators engaging in microbiota research. The high degree of interindividual variation in species content means that all but the most significant trends may be masked by differences between individuals. Many studies have therefore attempted to use each individual as their own control but results in these cases may also be affected by temporal or spatial fluctuations in species abundance.

Despite these inter- and intraindividual variations, however, the microbiota remains relatively functionally stable and generally carries out a basic set of biochemical reactions [11]. This functional stability is reflected in recent results from metagenomic studies, which indicate that, regardless of species composition, the collective genomes of each individual's microbiota (termed the "microbiome") appear to encode a remarkably conserved set of functions [29]. Indeed, the gut microbiome may encode over 100 times more unique genes than the human genome and many of these functions are complementary, endowing us with capabilities that we have not had to evolve ourselves [30].

Beneficial Activities of the Microbiota

Under normal circumstances the microbiota may be considered to be either commensal or beneficial to human health [1]. The primary activities of the gut microbiota are the breakdown of complex substrates that escape digestion in the small intestine and the fermentation of these to SCFAs and gases [43]. Ninety-five percent of bacterially derived SCFAs are absorbed by the colonic mucosa [44] meaning that the human host extracts energy from otherwise inaccessible food components. Humans on a western diet may yield 0.5–0.6 M of SCFA, with a total energy value of 140–180 kcal, per day [11]. While this energy was doubtless of great importance to human health throughout history, in the modern westernized world characterized by a surplus of food the gut microbiota has instead been implicated in the development of obesity [45].

Acetate, propionate, and butyrate are the predominant SCFAs produced by the gut microbiota [43]. Of these, butyrate has received the most attention as the colonic epithelium derives as much as 60–70% of its energy needs from this SCFA and there is some evidence that it may be both anti-inflammatory and anti-carcinogenic [46]. The main butyrate-producing bacteria found in the human

colon appear to belong predominantly to the *Roseburia* and *Faecalibacterium* genera of the *Firmicutes* phylum [46].

Aside from their roles in the digestion of food and production of SCFAs the colonic microbiota may have a variety of other potentially beneficial effects upon human health. This includes the synthesis of nutrients such as vitamins B and K, folate and phytoestrogens [25, 47] and the metabolism of potentially harmful substances such as bile acids, bilirubin, cholesterol, nitrosamines, heterocyclic amines, and oxalate [10]. The sheer volume and diversity of the microbiota is also a formidable barrier to colonization of the gut by foreign pathogens and indeed some pathogens such as *Salmonella* species appear to have evolved mechanisms to overcome this colonization resistance [48]. The dialogue between host and gut microbiota along the mucosa also seems to play a part in the development and maintenance of normal mucosal immunity [49].

However, a balanced relationship must exist between bacteria and their human hosts as the microbiota is a major source of antigens, including lipopolysaccharides, peptidoglycan, lipoproteins, flagellin, and unmethylated CpG-containing DNA, all of which can activate both innate and adaptive immune responses [50, 51]. A disruption in this homeostasis threatens the state of immune tolerance and may result in gut inflammation.

The Microbiota and Inflammatory Bowel Disease

While we have yet to fully elucidate the mechanisms behind the inflammatory response seen in ulcerative colitis (UC) and Crohn's disease (CD) the current generally accepted disease hypothesis is that the chronic inflammation of IBD results from a genetically dysregulated host immune response directed at the gut microbiota or to metabolites derived from the microbiota [52].

A number of lines of evidence support this hypothesis and implicate the microbiota in disease pathogenesis and progression. Animal models of colitis typically require the presence of gut bacteria to initiate inflammation [53], broad-spectrum antibiotics have some efficacy in the treatment of IBD [54], fecal stream diversion has been shown to prevent the recurrence of symptoms in CD sufferers while re-infusion of intestinal contents into the excluded ileum induces mucosal lesions [55], patients with IBD have increased intestinal mucosal secretion of IgG-type antibodies against a broad spectrum of commensal bacteria [18], higher concentrations of bacteria are observed in the mucosal layer of diseased patients and this concentration increases with severity of disease [56], and genome-wide association studies have identified numerous polymorphisms in host genes involved in bacterial recognition and clearing [57].

Given these tantalizing findings, the microbiology of IBD is an active area of research with a large body of literature on the subject. A number of studies have attempted to characterize gut microbial communities in IBD and to identify changes in comparison with healthy individuals. It should of course be acknowledged that such comparative studies can only demonstrate associations and that it is difficult to differentiate between changes that may play a role in disease pathogenesis and those which may simply have occurred as a result of disease. Indeed, the gut in IBD is likely to be a less stable environment than that of healthy individuals with a number of factors, such as increased drug exposure, regular bouts of diarrhea, dietary interventions, localized release of inflammatory and antimicrobial compounds and changes in mucosal structure, likely to result in altered conditions for bacterial growth [58, 59].

Nonetheless, the results of comparative studies have revealed a small number of recurring themes. Individuals with IBD have repeatedly been shown to have reduced bacterial diversity in their intestines, reduced temporal stability in bacterial community structure and often these communities cluster separately when compared to healthy controls [30, 58, 60–64] (Fig. 5.3).

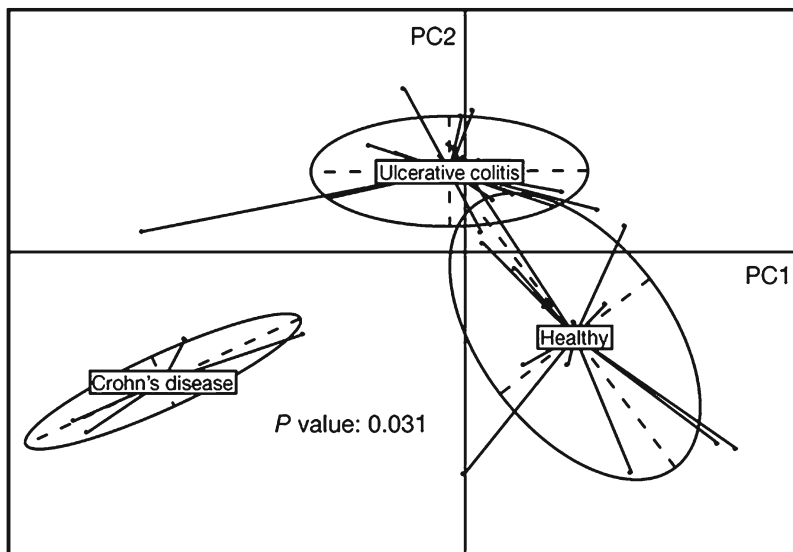


Fig. 5.3 Bacterial community structure differentiates IBD patients from healthy individuals. Results were generated as part of an in-depth metagenomic analysis of fecal samples from cohorts of healthy, CD and UC patients [30]. Principal component analysis based on the abundance of 155 bacterial species indicates that there is a distinct separation between the microbiota found in IBD sufferers (particularly CD patients) and those found in healthy individuals. (Reprinted by permission from Macmillan Publishers Ltd: Nature. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing:464(7285):59–65. © 2010)

While there do, therefore, appear to be some broad reproducible differences between the microbiota in IBD patients and those from healthy individuals, compositional comparisons that aim to identify putative etiological agents have unfortunately generated inconclusive and inconsistent results. Individual species, or groups of species, such as *Mycobacterium avium* subspecies *paratuberculosis*, sulfate-reducing bacteria, *Yersinia*, *Listeria* or adherent-invasive *Escherichia coli* have been implicated in disease etiology [65–67] but these organisms are not ubiquitous in IBD patients and some may also form part of the microbiota in healthy individuals [66]. Therefore, as yet, there is no definitive evidence to link any of these species to IBD pathogenesis.

Driven by technological advances, focus in recent years has to some extent shifted instead onto the microbiota as a whole. The “dysbiosis” hypothesis implicates multispecies assemblages in IBD pathogenesis and postulates that disease development and progression is linked to shifts in microbiota composition to a state where there is a reduction in beneficial or protective species and an increase in more harmful species or groups [68].

While results of these whole microbiota comparisons have also generated inconsistent results there appears to be a general trend toward reductions in components of the *Firmicutes* phylum in IBD, sometimes, but not always, with concurrent increases in *Bacteroidetes* and facultative anaerobes such as *Enterobacteriaceae* [63, 64, 68–72]. The increase in *Enterobacteriaceae* appears to be most pronounced in patients with CD but it is unclear whether or not this increase simply reflects the fact that this group seems particularly well adapted for growth in the presence of inflammation [48, 73].

While many studies aim to identify potentially pathogenic members of the microbiota, one member of the *Firmicutes* phylum, *Faecalibacterium prausnitzii*, has been implicated instead in protection from IBD development [74]. This species appears to be commonly diminished in individuals

with IBD [63, 64, 75] and furthermore appears to release a (currently unidentified) soluble factor that inhibits proinflammatory epithelial cell responses *in vitro* and attenuates inflammation in mouse models of colitis [74, 76].

At present, however, no individual species or overall bacterial composition has been conclusively shown to be involved in IBD pathogenesis, although the broad differences in bacterial diversity and overall structure may be important for disease maintenance and severity [77].

Conclusions and Future Prospects

It is clear that the intestinal microbiota has profound effects on host health. There is good evidence for the involvement of the microbiota in IBD development but the inherent complexity and variability in species composition and abundance between, and within, individuals means that study of the microbiota remains challenging. Previous IBD studies have primarily been based on small patient cohorts, have focused only on the bacterial component of the microbiota, and have used a variety of different methodologies and sampling protocols. This limits the scope for cross-comparison between studies and unfortunately does not take into account the role that factors such as host genotype and diet play in shaping the overall microbiota. Furthermore, recent evidence implicating a viral trigger in one model of IBD indicates that all constituent members of the microbiota should be considered, not just the bacteria [78]. If IBD is combinatorial in etiology, with many different combinations of genetic and environmental causes leading to similar therapeutic responses then, to overcome the difficulties outlined above, it is clear that large-scale comprehensive studies, incorporating as much metadata as possible, offer the best chance of increasing our knowledge. Given the functional redundancy of the microbiota, which means that similar functions may be encoded by multiple species, it may also be that we will be better served in the future by looking at the complete gene complement of the microbial community as a whole, not just species composition. Recent advances in DNA sequencing technology mean that studies such as this are already possible [30].

Regardless, the microbiology of IBD remains an active area of research worldwide and forms a major part of the current large-scale Human Microbiome Project and MetaHIT initiatives [79]. With this in mind, it is hoped that further analysis of the complex interplay between host and microbes will yield important insights into the role that the intestinal microbiota plays in the development of IBD.

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Chapter 6

Microbial Recognition and Pathogen-Associated Molecular Pattern Receptors in Inflammatory Bowel Disease

Kenya Honda, Koji Atarashi, and Junko Nishio

Keywords Toll-like receptor • NOD • C-type lectin • Autophagy • Antimicrobial proteins • IgA • Commensal bacteria

Introduction

The intestinal mucosa is routinely subjected to challenges by invading pathogens, such as bacteria, viruses, fungi, protozoa, and helminths. At the same time, the intestinal mucosa allows the presence of large numbers of harmless commensal bacteria in the lumen. Therefore, the gut mucosa has evolved multiple layers of protection to prevent the translocation of pathogenic as well as commensal microbes. The intestinal epithelium is covered with layers of mucus, which is composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells. In the colon, the inner layer of mucus gel is densely packed and firmly attached to the epithelium, and creates a critical barrier that prevents large particles, including most bacteria, from directly contacting the epithelial cells. The intestinal epithelium is also tightly sealed by tight junctions (TJs) and subjacent adherens junctions (AJs), which also play a very critical role in the prevention of invasion of microbes. The small intestinal epithelium contains Paneth cells at the base of the crypts, which contribute to innate immunity by secreting a diverse repertoire of antimicrobial proteins [1, 2]. Beneath the epithelium, the lamina propria (LP) contains dendritic cells (DCs), which extend their dendrites between epithelial cells to continuously monitor gut lumen content and activate LP lymphocytes [3]. LP contains large numbers of effector lymphocytes even in the absence of inflammation. These include IgA-producing plasma cells and CD4⁺ T cells dominated by a T helper type 1 (Th1) or Th17 phenotype. These in toto constitute the firewall that prevents systemic penetration and spread of commensal and pathogenic microbes.

Intestinal epithelial cells and innate immune cells express a number of receptors that recognize conserved moieties on microbial molecules, called pathogen-associated molecular patterns (PAMPs). The receptors of PAMPs, called pattern-recognition receptors (PRRs), include complement receptors, scavenger receptors, mannose-binding receptors, Toll-like receptors (TLRs), nucleotide-binding domain (NOD)-like receptors (NLRs), and C-type lectin receptors (CLRs). It is well established that PRRs recognize PAMPs from pathogens that invade across the barrier systems.

K. Honda, MD, PhD (✉) • K. Atarashi, PhD • J. Nishio, MD, PhD
Department of Immunology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan
e-mail: Kenya@m.u-tokyo.ac.jp

PRRs elicit signals for innate and adaptive immune responses, thereby eliminating pathogens without significant spread beyond the intestine.

The microbial ligands recognized by PRRs are not unique to pathogens and are also produced by commensal microorganisms. The intestinal mucosa should confine commensal microorganisms to the lumen of the gut and prevent their translocation into the systemic circulation and mounting robust immune responses against them during the steady state. It was considered that inflammatory responses to commensal bacteria is completely prevented owing to the sequestration of microflora by physical barrier systems. Recent results, in contrast, suggest that constitutive signaling through PRRs by commensal bacteria is operative and is rather required for epithelial barrier functions and normal host-commensal homeostasis. Indeed, the continuous sensing of commensal bacteria through PRRs facilitates the epithelial cell barrier functions and the production of antimicrobial proteins and IgA. The appropriate discrimination by and the well-controlled responses of PRRs in the gut to commensals and pathogens are vital, and the aberrant activation of PRRs against commensal bacteria is one of the most critical etiologies of inflammatory bowel diseases (IBDs). In this chapter, we will discuss the activation and regulation of PRR signaling, and their relationship with IBD.

TLRs

TLR Signaling

The family of TLRs consists of up to 14 germline-encoded transmembrane receptors in mammals (10 functional genes in humans and 12 in mice) [4]. Each TLR forms hetero- or homo-dimers and recognizes various PAMPs derived from bacteria, viruses, fungi, and/or protozoa. TLRs are differentially distributed within a cell. TLR1, TLR2, TLR4, and TLR6 are expressed on the cell surface, whereas TLR3, TLR7, TLR8, and TLR9 are expressed in endosomes/lysosomes and require endosomal acidification for their ligand recognition. All TLRs contain intracellular Toll-interleukin-1 (IL-1) receptor (TIR) domains, which transmit downstream signals via the recruitment of one or more TIR-containing adaptor proteins, which include MyD88, TIR domain containing adaptor protein (TIRAP; also called MAL), TIR domain containing adaptor-inducing interferon (TRIF; also called TICAM1), and Trif-related adaptor molecule (TRAM; also called TICAM2). Signaling through TLRs can be broadly categorized into two pathways: the MyD88-dependent pathway and the TRIF-dependent pathway. All TLRs, except TLR3, activate the MyD88 pathway, whereas TLR3 and TLR4 activate the TRIF pathway. MyD88 is linked to the activation of IL-1 receptor-associated kinases 1 (IRAK1), IRAK4, tumor necrosis factor receptor-associated factor 6 (TRAF6), and transforming growth factor- β -activated kinase 1 (TAK1). The signaling pathway through TRIF leads to the activation of TANK-binding kinase 1 (TBK1). Both MyD88- and TRIF-dependent pathways are linked to the activation of NF- κ B and mitogen-activated protein kinase (MAPK), but the activation of interferon regulatory factor 3 (IRF3) is limited to the TRIF-TBK1 pathway. Through MyD88 and/or TRIF, TLRs induce the production of cytokines, including TNF α , IL-6, IL-12, IFN- β , and IL-8, and eventually lead to the recruitment and differentiation of antigen-specific adaptive immune cells. Therefore, TLR signaling governs the entire host responses, innate and adaptive, against invading pathogens in the intestinal mucosa. Indeed, mice deficient in *Myd88* or *Trif* are highly susceptible to infection by various pathogenic microorganisms. Furthermore, *Myd88* or *Trif* deficiency results in serum IgG priming against intestinal commensal bacteria [5], suggesting that without the TLR system, even commensal bacteria can translocate into the systemic circulation.

TLR4

TLR4 was identified as the first mammalian homolog of *Drosophila* Toll [6]. TLR4 forms a complex with MD2 on the cell surface and serves as a critical receptor for bacterial lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria. LPS-binding protein (LBP) and CD14 are involved in TLR4-mediated LPS recognition. LBP is a soluble plasma protein that binds LPS, and CD14 binds LBP and delivers LPS-LBP to the TLR4–MD2 complex. TLR4 activates two distinct pathways controlled by the TIRAP-MyD88 and TRAM-TRIF pairs of adaptor proteins, which elicit the production of proinflammatory cytokines and type I IFN, respectively. Normal intestinal epithelial cells (IECs) express low levels of TLR4 and CD14 [7, 8]. However, it has been shown that TLR4 and CD14 are upregulated in IECs of IBD patients [9, 10]. Therefore, a dysregulated expression of TLR4 and CD14 in IECs may be crucial in IBD pathogenesis. On the other hand, variant alleles of the *TLR4* gene, which results in impaired LPS signaling, have been found in some IBD patients [11, 12]. Therefore, these lines of evidences suggest that the abrogation of signaling as well as an excessive signaling of TLR4 can be both causative in the disease process of IBD.

TLR2

TLR2 recognizes peptidoglycan (PGN), lipoteichoic acid (LTA), lipoproteins, lipoarabinomannan, and zymosan. TLR2 forms a heterodimer with TLR1 or TLR6. The TLR2–TLR6 heterodimer recognizes diacylated lipopeptides, PGN, or zymosan, whereas the TLR1–TLR2 heterodimer recognizes triacylated lipopeptides. Not only TLR1 or TLR6, but also CD36 and dectin-1 interact with TLR2. Using several coreceptors, TLR2 mediates diverse signaling. TLR2 transmits signaling solely through MyD88. Although MyD88-dependent signaling is generally known to induce a Th1 response by driving IL-12 production, TLR2–MyD88 signaling in DCs can induce a Th2-biased immune response, through the production of IL-10 and IL-13. TLR2 signaling is also unique in terms of triggering the induction of forkhead box P3 (Foxp3)-expressing regulatory T (Treg) cells. In DCs, TLR2 stimulation by zymosan elicits the activation of extracellular regulated kinase (ERK) to express the retinoic-acid-metabolizing enzyme retinaldehyde dehydrogenase (Raldh). Raldh enzymatically converts vitamin A to retinoic acid, which acts on CD4⁺ T cells to differentiate into Treg cells [13]. It has also been shown that polysaccharide A (PSA) from *Bacteroides fragilis* activates TLR2 perhaps on DCs and directs the development of IL-10-expressing Foxp3⁺ Treg cells in intestinal LP [14]. Collectively, TLR2 contributes to both activation and suppression of the intestinal immune system, and is therefore a highly relevant molecule for the pathogenesis of IBD.

TLR5

TLR5 recognizes the bacterial protein flagellin, which is required for flagellar filament assembly and motility. Although TLR5 recognizes an evolutionarily conserved site of flagellin, α and ε *Proteobacteria*, including the human pathogens *Campylobacter jejuni*, *Helicobacter pylori*, and *Bartonella bacilliformis*, are known to possess unique flagellin molecules that escape from recognition by TLR5 [15]. A subset of commensal bacteria also possesses one or more flagella. In polarized epithelial cells, TLR5 is localized to the basolateral membrane and is thus segregated from the luminal contents that contain flagellin. Therefore, only when flagellin from enteric bacteria penetrates and reaches the basolateral side of the epithelium, will a rapid proinflammatory response be

evoked by TLR5. However, in IBD patients, flagellin from commensal bacteria can reach the basolateral side of IECs and can be recognized by TLR5, upon which aberrant and chronic immune responses are induced [16]. This is likely because these genetically susceptible individuals have a leaky bowel mucosa, allowing flagellin from commensals to obtain access to TLR5.

TLR9

In addition to cell wall components, microbial DNAs can also function as a PAMP. TLR9 recognizes unmethylated CpG DNA motifs that are frequently present in bacteria and viruses but are rare in mammalian cells. In response to CpG-containing DNA, B-cells robustly proliferate and secrete immunoglobulins. CpG DNA can also activate monocytes, macrophages, and DCs to secrete Th1-like proinflammatory cytokines, particularly IL-12, and upregulate MHC class II and costimulatory molecules, thereby stimulating T-cells and natural killer (NK) cells to produce IFN- γ , leading to an enhanced host defense against invading pathogens.

Requirement of Constitutive TLR Signaling in the Gut

Under steady-state conditions, IECs express low levels of TLR2, TLR4, and CD14; TLR5 is expressed on the basolateral surfaces of IECs; and TLR3, TLR7, TLR8, and TLR9 are expressed in intracellular endosomal organelles. Such regulated expression and location of TLRs contribute to the prevention of aberrant immune response against commensal bacteria, not just to completely avoid stimulation by commensal bacteria. Instead, accumulating evidence indicates that a constitutive activation of TLRs by commensal bacteria in IECs and hematopoietic cells occurs and is required for the maintenance of intestinal homeostasis (Fig. 6.1).

Maintenance of Epithelial Barrier Integrity

Constitutive TLR signaling by commensal bacteria is required for the maintenance of physical barrier functions of IECs. Wild-type mice treated with broad-spectrum antibiotics are highly susceptible to dextran sulfate sodium (DSS)-induced intestinal inflammation [17]. This is due to the defect of constitutive TLR signaling by commensal bacteria, because an oral administration of LPS or LTA restores the susceptibility of antibiotics-treated mice to severe injury of colonic epithelium by DSS. Mice lacking either *Tlr2*, *Tlr4*, *Tlr9*, or *Myd88* are highly susceptible to DSS colitis [17, 18]. TLR5 has an essential and nonredundant role in the maintenance of gut homeostasis. Mice lacking *Tlr5* develop spontaneous colitis, and bacterial translocation occurs in the spleen and liver of *Tlr5*^{-/-} mice [19].

TLR9 activation through the apical surface of epithelial cells by commensals prevents aberrant NF- κ B activation and inhibits inflammation [20]. In addition, TLR9 triggers IECs to produce type I IFN, which plays anti-inflammatory functions in mouse colitis models [21]. The absence of MyD88 signaling in IECs has been shown to be associated with a decreased production of cytoprotective factors such as IL-6, KC-1, and heat shock proteins in IECs [17]. TLR2 signaling plays critical roles in the maintenance of the functional TJ barrier integrity of the intestinal epithelial layer [22]. TLR2 signaling is also required for the stimulation of goblet cells to produce trefoil factor 3 (TFF3), which confers IECs resistance to inflammatory stress-induced damage [23]. *Tlr2*^{-/-} mice

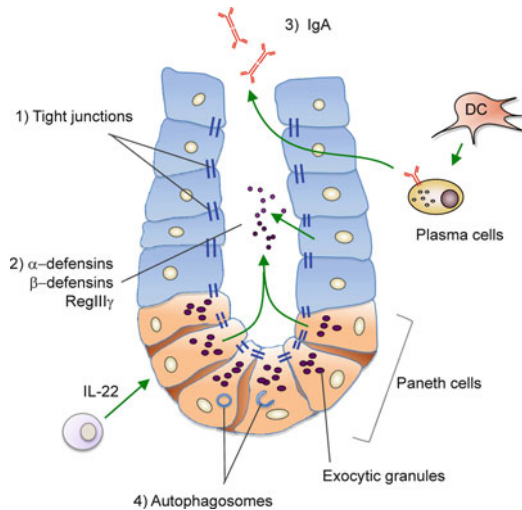


Fig. 6.1 Mucosal barrier systems regulated by PRRs. The constitutive activation of PRRs in/on IECs, Paneth cells and hematopoietic cells by commensal bacteria under normal steady-state conditions is required for various aspects of mucosal barrier systems. (1) The constitutive activation of PRRs by commensals is required for the maintenance of the functional tight junction barrier integrity of the intestinal epithelial layer. (2) The direct recognition of commensal bacteria by PRRs in Paneth cells and IECs leads to the production of antimicrobial proteins, including α -defensins, β -defensins, and RegIII γ . A subset of hematopoietic cells can recognize PAMPs and produce IL-22, which acts on IECs to produce antimicrobial proteins. (3) PRR signaling in DCs, particularly in iNOS⁺ DCs or TLR5⁺ DCs, promotes the differentiation of B cells into IgA-producing plasma cells. (4) The constitutive PRR signaling in Paneth cells is likely required for the induction of autophagy. A defect of autophagosome formation in Paneth cells results in an impaired secretion of granule contents containing antimicrobial peptides and other proteins

are susceptible to *Citrobacter rodentium*-induced colitis and exhibit marked mucosal alterations, characterized by severe mucosal ulcerations and crypt destruction [24]. These *Tlr2*^{-/-} phenotypes are associated with increased epithelial cell death, defective localization of zona occludens (ZO)-1 and claudin-3 to TJs, and increased transepithelial permeability of intestinal mucosa. These findings all support the concept that TLRs play an important role in maintaining the functional integrity of mucosal barriers (Fig. 6.1).

Production of Antimicrobial Proteins

IEC-derived antimicrobial proteins are members of diverse protein families, including α -defensins [human defensin 5 (HD5) and HD6, and murine cryptidins], β -defensins [human β -defensin 2 (HBD2) and mouse β -defensin 3 (mBD3)], cathelicidins [human LL37 and mouse cathelicidin-related antimicrobial peptide 4 (mCRAMP4)], C-type lectins [human hepatointestinal pancreatic/pancreatitis-associated protein (HIP/PAP) and mouse regenerating islet-derived 3 (RegIII)], and lipocalin2. Most of these proteins kill pathogenic bacteria directly through enzymatic attack of the bacterial cell wall or by disrupting the bacterial inner membrane. At the same time, antimicrobial proteins play key roles in shaping the composition of the intestinal microbiota and in the modulation of mucosal immune responsiveness [25].

The expression of a subset of antimicrobial proteins is controlled by bacterial signals through TLRs (Fig. 6.1). For example, the *Myd88*-dependent TLR activation in Paneth cells by commensals plays a critical role in the production of RegIII γ [26], which kills Gram-positive bacteria, including

vancomycin-resistant *Enterococcus* (VRE) [27, 28]. There are lines of evidence suggesting that the expressions of α - and β -defensins are regulated by TLR signaling in IECs and Paneth cells [26]. In addition, the production of antimicrobial proteins is indirectly regulated by TLRs via cytokines, such as IL-22. The TLR5-mediated recognition of commensal bacteria in hematopoietic cells leads to the production of IL-22 [28]. The produced IL-22 then acts on IECs to produce RegIII γ and lipocalin2 [29].

IgA Production

Mucosal IgA plays a critical role in the regulation of gut microbial ecology and in the determination of the nature of intestinal bacteria [30]. The development of IgA-producing B cells is regulated by TLR signaling activated by commensal bacteria (Fig. 6.1). Microbial TLR ligands, such as LPS, can substitute for the CD40 ligand (CD40L) and act directly on B cells along with transforming growth factor- β (TGF- β) to initiate IgA class switching [31]. In addition to engaging TLRs on B cells, microbial products stimulate DCs to produce B-cell activating factor (BAFF; also known as BLyS) and a proliferation-inducing ligand (APRIL). BAFF and APRIL are functionally related to CD40L and promote IgA class switching in a CD40L-independent manner. It has been shown that BAFF and APRIL are expressed in inducible nitric oxide synthase (iNOS)-expressing DCs in intestinal LP [32]. The iNOS⁺ DCs express gaseous nitric oxide, which induces BAFF and APRIL via an unknown mechanism. The expression of iNOS in DCs is regulated by TLRs, and mice deficient in TLR signaling have a reduced production of luminal IgA [32]. TLR5⁺CD11c^{hi}CD11b^{hi} cells are another subset of LP DCs that preferentially induce IgA class switching [33]. Upon stimulation by flagellin, TLR5⁺ DCs express Raldh2, which produces retinoic acid from vitamin A. Retinoic acid then acts on B cells to differentiate into IgA-producing plasma cells.

TLR Signaling and Autophagy

Autophagy is an evolutionarily conserved degradation system, which orchestrates the engulfment of cytoplasmic proteins into double-membraned vesicles (autophagosomes) and coordinates the fusion of the autophagosomes with lysosomes. Autophagy is involved in a housekeeping role in removing misfolded or aggregated proteins, and clearing damaged organelles, such as mitochondria [34]. Moreover, several studies have shown that autophagy plays a critical role in host defense responses by promoting the elimination of intracellular pathogens [35, 36]. Autophagy also contributes to MHC class II-restricted endogenous antigen presentation and controls T-cell development.

Recent genome-wide association studies have revealed a strong association between ATG16 autophagy-related 16-like 1 (*ATG16L1*) gene and Crohn's disease [37, 38]. Using hypomorphic *Atg16L1*^{HM} mice, in which the *Atg16L1* expression level is very low, it has been shown that autophagy plays a key role in the granule exocytosis pathway in Paneth cells [39]. Crohn's disease patients homozygous for the *ATG16L1* risk allele have also shown abnormalities in the secretion of granule contents in Paneth cells [39].

TLRs can promote the induction of autophagy (Fig. 6.1). The stimulation of macrophages with TLR ligands enhances the redistribution of microtubule-associated protein 1 light chain 3 (LC3), a critical molecule for the induction of autophagy [40]. Engagement of TLR4 recruits not only MyD88 but also Beclin-1 into the TLR4-signaling complex [41]. Beclin-1 is a key component molecule of a class III phosphatidylinositol 3-kinase complex (PI3KC3) that initiates the formation of

autophagosomes by helping to localize other autophagy proteins to the pre-autophagosomal membrane [41]. TLR-mediated induction of autophagy, in turn, is involved in the regulation of TLR-mediated inflammation. *Atg16l1*-deficient macrophages produce high amounts of IL-1 β and IL-18 via inflammasome activation. Mice lacking *Atg16l1* in hematopoietic cells are highly susceptible to DSS-induced colitis [42]. Therefore, constitutive TLR signaling in various mucosal cells is likely required for facilitating the induction of autophagy, which regulates various immune cell functions and Paneth cell exocytosis pathways.

Regulation of TLR Signaling

Above we described the importance of the constitutive activation of TLRs by commensal bacteria under normal steady-state conditions for the maintenance of IEC barrier integrity, production of antimicrobial proteins and IgA, and induction of autophagy. However, if the constitutive TLR signaling is not properly regulated, harmful inflammation may occur. In particular, inflammatory cytokines induced by TLRs, such as IFN- γ and TNF- α , can act as a precipitating factor for IBD by modifying tight junction function in IECs and increasing flux across the leak pathway. To avoid unnecessary inflammation and cytokine production, several molecules function as negative regulators of TLR signaling pathways in intestinal LP macrophages, DCs, and IECs [43].

An example is A20. A20 is a ubiquitin-modifying enzyme that is rapidly induced following TLR stimulation and terminates TLR-dependent responses by removing ubiquitin moieties from the signaling molecule TRAF6 [44]. *Tnfaip3* (the gene encoding A20)-deficient mice exhibit severe inflammation of multiple organs including the intestine. Spontaneous inflammation in *Tnfaip3*-deficient mice is rescued by introducing the deficiency of MyD88. In addition, the depletion of intestinal bacteria by treatment with broad-spectrum antibiotics diminishes the inflammation in *Tnfaip3*-deficient mice [45]. Thus A20 is a key negative regulator of intestinal bacteria- and TLR-dependent inflammation.

Another example is the single immunoglobulin IL-1 receptor-related molecule (SIGIRR), which contains an intracellular Toll/interleukin-1 receptor (TIR) domain [46]. SIGIRR is expressed on DCs and IECs and inhibits IL-1 and TLR signaling by interacting with TLR through its TIR domain. Mice lacking *Sigirr* are highly susceptible to intestinal inflammation induced by DSS, and IECs from *Sigirr*-deficient mice show an increased commensal bacteria-dependent cytokine production [47]. Thus, SIGIRR plays a critical role in fine-tuning the modulation of TLR signaling in the intestine.

Peroxisome proliferators-activated receptor- γ (PPAR γ) is a member of the nuclear receptor family, which is highly expressed in IECs [48]. PPAR γ has been shown to suppress the expression of a subset of TLR-dependent genes by interacting with transcription factors downstream of TLR, such as NF- κ B [49]. The presence of *Bacteroides thetaiotaomicron*, a prevalent commensal anaerobic bacterium of the human intestine, leads to nuclear export of the RelA subunit of NF- κ B in IECs. This anti-inflammatory action of *B. thetaiotaomicron* is mediated by PPAR γ [50]. Moreover, PPAR γ is a key receptor mediating the effect of 5-aminosalicylic acid (5-ASA), which is widely used for the treatment of IBD [51].

TLR-mediated responses are also counteracted by inhibitory cytokines such as IL-10. IL-10 is produced by Treg cells, DCs, and macrophages in the intestine in the steady state. *Il10*-deficient mice, and mice with myeloid-cell-specific deficiency for STAT3, an essential signaling molecule acting downstream of the IL-10 receptor (LysM-*Stat3* ko), spontaneously develop intestinal inflammation [52, 53]. In these mutant mice, LP macrophages are in the state of constitutive and aberrant activation, and the blockade of TLR signaling by introducing a deficiency in TLR4 or MyD88 abrogates this intestinal inflammation [54, 55], indicating that IL-10 acts on myeloid cells and induces STAT3 activation to suppress excessive inflammation during TLR responses.

In addition to A20, SIGIRR, PPAR γ , and IL-10, TLR signals are regulated by many other molecules, including IRAK-M, suppressor of cytokine signaling-1 (SOCS1), ST2, and I κ BNS. These proteins can restrict the duration and/or intensity of TLR signals and modulate the cellular outcome of TLR signaling, thereby helping to determine whether TLR signals lead to homeostatic or inflammatory responses.

TLR signaling in the mucosa exerts its appropriate function only when the integrity of the epithelial cell barrier is maintained. This was demonstrated by analyses of mice with intestinal epithelial-cell-specific ablation of NEMO (also called I κ B kinase- γ (IKK γ)), or IKK α and IKK β , both IKK subunits essential for NF- κ B activation [56, 57]. These conditional knockout mice spontaneously develop severe chronic intestinal inflammation. NF- κ B deficiency leads to apoptosis of IECs, impaired expression of antimicrobial proteins, and translocation of bacteria into the mucosa. Intestinal inflammation is prevented by crossing these mice with *Myd88*-deficient mice. These findings support a model in which chronic activation of TLR by continuous bacterial translocation triggers the expression of proinflammatory cytokines, such as TNF α and IL-1 β , which causes further destruction of the epithelium and results in chronic intestinal inflammation. That is, the breakdown of the epithelial cell barrier, along with constitutive and aberrant TLR activation, may be the major underlying pathogenic mechanism of IBD.

NLRs

Nucleotide oligomerization (NOD)-like receptors (NLRs) are another family of PRRs. The NLRs consist of three subdomains: the N-terminal protein–protein interaction domain, the central nucleotide-binding oligomerization (NOD) domain that mediates self-oligomerization during activation, and the C-terminal leucine-rich repeat (LRR) domain [58]. The NLR family proteins are classified into three subgroups on the basis of the N-terminal domains. The first subgroup includes NOD1 (CARD4) and NOD2 (CARD15) proteins that carry a N-terminal caspase recruitment domain (CARD). The second subgroup includes NALP1 (also known as NLRP1 or CARD7) and NALP3 (also known as NLRP3 or CIAS1), which have a pyrin domain (PYD). IPAF and NAIP are classified into the third subgroup because they are evolutionarily separate from the other two subgroups. IPAF contains a CARD, whereas NAIP has three BIR domains [59]. NLRs have been implicated in the recognition of several bacterial components as well as environmental danger signals such as adenosine 5'-triphosphate (ATP), urate crystals, and silica [60]. Among these NLR family members, NOD2 and NOD1 have been well characterized and found to be involved in immune responses in the intestinal mucosa [61].

NOD2

NOD2 is a sensor of PGN through the recognition of muramyl dipeptide (MDP), which is common to both Gram-positive and Gram-negative bacteria [61]. Upon recognition of MDP, NOD2 forms oligomers and recruits receptor-interacting protein (RIP2, also known as RICK). RIP2 undergoes polyubiquitination and mediates the recruitment and activation of TAK1, resulting in the activation of NF- κ B and induction of inflammatory cytokines [62]. Therefore, NOD2 activates similar signaling pathways to TLRs, and many studies have shown that NOD proteins synergize with TLRs in macrophages and DCs. On the other hand, NOD2 has been shown to play a regulatory role in TLR signaling. For example, NOD2 was shown to negatively regulate TLR2 signaling [63–65].

NOD2 is expressed in monocytes and Paneth cells. NOD2 is required for the expression of antimicrobial proteins in Paneth cells. Indeed, *Nod2*-deficient mice are susceptible to oral infection by

Listeria monocytogenes and show defective expression of antimicrobial α -defensins, such as defensin- α -related sequence (Defa-rs) 10 and Defcr4, in Paneth cells [66]. *Nod2*^{-/-} mice also show an increased amount of commensal bacteria as well as a reduced capability to clear newly colonizing bacteria, suggesting that NOD2 also plays an important role in maintaining the balance of commensal flora [67].

NOD2 gene mutations are associated with an increased susceptibility to Crohn's disease in Western (but not Asian) populations [68, 69]. There are three major polymorphisms associated with Crohn's disease: R702W, G908R, and the frame-shift deletion mutation L1007fs. Although in vitro assays suggest that these *NOD2* mutations result in a loss of function [70], whether these mutations predispose an individual to Crohn's disease in vivo through a loss or a gain of function remains an issue. In order to explain the discrepancy between the loss-of-function phenotype of patient monocytes and the reality of inflammation developing in the intestine, several possible hypotheses are proposed [59, 71]. The most favored explanation is that *NOD2* variants cause a defect in mucosal barrier function, including a defect in the production of α -defensins by Paneth cells, which leads to an enhancement of bacterial translocation and subsequently evokes innate and adoptive immune responses [72–74]. Another hypothesis is the inappropriate hyper-responsiveness against commensal bacteria [75]. This scenario is based on evidence that NOD2 might be a negative regulator of inflammation. Macrophages from *Nod2*^{-/-} mice display an enhanced TLR2-driven production of IL-12, leading to differentiation of Th1 cells [64]. In addition, NOD2 contributes to IL-10 production in macrophages by acting with TLR2 [76]. Further investigation will be required to elucidate the mechanisms underlying the NOD2 mutation-directed disease development.

NOD1

NOD1 detects diaminopimelate (DAP)-containing PGN, which is found mostly in Gram-negative bacteria [77]. Similarly to NOD2, NOD1 mediates the RIP2-dependent NF- κ B pathway. However, in contrast to the restricted cellular expression of NOD2, NOD1 is expressed more ubiquitously, including IECs. Therefore, NOD1 is postulated to play a different role from NOD2 in the intestinal immune system. In vitro, NOD1 is required for sensing *Shigella flexneri* and *Escherichia coli*. In vivo, *Nod1*-deficient mice are highly susceptible to infection by *Helicobacter pylori* [78]. *H. pylori*-induced production of β -defensins is abolished in *Nod1*^{-/-} mice, indicating that NOD1 plays a critical role in the production of β -defensins.

Constitutive NOD1 signaling by PGN from commensal Gram-negative bacteria, such as genus *Bacteroides*, is required for the induction and maintenance of gut-associated lymphoid tissues (GALT), particularly isolated lymphoid follicles (ILFs) [79]. Conversely, in the absence of ILFs, the composition of the intestinal bacterial community is profoundly altered, because ILFs are a critical structure for the generation of IgA-producing plasma cells. It has also been shown that NOD1 activation by intestinal microflora under basal conditions is required for the systemic priming of the innate immune system, particularly for the activation of circulating neutrophils [80]. Although the association of *NOD1* gene with IBD remains controversial [64, 65, 81–83], it is clear that NOD1 recognizes intestinal commensal and pathogenic bacteria and plays critical roles in the regulation, activation, and organization of both local and systemic innate and adaptive immune systems.

C-Type Lectin Receptors

C-Type lectin receptors (CLRs) are another family of PRRs that bind to carbohydrate structures on microbes. Several CLRs, such as dectin-1 and DC-specific intercellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN), have been implicated in intestinal immunity.

Dectin-1

Dectin-1 (also known as CLEC7A) is expressed on myeloid cells and is involved in the recognition of exogenous β -glucan, a component of fungal and bacterial cell walls [84]. Dectin-1 signaling in DCs and macrophages facilitates their maturation, phagocytosis, and production of cytokines, thereby playing a critical role in the host defense against pathogens, including *Candida albicans*, *Aspergillus fumigates*, and *Pneumocystis carinii*. Upon ligand binding, dectin-1 recruits spleen tyrosine kinase (Syk) through a cytoplasmic tyrosine motif resembling the immune receptor tyrosine-based activation motif (ITAM) [85]. Syk activates a downstream signaling cascade leading to the activation of the key adaptor molecule caspase-activating recruitment domain 9 (CARD9), which acts with B-cell lymphoma 10 (Bcl10) and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (Malt1) to induce the activation of NF- κ B and MAPK [86]. A specific dectin-1 ligand, curdlan, has been shown to activate DCs to induce the differentiation of Th17 cells through production of IL-6 and IL-23 [87]. Dectin-1 is also involved in the production of IL-10. IL-10 production downstream of dectin-1 requires signaling through the ERK pathway.

An increased dectin-1 expression level on macrophages has been observed in the inflammatory lesions of IBD patients [88]. Mutations in *CARD9*, an essential signaling adaptor molecule for dectin-1, are reported to be associated with susceptibility to IBD [89]. Thus far, although no significant association between mutations in CLEC7A (the gene for dectin-1) and susceptibility to IBD has been reported, considering that dectin-1 signaling promotes Th17 differentiation as well as IL-10 production, dectin-1 may participate in the pathogenesis of IBD.

DC-SIGN

DC-SIGN (the mouse homolog is SIGNR1) was first identified as a DC-specific adhesion receptor for ICAM-3 expressed on T cells [90]. DC-SIGN then attracted attention as a receptor for some viruses, including HIV. DC-SIGN is now known to be a receptor expressed on DCs for mannose-type carbohydrates on the surfaces of a wide range of pathogens, including viruses, bacteria, and fungi. DC-SIGN signaling in DCs facilitates their migration, antigen capturing, and T-cell priming. DC-SIGN cross-linking activates the serine/threonine protein kinase Raf-1, which induces the phosphorylation and acetylation of the NF- κ B subunit RelA [85]. This signaling cascade increases IL-10 production in cooperation with TLRs [91]. Interestingly, it has been shown that the specific recognition of mannosylated antigens by SIGNR1 expressed by LP DCs unleashes a cascade of tolerogenic events involving the secretion of IL-10, which promotes the generation of IL-10-producing regulatory T cells (Tr1 cells) [92]. Therefore, DC-SIGN may be a critical PRR for the regulation of immune responses in the intestine.

The gene coding DC-SIGN is located in the region 19p13, which is a linkage region for IBD identified by genome-wide analysis of Canadian families [93]. A promoter variant of this gene (−336A>G; rs4804803) has been associated with susceptibility to celiac disease [94]. Thus, DC-SIGN may contribute to the pathogenesis of IBD.

Conclusion and Perspectives

Considerable progress has been made in the understanding of the roles of PRRs in the induction of inflammation against pathogens: In isolated individual cells, PRR signaling and consequences appear rather simple. Thus, one might envisage that this can be applied to in vivo situations, in

which the activation of PRRs only occurs when intestinal bacteria accidentally invade the mucosa, which simply results in inflammation. However, we now know that the actual and entire picture of the intestinal innate immune system is not so simple. Not only pathogenic bacteria, but also commensal bacteria continuously provide activation signals to PRRs. The constitutive activation of host cells, particularly IECs, results in a continuous production of tissue repairing factors, antimicrobial proteins, and IgA for maintaining intestinal homeostasis; without which intestinal barrier injury and bacterial translocation may occur. The PRR signaling is also required for the control of components of intestinal microflora to form a mutually beneficial relationship. The dysbiosis of commensal microbes is now known to be a critical instigator of IBD. Therefore, further investigation is clearly required for understanding the *in vivo* signaling of PRRs and for developing new strategies for treatment of IBD.

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Chapter 7

Inflammasomes in Inflammatory Bowel Disease

Till Strowig and Richard A. Flavell

Keywords Inflammasome • NLRP3 • Caspase-1 • IL-1 β • IL-18 • Autoinflammatory diseases

Introduction

Innate immunity serves as the first line of defense against pathogens and plays additionally an important role in shaping adaptive immune responses [1]. It is mediated by various cell types such as granulocytes and phagocytes, as well as soluble factors such as the complement system and antimicrobial peptides. The innate immune system distinguishes between self and non-self using pattern recognition receptors (PRR) that recognize conserved structures in viruses, bacteria, and fungi. These germline-encoded PRRs include Toll-like receptor (TLR) family [2], C-type lectin receptors [3], RIG-I-like receptors [4], and NOD-like receptors (NLR) [5]. Activation of these receptors by their cognate ligands leads to the activation of powerful pro-inflammatory pathways that orchestrate protective anti-microbial responses, but these may also act pathological in chronic inflammatory diseases. In this chapter, we will focus on a group of proteins that form so-called inflammasomes, which control the maturation and secretion of IL-1 β and other pro-inflammatory cytokines, and their roles in health and disease.

Inflammasomes as Platforms for Activation of Inflammatory Caspases

IL-1 α/β , originally called endogenous pyrogens, are important mediators of inflammatory responses regulating fever, pain threshold, vasodilatation, hypotension, and leukocyte adhesion [6]. It was noted more than 20 years ago, that IL-1 β is produced as a pro-protein and needs processing by proteases to become biologically active. Caspase-1, a member of a family of proteases that is produced as catalytically inactive zymogen requiring proteolytic activation (caspases), has been identified as main regulator of IL-1 β processing [7]. Due to its ability to cleave cytokines, caspase-1 is termed an inflammatory caspase. This group additionally includes human and murine caspases-12,

T. Strowig, PhD • R.A. Flavell, PhD (✉)
Department of Immunobiology, Yale University School of Medicine, 300 Cedar Street,
TAC S-569, New Haven, CT 06520, USA
e-mail: richard.flavell@yale.edu

human caspases-4 and -5, as well as murine caspase-11 [8]. The activation of caspase-1 is regulated by multimeric high molecular weight protein complexes that were coined as “inflammasomes” [9]. Similar to the apoptosome, these complexes integrate multiple cellular signals serving as a switch and resulting in the activation of downstream effects. The cascade is initiated by detection of a stress situation by a sensor protein, which subsequently multimerizes and assembles into a large complex including effector proteins. Inflammasomes built around the sensor proteins Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain-containing protein 1 (NLRP1), NLRP3, and NLRC4, all NLR family members, as well as absent in melanoma 2 (AIM2), a PYHIN family member, have been identified to date [9–12] (Fig. 7.1a). Their assembly leads to the activation of pro-caspase-1 that undergoes autocatalytic cleavage in the cytosol possibly in trans. Active caspase-1 is a heterodimer of a 10- and 20-kDa subunit that cleaves another cytokine, IL-18 in addition to pro-IL-1 β . Furthermore, caspase-1 is involved in the cleavage of other cellular substrates as well as in the regulation of cellular process such as cell death, membrane repair, and unconventional protein secretion by mechanisms that have not been fully defined [13].

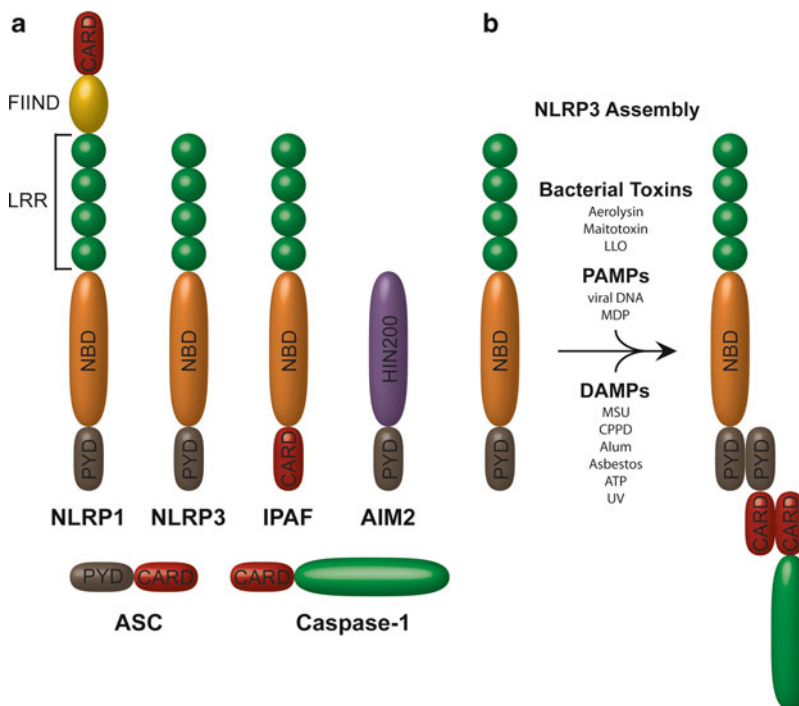


Fig. 7.1 Inflammasomes are molecular complexes regulating caspase-1 activity. **(a)** The NLR proteins NLRP1, NLRP3, and NLRC4, as well as the PYHIN protein AIM2 have been demonstrated to form distinct inflammasomes via their interaction with ASC and caspase-1. **(b)** Upon sensing exogenous PAMPs or endogenous danger signals NLRP3 forms a high molecular weight complex with ASC and caspase-1 leading to cleavage of pro-IL-1 β and other substrates. *CARD* (red) Caspase activation and recruitment domain, *FIIND* (yellow) function to find motif, *LRR* (green) Leucine-rich repeat, *NBD* (orange) nucleotide-binding domain, *PYD* (brown) pyrin domain, *HIN200* (violet) hematopoietic interferon-inducible nuclear antigens with 200 amino acid repeats domain; *LLO* listeriolysin O, *MDP* muramyl dipeptide, *MSU* monosodium urate, *CPPD* calcium pyrophosphate dehydrate

The NLRP3 Inflammasome: A Prototypical Inflammasome

The NLRP3 inflammasome is the best-studied inflammasome with over 600 studies published to date. A role for NLRP3 in the regulation of IL-1 β production was initially identified in a genetic screen of patients suffering from a rare autoinflammatory disease called Familial cold autoinflammatory syndrome (FCAS) with patient-specific mutations in NLRP3 leading to an increased production of IL-1 β by human monocytes [14, 15].

NLRP3 is a multidomain protein containing a leucine-rich repeat domain (LRR) near the C-terminus, a NACHT nucleotide-binding domain (NBD) in the center, both typical of NLR proteins, and a Pyrin domain (PYD) at the N-terminus [8] (Fig. 7.1a). The LRR domain is thought to play a role in autoregulation, the recognition of pathogen-associated molecular patterns (PAMPs), and protein–protein interactions. It is a domain that is shared with other PRRs such as the TLR family. The NBD domain can bind nucleotides and is possibly involved in the induction of conformational change and self-oligomerization. The PYD domain is responsible for recruitment of the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) that contains a PYD and a CARD domain via homotypic interaction of their PYD domains. Finally, ASC binds caspase-1 via their CARD domains. Similarly, AIM2 requires ASC to bind caspase-1, while NLRP1 and NLRC4 contain CARD domains that are able to bind caspase-1 directly.

Activation of the NLRP3 Inflammasome

A broad range of endogenous and exogenous stimuli can induce the activation of the NLRP3 inflammasome [8](Fig. 7.1b). Exogenous stimuli are of a diverse nature ranging from PAMPs to bacterial toxins, live viruses, bacteria, and fungi as well as aggregated microbial-derived molecules. Endogenous stimuli include stress-associated molecules, also called danger-associated molecular patterns (DAMPs) or alarmins, such as extracellular ATP, or aggregated endogenous molecules forming crystals such as uric acid that forms monosodium urate (MSU) crystals. The diversity of ligands activating the NLRP3 inflammasome is in striking contrast to other PRRs that usually recognize more confined structural motifs [2]. Hence, several models have been proposed to explain how all these substances could activate the NLRP3 inflammasome [16, 17]. One requirement for NLRP3 inflammasome assembly that is agreed upon in all models is a low-intracellular K⁺ concentration [18]. Changes in intracellular K⁺ concentrations are frequently observed across evolution from plants to mammals as an early response to stress on a cellular or organism level. K⁺ efflux from cells can be mediated by either transport through opened ion channels or the result of membrane pores. But, K⁺ efflux itself is not sufficient to activate the NLRP3 inflammasome. One model that attempts to unify the diverse NLRP3 agonists is based on the direct recognition of specific microbial substances by NLRP3 that can enter the cell after the formation of pores in the cytoplasmic membrane [19]. It involves sensing of extracellular ATP released as a consequence of stress or injury by the P2X7 protein leading to the recruitment and hemi-channel formation by the protein pannexin-1. Microbial molecules such as MDP, as well as K⁺, could simultaneously transverse through this channel, making this an attractive model, yet, many NLRP3 inflammasome activators would not fit through such a channel since they are too big. Hence, the channel model is not able to provide a general model for NLRP3 activation, but might hold true in some cases. To address the issue of large activator, an alternative model proposes that during phagocytosis of large particles spontaneous rupture of phagosomes and lysosomes occurs leading to the release of vesicle contents into the cytoplasm that proteolytically activate the inflammasome [20]. Indeed, inhibitors of cathepsin B, a lysosomal protease, block activation of the human NLRP1 inflammasome supporting this

model [21]. Similarly, it has been described that TLR9 requires proteolytic cleavage before it can recognize its cognate ligands [22]. However, it remains controversial if this is a general mechanism of inflammasome activation as a different group found conflicting results using cathepsin B-deficient mice [23]. This result could indicate that other lysosomal proteases are involved in proteolytic activation of the NLRP3 inflammasome in mice, which has to be investigated or that the chemical cathepsin B inhibitors had off-target effects. A third model is based on the formation of reactive oxygen species (ROS) as a result of cellular stress which then activates the NLRP3 inflammasome [16]. Strikingly, many examined NLRP3 activators induce the production of ROS from various sources, potentially also from mitochondria [24, 25]. Indeed, blockade of a pathway that is involved in the degradation of damaged mitochondria, called autophagy, increases inflammasome activation correlating with the increased production of ROS [26]. Moreover, chemical inhibition of ROS inhibits NLRP3 inflammasome activation [24]. A potential molecular mechanism that links ROS production with inflammasome activation is thioredoxin-interacting protein (TXNIP) that interacts with NLRP3 in a ROS-dependent manner [27]. In undisturbed cells, TXNIP is bound to thioredoxin, but binds to the LRR domain of NLRP3 in ATP-stimulated cells activating the NLRP3 inflammasome. But, the exact mechanism of this process and the source of ROS upon stimulation with various agonists have not been identified. Another question is why some stimuli that induce ROS such as Tumor necrosis factor signaling do not activate the inflammasome pointing at the existence of distinct spatial and temporal requirements for this process. Finally, it remains unclear if the different proposed models are mutually exclusive or if these pathways can interact at different levels regulating this important multiprotein complex.

Regulation of Immune Responses by Inflammasomes

Inflammasomes are well-known for their function in regulating the levels of the two important immunoregulatory cytokines IL-1 β and IL-18. Both of these cytokines have powerful effects on local and systemic innate responses such as induction of IL-6 by IL-1 β or promoting IFN- γ production in NK cells by IL-18, but in addition they influence directly the quality and quantity of the adaptive immune responses [6, 28]. This is achieved via the regulation of CD4 $^{+}$ T helper cells polarization by both IL-1 β and IL-18. In particular, IL-1 β (beta) plays a role in the polarization of Th17 cells, a subset that has been identified as a potential mediator of inflammatory and pathological conditions [29]. IL-1 β can assist in the development of Th17 cells and cooperates with IL-23 in the induction of IL-17-producing γ/Δ T cells in mice [30, 31]. Similarly, human Th17 cells require IL-1 β for their differentiation, supporting a role for inflammasome-derived IL-1 β in Th17 biology in both man and mouse [32]. In contrast, the role of IL-18 in regulating adaptive responses seems to be dependent on the cytokine environment [28]. Its first recognized function was the polarization of Th1 cells in combination of IL-12, but subsequently it was shown to be able to induce Th2 responses by itself or in combination with IL-2 [33, 34]. Thus the role of IL-18 in adaptive immune responses is complicated and heterogeneous. Mice deficient in components of the NLRP3 inflammasome, IL-1 β or IL-18 are characterized by distinct phenotypes in many T cell-mediated inflammatory models such as contact hypersensitivity, experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, or anti-tumor responses [35–37]. Moreover, NLRP3 inflammasome-deficient mice are unable to successfully raise adaptive Th2-type immune responses after vaccination with antigens formulated with alum adjuvants, the most commonly employed adjuvant in vaccines for humans [38, 39]. Altogether, these studies highlight the importance of the inflammasome in regulating adaptive immune responses with immediate impact on many human inflammatory diseases.

Inflammasome Activation in Pathological Conditions

While inflammasomes have been recognized to perform important regulatory functions that protect the host against pathogenic challenges, it has become clear that they also play critical roles in diverse pathological conditions ranging from responses to environmental toxins, sterile inflammation and chronic autoinflammatory diseases. In particular, mutations in inflammasome-associated genes have highlighted their importance in the development of inflammation. Examples of these diseases are the chronic autoinflammatory diseases, which are a group of inherited autosomal diseases characterized by intermittent episodes of rash, arthralgia, and fever. These include FCAS, Muckle–Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID) that are all caused by missense mutations in the NBD of NLRP3. It is thought that these mutations lead to a gain-of-function causing excessive production of IL-1 β that triggers episodes of disease [12, 40]. Accordingly, patients with these diseases respond well to therapies with IL-1Ra, a clinically approved antagonist of IL-1 α/β marketed as Anakinra [41, 42]. Two other autoinflammatory diseases that respond at least partially to this treatment are familial Mediterranean fever (FMF) and pyogenic arthritis pyoderma gangrenosum, and acne syndrome (PAPA) which are both characterized by overt inflammasome activation [43]. These diseases are driven by distinct genetic loci encoding pyrin and proline-serine-threonine-phosphatase-interacting protein 1 (PSTPIP1), respectively [44, 45]. Similar to the mutations in NLRP3 in MWS, FCAS, and NOMID, the mutations in pyrin and PSTPIP1 alter their functions in regulating inflammasome function and hence IL-1 β production [46–48]. Notably, disease-associated polymorphisms are also found in other inflammasome sensor proteins. Several single-nucleotide polymorphisms in NLRP1 are associated with vitiligo and a range of other autoinflammatory diseases including type I diabetes and Addison's disease [49, 50]. In addition to genetically defined diseases, the NLRP3 inflammasome is involved in a range of other disorders via recognition of exogenous and endogenous danger signals. Animal models revealed that the NLRP3 inflammasome mediates recognition of MSU and calcium pyrophosphate dehydrate (CPPD) crystals, which are commonly found in gout and pseudogout, respectively [51]. These crystals induce the production of IL-1 β in macrophages, thereby inducing or sustaining an inflammatory cascade. Similarly, the NLRP3 inflammasome plays a role in mouse models of the serious human pulmonary fibrotic disorders, asbestosis, and silicosis [24, 25]. And more recently, the NLRP3 inflammasome was further suggested to be involved in metabolic diseases. Two studies found that NLRP3 was responsible for the increased production of IL-1 β (beta) observed in type 2 diabetes via recognition of either ROS produced as response to high glucose concentrations or aggregation of the islet amyloid polypeptide, respectively [27, 52]. Another study found that the NLRP3 inflammasome can be activated by cholesterol crystals found in atherosclerotic plaques and that NLRP3-deficient mice had markedly decreased early atherosclerosis [53]. Altogether, the recognition of the importance of inflammasome function in these diseases has opened the door to develop new therapeutic approaches with the availability of several inhibitors of IL-1 β in clinical practice and further in clinical testing [54–56].

The Role of Inflammasome in Intestinal Homeostasis and Immunity

The immune system in the intestine has to maintain a fine balance between pro-inflammatory and tolerogenic signals to prevent autoinflammation in the local environment. One can imagine that this is particularly difficult to achieve in the large intestine which contains up to 10^{12} bacteria/g fecal content. Many PRRs including components of inflammasomes are constitutively expressed in both hematopoietic and nonhematopoietic cells. Consequently, in addition to other pro-inflammatory cytokines such as TNF α , and IL-6, IL-1 β plays an important role in regulating

intestinal inflammation. Early studies using caspase-1-deficient mice and pharmacological inhibitors of caspase-1 suggested a pathological role for inflammasomes in chemically induced colitis [57, 58]. Similarly, upon induction of experimental chemical colitis in mice, IL-1 β -deficient mice are characterized by reduced severity of disease. IL-18 on the other hand seems to be more important in regulating intestinal homeostasis as IL-18-deficient mice are characterized by an exacerbated disease due to defects in tissue repair [59, 60]. Notably, similar phenotypes were observed in ASC- and caspase-1-deficient mice in several studies indicating that the role of inflammasomes in regulating tissue repair is dominant over its pro-inflammatory role after tissue injury in the intestine [61–64]. Interestingly, somewhat conflicting results were obtained using NLRP3-deficient mice. Most studies found increased autoinflammation in these mice indicating that the NLRP3 inflammasome is responsible for protective IL-18 production [61–63]. One study, however, found an ameliorated phenotype that was attributed to decreased levels of IL-1 β [65]. Other conflicting results reported in these studies relate to the location of NLRP3 activity. While one study found that NLRP3 is required in nonhematopoietic cells [62], another study found it to be required in hematopoietic cells for its protective function [61]. Differences between these studies could be in part explained by slight variations in the experimental procedures and conditions such as duration of treatment and concentration of chemical used to induce colitis, but it is likely that other factors might play a significant role. Indeed, it was recognized that antibiotic treatment of NLRP3-deficient mice reduced the severity of disease in one study and another study reported differences in the microflora between NLRP3-deficient and control mice [62, 63]. Since the crosstalk between commensal bacteria and the immune system also provides signals that regulate epithelial cell maturation and turnover, it is possible that differences in the microbiota could contribute to these variations. Overall, stimulation of inflammasome activity by members of the gut flora or by danger signals released from damaged intestinal cells seems to be crucial for the regulation of mucosal repair upon tissue injury.

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Chapter 8

Antimicrobial Peptides in Inflammatory Bowel Disease

Charles L. Bevins

Keywords Antimicrobial peptide • α (alpha)-Defensin • Lysozyme • sPLA2 • Secretory phospholipase • NOD2 • Paneth cell • Microbiota • Crohn's disease • Ulcerative colitis • Dysbiosis • Salmonella • Hepatocarcinoma-intestine-pancreas • Pancreatitis-associated protein • Cryptdin-related sequences • *Candidatus arthromitis* • Segmented filamentous bacteria • Matrix metalloproteinase-7 • Tcf-4 • X-box-binding protein 1 • KCa 3.1

Introduction

The causes of Crohn's disease (CD) and ulcerative colitis (UC), the two major idiopathic inflammatory bowel diseases (IBDs) are incompletely understood, despite having been defined many decades ago on the basis of their clinical manifestations [1–3]. For both UC and CD, most theories on pathogenesis include intestinal microbes in either initiating and/or perpetuating mucosal inflammation in genetically susceptible hosts [4–7]. Abnormalities of intestinal microbes found in IBD include alterations in the composition (dysbiosis), adherence of bacteria to the mucosal surface, defective clearance and the presence (or absence) of particular bacterial species [6, 8]. Evidence supports that host factors have a major role in regulating intestinal microbes. Antimicrobial peptides are a class of innate immune system mediators that serve as endogenous antibiotics [9] and likely constitute a major type of host factor to regulate these microbes [10–12]. This chapter will provide an overview on the proposed biological role of intestinal antimicrobial peptides relevant to IBD pathogenesis and develop a hypothesis to link altered expression of antimicrobial peptides in the pathogenesis of CD.

Intestinal Microbiota in IBD

The intestine harbors an astonishingly numerous and diverse collection of microbes. Most studies have focused on bacteria, because of their high abundance, but archaea, fungi and viruses contribute also to the intestinal microbiome [13, 14]. Humans typically harbor approximately 10^{14} bacteria in

C.L. Bevins, MD, PhD (✉)
Department of Microbiology and Immunology, University of California, Davis School of Medicine,
Davis, CA 95616, USA
e-mail: clbevins@ucdavis.edu

their intestines – an order of magnitude more bacteria than the total number of human cells in the body [15]. The intestinal bacteria are quite diverse, with a thousand or more different bacterial species represented [16]. Most of these bacteria are resident organisms (microbiota), but also include food- and water-borne organisms in transit through the intestine. Opportunistic and potentially virulent pathogens may be found in both resident and transiting groups.

The gut microbiota occupies anatomical and physiological niches that are vital to many physiological and homeostatic functions of the host [17]. For example, the commensal microbiota have important roles in nutrition, by fermenting indigestible components in the diet and synthesizing vitamins, amino acids and short-chain fatty acids; in host defense, through colonization resistance; and in the development and regulation of the mucosal immune system [17–24]. Despite these host-beneficial effects, the microbiota poses a perpetual threat of microbial disease owing to the sheer numbers of bacteria and the close contact with the mucosal surface. As a countermeasure, the host relies on multifaceted defenses that involve both the innate and adaptive immune systems. The intestinal epithelium serves as a critical component of the innate immune system, both by providing a physical barrier and by secreting various antimicrobial factors, including antimicrobial peptides [10, 21].

In IBD, the microbiota loses diversity and has altered composition [8]. Clinical observations support that intestinal bacteria are critical in IBD pathogenesis [25]. The presence of granulomas associated with CD has suggested one or more specific pathogens as potential causative agents. Although some data support that specific pathogens could cause CD [8, 26], this is not a widely held hypothesis [8]. More favored are hypotheses invoking detrimental influences of altered composition, termed dysbiosis [4]. The dysbiosis in Crohn's disease includes increases in *Enterobacteriaceae* [27], a change that may be linked intimately to mucosal inflammation [28–30]. In addition, the dysbiosis is accompanied by a striking increase in mucosally adherent bacteria [31–34]. There is also a decrease in some populations that are considered protective, such *Faecalibacterium prausnitzii* [35]. Finally, the ability of intestinal bacteria to incite and perpetuate intestinal inflammation has been demonstrated in a variety of rodent models [28, 29, 36–39]. Together, accumulating evidence points to a central pathogenic role for intestinal microbes in IBD, although multiple mechanisms are likely and many details remain unresolved [6, 8, 40, 41].

The Host Shapes the Microbiota

The intestinal microbiota is a complex ecosystem whose composition depends on both endogenous and exogenous factors. The microbes themselves influence their own environment (pH, nutrients, etc.), which in turn affects microbe composition. The exogenous factors come from the environment and from the host. Environmental factors include: ingested microbes from food- and water-born sources, maternal sources of microbes [42, 43], dietary nutrients [44, 45], and antibiotic exposure [46]. These factors can have longstanding significant influence on composition of intestinal microbiota. In addition to these determinants, host factors likely have an overarching influence on the make-up of the microbiota [11, 47, 48].

Experimental evidence supports the idea that all metazoans shape the composition of their colonizing microbiota. Studies involving transplantation of intestinal microbiota from mice and zebra fish into their reciprocal germ-free host (i.e. zebra fish microbiota into germ-free mice and vice versa) resulted in dramatic shifts in composition, whereby the recipient host selected microbes that much more closely approximated their own native microbiota than composition observed in the transplanted microbiota [47]. In studies of non-human primates, the phylogeny of the host superseded both diet and environmental influences in determining fecal microbial community structure [43]. No doubt, there are many host factors that influence the make-up of the colonizing microbes, and we are only beginning to unravel an understanding of these factors.

The host immune system is likely to be a key driving force in shaping the composition of the colonizing microbiota under baseline, as well as inflammatory conditions. This idea is supported in studies of the basal metazoans, such as *Hydra* [49], as well as in mammals. In humans and other mammals, immunoglobulin-A (IgA) and defensins are two implicated effector molecules of the intestinal mucosal immune system. IgA is proposed to influence the composition of the intestinal microbiota [50]. The absence of IgA results in notable changes in microbial colonization [51], and there is a dynamic interaction between intestinal IgA and microbial colonization [52, 53]. Defensins are the second host factor that shapes the structure of the microbial community. Experimentally, Paneth cell α (alpha)-defensins have significant impact on the composition of intestinal microbiota [11]. This concept will be detailed below.

Complementary evidence for the role of host factors in altering the composition of the microbiota stems from analysis of the acute response to enteric pathogens. Interestingly, an important aspect of virulence for some enteric pathogens involves eliciting a mucosal inflammatory response, which then creates an environment favoring the pathogen as it strives to out-compete the residing microbiota. For example, *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) induces intestinal inflammation that can impede the commensal microbiota via various mechanisms. Concurrently, this pathogen uses multiple virulence factors to evade (and even profit from) the host-generated lethal antimicrobial inflammatory milieu [30, 54, 55]. One factor released from epithelial cells during the mucosal inflammatory response is lipocalin-2 (also known as siderocalin, neutrophil gelatinase-associated lipocalin, or 24p3), a 23-kDa antimicrobial “peptide.” As discussed below lipocalin-2 sequesters bacterial siderophores, the bacterial proteins used for iron acquisition, thereby suppressing bacterial growth. However, *S. typhimurium* expresses a variant siderophore (called salmochelin) that is not recognized or inhibited by lipocalin-2 [56]. Thus, during *S. typhimurium* infection, the elicited lipocalin-2 selectively suppresses growth of the indigenous microbiota dependent on siderophore-acquisition of iron, providing an advantage to *S. typhimurium* [57]. Similarly, *Citrobacter rodentium* triggers intestinal inflammation that reduces the abundance of many commensal bacteria, and in turn, enhances pathogen growth [28]. An analogous virulence strategy involving defensin peptides has been proposed for *Helicobacter pylori* [58]. Together, these data support that host factors, including antimicrobial peptides, mold the composition of the microbiota, both in baseline and in inflammatory conditions.

Antimicrobial Peptides

Antimicrobial peptides are among the most ancient elements of the immune system, and arguably are the most widely utilized host-defense effector molecules found in nature [9]. Antimicrobial peptides are ribosomally synthesized antibiotics, differentiated from other antibiotics in nature, such as penicillin, which are typically products of secondary metabolism of microorganisms. Studies of lower organisms have established that antimicrobial peptides are key to both host defense [59] and mucosal colonization by beneficial bacteria [49]. Most often, a size of about 100 amino acids differentiates “peptide” from “protein,” but this size limit is rather arbitrary. Rather than size, the characteristic of being a gene-encoded, ribosomally synthesized antibiotic is most germane to the antimicrobial peptide designation [9]. Therefore, several small proteins fall under the umbrella of antimicrobial peptide in this discussion (Table 8.1). So defined, intestinal antimicrobial “peptides” function by mechanisms that include membrane disruption, inhibition of cell wall biosynthesis, cell wall degradation and vital nutrient scavenging.

Defensins are a major family of antimicrobial peptide of mammals [60–62], and in vivo models have provided compelling support for their antimicrobial functions in the intestinal tract [11, 63, 64]. Characteristically, defensins are 18–40 amino acids in length and contain three intramolecular

Table 8.1 Major antimicrobial peptides of the human small intestine and colon

Antimicrobial	Size	Antibacterial activity	Cellular source
α -defensin 1–4	3.4 kDa	Gm+ and Gm–	Neutrophils
α -defensin 5	3.6 kDa	Gm+ and Gm–	Paneth cells
α -defensin 6	3.7 kDa	No direct activity	Paneth cells
β -defensin 1–3	4 kDa	Gm+ and Gm–	Colonocytes
Lysozyme	14 kDa	Gm+ > Gm–	Paneth cells, mØ
sPLA2	16 kDa	Gm+ > Gm–	Paneth cells, colonocytes, mØ
Reg3A/HIP/PAP	16 kDa	Lectin, Gm+	Paneth cells, enterocytes
Lipocalin 2	24 kDa	Selectively bacteriostatic (sequesters chelated iron)	Colonocytes

disulfide bonds [60, 61]. In the course of evolution, the genes encoding defensins have undergone duplication and diversity, accompanied by relatively rapid changes in primary sequence attributed to host's interactions with microbes in the internal and external environment [65]. Together these sequence changes have resulted in much diversity in the defensin family between species. However, a conserved sequence feature is the alignment of the six cysteines that participate in the three-disulfide bonds, which helps define the two subfamilies of defensins expressed in humans. One of these sub-families, α (alpha)-defensins, is expressed most highly in neutrophils and in Paneth cells. In both of these cell types, α (alpha)-defensin expression is constitutive and part of the cellular differentiation of these cells as they mature. While this expression pattern in neutrophils and Paneth cells is observed in marsupials, glires (including rats) and some other mammals, mice are a striking exception, where defensins are expressed in Paneth cells (where they are called “cryptdins,” to emphasize *crypt defensins*), but not neutrophils [60, 62]. Paneth cells granules contain proteolytic enzymes that mediate processing of α (alpha)-defensin precursors [63, 66]. In humans, Paneth cell trypsin is the processing enzyme of pro- α (alpha)-defensins, while in mice matrix metalloproteinase (MMP)-7, also called matrilysin, serves this function [63, 66]. The β (beta)-defensin subfamily is expressed in many epithelial cells, including colonocytes. In the intestine, epithelial expression of β (beta)-defensin is inducible, except for the apparent constitutive expression of HBD-1. In summary, in the human intestine, α (alpha)-defensins are highly expressed in Paneth cells of the small intestine, and β (beta)-defensins are expressed in the colon. At both locations, infiltrating neutrophils also carry an abundant stockpile of α (alpha)-defensins.

Both α (alpha)- and β (beta)-defensins typically have bactericidal activity against both Gm- and Gm+ bacteria. Some defensins also have activity against fungi, viruses and protozoa [61, 67]. Many defensins kill their target microbes by disrupting membrane integrity, but recent studies find that some defensin peptides interfere specifically with lipid II function and thereby block bacterial wall biosynthesis [68–70]. Curiously, while Paneth cell HD5 has potent bactericidal activity, the second Paneth cell α (alpha)-defensin, HD6, does not have cidal activity against any bacteria tested to date [71]. Identification of the biological function of HD6 is atopic of active investigation. Besides their antimicrobial activity, some defensins have additional activities that suggest their capacity to help coordinate host defense responses in the intestine [61, 62]. For example, human β (beta)-defensin-1 and -2 have chemoattractant activity for cells expressing the chemokine receptor CCR-6, including dendritic cells [72, 73]. Human α (alpha)-defensin 5 is a potent lectin [74] and can neutralize bacterial exotoxins [74]. Certain other α (alpha)-defensins promote ion fluxes in epithelial cells [75, 76]. These and other activities of intestinal defensins suggest a broad role where these peptides likely contribute in several ways to innate immunity.

Lysozyme is an enzyme that specifically hydrolyzes peptidoglycan. This enzyme is very abundant in the surface-lining fluid of many organs, including the intestine where it is chiefly made by Paneth cells.

In addition, lysozyme is abundant in macrophages and neutrophils. Its high concentration in these cells and at mucosal surfaces supports that lysozyme has a substantial role as an antimicrobial agent *in vivo*, even though specific activity *in vitro* is modest. In addition to its role as an antimicrobial agent, analysis of knockout mice points to another key *in vivo* role for lysozyme – an ability to impede peptidoglycan accumulation in tissue [60]. By enzymatic degradation of peptidoglycan, lysozyme activity appears to help avoid prolonged inflammatory responses that otherwise would result from persistence of bacterial cell wall antigens [60]. Whether eliminating peptidoglycan from crypts and intestinal tissue, or antibacterial activity against luminal microbes is the chief role for lysozyme has not been determined.

Phospholipase A2 enzymes are a large family of catalytic molecules that hydrolyze the fatty acid ester bond at position sn-2 of membrane phosphotriglycerides. Paneth cells granules contain abundant quantities of one specific member of this family that has selective activity on bacterial membranes [77, 78]. Colonic epithelial cells also express this enzyme, which is named group IIA secretory phospholipase A2 (PLA2G2A, or simply sPLA2) [79]. Like lysozyme, sPLA2 is not only expressed in epithelial cells, but also in macrophages [78]. Bacterial membranes, rich in phosphatidylglycerol and phosphatidylethanolamine, are the key targets of sPLA2, but the enzyme can cleave other phosphotriglyceride substrates [78, 80]. The enzyme is bactericidal, with preferential activity against Gm⁺ bacteria [80, 81]. Interestingly, genetic evidence found that the gene encoding sPLA2 is a key modifier gene in mouse lines that spontaneously form intestinal adenomas and tumors [82, 83], but the mechanism leading to increased neoplastic growth is unknown [84].

An interesting C-type lectin with a single carbohydrate recognition domain is expressed in the human intestine, named Reg3 α (alpha) (also called hepatocarcinoma-intestine-pancreas (HIP) or pancreatitis-associated protein (PAP)). Like its mouse ortholog, Reg3 γ (gamma), this lectin binds peptidoglycan and is bactericidal against Gm⁺ bacteria [85–87]. As with some other lectins, the polymeric repeating glycan character of its substrate, the intact bacterial cell wall, contributes to high-affinity binding [88]. This lectin is abundantly expressed in Paneth cells and enterocytes [89, 90, 85].

Lipocalin-2 binds bacterial siderophores, which are the catechol-related iron chelators secreted by bacteria to acquire iron from their environment. Sequestering these iron chelators prevents this mode of bacterial iron acquisition and inhibits bacterial growth [91]. Lipocalin-2 is inducibly expressed by many cells, including liver, macrophages, and epithelial cells of the lung and intestine [57, 92, 93]. In the intestine, lipocalin-2 may selectively inhibit growth of siderocalin-dependent bacteria at the mucosal surface.

Mouse Paneth cells express certain antimicrobial peptides that have not been identified in humans, including a group of peptides called cryptdin-related sequences (CRS) [94, 95]. The genes encoding CRS peptides are homologs of α (alpha)-defensins [96]. The CRS peptides are highly expressed, cationic and cysteine-rich, like α (alpha)-defensins, and they have similar antimicrobial activity, but differ in many structural features [62]. Angiogenin-4 is another antimicrobial peptide that does not have a clear human ortholog. Originally identified as a Paneth cell product induced when germ-free mice were colonized [97], angiogenin-4 belongs to a subfamily of ribonuclease enzymes that have antibacterial and antiviral activities [98].

Before leaving the topic of intestinal antimicrobial peptides, it must be mentioned that the host is not the sole source of antimicrobial peptides in the intestinal lumen. While antimicrobial peptides in the intestinal tract are chiefly derived from the host epithelium, some Gm⁺ and Gm[–] bacteria also secrete ribosomally synthesized antimicrobial peptides called bacteriocins [99–101]. Bacteria seem to use these peptides to establish or protect an environmental niche. The bacterial strains producing bacteriocins will affect, like their host-derived counterparts, the composition of complex bacterial communities, adding considerable complexity to the dynamics of the intestinal microbiota [99, 101].

Paneth Cells: An Antimicrobial Peptide Factory

Paneth cells are epithelial cells with intensive secretory activity. Their large secretory granules contain massive concentrations of antimicrobial peptides [102, 103]. The cells reside in small clusters at the base of the small intestinal crypts. They discharge their granules into the crypt lumen, and their contents diffuse from the crypt to target microbes at the intestinal surface and in the lumen, including both resident and newly acquired microbes (see Fig. 8.1). In so doing, Paneth cell antimicrobials can maintain host sovereignty over its epithelial surface, provide protection from food and water-borne pathogens and help shape the composition of the endogenous microbiota [104]. In vivo studies in mouse models have provided strong support for these proposed antimicrobial functions [11, 63, 64, 87, 105]. The biological activities of Paneth cell antimicrobial peptides may be evident not only in the small intestine, but also in the colon, as luminal contents transit via peristalsis [106].

A direct role of Paneth cell antimicrobials in regulating the small intestinal microbiome was investigated using complementary mouse models [11]. In α (alpha)-defensin-deficient mice (via processing enzyme MMP7 knockout), there was decreased abundance of Bacteroidetes and increased Firmicutes, as compared to wild-type littermate controls. In HD5 transgenic mice, the abundance of Bacteroides species increased and the Firmicutes dropped inversely. Thus, Paneth cell α (alpha)-defensin expression had significant impact on the colonizing microbiota under baseline conditions. In addition to these shifts in the dominant phyla of the small intestinal microbiota, transgenic expression of HD5 also eliminated colonization by *Candidatus arthromitis*, also referred to as segmented filamentous bacteria (SFB) [11]. SFB is a bacterium that directly contacts the surface of the small intestinal epithelium. The loss of SFB in the HD5 transgenic mice resulted in a significant decrease of Th17 cells in the small intestinal lamina propria, highlighting that Paneth cell antimicrobials can indirectly affect lymphocyte populations in intestinal tissues [11]. Interestingly, a converse SFB increase was reported in a mouse model that had marked Paneth cell dysfunction [107]. Together these data support that Paneth cell antimicrobials have an important homeostatic role in regulating the small intestinal microbiome. Furthermore, in their capacity to regulate the

Roles of Antimicrobial Peptides

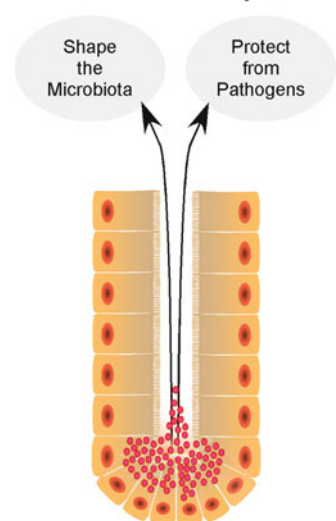


Fig. 8.1 Proposed roles for enteric antimicrobial peptides. The two principal roles for antimicrobial peptides in the intestine, such as Paneth cell α -defensins, are to protect the host from ingested pathogens and to help shape the composition of the colonizing microbiota. Depicted here in cartoon form is the release of secretory granules, chock full of antimicrobial peptides, from Paneth cells in the crypts of the small intestine

composition of the microbiome, Paneth cell antimicrobials can indirectly alter the abundance of subsets of lymphocytes in the lamina propria. Taken together, it seems clear that genetic or acquired abnormalities in Paneth cell function may have negative impact on host–microbe homeostasis.

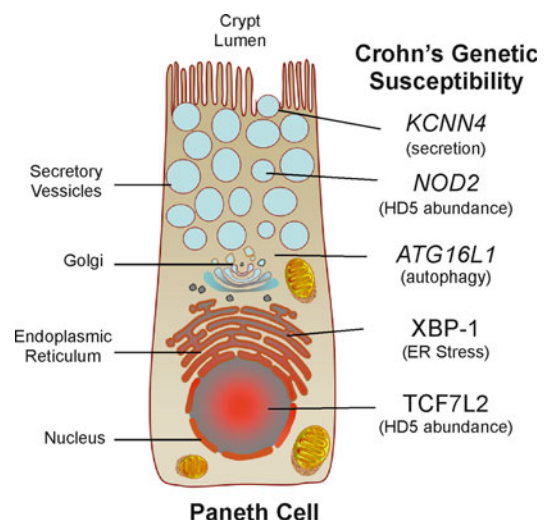
While antimicrobial peptides are the most abundant component of the secretory granules, other secretory peptides and proteins impart additional biological roles for Paneth cells [102]. Many molecules that have likely roles in innate immunity, or bridge between innate and adaptive immunity, are expressed in Paneth cells [28, 102, 103, 108–111]. For example, Paneth cells express *NOD2*, the gene encoding nuclear oligomerization domain 2 protein, an intracellular receptor for muramyl dipeptide of bacterial cell walls [108]. New data reveals that Paneth cells also express several molecules that serves as ligands for the adjacent intestinal stem cells, providing them with vital trophic factors [112]. It is likely that these cells have a much more fundamental and sophisticated role in intestinal homeostasis than currently acknowledged.

Paneth Cells and Crohn's Disease

CD often involves the distal portions of the small intestine where Paneth cells are abundant [1, 3, 113]. CD is associated with dysbiosis and abnormal bacterial adherence to the intestinal mucosal surface [4]. The identification of several CD susceptibility genes has placed a focus of CD pathogenesis directly on Paneth cells and their antimicrobial products [113]. Although each of these genetic susceptibility genes is found in only a subset of patients with CD, and all of these genes are expressed in other cells in the body, a common thread connecting them suggests Paneth cell dysfunction leads to an antimicrobial peptide deficiency, which underlies disease pathogenesis in pre-disposed hosts (see Fig. 8.2).

Wehkamp and colleagues found reduced Paneth cell α (alpha)-defensin expression in ileal CD compared to controls [90, 114]. Similar reduced expression of defensins was not observed in either ulcerative colitis or Crohn's disease of the colon [90]. The decrease in Paneth cell α (alpha)-defensin expression was independent of intestinal inflammation [90]. This suggests that reduced α (alpha)-defensin expression is not the result of inflammation, but rather an intrinsic and early event in the pathophysiology of CD (see Fig. 8.3). Individuals with 1007fs (SNP13) *NOD2* mutations, have especially low

Fig. 8.2 Genetic associations implicating Paneth cells in Crohn's disease pathogenesis. Paneth cells are quintessential secretory cells of the small intestinal epithelium. Their functions contribute to intestinal homeostasis. Several genetic susceptibility factors for IBD may have their phenotypic underpinnings in Paneth cell dysfunction. These include *KCNN4* (encoding KCa3.1) [133], *NOD2* [122, 123], *ATG16L1* [126], *XBP1* [40], and *Tcf7-L2* (encoding Tcf-4) [119]



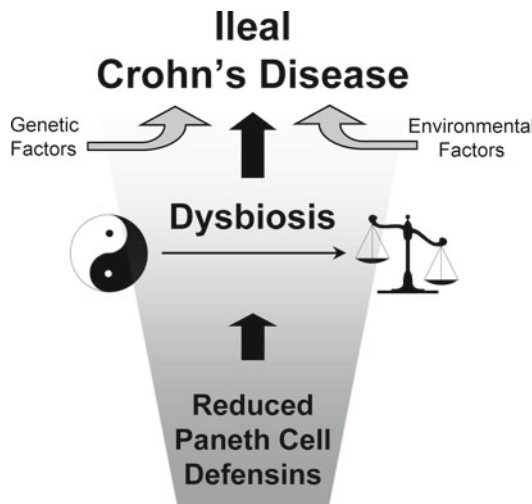


Fig. 8.3 Hypothesis on the possible link of Paneth cell for- ∞ and ileal Crohn's disease. Reduced expression of Paneth cell for- ∞ is characteristic of Crohn's disease of the ileum, but not ulcerative colitis or Crohn's disease of the colon [90]. Genetic and environmental factors may reduce Paneth cell expression of defensins. The resulting for- ∞ deficit is thought to contribute to dysbiosis. In turn, the dysbiosis, when combined with genetic susceptibility factors and environmental triggers, leads to Crohn's disease

α (alpha)-defensin levels, which are even lower than others with ileal CD but without this specific genetic variant [90]. This specific genetic polymorphism in *NOD2* is linked to more significant ileal involvement [2, 115, 116]. Additional studies show that deficiency of Tcf-4 is associated with ileal CD [117]. Tcf-4 is an integral transcription factor that drives both Paneth cell differentiation and α (alpha)-defensin expression [118]. The decrease in Tcf-4 expression was not dependent on an abnormal *NOD2* genotype, and was independent of the degree of tissue inflammation, again suggesting that α (alpha)-defensin-deficiency may be a common predisposing event in CD pathogenesis. A genetic association of *TCF4* with ileal CD provides evidence that a decrease in Paneth cell α (alpha)-defensins is a primary factor in disease pathogenesis for some individuals [119]. Despite the aggregate of supportive data, the role of reduced α (alpha)-defensin expression is a subject of some residual controversy [120, 121].

NOD2 was the first susceptibility gene identified for IBD and is especially linked to CD [122, 123]. Epithelial expression of *NOD2* in the small intestine is restricted to Paneth cells [124]. *Nod2*^{-/-} mice have reduced Paneth cell α (alpha)-defensin expression [108] and are deficient in immune response to enteric pathogens, including *Listeria monocytogenes* [108] and *Helicobacter hepaticus* [39]. Interestingly, *Nod2*^{-/-} mice develop granulomatous lesions in the ileal mucosa when challenged with *H. hepaticus*, a phenotype that is rescued by transgenic expression of HD5 in the mouse Paneth cells [39]. In addition, *Nod2*^{-/-} mice have alterations in their small intestinal microbial microbiota [125].

Other CD susceptibility genes are associated with dysfunction of Paneth cell secretory pathways, rather than antimicrobial expression. One such susceptibility gene is *ATG16L1*, which is homologous to the yeast autophagy gene ATG16 [126]. Autophagy is an essential cellular process for renewal and homeostasis, whereby organelles and other components are recycled following targeting to lysosomes for degradation [127, 128]. Mouse strains with reduced expression of the gene encoding ATG16L1 resulted in abnormalities in Paneth cell granule form and function [129]. CD patients with the *ATG16L1* mutation have similar abnormalities of their Paneth cell granules [129], and have alterations in their microbiota [130]. The phenotype in mice with hypomorphic expression

of *Atg16L1* is triggered by viral infection [131]. Interestingly, viral triggers have been implicated in human IBD [132].

A second gene associated with CD susceptibility may have pathogenic roots in Paneth cell secretion. *KCNN4*, which encodes for the calcium-activated potassium channel KCa3.1, was identified in a CD susceptibility genome-wide association study [133]. KCa3.1 is expressed in Paneth cells where it is involved in granule secretion [134]. One might speculate that disruption of the KCa3.1 channel would disrupt Paneth cell granule secretion, and result in deficiency of α (alpha)-defensins and other Paneth cell antimicrobials.

Finally, variants of the transcription factor X-box-binding protein 1 (XBP1) have been associated with increased risk for CD [40]. XBP1 is involved in maintaining endoplasmic reticulum function, especially important in intensive secretory cells, such as Paneth cells. Selective deletion of XBP1 in intestinal epithelial cells leads to Paneth cell (and goblet cell) dysfunction and results in intestinal inflammation in mice [40]. Genetic variations in the gene encoding XBP1 are associated with IBD susceptibility, and some uncommon variants may also predispose to CD [40].

Together, these puzzle pieces merge to implicate Paneth cells, and their arsenal of antimicrobial peptides in the pathogenesis of CD [113]. It seems likely that the aforementioned susceptibility genes may manifest part of their phenotypic effects through compromise of Paneth cell function. The aberrant function may lead to chronic alterations in microbiota, establishing dysbiosis, or limitations in the ability of these cells to successfully cope with acute microbial challenges.

Conclusions

The human intestine is colonized by a diverse, abundant and dynamic microbial ecosystem. These microbes are key to physiological homeostasis and proper balance of the immune system. The interactions between antimicrobial peptides, and this intestinal microbiota may have significant impact in both health and disease, and many aspects of this dynamic interplay are areas of current investigation. Paneth cell antimicrobial peptides, including α -defensins, are likely fundamental host factors that determine the composition of the colonizing microbiota. Perturbations of Paneth cell α -defensin expression may be a key factor in the pathogenesis of ileal Crohn's disease through interactions with the intestinal microbiota. Future studies to further define the function(s) and regulation of enteric antimicrobial peptides, will enhance our understanding of normal intestinal physiology and homeostasis. This knowledge may provide new therapeutic targets for treating inflammatory diseases in the intestine.

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Chapter 9

Vascular Responses to Intestinal Inflammation in Inflammatory Bowel Disease

D. Neil Granger and Norman R. Harris

Keywords Leukocyte-endothelial cell adhesion • Blood flow • Angiogenesis • Thrombosis • Endothelial barrier dysfunction

Introduction

It is now well recognized that the blood and lymph vascular systems play an important role in the genesis and perpetuation of an inflammatory response. Evidence accumulated from decades of clinical and basic research has revealed that the vasculature of the inflamed intestine undergoes a variety of functional and structural changes that influence the quality and intensity of the inflammatory and tissue injury responses, and sets the stage for tissue repair and regeneration. All segments of the vascular tree (arterial, capillary, venous) exhibit characteristic changes in response to inflammation. These include an altered reactivity of arterioles to vasodilators and vasoconstrictors, impaired capillary perfusion, adhesion of leukocytes and platelets to venular endothelium, enhanced coagulation and thrombus formation, increased vascular permeability with accelerated fluid and protein filtration, and proliferation of blood and lymphatic vessels. Since virtually every cell that either resides within or courses through the inflamed gut is activated, it is generally believed that multiple cell types and mediators activate the signaling mechanisms that account for the altered vascular function that accompanies inflammation. This chapter provides a brief summary of the vascular responses to gut inflammation and addresses the potential mechanisms that underlie these diverse vascular changes (Fig. 9.1) that contribute to the pathogenesis of inflammatory bowel diseases (IBDs) [1–4].

Vasomotor Dysfunction

Intestinal blood flow can be profoundly altered by chronic gut inflammation. However, the nature and magnitude of the change in intestinal blood flow appear to be dependent on the stage of progression of the inflammatory response. During the early “exudative” phase of colonic inflammation in

D.N. Granger, PhD (✉) • N.R. Harris, PhD
Department of Molecular and Cellular Physiology, Louisiana State University Health
Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932, USA
e-mail: dgrang@lsuhsc.edu

of the response detected in normal arterioles. The impaired vasodilatory response appears to result from a diminished capacity of arteriolar endothelial cells (EC) to produce nitric oxide (NO), rather than prostacyclin. The NO deficiency results from an excessive production of superoxide, which rapidly reacts with NO and inactivates it, thereby inhibiting smooth muscle relaxation [2, 6, 12].

The changes in colonic blood flow during IBD may also reflect an altered production of endogenous vasoconstrictors [8, 13]. There are several reports describing increased levels of thromboxane synthase and/or thromboxane B₂ in the colon of IBD patients, with a correlation noted between thromboxane synthase and disease activity. Although some clinical trials have targeted thromboxane synthase as a potential therapeutic locus for IBD, it remains unclear whether thromboxane-induced vasoconstriction contributes to disease progression because thromboxane synthase inhibitors showed little effectiveness in promoting the remission of IBD. Angiotensin II and endothelin-1, other endogenous vasoconstrictors generated by the gut, are also detected at higher levels in the colonic mucosa of CD patients [13]. Endothelin-1 has been implicated in the tissue hypoxia that accompanies the ischemic phase of IBD progression [14].

Leukocyte– and Platelet–Endothelial Cell Adhesion

The adhesion of leukocytes to vascular endothelium is a hallmark of the inflammatory response. This adhesive interaction between blood cells and the vessel wall largely occurs in postcapillary venules and is critical for the recruitment of inflammatory cells to sites of infection and/or tissue injury. An important determinant of whether leukocytes adhere to vascular endothelium is the pro-adhesive force that is generated by adhesion molecules expressed on the surface of activated leukocytes and endothelial cells (EC). Table 9.1 summarizes some of the EC adhesion glycoproteins that have been implicated in the recruitment of leukocytes in inflamed colonic venules, their ligands (counter-receptors) on leukocytes, and the type of adhesive interaction mediated by the glycoprotein pairs. Studies in animal models of IBD have demonstrated a time-dependent increase in the expression of P- and E-selectins, ICAM-1, VCAM-1, and MAdCAM-1, in the vasculature of the inflamed colon [1, 15, 16]. Similar findings have been reported for mucosal biopsy samples derived from patients with active (but not quiescent) ulcerative or Crohn's colitis. This upregulation of EC adhesion molecules is accompanied by the recruitment of large numbers of rolling, firmly adherent, and emigrated (extravasated) leukocytes within (and surrounding) inflamed colonic venules. The critical importance of adhesion molecules in the genesis of experimental IBD is evidenced by a number of studies demonstrating attenuated inflammatory and tissue injury responses in mice that are genetically deficient in specific leukocyte or endothelial cell adhesion molecules, and in animals treated with blocking antibodies directed against these adhesion glycoproteins [1, 15, 16].

A variety of factors contribute to the modulation of leukocyte–endothelial cell adhesion during gut inflammation either by affecting the intensity of adhesion molecule expression and/or the ability of

Table 9.1 Endothelial cell adhesion molecules: ligands and functions

Adhesion molecule	Leukocyte receptors	Adhesion response
P-selectin	L-selectin, PSGL-1	Rolling
E-selectin	L-selectin	Rolling
ICAM-1	CD11/CD18	Adherence, emigration
VCAM-1	VLA ₄	Adherence, emigration
MAdCAM-1	alpha4beta7	Adherence, emigration
PECAM-1	PECAM-1	Emigration

ICAM-1, intercellular cell adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand; VCAM-1, vascular cell adhesion molecule-1; VLA₄, very late antigen-4, MAdCAM-1, mucosal addressin cell adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule-1

these glycoproteins to sustain strong adhesive interactions between the blood cell and vascular endothelium. Nitric oxide, adenosine, and prostacyclin produced by EC tend to prevent leukocyte–EC adhesion while reactive oxygen species (superoxide, hydrogen peroxide) produced by activated leukocytes and EC appear to promote leukocyte adhesion [15, 16]. These endogenous regulators of leukocyte adhesion exert either a direct (transcription-dependent) or an indirect (i.e., altering the production of inflammatory cytokines) effect on the production/expression of EC adhesion molecules. Vasoactive agents (e.g., nitric oxide) can also influence the quality of the adhesive interaction between glycoproteins expressed on leukocytes and EC by altering shear forces generated by the movement of blood through the microvasculature. It has been noted that the number of leukocytes recruited in inflamed venules is inversely related to the wall shear rate, suggesting that it is easier for leukocytes to create strong adhesive bonds with ECs at low shear rates and that high shear rates are likely to prevent the creation of such bonds. This dependency of leukocyte adhesion on shear rate suggests that the changes in blood flow associated with the early and late stages (discussed earlier) of inflammation may significantly influence the intensity of leukocyte recruitment during IBD [15–17].

Platelets also accumulate in the microvasculature of the inflamed colon. The adhesion of platelets to the walls of colonic venules appears to reflect platelet binding to activated ECs rather than adhesion to the collagen-rich subendothelial surface of damaged blood vessels [18, 19]. Intravital microscopic evaluation of platelet binding in inflamed colonic venules has revealed that a relatively small proportion (<10%) of the platelets bind directly to ECs, with the remainder attaching to leukocytes that are already bound to the vessel wall. P-selectin blockade effectively inhibits both the leukocyte-dependent and -independent components of platelet adhesion in colonic venules of colitic mice, suggesting that P-selectin (whether expressed on the platelet or EC) contributes to both recruitment processes [19]. While platelet binding to EC is not altered by ICAM-1 or CD18 immunoblockade, the accumulation of leukocyte-bound platelets is dramatically reduced. The findings are consistent with a model wherein leukocytes require P-selectin to roll on venular endothelium and subsequently establish firm adhesion via CD18–ICAM-1 interactions. The adherent leukocytes, which constitutively express PSGL-1, then create a platform onto which platelets can bind using P-selectin. Similar P-selectin (platelet-associated)–PSGL-1 (leukocyte-associated) interactions also likely account for the platelet leukocytes that are detected in blood of IBD patients. The pathophysiological significance of platelet recruitment into inflamed microvessels remains unclear, but may relate to the ability of activated platelets to produce and release a variety of proinflammatory mediators, as well as the ability of platelets attached to neutrophils to enhance superoxide production by the latter cells [20].

Coagulation and Thrombosis

Additional consequences of the altered platelet function that occurs during IBD include hyperreactivity, hyperaggregability, and an increased propensity for thrombus formation in large and microscopic blood vessels [3, 21–25]. Intravascular platelet aggregates are detected in mucosal biopsies from patients with ulcerative colitis, and the number of platelet aggregates in mesenteric venous blood is increased in IBD. The enhanced reactivity and aggregability of platelets in IBD are accompanied by evidence of significant systemic activation of the coagulation system. Activation of the coagulation system is manifested in IBD patients as changes in blood levels of a number of soluble circulating biomarkers of a procoagulant state, including thrombin–antithrombin (TAT) complexes, fibrinogen, von Willebrand factor, and clotting factors V, VII, and VIII. The anticoagulant role of ECs is diminished during inflammation, which is reflected in an increased expression of tissue factor, downregulation of anticoagulant protein C pathway, and the inactivation of nitric oxide by superoxide. Collectively, the hemostatic alterations that accompany inflammation tip the balance

between pro- and anticoagulant mechanisms in favor of thrombus formation. This combination of a hypercoagulable state and platelet hyperreactivity/-aggregability in IBD likely accounts for the fact that vascular beds distant from the inflamed bowel are vulnerable to thrombosis [3, 21–25].

Systemic thromboembolic events (TE) are a significant cause of morbidity and mortality in IBD patients. Clinical studies have revealed that the incidence of TE events in IBD patients is ~6.2%, with a 3.6-fold increase in the risk for TE complications compared to the general population [3, 26, 27]. A much higher incidence of systemic TE (41%) is predicted from postmortem studies, suggesting that many cases are not detected. Both the arterial and venous circulations appear to be involved in IBD-associated TE. Venous complications occur more frequently, but the mortality associated with arterial thrombosis is comparable to that noted when veins are involved. TE is most commonly manifested as deep vein thrombosis or pulmonary embolism, although thromboses have been detected in other regional circulations, including brain, retina, and liver [3]. While some clinical studies suggest that the risk of TE may correlate with disease severity and the extent of colon involvement, other studies have demonstrated that up to one-third of TE complications occur during disease quiescence, suggesting that the procoagulant tendency may be independent of disease activity. No single biomarker of coagulation appears to reliably correlate with the occurrence of TE in IBD.

Many of the hemostatic alterations reported in patients with IBD have been recapitulated in animal models [3]. These include increases in blood levels of fibrinogen and TAT complexes, thrombocytosis, and a reduced capacity for protein C activation [28, 29]. Mice with experimental colitis also exhibit an increased vulnerability to microvascular thrombus formation in extraintestinal tissues, a response that is largely confined to arterioles [3, 28]. Tissue factor activation, an impaired protein C pathway, and thrombin have all been implicated in the enhanced extraintestinal thrombosis in experimental IBD models. While the chemical and/or cellular signals produced by the inflamed gut that initiates this distant organ thrombogenic response remain undefined, there is evidence implicating the cytokines, interleukin-1 β , and tumor necrosis factor- α (TNF α) [3, 30]. Immunoblockade of both cytokines appears to largely prevent the enhanced extraintestinal thrombosis associated with DSS-induced murine colitis. The DSS model has also been useful in demonstrating a role for pro-(tissue factor) and anticoagulant (activated protein C) in modulating the initiation and perpetuation of gut inflammation, findings consistent with the view that inflammation and coagulation are interdependent processes that can initiate a vicious cycle wherein each process propagates and intensifies the other [31].

Angiogenesis and Vascular Permeability

There is a growing body of evidence that IBD is associated with the development of new blood (angiogenesis) and lymph (lymphangiogenesis) vessels from the existing vascular network and that the angiogenic response tends to prolong and intensify the inflammatory response [4, 9, 31, 32]. Mucosal biopsies from inflamed bowel in IBD patients have revealed a significant increase in the density of microscopic blood vessels, compared to normal bowel samples. Similar evidence for increased vascularity has been reported in different models of experimental colitis. A dependency of the inflammatory response on angiogenesis is supported by animal studies that demonstrate significant positive correlations between angiogenic and inflammatory disease activity [32, 33]. Further support is provided by reports that describe diminished disease activity, tissue injury, and colonic inflammation in mice treated with (or that genetically overexpress) anti-angiogenic agents (e.g., soluble VEGF-1 receptor), while a worsening of disease activity, injury, and inflammation is noted in colitic animals treated with (or that genetically overexpress) pro-angiogenic agents such as vascular endothelial growth factor-A (VEGF-A) [32]. Such findings have led to the proposal that angiogenesis may be a novel and effective therapeutic target for IBD.

Angiogenesis is a complex process that begins with an initiating event like inflammation, which involves the activation of different cell populations that release angiogenic factors such as VEGF, platelet derived growth factor (PDGF), fibroblast growth factor (FGF), TNF α , and adenosine [16, 32, 33]. This is followed by a proliferation/invasion phase that involves changes in the vessel wall (e.g., increased vascular permeability, dissolution of the basement membrane) that allow for the migration and proliferation of ECs. The final stage (maturation/differentiation) includes tube formation and restoration of a normal vessel wall. The rate of angiogenesis is governed by the balance between angiogenic and angiostatic factors produced by the tissue. During inflammation, this balance favors angiogenesis. Because sites of inflammation are often hypoxic, the low tissue oxygen tension also contributes to angiogenesis by virtue of its induction of hypoxia-inducible factor, which elicits the transcription-dependent production of VEGF and FGF [14]. The leukocytes, platelets, and macrophages that are recruited and activated in inflamed tissues are also capable of producing large quantities of pro-angiogenic factors, including VEGF and cytokines.

Inflammation is generally associated with impaired endothelial barrier function and an increased vascular permeability [34, 35]. While this response is a critical component of angiogenic response, it is not confined to those regions of the vascular bed that sprout new blood vessels. Inflamed post-capillary venules exhibit an increased permeability to plasma proteins which leads to an increased tissue oncotic pressure, withdrawal of fluid from the intravascular compartment (capillaries and venules), increased interstitial fluid volume, and ultimately interstitial edema (Fig. 9.1). Studies in animal models of IBD support the view that substances released by recruited inflammatory cells, and resident mast cells and macrophages mediate the endothelial cell contraction and diminish the barrier function noted in the inflamed gut [36]. Candidate mediators include histamine, VEGF, cytokines (interleukin-8), lipid mediators (e.g., platelet activating factor), leukocyte-derived proteases (e.g., elastase), and reactive oxygen and nitrogen species.

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Chapter 10

Immunobiology of Dendritic Cells in Inflammatory Bowel Disease

Daniel C. Baumgart

Keywords Dendritic cells • Myeloid dendritic cells • IBD • Crohn's disease • Ulcerative colitis • Inflammatory bowel disease • Human

Introduction

Breakdown of immunological tolerance toward the commensal microflora in genetically susceptible individuals is believed to be the key event in the pathogenesis of inflammatory bowel disease [1, 2].

Dendritic cells control the critical balance between anergy and immunity due to their functional dichotomy of being either the most potent antigen presenters or effective inducers of (peripheral) tolerance. Due to their expression of the entire spectrum of pattern recognition receptors (PRRs), such as TLR and NOD they can sense virtually all microbial-associated molecular patterns (MAMPs). This puts them in a pivotal position for understanding the distinct innate and adaptive immune responses that intestinal microbiota induce in inflammatory bowel disease. Following microbial recognition and interpretation, dendritic cells direct the innate and adaptive immune response of effector cells toward regulation vs. inflammation [3, 4].

Thus, dendritic cells are considered key for the initiation, perpetuation and control of inflammation in inflammatory bowel disease. Animal and in vitro studies suggest that DC falsely recognize commensal bacteria and may induce a T_H1 or T_H17 , proinflammatory immune response normally directed at pathogens. Activated DC have been demonstrated in IBD animal models which may prolong the survival of activated T cells thereby maintaining inflammation [5–8].

Human dendritic cell populations in Crohn's disease and ulcerative are insufficiently characterized. This is due to their generally low frequency in accessible tissues and further complicated by our still incomplete understanding of human dendritic cell origins and ontogeny and the resulting problem to unequivocally identify the human counterparts of the various murine dendritic cell subsets [9–11].

This chapter focuses exclusively on human dendritic cells in human inflammatory bowel disease, while the data on inflammatory bowel disease animal model-derived dendritic cell data has been extensively reviewed elsewhere [12].

D.C. Baumgart, MD, PhD (✉)

Division of Gastroenterology and Hepatology, Department of Medicine,
Charité Medical Center – Virchow Hospital, Medical School
of the Humboldt-University of Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: daniel.baumgart@charite.de

Distribution, Phenotype, and Function of Human Dendritic Cells in Inflammatory Bowel Disease

Peripheral (Circulating) Blood Dendritic Cells

A number of studies have looked at peripheral blood dendritic cells from patients with inflammatory bowel disease. We and others found an activated (mature) phenotype indicated by higher expression of CD40, CD83, or CD86 and on freshly isolated myeloid dendritic cells from Crohn's patients and ulcerative colitis compared with healthy controls [13, 14]. Myeloid dendritic cells from Crohn's and ulcerative colitis patients show an exaggerated response to lipopolysaccharide, stimulate T cells significantly more in mixed lymphocyte reactions and are important producers and secretors of key inflammatory cytokines such as IL-1 [15], IL-6 [13], IL-8 [13, 15], TNF- α [13], and nitric oxide [16].

The frequency of peripheral blood dendritic cells is already low compared with primary lymphatic organs such as the spleen or mesenteric lymph nodes [17]. Data from our group demonstrated a decreased frequency of both plasmacytoid and myeloid dendritic cells in IBD patients that strikingly correlates with disease activity [18]. This peripheral depletion and their accumulation at the sites of inflammation (see below) suggest recruitment of dendritic cells to the gut in active inflammatory bowel disease. In fact their expression of transmigration, gut homing and chemokine/chemoattractant receptors such as intercellular adhesion molecule-1 (ICAM-1), the integrin $\alpha\beta 7$ (CD49d) [18], CCR6 [19], and CCR7 (CD197) [20, 21] has been shown and supports this hypothesis [13, 22, 23]. It is conceivable that dendritic cells are attracted to the mucosa and mesenteric lymph nodes by the stromal expression of chemokines or chemoattractants such as lymphotactin [24], CXCL-8 [25], CXCL9 [25], CXCL10 (IFN- γ induced protein 10 kD [IP-10]) [25] and CXCL13 (also called B-cell-attracting chemokine 1 [BCA-1] or B-lymphocyte chemoattractant [BLC]) [26, 27] as well as CCL19 [21, 28], CCL20 [21, 29] and CCL21 [21, 28, 30].

More recent research aimed potentially therapeutically exploitable modulation of human peripheral blood dendritic cells. One approach, based on the evolving concept that retinoic acid enhances the transforming growth factor TGF β -dependent conversion of naive T cells into regulatory T (Treg) cells, used peripheral blood monocyte-derived DC (MoDC) with or without synthetic retinoic acid (Am80). The authors found that (Am80-MoDC) compared with conventional MoDC showed macrophage-like adherent phenotype and lacked the expression of the typical DC marker CD1a. Am80-MoDC-produced less IL-12p70 and revealed less polarizing ability toward Th1 by allogeneic mixed lymphocyte reaction with naive T cells [31]. Another group reported that MoDC and circulating CD1c⁺ myeloid DC conditioned with supernatants from intestinal epithelial cells from healthy donors promoted the differentiation of tolerogenic DC able to drive the development of adaptive Foxp3⁺ Treg cells [32].

Mucosal Dendritic Cells

Initially, a subpopulation of gut mononuclear phagocytes were suspected to be dendritic cells based on their long dendritic cytoplasmic projections [33]. The studies employing a marker that identifies interdigitating cells antigen presenting (RFD1) hypothesized distinct phenotypic change in macrophages away from an interdigitating phenotype toward mature tissue macrophages (RFD7) in actively inflamed intestinal segments and pouches [34–36]. A later study showed that aphthoid lesions in the colonic mucosa of Crohn's disease patients contained densely aggregated CD68⁺ macrophages surrounded by numerous ICAM-1, HLA-DR, ID-1-positive dendritic cells [22].

With the availability of a novel dendritic cell marker DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN), two recent histochemical studies identified additional distinct populations of DC present in Crohn's disease patients: a DC-SIGN-positive population that was present scattered throughout the mucosa, and a CD83-positive population that was present in aggregated lymphoid nodules and as single cells in the lamina propria [37, 38]. Another immunohistochemical study reported M-DC8⁺ myeloid dendritic cells localized in the T-cell area in the subepithelial dome region of Peyer's patches inflamed ileal mucosa of patients with active Crohn's disease [39]. Interleukin-27, a newly described member of the IL-12 family, is a heterodimeric cytokine composed of two subunits, p28 and Epstein–Barr virus-induced gene 3 (EBI3) and mainly produced by activated dendritic cells and monocytes. EBI3-expressing dendritic cells were described in the lamina propria of Crohn's patients with a pattern of reactivity was similar to control mucosa [40].

Bone loss is a major clinical problem in Crohn's disease. Receptor activator of NF- κ B ligand (RANKL) and its receptor RANK are potentially associated with nonsteroid attributable bone loss and controlled by proinflammatory cytokines. RANK, CD68, S100⁺ (myeloid dendritic) cells were increased in inflamed areas of colonic mucosa from Crohn's disease patients [41].

The accumulation of mucosal dendritic cells is not restricted to Crohn's disease. Immunohistochemical studies in ulcerative colitis patients revealed an increased number of follicular dendritic cells and myeloid cells dendritic cells basal lymphocyte aggregates of colonic lamina propria and inflamed crypts of ulcerative colitis patients [19, 21, 42, 43].

These early immunohistochemical data have been corroborated by a number of functional flow cytometry studies with different marker panels focused on myeloid dendritic cells. Several studies reported an increased frequency of activated, i.e., CD40⁺, CD83⁺, and/or CD86-expressing myeloid, dendritic cells in the inflamed mucosa of both Crohn's and ulcerative colitis [13–15, 44–46].

Knowing their mature state it is perhaps not surprising that mucosal myeloid dendritic cells are also an important source of inflammatory cytokines such as TNF- α , IL-6, IL-8(CXCL8), IL-12, IL-17, IL-23, and IL-27 mostly in patients with Crohn's disease [25, 39, 40, 46, 47]. However, the production of IL-12 and more recently also IL-23 in Crohn's disease has been called into questions by a study of mucosal monocyte-derived dendritic cells in children with Crohn's disease [48].

Mesenteric Lymphnode Dendritic Cells

Dendritic cells have been linked to a hallmark feature of Crohn's disease – granuloma formation [49–51]. Early immunohistochemical studies in granulomatous lymphadenitis in Crohn's patients investigated the cellular composition of the granulomas in mesenteric lymph nodes and found an increased number of interdigitating reticulum cells with characteristics of antigen-presenting cells that we would now classify as dendritic cell subsets [52].

More recent studies using more refined markers identified mature myeloid CD83⁺ dendritic cells, DC-SIGN-positive dendritic cells and CD141⁺ dendritic cells in mesenteric lymph nodes clustered around T cells of Crohn's disease patients [37]. Another study that looked at IL-27-expressing myeloid dendritic cells demonstrated that granulomas present in the intestinal wall or in mesenteric draining lymph nodes of Crohn's disease patients stained positive with both anti-EBI3 and anti-p28 antibodies localized to the cytoplasm of epithelia and multinucleate giant cells [40].

The expression of chemokines that regulate homing of T cells and promote recirculating T-cell and dendritic cell interactions, CCL19 and CCL21, was studied in surgically resected mesenteric lymph nodes from controls and patients with Crohn's disease and ulcerative colitis. Mesenteric lymph nodes from Crohn's disease patients displayed an increased expression of CCL21 and CCL19, mainly in high endothelial venules (HEV), mature DC, and lymphatic vessels. CCR7 mRNA was

increased in T-cell areas. This data indirectly supports the hypothesis of homing T cells and mature myeloid dendritic cells to mesenteric lymph nodes in Crohn's disease [28].

More recently, CD103⁺ DCs described in the mesenteric lymph nodes draining the normal small intestine, although the percentage of showed considerable variation. Human CD103⁺ mesenteric lymph node DC displayed a more mature phenotype compared with their CD103⁻ counterparts, as judged by expression of CD40 and CD83. In mixed lymphocyte reactions with allogeneic peripheral blood lymphocytes human CD103⁺ mesenteric lymph node DC induced significantly higher levels of CCR9 on responding CD8⁺ T cells than their CD103⁻ counterparts. Both, CD103⁺ and CD103⁻ populations induced similar expression of $\alpha 4\beta 7$. No difference was seen with their counterparts isolated from mesenteric lymph nodes from patients with Crohn's disease. The induction of CCR9⁺ and $\alpha 4\beta 7$ ⁺ by human CD103⁺ mesenteric lymph node DC was dependent on RAR signaling as gut-homing receptor expression was inhibited by addition of LE540 to the cultures [53]. Human CD103⁺ DC also express leucine-rich repeat kinase 2 (LRRK2), an IFN- γ target gene and its expression increased in intestinal tissues upon Crohn's disease inflammation. LRRK2 expression enhances NF- κ B-dependent transcription [54].

When stimulated with exogenous bacterial derivative, mesenteric lymphnode myeloid DC from Crohn's disease produce a higher amounts of IL-23 and a lower amount of IL-10 compared with controls and induce stronger Th1 immune responses in mixed lymphocyte reactions compared with those from ulcerative colitis and normal controls [47].

Dendritic Cells in Extraintestinal Tissues in Inflammatory Bowel Disease

The distribution of DC in Crohn's disease patients is not restricted to classical lymphatic spaces and compartments. Adipose tissue is reported to contain monocyte-like pre-adipocytes, which may mature into macrophages, contributing to local inflammation. Myeloid dendritic cells originate from monocytes [10]. Accumulating dendritic cells enriched from "creeping fat" were potent stimulators of primary proliferation of allogeneic T cells in mixed leukocyte reactions in one study [55]. Another study investigating corneal changes of patients with Crohn's disease using confocal microscopy found that patients with Crohn's disease had a lower corneal density of dendritic cells [56].

Interaction of Human Dendritic Cells with Microbial Antigens in Ulcerative Colitis and Crohn's Disease

Interaction of Microbial Antigens

Both peripheral blood [13] and mucosal [38, 57–59] dendritic cells from patients with Crohn's disease and ulcerative colitis express the microbial pattern recognition receptors TLR2 and/or TLR4. Myeloid DC from patients with inflammatory bowel disease have been shown to respond with an exaggerated immune response to lipopolysaccharide – the principal ligand of TLR4 [13]. Single-nucleotide polymorphisms (SNPs) in the TLR4 receptor have been reported to be associated with Crohn's disease in some cohorts and were indeed shown to modulate dendritic cell response toward TLR4 agonists to the generation of strongly polarized Th1 responses against common commensal microorganisms [60].

Myeloid dendritic cells also express the intracellular pattern recognition receptor NOD2. A study comparing NOD2-dependent genome-wide expression profiles of muramyl dipeptide (MDP) – a derivative of bacterial peptidoglycan – stimulated MoDC from Crohn's disease NOD2 SNP carriers and controls showed that the transcription of pathogen response genes was absent [61].

There is evidence that these expression profile differences translate into actual functional defects. One group was able to link the development of Th17 through the NOD2 ligand. The role of NOD2 in this IL-23-IL-1-IL-17 axis could be confirmed in NOD2-deficient DCs from selected Crohn's disease patients [62]. These data were corroborated by the work of several other groups that NOD2 mutant MoDC from Crohn's disease patients were unable to react appropriately to MDP and promoted the development of Th1 cells [60, 63, 64].

NOD2 triggering by MDP normally induces autophagy in myeloid DC and requires receptor-interacting serine-threonine kinase-2 (RIPK-2), autophagy-related protein-5 (ATG5), ATG7 and ATG16L1 but not NLR family, pyrin domain containing-3 (NALP3) [65]. Two studies showed that NOD2-mediated autophagy is required for both bacterial handling and generation of major histocompatibility complex (MHC) class II antigen-specific CD4⁺ T-cell responses in DC and that DC from individuals with Crohn's disease expressing Crohn's disease-associated NOD2 or ATG16L1 risk variants are defective in autophagy induction, bacterial trafficking, and antigen presentation [65, 66].

Therapeutic Modulation of the Microbial Response

Based on diversity of the commensal flora in the human gut, the importance of microbial antigen recognition and preliminary evidence that the intestinal dendritic cell function may be influenced by the composition of the commensal microbiota encouraged pilot studies with different probiotic microbial species to exploit this important aspect of the immune response therapeutically [67].

In one study, human MoDC were cultured in vitro with different *Lactobacilli* species. *Lactobacillus reuteri* and *Lactobacillus casei*, but not *Lactobacillus plantarum* primed MoDC to drive the development of IL-10-producing Treg through binding of the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) [68]. However, these findings are partially contradicted by a study where *Lactobacillus rhamnosus*-treated MoDC decreased T-cell proliferation and cytokine production of IL-2, IL-4, and IL-10 [69].

Not only bacteria have therapeutic potential with IBD DC. Culture of primary human myeloid CD1c⁺CD11c⁺CD123⁻ myeloid DC in the presence of *Saccharomyces boulardii* culture supernatant (active component molecular weight <3 kDa, as evaluated by membrane partition chromatography) reduced significantly the expression of CD40, CD80, and CCR7 (CD197) induced by the prototypical microbial antigen and TLR4 ligand lipopolysaccharide. Moreover, secretion of TNF α and IL-6 were notably reduced, while the secretion of IL-10 increased. Finally, Sb supernatant inhibited the proliferation of naive T cells in a mixed lymphocyte reaction with myeloid DC [20].

A different approach was taken by another group of investigators based on the fact that some *Bifidobacteria* species are immunoregulatory, induce increased dendritic cell interleukin IL-10 release in vitro, and that administration of fructooligosaccharides increases fecal and mucosal bifidobacteria. They found the percentage of IL-10 and TLR2- and TLR4-positive dendritic cells in fructooligosaccharide-treated Crohn's disease patients [58]. Unfortunately, these data could not be validated in a randomized trial [70] (see Fig. 10.1).

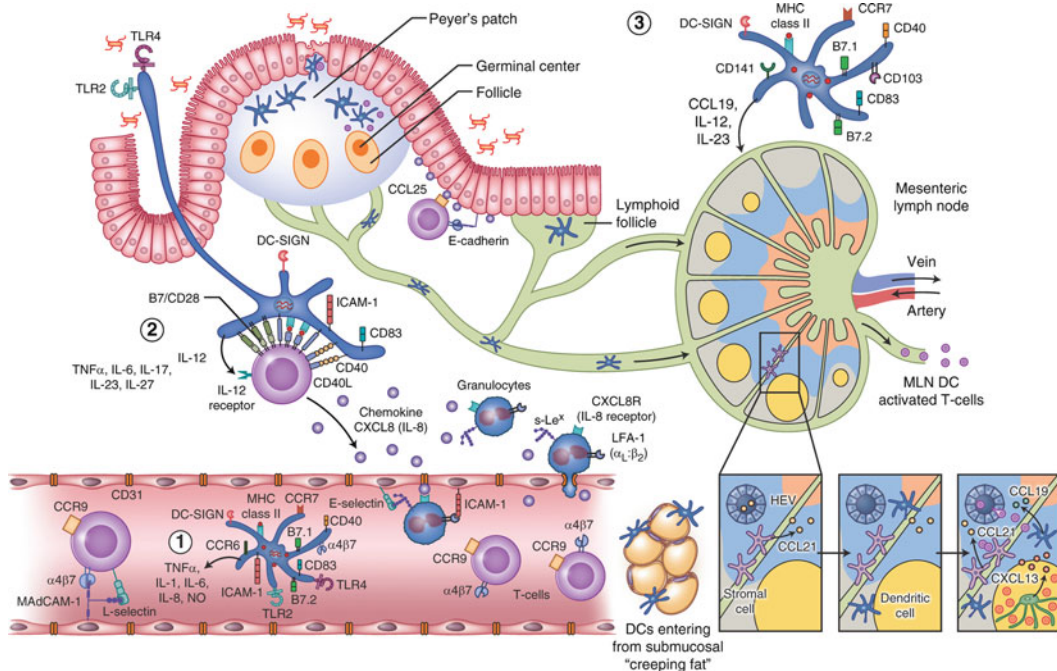


Fig. 10.1 Model of the three major myeloid dendritic cell populations and their hypothesized (dys)function in human inflammatory bowel disease. (The majority of pathways were identified for Crohn's disease and to a lesser degree for ulcerative colitis. See text and references for details. For clarity, no distinction between Crohn's disease and ulcerative colitis was made and to bring out cellular details this cartoon is not to scale.) (1) Peripheral blood myeloid dendritic cells: In IBD patients these cells display an activated phenotype and have been demonstrated to express HLA-DR, CCR6, CCR7(CD197), CD80, CD83, CD86, CD40, ICAM-1 (CD49d), TLR2, and TLR4. In acute flares circulating dendritic cells evade the peripheral blood pool and probably migrate to the mucosal sites of the inflammation. Their secretion of chemokines such as CXCL8 (IL-8) attracts innate immune cells such as granulocytes expressing its cognate receptor CXCR8 and homing CCR9⁺ $\alpha 4\beta 7^{+}$ T lymphocytes that transmigrate into the tissue mediate, amplify, and perpetuate the inflammation. (2) Mucosal myeloid dendritic cells: In IBD they show an activated phenotype as well. These cells inspect molecular microbial-associated patterns (MAMP) of the intestinal microbiota with their own TLR2 and TLR4 receptors directly through their epithelial layer-penetrating dendrites or are fed microbial antigens via M-cells in Peyer's patches that transport them into the lamina propria. Moreover, antigens are recognized by intracellular NOD. In the small intestine this happens in the subepithelial dome area of Peyer's patches or isolated lymph follicles in other parts of the small and large intestine. DC-SIGN and CD83-expressing myeloid dendritic cells have been described in isolated lymph follicles as well. Misinterpretation of MAMPs perhaps due to single-nucleotide polymorphisms of TLR4 and NOD2 reported in IBD and absent tolerogenic effects of TGF β and retinoic acid on mucosal dendritic cells normally secreted intestinal epithelia triggers immunity against the normally tolerated flora. The distinct proinflammatory cytokine profile (TNF α , IL-6, IL-17, IL-23, and IL-27) of myeloid mucosal dendritic cells in IBD promotes the development of inflammatory Th1 and Th17 T lymphocytes and attracts other leukocytes to transmigrate through the endothelial layer into the lamina propria. (3) Mesenteric lymph node myeloid dendritic cells: Stromal cells and high endothelial venules (HEV) secrete the chemokine CCL21 and attract circulating dendritic cells that recognize it through their expression of its native receptor CCR7. Mucosal dendritic cells migrate along the chemokine gradient via efferent lymphatic vessels from the lamina propria into the afferent lymphatic of the mesenteric lymph node (MLN). In the paracortex mesenteric myeloid lymph node dendritic cell function depends on their expression of CD103. Once arrived in the T-cell zone CD103⁺ dendritic cells secrete CCL19 that increases via feedback loop the expression of CCR7 – the CCL19 receptor on dendritic cells – and also attract CCR9⁺ $\alpha 4\beta 7^{+}$ T lymphocytes to arrive through HEV from the blood. These T cells start to proliferate once they recognize their cognate antigens presented by the mesenteric lymph node dendritic cells and leave the lymph node activated through the efferent lymphatic further aggravating inflammation. The expression of CCL19 and CCL21 suggests that B cells are also attracted to mesenteric lymph nodes which may induce follicular dendritic cells that secrete CXCL13 (BCA-1 or BLC) that drives more B-cell migration. While the chemokine expression has been described in IBD, the interaction is speculative

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Chapter 11

Immunobiology of T Cells in Inflammatory Bowel Disease

Scott B. Snapper and Deanna D. Nguyen

Keywords CD4+ T cells • CD8+ T cells • TCR $\gamma\delta$ cells • Regulatory T cells • Effector T cells • T-cell plasticity • Tr1 cells • Th3 cells • Th1 cells • Th2 cells • Th17 cells • iT_R35 • Inducible regulatory T cells • iTregs • Naturally occurring regulatory T cells • nTregs • NK-T cells • Foxp3 • Scurfy mice • IPEX • IL-10 • TGF- β • IL-35 • IL-5 • IL-13 • IL-17 • CCR9 • $\alpha 4\beta 7$ • Natalizumab • Vedolizumab

Introduction

The adaptive immune system plays a central role in the pathogenic process in inflammatory bowel disease (IBD). In a healthy state, both CD8+ and CD4+ subsets of T cells are present in the intestines in small numbers; the former are found mostly in the epithelial layer while the latter are seen exclusively deeper in the lamina propria. In an inflammatory state, however, the number of CD4+ T cells is often highly magnified. Early research efforts were largely centered on T lymphocytes given evidence that CD4+ T cells from colitic animals and human IBD patients expressed activation markers and produced proinflammatory cytokines [1–5] and that adoptive transfer of CD4+ T cells from several animal models of colitis into immunodeficient mice could transfer disease [6–8]. While still controversial, it seems likely in most IBD patients that altered T-cells are not the critical initiating signal driving inflammation, but rather are an essential mediator and propagator of disease resulting from aberrant innate immune function [1].

T lymphocytes are broadly classified as regulatory or effector T cells. The latter is crucial in the protective immune response against infections. In contrast, the function of regulatory T cells is to maintain mucosal homeostasis by limiting the immune response and by disarming any self-reactive effector T cells that escape negative selection in the thymus. Both T-cell subsets are present in the gut-associated lymphoid tissue. Thus, inflammation can result from an imbalance of the number and/or function of these two T-cell arms of the homeostatic T-cell equation. Most studies on the role of

S.B. Snapper, MD, PhD (✉)
Gastroenterology, Children's Hospital and Brigham and Women's Hospital, 300 Longwood Avenue,
Boston, MA 02115, USA
e-mail: scott.snapper@childrens.harvard.edu

D.D. Nguyen, MD
Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital,
Boston, MA, USA

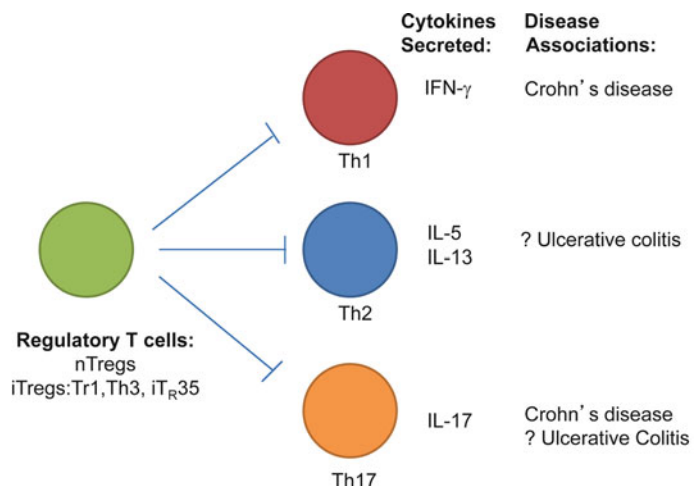
T lymphocytes in IBD pathogenesis have centered on the CD4⁺ T-cell subset, which will be the main focus of this chapter; however, other T-cell subsets will be briefly covered at the end of the chapter.

Effector T Cells and Their Cytokines

Constantly exposed to the luminal microbiota, effector T cells are critical for protecting against intestinal pathogens. Effector CD4⁺ T cells are characterized by their cytokine secretion profile to be Th1, Th2, or Th17 cells. Interferon- γ and TNF- α are the predominant cytokines produced by Th1 cells, whereas Th2 cells produce interleukin (IL)-4, IL-5, and/or IL-13. Th17 cells secrete IL-17, a phenotype that is perpetuated by IL-23. In a physiologic state, Th1 and Th2 cytokines are necessary to restrain pathogens; the latter are secreted in response to parasitic infections, whereas Th1 cells usually develop as a response to the presence of intracellular bacteria and viruses. IL-17, on the other hand, plays a role in protection against pathogenic bacteria, viruses, and fungi, namely by recruitment of other immune subsets to mucosal sites [9].

However, inappropriately activated effector T cells can lead to pathology. Crohn's disease was classically considered a Th1-associated disease [3, 5], but more recently, Crohn's disease has been re-classified as a mixed Th1/17 phenomenon where mucosal tissue from Crohn's patients have been found to produce IL-17 in addition to TNF- α and interferon- γ [10, 11]. Ulcerative colitis, on the other hand, has been described by some but not all studies to be a Th2-associated disease with elevated levels of IL-5 and IL-13 (but not the classic Th2 cytokine IL-4) detected in diseased mucosa [4, 11]. More recently, IL-17 has also been detected in UC tissues [10, 11]. The association of Crohn's disease and ulcerative colitis with particular T helper cell subsets are summarized in Fig. 11.1. These cytokines are likely crucial to the pathogenicity of disease given blockade of TNF- α is effective in treating human and mouse IBD, and neutralization of even other cytokines has been demonstrated to be protective against colitis development in animal models. For example, anti-interferon- γ antibody administration has abrogated disease in the CD45RB^{hi} T-cell transfer model of disease [12] and anti-IL-4 antibody administration highly attenuated spontaneous colitis in Wiskott–Aldrich Syndrome protein-deficient mice [8]. In addition, support for a role of the IL-17/IL-23 axis in the pathogenesis of UC and Crohn's disease comes from data demonstrating IL-23R as a susceptibility gene for both diseases in genome-wide association studies [13]. Please refer to Chaps. 15 and 16 on Th1/2/17 cells and animal models of IBD, respectively, for further details.

Fig. 11.1 Effector and regulatory T-cell populations controlling IBD. Depicted are various effector T-cell populations (Th1, Th2, and Th17) and their cytokine secretion patterns as well as potential IBD disease associations. Regulatory T cells (nTregs and iTregs) can suppress effector T-cell function employing diverse mechanisms including anti-inflammatory cytokine secretion (e.g., IL-10, TGF- β , and IL-35)



Regulatory T Cells and Their Cytokines

The pro-inflammatory capacity of effector cells are kept in balance by regulatory T cells. These cells are classically hypoproliferative, hyporesponsive, and potent in their suppressive capacity to inhibit proliferation and activation of nonregulatory T-cell counterparts. Regulatory T cells are classically divided into at least two distinct populations (summarized in Fig. 11.1): naturally occurring regulatory T cells (nTregs) that are generated in the thymus and “induced” regulatory T cells (iTregs) that are generated in peripheral tissues or are generated in vitro in the presence of certain anti-inflammatory cytokines. Both populations are likely essential, with distinct and nonredundant functions in maintaining mucosal homeostasis [14].

Naturally Occurring Tregs

Easily detected in the peripheral blood and peripheral immune compartments, such as the spleen, lymph nodes, and the intestines, nTregs express Foxp3, a member of the fork-head family of transcription factors that is crucial for their development and function. Scurfy mice, which lack Foxp3 and are devoid of nTregs, die soon after birth from autoimmunity, a phenotype that can be rescued by transfer of regulatory T cells within a few days after birth [15]. Similarly, humans without functional Foxp3 develop immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and suffer from multiple autoimmune manifestations, including enteropathy [16–18]. In the mouse, Foxp3 is a reliable marker of Tregs. However, in human cells, CD4+ T cells can transiently express low levels of Foxp3 when activated although without suppressive properties, thus, making Foxp3 an ambiguous marker of nTregs [19]. Some cell surface markers have been described to be inconsistently expressed by nTregs, such as GITR, CD25, and CTLA-4 [20, 21]. Unfortunately, these cell surface markers are not specific to nTregs and, therefore, leave room for better markers for identification of nTregs.

Despite the fact that they make up only 5–10% of the total CD4+ cell population, they are key to keeping immunologic homeostasis. In the mouse, co-transfer of nTregs with CD45RB^{hi} T cells into immunodeficient mice (i.e., mice that lack lymphocytes) not only protect against colitis but can also reverse ongoing colitis [22, 23]. In fact, transfer of nTregs into susceptible animals can prevent not only colitis but other autoimmune diseases, such as experimental autoimmune encephalopathy (EAE, a model of multiple sclerosis), nonobese diabetic (NOD) mice (a model of type I diabetes), or systemic lupus erythematosus [24–26]. Along the same line, mice depleted of CD25+ T cells, most of which are Tregs, develop significant autoimmune diseases [27]. In the in vitro setting, nTregs can potently suppress proliferation of co-cultured naïve T cells [20, 28].

Naturally occurring Tregs function partly through secretion of a multitude of anti-inflammatory cytokines, including IL-10, TGF- β , and the more recently identified IL-35 [29]. Tregs deficient in TGF- β or WT Tregs transferred with neutralizing antibodies to TGF- β were not able to suppress disease in an adoptive cell transfer model of colitis [30, 31]. The data regarding IL-10 are not as clear; Tregs deficient in IL-10 or WT Tregs transferred with neutralizing antibodies to IL-10R are defective in colitis protection in some, but not all, settings [6, 32]. On the other hand, IL-35, which is a heterodimer of Epstein–Barr-virus-induced gene 3 (EBI3) and IL-12 α , appears crucial to the regulatory function of Tregs, since EBI3-deficient and IL-12 α deficient Tregs lose their suppressive properties both in vitro and in vivo in a model of colitis [29]. Whether or not all three anti-inflammatory cytokines are necessary for mucosal homeostasis in a normal mouse is not clear, but they all certainly play important roles in the suppressive function of Tregs in different settings [33].

Inducible Tregs

In addition to nTregs that are generated in the thymus, regulatory T cells can be “induced” in vivo outside of the thymus in peripheral lymphoid tissue under tolerogenic conditions. Observed by transferring Foxp3 cells from reporter animals into immunodeficient mice and identified by the subsequent expression of Foxp3, these inducible Tregs (iTregs) were demonstrated to be as suppressive as nTregs in an in vitro suppression assay [14]. In addition, antigen administration at low dose can lead to oral tolerance, a condition of systemic and/or local immunological tolerance, through the generation of iTregs that secrete both TGF- β and IL-10 [34].

Similarly, iTregs can also be induced ex vivo in the cell culture system when naïve T cells are stimulated through the TCR in the presence of different anti-inflammatory cytokines. For example, Th3 cells can be generated in vivo by exposure to oral antigen at low dose or in vitro by stimulating naïve T cells in the presence of TGF- β , along with other cytokines. Tr1 cells result when naïve T cells are stimulated in the presence of IL-10 either in vitro or in vivo. Th3 cells secrete abundant amounts of TGF- β and have been described to express Foxp3. They can protect against colitis alone but also synergize with nTregs in an adoptive cell-transfer model of colitis [35]. Tr1 cells, which secrete large amounts of IL-10 and some TGF- β but do not express Foxp3, also have been shown to prevent a similar model of colitis by adoptive cell transfer [36, 37]. Although Tr1 cells certainly can be generated in vitro from both human and murine naïve T cells [36], they can also be found in the GALT of normal mice [38] and have been detected in humans in the setting of transplantation, autoimmune diseases, and exposure to allergens (reviewed in [39]).

Recently, a new class of Tregs, iT_R35 cells, were found to be generated in the setting of IL-35 stimulation [40]. Culture of either mouse or human CD4⁺ T cells with IL-35 leads to iT_R35 cells, which are themselves potent secretors of IL-35. These cells are highly effective suppressors of T-cell proliferation in vitro and autoimmune diseases in vivo [40], as shown in models of both multiple sclerosis (EAE) as well as colitis (CD45RB^{hi} cell transfer) [40]. Unlike other inducible regulatory T cells populations, iT_R35 cells appear stable and retain suppressive properties in vivo after transfer into recipient mice [40].

Plasticity of T Cells

T cells are able to adapt and change their capacity to produce different cytokines, and therefore, their phenotype is not always permanent. Tregs co-expressing Foxp3 and IL-17 have been described at mucosal sites and Tregs can be induced to express IFN- γ potentially leading to tissue damage by certain microbial infections (reviewed in [41] and [42]). In fact, cells that were previously Tregs have been demonstrated to lose their Foxp3 expression, gain a memory T-cell phenotype, produce inflammatory cytokines, and lead to diabetes in an adoptive cell transfer model [43]. Thus, it has been hypothesized that in rare circumstances, the instability of Treg phenotype may lead to autoreactive properties that lead to autoimmune manifestations.

Less Conventional T-Cell Subsets

In addition to CD4⁺ T cells, other T-lymphocyte subsets have potential effector or regulatory properties. In the lamina propria, CD8⁺ cells possess regulatory function and have been noted to be decreased in IBD tissues compared to controls [44, 45]. Likewise, in the intraepithelial compart-

ment, TCR $\alpha\alpha$ + CD8+ T cells have been shown to be protective in animal models of colitis using adoptive cell transfer [46, 47], whereas TCR $\gamma\delta$ + cells appear to limit the extent of injury and promote tissue healing in a chemically induced model of colitis [48]. However, others have demonstrated a potential pathogenic role of TCR $\gamma\delta$ + cells given absence of these cells in a transgenic colitis model leads to attenuated disease [49]. These findings may have implications for the pathogenesis of ulcerative colitis given higher frequency of these TCR $\gamma\delta$ + cells has been noted in colonic tissue from UC patients [49]. Another less common subset of T cell is NK-T cells, which express surface markers and possess certain functional properties typical of both NK cells and T cells. In addition to expressing components of the T-cell receptor complex, CD3, they express semi-invariant CD1d-restricted $\alpha\beta$ TCRs recognizing glycolipid antigens. Some regard these cells as potentially pathogenic in IBD. In fact, Fuss et al. demonstrated circumstantial evidence for NK-T cells being the source of high IL-13 levels noted in samples from patients with active UC, suggestive of a pathogenic role of these cells [4]. Likewise, in the oxazolone-induced murine model of ulcerative colitis, depletion of NK-T cells led to protection against disease [50]. However, in a chemically induced model of colitis (with dextran sodium sulfate), NK-T cells have been demonstrated to possess a regulatory function [51]. Although these less conventional T-cell subsets typically have lower frequencies when compared with the more conventional CD4+ T cells, their pathogenic/regulatory properties may be critical and they likely contribute, at least in part, to overall mucosal homeostasis.

The Role of T Cells in Colitis

T cells have been long recognized to be central to the mucosal inflammatory process and thought early on to even be the instigator of disease given the marked expansion of activated CD4+ T cells in the lamina propria in both inflamed mouse and human intestinal tissues [1, 2, 52]. In fact, CD4+ T cells from intestinal samples isolated from Crohn's and UC patients were found to be resistant to apoptosis [53], which may be one mechanism for such seemingly inadequate controlled expansion of T cells. The ability to reduce colitis by adoptively transferring CD4+ T cells from a colitic mouse to an immunodeficient host in many animal models also implicated the colitogenic potential of CD4+ T cells [6–8]. Even transfer of CD4+ T cells depleted of CD25+ regulatory T cells from wild-type animals could lead to disease in a lymphocyte-deficient host [22], demonstrating the pro-inflammatory nature of effector T cells and its usual tight regulation by regulatory T cells in a homeostatic state.

Similar to that seen in a variety of murine models of IBD, CD4+ T cells isolated from patients with Crohn's disease or ulcerative colitis express large quantities of pro-inflammatory cytokines [3, 5, 10]. Interferon- γ , tumor necrosis factor- α , and IL-17 have been demonstrated repeatedly to be elevated in Crohn's disease mucosa, whereas IL-5 and IL-13 (and IL-17 recently) have been associated with UC. Since targeting of these overabundant cytokines appear to ameliorate disease activity in some IBD patient populations, T cells certainly act, at least in part, as a mediator of disease.

More recent data, however, suggest that the initial aberrant signal may derive from defects in the innate immune system (such as an epithelial cell, macrophages, dendritic cells, NK cells, etc.). Polymorphisms in genes (e.g., NOD2, *ATG16L1*, and *IRGM*) that code for proteins important in recognizing and handling intracellular microbes, processes that occur in innate immune cells, have been recognized to be associated with Crohn's disease risk in genome-wide association studies [13]. Data from animal studies have also implicated professional antigen-presenting cells or epithelial cells in playing a more direct role in colitis induction [32, 54]. Nonetheless, T cells are still thought to be an important mediator of disease, contributing to either the initiation or perpetuation of disease [1].

In this latter context, once activated by soluble factors and/or by cell–cell contact, T cells are able to expand and secrete chemokines and cytokines that recruit and activate, respectively, other immune cell subsets to perpetuate an inflammatory signal.

One may hypothesize that T-cell suppression is diminished in IBD either from a qualitative or quantitative defect in Treg function. However, a defect in Treg numbers appears not to be a principal cause of IBD, since their proportions are noted to be increased in inflamed IBD tissue when compared to noninflamed samples from IBD patients or samples from healthy control patients [55]. In fact, during periods of active disease, fewer Tregs are seen in the peripheral blood and more are found to the mucosa, suggesting an increase (through homing) or retention of Treg to sites of inflammation. Moreover, peripheral blood, mesenteric lymph node, and mucosal Tregs when removed from IBD patients retain *in vitro* suppressive function [55–57]. Thus, Tregs from IBD patients have the capacity for functional activity. However, the increase in Treg proportions in inflamed mucosa from IBD patients appear to be quantitatively less than that seen in non-IBD inflammatory control samples (those with diverticulitis), suggesting the increase in Treg numbers seen in IBD is not as robust as seen in other inflammatory disorders. Nonetheless, despite the presence of regulatory T cells in IBD-associated mucosa, these cells are not sufficient *in situ* to control inflammation.

However, in order to effect the damage in the gut, effector T cells need to adhere and migrate into intestinal mucosa, a process which depends on the recognition between adhesion receptors expressed on the T-cell surface and their ligands on the gut vascular endothelial surface. CCR9 and $\alpha 4\beta 7$ are the two most well-characterized gut-homing receptors on T cells, and blocking the interaction between these receptors and their ligands on endothelial cells prevents T cells from entering the gut. This serves as the basis for the development of biologic therapies for Crohn's disease such as natalizumab, which is a monoclonal antibody against the integrin $\alpha 4$, and vedolizumab (*i.e.*, MLN0002), a monoclonal antibody against $\alpha 4\beta 7$. Both interfere with T-cell homing to the gut and consequently decrease inflammatory activities leading to clinical efficacy in Crohn's disease [58, 59] with the use of either antibody as well as in ulcerative colitis with vedolizumab [60]. Other agents blocking such signals crucial to T-cell gut homing are also under development.

Regulatory T Cells as Potential Therapy for IBD

Given the potent suppressive properties of regulatory T cells, one potential therapeutic option for IBD is quantitative enhancement of Treg numbers and/or functional capacity [61]. One approach is to isolate Tregs from a patient's peripheral blood, followed by augmentation of cell function/numbers *in vitro*, and subsequent infusion of these cells into the patient. However, this idea has been hindered by difficulty in Treg expansion given their tendency for hypoproliferation, poorly defined surface markers, and uncertain stability and longevity *in vivo*. One potential way of overcoming these difficulties is to generate and expand inducible Tregs *in vitro* from naïve T cells, such as iT_{R35} cells, Tr1 cells, or regulatory CD8+ cells [36, 40, 45]. Even then, it is difficult to purify some of these populations due to the lack of specific cell surface markers. In addition, this technique will still require peripheral blood cell isolation; immediate processing or cryopreservation; *in vitro* culture and stimulation; vigorous testing for function, purity, and viability; and reinfusion of newly induced Tregs, posing technical challenges to avoid infection and maintain quality control. An alternative option is to pharmacologically expand the already existing pool of Tregs or induction of an iTreg population. The latter could be done with an agent such as oral anti-CD3 antibody, which has been shown to expand a particular type of Tregs and has been shown to suppress EAE, SLE, and diabetes in mice [62, 63]. Such approaches and others to induce iTregs or expand pre-existing Treg cell populations are under clinical development.

Summary

T cells have been clearly implicated as a mediator and propagator of disease in many animal models of IBD. In patient samples, T cells secrete an abundance of proinflammatory cytokines suggesting a central role of T cells in the pathogenesis of disease. Despite the large body of knowledge regarding the role of T cells in mucosal homeostasis, much still remains to be understood with regard to how these T-cell subsets interact with each other and with other immune cell subsets to lead to IBD and how these interactions can be repaired to restore intestinal homeostasis.

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Chapter 12

Immunobiology of B Cells in Inflammatory Bowel Disease

Atsushi Mizoguchi and Atul K. Bhan

Keywords Autoantibodies • Breg • Colitis • IL-10 • IL-12 • Inflammatory bowel disease • Regulatory B cells • T-cell receptor knockout mice

Introduction

Unlike T cells, the role of B cells in the pathogenesis of inflammatory bowel disease (IBD) is poorly understood [1–3]. In this chapter, the impact of B cells on IBD pathogenesis will be discussed in relation to the known functions of B cells: antibody production, antigen presentation and interaction with T cells, and cytokine production (Fig. 12.1). Since most of the information regarding the immunopathogenesis of IBD in the last two decades has been obtained by the use of experimental models, the emphasis will be on IBD models [4–6] rather than on human studies.

Antibody Production

B cells in the intestine are primarily located in the lymphoid follicles and as plasma cells in the lamina propria [7]. The activation of mucosal B cells occurs in the lymphoid follicles and mesenteric lymph nodes (MLN) with subsequent migration and differentiation to predominantly IgA secreting plasma cells in the lamina propria. The circulating B cells with activated phenotype (TLR2⁺) may either represent activated mucosal B cells or B cells activated in circulation perhaps by translocated enteric bacteria/bacterial antigens capable of migrating to mucosal sites [8]. The histological evidence of prominent lymphoid follicles and lymphoplasmacytic infiltrate in the inflamed intestine suggests the involvement of B cells in IBD, in particular ulcerative colitis (UC). Furthermore, the frequent presence of several types of circulating antibodies reactive with both microbial antigens and self-antigens in IBD supports the notion that dysregulated immune response to normal enteric microorganisms represents the primary pathogenic event in IBD. The antibodies include anti-Saccharomyces cerevisiae antibodies (ASCA), anti-neutrophilic cytoplasmic antibodies (ANCA), and antibodies to outer membrane porin (OMP), Pseudomonas fluorescens-related sequence

A. Mizoguchi, MD, PhD • A.K. Bhan, MBBS, MD (✉)
Department of Pathology, Harvard University, Massachusetts General Hospital,
Immunopathology Unit, Warren 501, 55 Fruit Street, Boston, MA 02114, USA
e-mail: abhan@partners.org

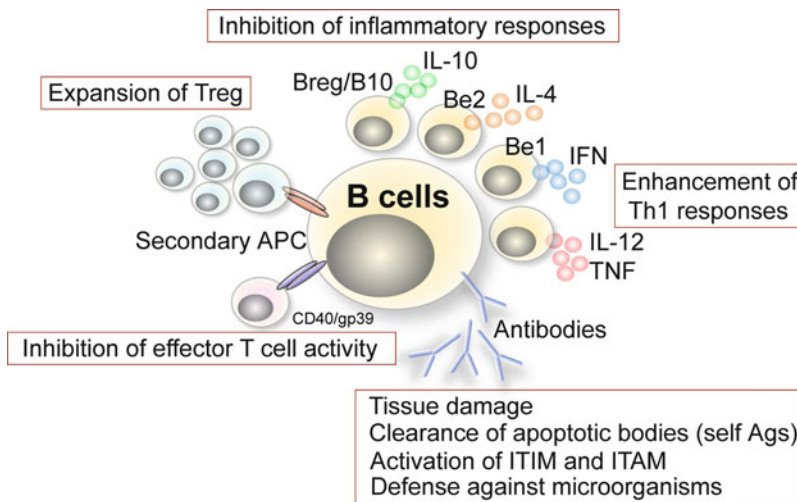


Fig. 12.1 Functional diversity of B cells in IBD. Immunoglobulins (Igs) produced by B cells may have both deleterious and protective roles in IBD. Binding of autoantibodies to colonic tissues or IgG Fc fragment-mediated ITAM-dependent activation of immune responses could result in tissue damage. Antibodies could provide protection by altering the diversity of enteric microorganisms that are required for the development of IBD and by helping the clearance of apoptotic bodies that may serve as a source of self-antigens for eliciting autoimmune responses. Furthermore, IgG may suppress immune response through the ITIM by binding to inhibitory FcγRIIb receptors expressed on immune cells. B-cell subsets could modulate inflammatory responses depending on their distinct cytokine production profiles. IL-10-producing B cells (“Breg”) inhibit chronic colitis progression. B-cell subsets producing IL-12p70 or IFN-γ may have a pathogenic effect in CD, but a beneficial role in UC. B cells may also regulate immune responses by serving as a second line of APCs, by enhancing expansion of CD4⁺ Foxp3⁺ Tregs, and by inhibiting proliferation of effector CD4⁺ T cells in a contact-dependent manner

I2, and Cbir (see below), and anti-carbohydrate antibodies (ALCA, ACCA, and AMCA) [9, 10]. However, most of the studies performed with circulating antibodies have focused on their diagnostic or prognostic utility rather than their role in IBD pathogenesis [9, 10]. ASCA are detected frequently in CD, whereas seropositivity for ANCA predominates in UC.

The normal IgA dominant immune response at the mucosal sites is skewed toward IgG in chronically inflamed mucosa of IBD [11]. The isolated cells from the inflamed mucosa have been shown to secrete antibodies to bacteria to *Escherichia coli* strains [11, 12] as well as antibodies against colonic epithelial antigens [13]. Antibodies to *E. coli* are more often detected in CD, whereas anti-colonic epithelial antibodies are particularly seen in UC. The colonic epithelial antigens that are reactive with antibodies include tropomyosin (40 kDa) isoforms (TM1 and TM5) and 200 kDa colon epithelial protein [14]. Studies performed in Per Brandtzaeg’s laboratory have provided evidence for a pathogenic role of antibodies in UC by showing complement activation in relation to IgG1 deposited at the apical aspect of the colonic epithelium [11, 15]. It is not clear whether these findings represent primary pathogenic events or a secondary phenomenon due to local immune response in the setting of chronic inflammation and tissue injury. In any case, the locally produced antibodies help maintain epithelial barrier, and may play a role in regulating enteric flora repertoire and excluding invading microorganisms. Decreased J-chain production with resultant reduced secretion of dimeric IgA has been reported in IBD [11, 16]. However, IgG antibodies, because of their phagocytotic enhancing properties, are likely to be more efficient than IgA in removing invading microorganisms and antigens, and could compensate for abnormality of the IgA response in IBD [11]. They could also be involved in the pathogenesis of IBD (see discussion of IgG Fcγ receptors below) [17].

Circulating autoantibodies and antimicrobial antibodies have also been reported in experimental models of IBD [5, 18–20]. The IL-4-mediated spontaneous colitis in T-cell receptor α knockout

(TCR α KO) mice resembles ulcerative colitis, and is associated with expansion of MLN B cells, increased production of antibodies (ANCA, anti-nuclear, and anti-tropomyosin), and alteration of polyclonal to an oligoclonal immune response to cecal bacterial antigens [18, 19]. This raised the possibility that B cells or antibodies may be pathogenic in this model. However, B-cell-deficient TCR α KO mice developed more severe colitis (see below) [21]. Transfer of autoantibodies or purified immunoglobulin from TCR α KO mice to B-cell-deficient TCR α KO led to attenuation of colitis and decrease in the apoptotic cells, supporting the notion that autoantibodies may have a role in clearance of self-antigens released from apoptotic cells [21]. Our recent study also indicates that B-1 B cells, which represent a major source of natural IgM antibodies that provide first line of defense against microorganisms, are fully activated in conventional facility as compared to specific pathogen-free facility, resulting in the inhibition of colitis in TCR α KO mice [22]. These results support a role of B cells in the “hygiene hypothesis,” which is based on the observation that repeated childhood infections lead to decreased incidence of allergic diseases in adulthood [22].

The spontaneous colitis in C3H/HeJBir mice is associated with both B and T cells responses to selective enteric bacterial antigens; the colitis can be transferred with T cells. Unlike TCR α KO mice, the oligoclonal response to enteric bacteria is detected even in young C3H/HeJBir mice [20]. Serologic expression cloning of cecal bacterial antigens in C3H/HeJBir mice led to the identification of previously unknown microbial flagellins [23]. The flagellin, CBir1, was found to be the dominant antigen capable of inducing T-cell-mediated colitis. Interestingly, sera from about 50% of patients with Crohn’s disease are reactive with CBir1; the CBir1 sera reactivity identified a subset of patients with complicated CD [24].

It is well established that humoral immunity can be regulated by Fc fragments of IgG [17]. Although most receptors of IgG, Fc γ Rs are activating receptors due to the presence of immunoreceptor tyrosine-based activation motif (ITAM), Fc γ RIIB is the only Fc γ R that has been shown to have inhibitory functions through immunoreceptor tyrosine-based inhibitory motif (ITIM), which includes suppression of B cells, macrophages, dendritic cells, mast cells, and basophils [17]. Fc γ RIIB is involved in the pathogenesis of autoimmune disease, in particular lupus erythematosus. Recent studies indicate that intravenous immunoglobulin (IVIG) in autoimmune diseases and infliximab [anti-TNF α antibodies] in rheumatoid arthritis may partly act through Fc γ RIIB [25, 26]. Since Fc γ RIIB also effects anti-microbial immune responses [17], it is likely that this receptor may play an important role in the pathogenesis of IBD. Fc γ RIIB KO mice exhibit less distal colon inflammation during *Citrobacter rodentium* infection, probably due to increased phagocytic function of macrophages as compared to wild-type mice [27]. Granulomatous inflammation developing in B cell and IL-4 deficient TCR α triple knockout mice can be suppressed by the administration of Fc fragments of IgG [28]. The importance on Fc-mediated pathway in IBD is highlighted by recent genome-wide association studies identifying Fc γ RIIA an UC-associated gene [29].

Antigen Presentation and Interaction with T Cells

It has become increasingly clear that B cells have functional capabilities that are not directly related to secreted immunoglobulins. B cells have been shown to serve as “second line” of antigen presenting cells (secondary APCs) by conditioning the activity of effector memory T cells that have already been primed by professional antigen presenting cells such as dendritic cells [30, 31]. Indeed, B cells can suppress proliferation of effector CD4⁺ T cells in a contact dependent manner through the interaction of CD40 on B cells and gp39 on effector T cells [32, 33]; this interaction contributes to the suppression of colitis in TCR α KO mice [32]. This observation is supported by a study showing that forced ectopic overexpression of gp39 on B cells, leading to the impairment of interaction of CD40 (B cells) and gp39 (T cells), induces the development of colitis [34]. In G α i2 knockout mice, B cells

facilitate expansion of CD4⁺CD8 α ⁺ intraepithelial T cells and CD3⁺ CD4⁻ NKT cells with consequent suppression of colitis [35]. MHC class I-mediated antigen presentation is required for this B-cell-mediated induction of regulatory CD8⁺ T-cell subset capable of controlling colitis through the production of perforin [36].

Since autophagy is a cellular degradation system, which is used not only for the elimination of intracellular bacteria but is also involved in adaptive immune responses as well as MHC-dependent antigen presentation [37], it is likely that autophagy plays an important role in secondary APC function of B cells. Autophagy is also required for B cell development [38] and for B cells to induce tolerance of CD4⁺ T cells [39]. Genome-wide association studies have identified autophagy-related gene (Atg) 16L1 as a CD susceptibility gene [1] and a deletion polymorphism upstream of IRGM, a gene essential for autophagy, is also associated with the development of CD [40].

Recent studies suggest that regulatory B cells may function through interaction with regulatory CD4⁺ Foxp3⁺ (Treg) cells that are known to suppress a wide range of murine and human inflammatory responses [41]. B cells may enhance the expansion of Tregs either in a contact-dependent manner or a contact-independent manner through the production of IL-10 [33, 42, 43] or maximize their regulatory activity. Spontaneous colitis in mice expressing T-cell-specific dominant negative TGF β receptor II is exacerbated when they are crossed with B-cell-deficient mice, and the B-cell deficiency is associated with a significant reduction of Tregs [44]. These regulatory interactions support the findings of recent genetic studies in IBD patients, which highlight the significance of immune regulatory network to prevent the development of IBD [1].

Cytokine Production

The recent recognition of B cells as cytokine-producing cells represents a major advancement in understanding the function of B cells in inflammatory disorders. Both human and murine B cells can produce a spectrum of cytokines, especially under inflammatory conditions. The cytokines include IL-4, IFN- γ [45], IL-2, TNF- α [46], and IL-12p70 [47]. Therefore, like CD4⁺ T cells, B cells may be classified into functionally different subsets: IFN- γ -producing B effector 1 (Be1) and IL-4-producing B effector 2 (Be2) cells [45, 46].

Our studies in TCR α KO mice have identified a B-cell subset that regulates inflammation by the production of a regulatory cytokine IL-10; we have called these regulatory cells “Breg” [48]. As stated above, TCR α KO mice spontaneously develop a Th2-mediated chronic colitis, and B-cell-deficient TCR α double knockout mice develop much more exacerbated form of colitis as compared to TCR α KO mice indicating a protective role of B cells in this colitis model [21]. IL-10-producing B cells, which are characterized by high expression levels of CD1d, appear in the MLN of this model after, but not before, the development of colitis. Cell transfer studies conclusively showed that the inducible IL-10-producing B cells attenuate ongoing colitis [49]. A recent study using a reporter mouse system that expresses green fluorescent protein (GFP) when IL-10 expressions are induced confirms that a major source of IL-10 in the MLN under inflammatory condition is B cells [50]. Importantly, B-cell-specific deletion of IL-10 cannot cause spontaneous colitis [50], consistent with previous reports that IL-10-producing Breg is involved in controlling the progression, but not induction, of colitis [48].

Several other studies have also identified IL-10 producing B cells to suppress diverse inflammatory diseases including IBD, graft versus host diseases (GVHD), experimental allergic encephalomyelitis, and collagen-induced rheumatoid arthritis [48, 51–54]. As stated above, IL-10-producing regulatory B cells exist at a very low number in normal conditions and expand under inflammatory conditions [48, 51]; these cells function primarily to suppress ongoing inflammation rather than inhibit the initiation of inflammatory process. The regulatory B cells exhibit unique phenotypic characteristics. This includes expression of both immature transitional type 2 B cells and fully matured

marginal zone B cells [49, 53]. Although IL-10-producing regulatory B cells originate from B2 B cell lineage, some of these cells express CD5, a marker associated with B1 B cells [55, 56]. Like dendritic cells, high levels of MHC class II may be expressed by some regulatory B cells [42, 43].

IL-10-producing regulatory B-cell subsets may originate from either immature/naïve or activated memory B cells. Like regulatory T-cell subsets (Treg, Tr1, and Th3), it is likely that regulatory B cells also originate in the gut-associated lymphoid tissues (GALT) containing about 80% of activated B cells [6, 7]. Our studies in TCR α KO mice indicate that “Bregs” appear in the mesenteric lymph nodes (MLNs) only under intestinal inflammatory conditions [48, 49] and are capable of expanding throughout the body [35]. Bregs, which are phenotypically characterized by high expression levels of CD1d, represents immature/naïve B cells that are polyclonally activated, presumably by stimulation with enteric microorganisms. Functionally, Bregs attenuate ongoing colitis by inhibiting proinflammatory responses such as the production of IL-1 β [48, 49]. Recent studies have identified a spleen-specific IL-10-producing regulatory B cells termed “B10,” which are characterized by a CD1d^{high} CD5⁺ surface phenotype [55]. The B10 cells originate from memory follicular B cell pool and develops in an antigen-dependent manner [51, 55]. B10 cells, unlike Bregs, regulate the initiation, but not progression, of inflammatory conditions such as murine lupus and experimental autoimmune encephalomyelitis by downregulating the ability of dendritic cells to act as APCs for priming effector CD4⁺ T cells [55–57]. Another difference between B10 cells and Breg is that B10 cells are detected in the systemic circulation, but not in lymph nodes, where Bregs develop [55].

In addition to IL-10-producing Bregs, another unique B cell population, capable of producing IL-12p70 but not IL-10, is also generated in the MLN of TCR α KO mice during colitis development and participates in the attenuation of this Th2-mediated colitis [47]. Interestingly, a unique B-cell subset, which is characterized by high expression levels of MHC class II and its ability to produce IL-12p70 in response to a bacterial product CpG (toll-like receptor 9 ligand), has been identified in the colon of these mice [58]. These unique colonic B cells are recruited from immature/transitional and recirculating naïve B2 B-cell pools. Like Bregs, they are inducible; they exist in normal colon at very low number and expands during the recovery phase of intestinal inflammation [58]. IL-10 producing Bregs may have a wider role in inhibiting a large spectrum of inflammatory conditions, whereas IL-12p70 producing B cells may have a more limited role in suppressing Th-2-mediated colitis.

A protective role of IL-10-producing B cells has also been demonstrated in IBD experimental models with features of human CD. These models include G α i2 knockout mice in which the ability of regulatory B cells to produce IL-10 is impaired [59], CD45RB transfer model in TCR α KO mice [60], and mice expressing T-cell-specific dominant negative TGF β receptor II in which B cells regulate colitis in an IL-10-independent manner [44]. Polyclonally activated B cells have been shown to suppress an innate immune-mediated spontaneous colitis in nuclear factor of activated T cells (NFAT) C2-deficient RAG2 double knockout mice; this suppression is not dependent on IL-10 [61]. In contrast to above studies, a pathogenic role of B cells has been reported in ileitis developing in the SAMPl/Yit congenic mouse model and in the TCR β $\times$ TCR δ double knockout mouse model with the reconstitution of WT mouse-derived naïve CD4⁺ T cells [62, 63]. It is possible that the function of B cells differs depending on the site of inflammation: a pathogenic role in small intestine (ileitis), but a regulatory role in large intestine (colitis). Since a recent study indicates that the development of IL-10-producing regulatory B cells is impaired in SAMPl/Yit mice [64], a pathogenic role of B may be exhibited in the absence of regulatory B cells.

Human Mucosal and Regulatory B Cells

There have been a limited number of studies regarding the functional characterization of mucosal B cells in human IBD. In a recent study [8], circulating and mucosal tissue B cells (isolated from surgical resection specimens) from CD patients showed elevated levels of basal activation as indicated

by TLR2 expression, spontaneous IL-8 secretion, and increased levels of phosphorylated signaling proteins. Correlation between increased expression of TLR2 and IL-8 and clinical activity was observed in CD but not in UC. Whether the hyperactivated B cells reflect a pathogenic role or merely reflect a secondary response to microbes in diseased mucosa is not clarified in the study.

A unique phenotype of CD19⁺ CD24^{high} CD38^{high} CD1d^{high} CD5⁺ CD27⁻ of human IL-10 producing regulatory B cells in peripheral blood lymphocytes (PBL) has been reported [33]. These regulatory B cells require in vitro CD40 stimulation to exhibit IL-10 production and inhibit differentiation of Th1 cells in vitro. This regulatory capacity is lacking in patients with systemic lupus erythematosus [33]. A possible role of B cells in IBD has been suggested by B-cell depletion studies. In one study, UC was induced in a patient with Graves' disease after depletion of B cells through treatment with rituximab, a mouse/human chimeric anti-CD20 mAb [65]. In another study, administration of this antibody in a UC patient led to the exacerbation of colitis [66]. Interestingly, the exacerbation of colitis was associated with a reduction of IL-10 production in the colon, supporting the possible protective role of IL-10-producing Bregs in UC.

Concluding Remarks

It is now well established that dysregulation of mucosal immune response to enteric bacteria is the underlying factor in the development of IBD. B cells form an important component of mucosal immune system for maintaining an epithelial barrier, regulation of the enteric microflora diversity, and development of adequate immune response to both enteric floral and food antigens. A compelling case for regulatory B cells has been made in IBD experimental models; however, a pathogenic role of B cells has not been excluded. The presence of circulating antibodies to self-antigens and enteric bacteria in many patients indicates B cell involvement in human IBD. Whether or not, B cells play an important role in UC and CD pathogenesis has yet to be defined. Further understanding of the role of B cells in IBD would require functional characterization of human mucosal B cells in normal and diseased states. Genome-wide association studies of IBD may lead to the identification of B-cell-associated genes that may be candidate genes involved in the pathogenesis of chronic intestinal inflammation.

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Chapter 13

Immunobiology of Monocytes and Macrophages in Inflammatory Bowel Disease

Kevin J. Woollard

Keywords Monocytes • Macrophages • Heterogeneity • Innate immunity • Inflammatory disease

Monocytes

Monocytes represent 5–10% of peripheral blood leukocytes in humans and mice [1]. Recent new data has focused the field on the mechanisms and molecules mediating tissue monocyte recruitment, trafficking as well as their differentiation in a variety of tissues, both in the steady state and under an inflammatory response [1–3]. The two easily classified fates of monocyte differentiation can be defined by monocytes developing into macrophages or dendritic cells (DC) [1]. Simplified functional role of macrophages is to degrade material that they phagocytose, failing to present antigens or initiate a T-cell response, but release a plethora of cytokines upon activating signals and are shown to be important for the local clearance of dead or dying cells in both inflamed and noninflamed tissues [1, 2].

In contrast, DC have a weak proteolytic potential, but are able to engulf material from other cells, process and intracellularly retain antigens/peptides which can then be presented by major histocompatibility complex (MHC) molecules with corresponding co-stimulatory signals for priming T cells [4, 5]. After DC maturation the plasma membrane of DC turns over slowly allowing long duration of MHC (peptide) complexes that facilitate the signaling interactions with T cells [6]. In contrast, activated macrophages very rapidly renew plasma membrane proteins, including MHC-II, such that particular MHC/peptide complexes would be expected to have a rather short half-life at the cell surface [7]. Further, in generalized terms macrophages and DC have distinct migratory properties [8–10]. Macrophages remain in tissues, DC are, however, capable of migrating from peripheral tissues to lymphoid organs in order to interact with T cells and induce a classic immune response [11].

K.J. Woollard, Bsc, PhD (✉)
Division of Immunology, Infection and Inflammatory Diseases,
Centre for Molecular and Cellular Biology of Inflammation,
Kings College London, London SE1 1UL, UK
e-mail: kevin.woollard@kcl.ac.uk

Heterogeneity of Blood Monocytes

Heterogeneity and plasticity are hallmarks of cells belonging to the mononuclear phagocyte system (MPS) [12, 13]. More than 20 years of work into human monocytes have provided insights into the fate and function of monocyte subsets; however, these experiments are largely based on heterogeneous analysis *in vitro*. Study of monocyte subsets in a physiological context was restricted by the failure to characterize their mouse counterparts. However, in recent years groups, including ours, have focused their attention on characterizing mouse monocyte subsets using genetic labeling/targeting, adoptive transfer, gene expression arrays and the powerful technique of intravital microscopy to analyze monocytes, macrophages and DC *in situ* [14–16].

Antigenic differentiation of two monocyte subsets in mice was initially achieved after the observation that cells belonging to the monocyte phenotype (F4/80^{low}CD11b⁺) could be subdivided according to their expression of CCR2, CD62L, and CX3C-chemokine receptor 1 (CX3CR1) [8].

CX3CR1 was analyzed using a novel genetic reporter mouse with GFP “knocked-in” to one of the CX3CR1 alleles [2]. One monocyte subset expressed CCR2, CD62L, and only moderate amounts of CX3CR1, whereas the second did not express CCR2 or CD62L but expressed high amounts of CX3CR1. As expected, the CCR2⁺ subset migrated toward the CC-chemokine ligand 2 (MCP-1). Given the important role of CCR2 and its ligand MCP-1 in mediating recruitment within an inflammatory setting this subset was termed “inflammatory monocyte.” Dan Littman’s group subsequently extended data on the phenotypic differences between the two subsets particularly describing phenotypic expression patterns of the chemokine receptors [8]. However, Ly6C (part of the GR1 epitope) has been identified as an additional marker of CCR2⁺ monocytes in mice [3]. We distinguished functionality between the two subsets by adoptively transferring GFP-expressing monocytes from the CX3CR1 knock-in mice and studied cell fate and function in naïve and immunologically challenged recipient mice [2, 8, 15]. We discovered that the CCR2⁺CX3CR1^{low} monocytes were short lived after adoptive transfer and difficult to detect in the tissues of naïve recipients [8]. As expected by the expression of CCR2 and CD62L, (which are molecules well described to be involved in cell recruitment under an inflammatory response), these cells were rapidly recruited to sites of experimentally induced inflammation [8]. This again led to the term “inflammatory monocytes.”

After migration into the inflamed site, the CCR2⁺ monocytes unregulated expression of CD11c and MHC class II molecules, and some CD11c⁺MHC-II⁺ were recovered from the draining lymph nodes, indicating DC differentiation. Experimental evidence for the ability of CCR2⁺ monocytes to differentiate into DC was also obtained by transferring CCR2⁺ monocytes into MHC class I-deficient mice and showing that the transfected cells could prime naïve CD8 T cells [8]. By contrast, the CCR2-CX3CR1^{hi} monocyte population was found to persist for longer in animals after adoptive transfer and it was possible to recover GFP⁺ cells from the blood, spleen, lungs, liver, and brain of recipients for several days after transfer. Some of the donor cells that were recovered from the spleen had acquired a DC-like phenotype (CD11c⁺MHC-II⁺) following transfer [8].

However, limitations of fate mapping using CX3CR1^{gfp/+} mice make it difficult due to the differentiation of these cells that can lose or increase their GFP expression as they differentiate into tissue macrophages or DC [2], so fate mapping in the long term is not possible. Moreover, fate mapping is possible using adoptive transfer experiments as described. However, adoptive transfer of monocytes introduces several artifacts as the isolation and *ex vivo* manipulations significantly alter the activation status, differentiation, viability, and migratory properties of monocytes. Nonetheless, adoptive transfer methodologies yield consistent results with other methods to track monocytes in their physiological environment.

This second subset of mouse monocytes lacking expression of Ly6c (Gr1[−]), CCR2, and CD62L was termed “resident” in opposition to the “inflammatory” subset because they were found in both resting and inflamed tissue after the aforementioned adoptive transfer experiments. The function of

this resident CD115⁺Gr1⁻ subset remained unclear until the phenotypic utilization of increased CX3CR1 expression in the CX3CR1^{gfp/+} reporter mouse [15].

These CX3CR1^{gfp/+} reporter mice were selected as all monocytes but not PMNC express GFP. Further, the two main monocyte subsets described here, CD115⁺Gr1⁺ (CX3CR1^{lo}GFP^{lo}) and CD115⁺Gr1⁻ (CX3CR1^{hi}GFP^{hi}) can be distinguished from one another by confocal microscopy and flow cytometry based on GFP intensity [17]. However, as activated T cells and NK cells inside the blood vessels from CX3CR1^{gfp/+} mice also expressed GFP, we generated triple mutant mice Rag2^{-/-}, Yc^{-/-}, CX3CR1^{gfp/+}, devoid of all lymphoid cells and in which monocytes were the only GFP-expressing cells in the blood. (To obtain animals with B, T, and NK lymphocytes, the Rag^{-/-}, Yc^{-/-}, CX3CR1^{gfp/+} mice were reconstituted with normal lymphoid cells that do express GFP, by IV or IP of WT bone marrow) [15].

Intravital microscopy observation revealed that CD115⁺Gr1⁻GFP^{hi} monocytes within most blood vessels in the dermis exhibited a constitutive patrolling behavior in the steady state, via a long-range crawling on the luminal side of the endothelium with an average velocity of 12 mm min⁻¹ [15].

Surprisingly, we found that most CD115⁺Gr1⁻GFP^{hi} crawling cells stay within the blood vessels in the steady state. However, in response to tissue damage (e.g., aseptic wounding, irritants, and *Listeria monocytogenes* infection), CD115⁺Gr1⁻ monocytes extravasated rapidly within 1 h and invaded the surrounding tissues [15].

Similarly in mice, human peripheral blood monocytes show morphological heterogeneity, such as variability of size, granularity, and nuclear morphology [18, 19]. Human monocytes were initially identified by their expression of large amounts of CD14 (which is part of the receptor for lipopolysaccharide) [19]. However, subsequent identification of differential expression of antigenic markers enabled analysis of a further marker, CD16 (also known as FcγRIII) [20]. This defined two major subsets, the CD14⁺CD16⁻ monocytes, typically representing up to 90–95% of the monocytes in a healthy individual and the CD14^{dim}CD16⁺ monocyte population [21]. Like in the mouse these subsets differ in many respects, including adhesion molecule and chemokine receptor expression. CD14⁺CD16⁻ monocytes express CCR2, CD62L, and CD64, due to this expression profile they have been associated with the same “inflammatory” subset in the mouse [21]. Conversely, CD14^{dim}CD16⁺ monocytes lack CCR2 and have higher levels of MHC-II and CD32 [20]. Both subsets express the stated receptor for fractalkine, CX3CR1, but CD14^{dim}CD16⁺ monocytes express higher levels and as such have been associated to the “resident” subset in the mouse [21–23].

However, it is important to mention that considerable heterogeneity is known to exist in the CD16⁺ population [16]. For example, CD16⁺CD14⁺ have been shown to differentiate in vitro differently than CD14^{dim}CD16⁺ and has been reported to be the subset of CD16⁺ monocytes that express CCR5 [24, 25]. Within the CD16⁺ population differential expression of a relative of PSGL-1 (M-DC8) has also been reported [26].

A recent report from our group has shed light on defining human functional counterparts to mouse monocyte subsets [16]. Human CD14⁺ monocytes, which consist of CD16⁺ and CD16⁻ cells, resembled Gr1⁺ murine monocytes in gene expression analyses. They specialized in phagocytosis, production of ROS and the recognition of ligands for extracellular TLRs (such as the bacterial product LPS), to which they secrete inflammatory cytokines [16]. Among this subset, CD16 may be a marker of activation, as the CD14⁺CD16⁺ subset secreted the most TNF in response to bacterial cues, such as LPS [16]. In contrast, gene expression analyses indicated similarities between murine patrolling Gr1⁻ monocytes and human monocytes that lack CD14 (CD14^{dim}), which also express CD16. CD14^{dim} monocytes were found to patrol the endothelium of blood vessels after adoptive transfer (similarly to Gr1⁻ murine monocytes), in an LFA-1-dependent manner, and respond specifically to viruses and nucleic acid-containing immune complexes via a pro-inflammatory TLR7–TLR8–MyD88–MEK pathway [16]. CD14^{dim} cells may therefore be involved in the innate local surveillance of tissues with regard to cell death and viral infection, the scavenging of immune complexes and the pathogenesis of autoimmune diseases such as lupus [16].

Macrophage Heterogeneity

As discussed, within an immune or inflammatory response the fate of specific mouse monocyte subsets has been described. For example, a unique subset of differentiated monocytes exists in response to inflammation or infection termed TNF- α and iNOS producing (Tip)-DC [27]. These Tip-DC were named due to studies by the group of Parmer et al. and others, describing mice following *Listeria* infection, monocytes traffic from the BM to peripheral blood in a CCR2-dependent mechanism and differentiate via outside-in signaling involving MyD-88, into cells that produce TNF- α and NO (hence tip-DC) and that upregulate MHC-II antigens, CD80, CD86, and CD11c [10, 27].

Macrophage heterogeneity within the MPS system in response to cytokines and microbial products also exists in humans. These mononuclear phagocytes express specialized and polarized functional properties [28–30]. Mirroring the Th1/Th2 nomenclature, many groups refer to polarized macrophages as M1 and M2 cells [31]. Traditionally, M1 macrophages can be induced by IFN- γ alone or with microbial stimuli (LPS) or specific cytokines, for example, TNF and GM-CSF [2]. Moreover, IL-4 and IL-13 along with being inhibitors of macrophage activation have been shown to induce an alternative M2 form of macrophage [32]. M2 can be classed as activated macrophages that are not the classic M1 phenotype and along with IL-1 and IL-13, include macrophages stimulated with immune complexes, IL-10, glucocorticoid or steroid hormones [32]. Further a member of the TGF- β family has been shown to induce a M2 phenotype and interestingly CCR4 may play a role, as shown by M2 polarization in CCR4^{-/-} macrophages [29, 32]. Indeed M1 and M2 phenotypes have distinct chemokine and chemokine receptor profiles [32]. In general, the biology of a M2 phenotype has been shown to participate in polarized Th2 reactions, promote death and phagocytosis of parasites [32]. They have also been shown to have immunoregulatory functions and most intriguingly shown to be present in established tumors and promote tumor progression, tissue repair and remodeling [2, 33, 34]. Mouse macrophage phenotype corresponding to this M2 model has been postulated to be involved in models of human disease, including heart failure [35].

Tissue resident myeloid cells may also play an important role in innate immunity, in many organs including the skin, liver, brain, and intestines [11, 36–40]. However, only a few reports have described the putative origin of these cells and the functions of many of these resident subsets remain poorly described [11, 37, 41]. Many macrophages and DCs are short lived, and renew from the progeny of bone marrow hematopoietic stem cells (HSC) [1]. However, using langerhans cells (LCs) as an example, we have drafted a new paradigm proposing that LCs, a subset of resident myeloid cells, derive from an embryonic precursor and that LC homeostasis relies on in situ proliferation of differentiated cells in response to signals from neighboring stromal/epithelial cells [11, 14]. The signal(s) involved remain to be identified. Another challenge is to identify the embryonic precursor of LCs, with the exciting possibility that it might not be HSC-derived, but rather develops from yolk-sac (YS) macrophages [11]. Overall we can propose that YS and HSCs may contribute to the development of distinct subsets of macrophages and DCs, which may be involved in specific functions of tissue resident myeloid cells found in many organs of the body.

Conclusion

In conclusion many cells that make up the MPS include a vast repertoire of phenotypes and functions. Monocyte and macrophage heterogeneity development and functionality make up a complicated array of important and ever emerging part of immunological research, which already has shown to be important in many (but not exclusive to) clinical pathologies including cardiovascular

diseases [21, 42, 43], myocardial infarction [35], skeletal muscle injury [34], kidney disease including lupus nephritis [16, 44, 45], skin diseases [14], and intestinal-associated diseases [46–48]. Moreover, with the emergence of the developmental understanding of tissue-resident myeloid cells and independent functions of this unique subset, the continuation of research into the area of monocyte and macrophage immunity becomes increasingly important.

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Chapter 14

Immunobiology of Epithelial Cells in Inflammatory Bowel Disease

Stephanie Dahan, Vera K. Denmark, and Lloyd Mayer

Keywords Epithelial cells • Immune cells

Introduction

Intestinal epithelial cells (IECs) are nested between the lumen and a wide array of mucosal immune cells. This location initially raised the concept that IECs were an active partner in immune regulation. Considering the remarkable load of daily ingested foreign antigens and commensals, combined with the large number of lymphocytes resident in the mucosa, the intestine seems primed for an inescapable accident. Yet, despite these conditions, the normal state of the gut is one characterized by immune hyporesponsiveness. This suppressed state has implications for both normal and abnormal mucosal immune regulation, such as seen in intestinal inflammatory disorders. A frequent trademark of inflamed tissue resected from Crohn's disease and Ulcerative colitis patients is disruption of the intestinal epithelium, either because of enhanced epithelial differentiation or de-differentiation [1]. Normally, maintenance of the intestinal epithelium involves a dynamic process in which intestinal stem cells (ISCs) located at the crypt base undergo differentiation, proliferation, and migration along the crypt-villus axis. At the end of this journey, IECs undergo apoptosis. These processes appear to be dysregulated in the inflamed mucosa of IBD patients. More severe damage may manifest as either total epithelial destruction and ulceration, or dysplasia and progressive tumorigenesis. Because IECs and intestinal lymphocytes both express pattern recognition receptors (PRRs), exposure to the right ligand in the right environment has the potential to turn IECs into armed immune cells.

S. Dahan, PhD

Immunology Institute, Mount Sinai School of Medicine, 1425 Madison Avenue,
Box 1089, New York, NY 10128, USA

V.K. Denmark, MD

Division of Gastroenterology, Mount Sinai School of Medicine, New York, NY, USA

L. Mayer, MD (✉)

Immunology Institute, Mount Sinai School of Medicine, 1425 Madison Avenue,
Box 1089, New York, NY 10128, USA

Division of Clinical Immunology, Mount Sinai Medical Center, New York, NY, USA

e-mail: Lloyd.mayer@mssm.edu

IECs as Sentinels

The ability of IECs to respond to the enormous diversity of microorganisms relies upon a set of PRRs that recognize conserved bacterial and viral motifs. These receptors include lectins and adhesins, the nucleotide-binding oligomerization domain (NOD) family, and the toll-like receptors (TLRs) [2]. The innate immune system recognizes the presence of an infection and provides the necessary signals to elicit an adaptive immune response to the pathogen. In particular, TLR signaling is important for the maturation of antigen-presenting cells (APCs) that is necessary for the activation of T cells [3]. In response to their different and specific ligands, they activate signal transduction pathways, usually through the transcription factor NF- κ B that induces the expression of a variety of immune/proinflammatory responses. The expression of TLRs varies depending on the region within the intestinal tract (TLRs differ in the colon vs. the small intestine where there is a lower bacterial load) [4], the types of cells within the mucosa that express TLRs, and the regulation of TLR expression to specific compartments within cells [5, 6]. In general, TLR expression is carefully regulated to shut down a proinflammatory response to commensal organisms via the adaptor molecule TRIF [7, 8]. Some groups have reported that IECs do express several members of the TLR family apically and/or basolaterally, such as TLR5 involved in flagellin recognition [9]. By controlling which cells express which TLRs and in which cellular compartment, the intestine maintains tissue-specific homeostasis.

A landmark study using a mouse colitis model in which dextran sulfate sodium (DSS) acutely damages the intestinal epithelium demonstrated that MyD88 $^{-/-}$ mice (deficient in TLR, IL-1R, and IL-18R signaling) develop significantly enhanced colitis compared with WT mice [10]. In this context, innate signals are clearly protective and promote epithelial integrity [10–12].

The intestinal mucosa produces antimicrobial peptides called defensins that contribute to the maintenance of host immunity and protect against pathogenic flora. Defensins exert their effect by causing the formation of micropores in the bacterial membranes, leading to the loss of pathogen integrity. Defensins can be differentiated into two groups: α -defensins and β -defensins. Their type-expression is modulated along the different intestinal segments (with colonocyte and enterocyte differences). For instance, α -defensins (HD) – especially HD5 and HD6 – are synthesized by Paneth cells positioned in the crypts, while β -defensins (HBD) are produced by colonic epithelial cells [13, 14]. Evidence demonstrates that in CD there is an abnormal Paneth cell morphology and a defective expression of HD5 and HD6 with a release of a nonfunctional peptide, which forms an unfolded structure, probably due to a defective enzymatic digestion [15]. A decreased concentration of defensins is responsible for the presence of a less efficient mucus layer as a biochemical barrier for pathogenic bacteria (and possibly crypt dysbiosis). In addition to facilitating epithelial renewal, PRR activation may limit bacterial translocation by inducing the production of antimicrobial peptides, such as defensins and RegIII γ , by IECs [16–18].

The cause of deficient defensin production has not been clearly determined but the NOD2 and ATGL1 signaling pathways could be involved in this process. NOD2, an intracellular PRR recognizing muramyl dipeptide, has been implicated in the pathogenesis of Crohn's disease [19]. Between 19 and 37% of patients with CD carry one of three mutations in NOD2. In Paneth cells, this result translates into a failure to secrete defensins. In fact, NOD2 receptors are highly expressed in Paneth cells and an association between *NOD2* gene mutations and a reduced expression of HD5 mRNA in Paneth cells of CD patients has been found by Wehkamp et al. [20]. However, it should be pointed out that the primary cause of the α -defensin deficiency is epithelial cell loss [21]. In the IECs, the NOD2 mutations associated with Crohn's disease pathology may correlate with a failure to clear intracellular bacteria resulting in IEC dysfunction.

Recent studies also reported a role for NOD-like receptor (NLR)-mediated inflammasome activation in maintaining epithelial integrity [22–24]. Mice lacking some of the inflammasome signaling components exhibit enhanced DSS-induced colitis compared with WT controls [22–24].

The physical barrier function held by the epithelium is enhanced by the ability of certain epithelial cells, called goblet cells, to maintain the mucus layer at the epithelial surface. This mucus contains highly glycosylated proteins, named mucins and trefoil factors [25, 26]. It was postulated that glycosylated components could sort out commensal from pathogenic, allowing contacts between the flora and the epithelium in order to shape the underlying immune system. It is reported in the literature that TNF α stimulated HT29 Cl16E (a goblet cell line) secretes higher amounts of mucin than nonstimulated cells [27]. This might explain the increase in mucin release observed in the colonic mucosa of IBD patients [28].

These findings are consistent with a protective role of PRR signaling and epithelial secretions and a sentinel role of IECs. Indeed, acute epithelial damage in the microbe-rich gut may create a potent niche for immune cell activation.

IECs as Nonprofessional APCs

Recent advances have highlighted an observation that different specialized immune and nonimmune cells are capable of antigen presentation in the GI tract. This is required in order to achieve tolerance toward self as well as non-self harmless antigens while at the same time achieving immunity toward pathogenic microorganisms. Nonprofessional APCs like epithelial cells can either directly present antigen or release exosomes to potentiate DC presentation [29]. This suggests that several immune and nonimmune compartments need to be considered to fully understand the dynamics of immune regulation in the gut.

Antigen Presentation and Tolerance to Commensals

The apical mucous layer and glycocalyx in the intestine can act as the initial barrier and restrict the size of particles capable of being internalized by IECs; this restriction would favor antigen uptake by the modified “dome” epithelium (M cells), which overlie Peyer’s patches (PPs) [30]. The highly specialized M cells facilitate transport of macromolecules without notable or significant “cellular processing,” to the underlying lymphoid tissue, which contains several different types of APCs [31]. However, the surface area of the villous epithelium is exceptional, and IECs have a well-documented role in nutrient and soluble antigen uptake. IECs are exposed to a wide variety of antigens of diverse sizes and varying biochemical properties. Collectively, these observations highlight a physiological role for IECs in the uptake and processing of luminal antigens and pathogens [32]. Presentation of antigens associated with commensal microorganisms does not lead to the development of systemic tolerance. Indeed, if a commensal bacterium is introduced via systemic route, our organism is perfectly capable of initiating immunity toward it [33]. Barrier mechanisms (physical, biological, and chemical) and a generalized anti-inflammatory microenvironment in the gut help tolerate commensals locally [34]. However, antigen presentation of commensal-derived antigens can occur after transcytosis of commensals via M cells (and uptake by subepithelial APCs), that are scattered in the follicle-associated epithelium of PPs. This presentation leads to the induction of mucosal IgA responses that prevent bacterial entrance [33].

Antigen Presentation and Tolerance to Food Antigens

Tolerance to food is, by contrast, systemic [35, 36]. Oral exposure results in the generation of food antigen-specific T lymphocytes with regulatory activity (Treg) [37, 38]. These regulatory cells suppress the generation of antigen-specific effector lymphocyte responses, including pro-allergic helper T-cell type 2 (Th2) responses and IgE production.

In order for IECs to act as APCs, they must be able to internalize and process antigen. Normally, when in contact with soluble dietary antigens that escape proteolysis in the lumen, IECs take them up via fluid-phase endocytosis through the microvillous membrane. Antigens are then transported in small vesicles and larger phagosomes and digested when lysosomes combine to form phagolysosomes. Intact molecules that remain after digestion are deposited in the extracellular space via exocytosis [39]. As a result, approximately 2% of intact proteins reach the intestinal lymph and portal circulation under physiologic conditions [40]. IECs have been shown to readily internalize orally administered antigens [41]. IECs constitutively express MHC class II molecules. It is possible that IECs restimulate and favor the expansion of pre-existing primed Tregs in the gut, as recently proposed [42].

Antigen Presentation During Inflammation or Infection

The homeostasis of the gut is a result of a local noninflammatory microenvironment. It is achieved with the presence of immunomodulatory cells and the release by IECs or stromal cells of anti-inflammatory mediators like transforming growth factor- β (TGF- β), retinoic acid (RA), prostaglandin-E2, and thymic stromal lymphopoietin (TSLP). These mediators participate in the “education” of tolerogenic immune cells [43–45]. The homeostasis of the gut is abruptly interrupted during infection. The first cells sensing the presence of invaders are IECs that release a series of inflammatory mediators [46]. This results in the recruitment of inflammatory cells aimed not only at inactivating the pathogens, but also at initiating a protective adaptive immune response.

Both cytokine and chemokine production occurs within the epithelium. This production was initially documented in systems where bacterial invasion triggered a cascade of chemokines. Upregulation of epithelial-derived chemokines was also shown in IBD specimens. Similarly, a wide array of cytokines is secreted by IECs, including IL-6, IL-7, IL-15, IL-18, granulocyte macrophage colony-stimulating factor (GM-CSF) and TGF- β . These cytokines can play an inflammatory or regulatory role, depending upon the local environment. Once the infection is resolved, the immune homeostasis of the gut is re-established. Unfortunately, there are circumstances when inflammation is prolonged or an exaggerated response to the commensals is induced, potentially leading to the development, such as seen in IBD. In addition to the indirect transfer of information by IECs to the lymphoid tissue via chemokines, IECs can transmit information from the lumen by directly processing and presenting luminal microorganisms and antigens to antigen-specific lymphocytes. IECs constitutively express MHC class I throughout the entire bowel, and MHC class II predominantly in the small bowel. The expression of these molecules can be upregulated in response to proinflammatory signals, such as interferon- γ (IFN- γ), throughout the intestine. IFN- γ has also been shown to cause antigens to be sorted into MHC II-positive late endosomes in the epithelial cells [47]. Epithelial antigen processing occurs in a highly polarized fashion with apical antigens being sorted, processed, and presented exclusively basolaterally [48, 49]. Indeed, IEC lines express cathepsins and are able to process intact antigen into appropriate immunogenic peptides for CD4+ T-cell presentation.

Like other nonprofessional APCs, IECs do not express classical co-stimulatory molecules (CD80, CD86) mandatory for T-cell activation. However, IECs express novel members of the B7 family [50]. Additionally, IECs express a number of nonclassical MHC class I molecules that enable them to transmit luminal signals to the underlying lymphocytes, thereby make IECs a unique set of APCs in the gut. During inflammation, professional and nonprofessional APCs can function as potent restimulators of effector T cells thus exacerbating the inflammatory condition. Indeed, during inflammation IECs upregulate the expression of MHC II molecules [51]. This suggests an ongoing and upregulated antigen presentation, which can elicit a vicious cycle of uncontrolled inflammation. But the end effect is not chronic inflammation.

IECs and Their Suppressive Effect

A number of experimental systems have shown that altered regulation of intestinal T-cell immunity can result in chronic intestinal inflammation [52–57]. We know from these models that antigenic stimuli from the gut lumen may drive the expansion of both effector cells and regulatory T cells [58]. A lack of activation and/or expansion of regulatory cells could play a role in uncontrolled inflammation seen in IBD [59]. Distinct Tregs have been described, including CD4+ and CD8+ T-cell populations, many of which reside in the intestinal mucosa [60].

Tregs are functionally defined as T cells that suppress immune responses in two ways: via cell:cell contact or through the production of soluble factors such as IL-10 or TGF- β . These immunosuppressive mechanisms result in antigen nonspecific dampening of T-effector responses. The potential importance of CD4+ regulatory T cells in the gut comes from numerous observations made in mouse models of IBD and oral tolerance. While numerous mouse models have described the importance of CD4+ regulatory T cells in the gut immunity, their functional role in mucosal immune responses in humans is poorly understood. The in vitro and in vivo potentials to generate Tregs were examined in the human intestine [61, 62]. IECs may play a role in the regulation of immune responses toward luminal antigens. A subset of CD8+ T cells undergoes oligoclonal expansion in the intestinal mucosa, after interacting with a unique CEACAM5/CD1d complex expressed on the surface of IECs. This subset, which is regulatory in vitro, may play a role in the control of intestinal immune responses toward luminal antigens. A lack of expansion of these CD8+ Tregs is observed in IBD probably due to an impaired CEACAM5 expression [63]. Interestingly, patients with IBD cannot be tolerized to orally administered antigens [64, 65]. Together, this suggests that IEC activated CD8+ T cells with regulatory activity present in the LP of healthy individuals, but not in the LP of patients with IBD, play a central role in maintaining controlled inflammation in the intestinal mucosa.

Conclusion

Control of immune responses in the gut is critical for normal immune homeostasis (see Fig. 14.1 for summary). The dialogue between immune cells and IECs is disrupted in patients with chronic inflammatory bowel diseases, where alterations in cytokine secretion and Treg function promote rather than inhibit inflammation. Further understanding of the nature of IEC-T cell interactions and the immune defects observed in disease states will aid in the development of novel treatment strategies for IBD.

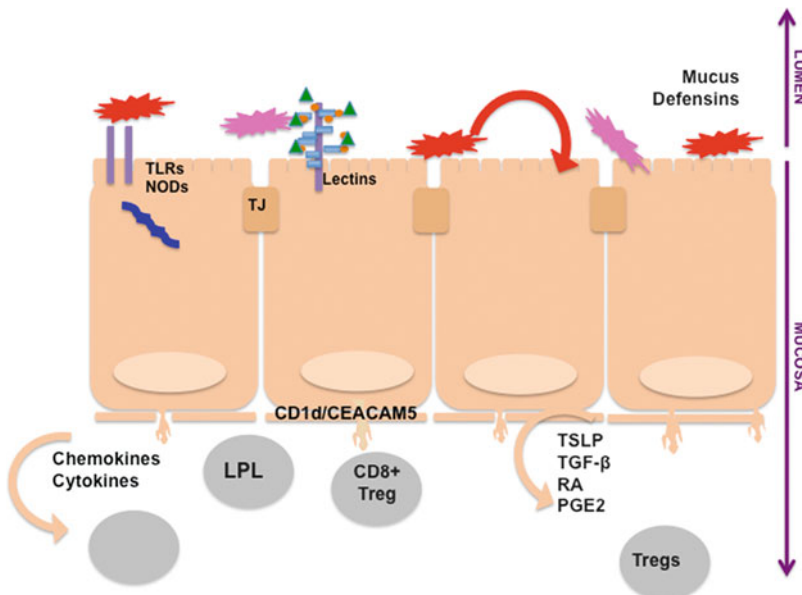


Fig. 14.1 The intestinal epithelial cell (IEC) plays a central role in gut homeostasis. It serves as a physical barrier between the intestinal lumen and the lamina propria by virtue of tight junction (TJ) seals, protective mucus and anti-microbial peptide production (defensins). IECs recognize conserved bacterial and viral motifs through a set of pattern recognition receptors (PRRs), such as lectins, toll-like receptors (TLRs) and the NOD family of receptors. Activation of these receptors leads to a variety of immune responses, usually through the activation of a transcription factor NF- κ B. This signal transduction cascade results in the IECs producing a wide array of cytokines that play inflammatory or regulatory roles, depending upon the local environment. For example, mediators like TGF- β , RA, PGE2 and TSLP participate in the “education” of tolerogenic immune cells, while proinflammatory cytokines like IL-6 and GM-CSF trigger a proinflammatory response by activating LPLs. In addition to cytokine production, IECs can act as antigen-presenting cells by directly processing and presenting luminal antigens to antigen-specific lymphocytes. This antigenic presentation may drive the expansion of both, effector and regulatory T cells. Specifically, a CD1d/CEACAM5 complex on the basal membrane of IECs activates a regulatory CD8+ T-cell population in the lamina propria, contributing to the control of inflammation in response to constantly encountered luminal antigens

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Chapter 15

The Th1, Th2, and Th17 Paradigm in Inflammatory Bowel Disease

Yasuhiro Nemoto and Mamoru Watanabe

Keywords Th1 • Th2 • Th17 • Helper T cells • Regulatory T cells • Effector T cells

Introduction

Inflammatory bowel disease (IBD) usually takes persistent course with life-long recurrence even if it once remitted, which makes this disease refractory [1]. This feature as if it possesses “pathogenic memory” suggests the importance of acquired immunity in the pathogenesis of IBD. CD4⁺ T cells play a central role in the acquired immune system. Above all, helper T (Th) cells secrete various cytokines, which regulate other immune cells. Although helper T cells have usually been classified as Th1 or Th2, Th17, which is a novel effector subset, and regulatory T cells (Treg), which regulates effector T cells negatively, have taken part in them recently and have made this system more complex [2]. Effectiveness of monoclonal antibody against TNF- α , which is one of effector cytokines secreted by Th1 cells, as the treatment for both Crohn’s disease (CD) and ulcerative colitis (UC) has changed natural history of IBD pathogenesis [3], and this has encouraged many scientists to develop more effective therapies targeting other cytokine pathways. Furthermore, Th17 is highlighted in the pathogenesis of IBD, since the significant association between genes involved in Th17/IL-23 pathway and IBD were reported. In this section, the role of Th1/Th2 and Th17 in the pathogenesis of IBD is discussed, which is essential for understanding of future biological treatments for IBD.

Differentiation of Helper T Cells

When CD4⁺ T cells are developed in thymus and migrate to the peripheral tissues, they are called naïve T cells, which secrete little cytokines and cannot exert efficient effector function. When antigens specific for their own T-cell receptors (TCR) are presented to naïve T cells by antigen-presenting

Y. Nemoto, MD, PhD • M. Watanabe, MD, PhD (✉)
Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University,
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
e-mail: mamoru.gast@tmd.ac.jp

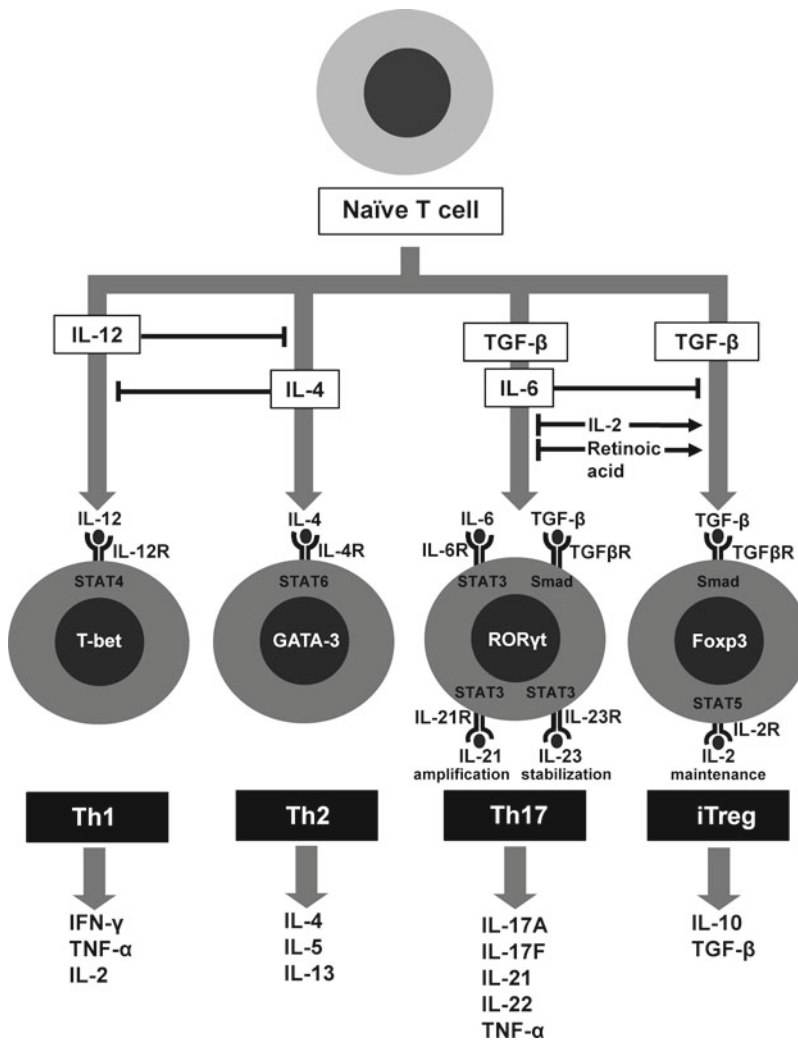


Fig. 15.1 Differentiation of helper CD4⁺ T cells. When antigens specific for their own TCR are presented to naïve T cells by antigen-presenting cells through MHC class-II/TCR signal with co-stimulatory signals, naïve T cells differentiate to helper T cells, which secrete characteristic types of cytokines and express specific transcription factors. Cytokines in the environment where the antigen presentation occurs determine the polarization of these helper T cells. Th1 cells differentiate in the presence of IL-12, and secrete IFN- γ , TNF- α , and IL-2. IL-12 activates STAT4 pathway through IL-12R and promotes transcription of T-bet, which is a master regulator for the Th1 differentiation. Th2 cells differentiate in the presence of IL-4, and secrete IL-4, IL-5, and IL-13. IL-4 activates STAT6 pathway through IL-4R and promotes transcription of GATA-3, which is a master regulator of the Th2 differentiation. Th17 cells produce IL-17A, IL-17F, IL-21, IL-22, and TNF- α . Th17 cells differentiate in the presence of IL-6 and TGF- β , through the expression of retinoic-acid-receptor-related orphan receptor (ROR)- γ t, which is a master regulator of Th17. While TGF- β drives Smad signaling, IL-6 activates STAT3 pathway, which promotes the expression of ROR γ t. IL-21 is an important cytokine for the amplification of Th17, while IL-23 is important for their stabilization. iTreg is derived from naïve T cells in the presence of TGF- β and the absence of IL-6 through the expression of Foxp3. Retinoic acid and IL-2 promote iTreg differentiation. In addition, IL-2 is an important survival factor of Treg. On the other hand, IL-6 prevents iTreg differentiation and promotes Th17 differentiation.

cells (APC) through MHC class-II/TCR signal with co-stimulatory signals, naïve T cells differentiate to helper T cells, which secrete characteristic types of cytokines and express specific transcription factors. Cytokines in the environment where the antigen presentation occurs determine the polarization of these helper T cells [4] (Fig. 15.1).

Th1 and Th2

Classically, helper CD4⁺ T cells were classified as Th1 or Th2. Th1 cells differentiate in the presence of IL-12 and secrete IFN- γ , TNF- α , and IL-2. Th1 cells play an important role in the cellular immunity against tumors and intracellular infections of virus and/or bacteria. IL-12 is secreted by innate immune cells, such as natural killer cells (NK), macrophages, and dendritic cells (DC). IL-12 activates STAT4 pathway through IL-12 receptor (IL-12R) and promotes transcription of T-bet and genes involved in the production of IFN- γ . In addition to IL-12, IFN- γ is also an important cytokine for Th1 differentiation, which is actually inhibited by neutralization of IFN- γ in vitro. Th1 cells express T-bet, which is a T-box family of transcription factors. T-bet is a master regulator for the Th1 differentiation. While transduction of T-bet in polarized Th2 cells converts them to Th1, its absence causes disorder of Th1 development in vitro and in vivo. T-bet prompts IL-12R expression and activates IFN- γ gene, which constitutes a positive feedback of Th1 polarization [4, 5].

Th2 cells differentiate in the presence of IL-4 and these cells secrete IL-4, IL-5, and IL-13. Th2 cells play an important role in humoral immunity against parasites and some allergens. IL-4 activates STAT6 pathway through IL-4 receptor (IL-4R) and promotes transcription of GATA-3, which is a master regulator of the Th2 differentiation. In the absence of GATA-3, Th2 development is inhibited in vitro and in vivo, while transduction of GATA-3 in the polarized Th1 cells converts them to produce IL-4. GATA-3 activates IL-4 genes, which form a positive feedback of Th2 polarization. IL-12 inhibits Th2 polarization, while IL-4 inhibits Th1 differentiation, which makes these subsets reciprocal [4, 5].

Th17

Recently, a novel Th subset distinct from Th1/Th2, named Th17, has been identified. Th17 cells produce L-17A, IL-17F, IL-21, IL-22, and TNF- α , and these cells play an important role in the protective immunity against the extracellular pathogens such as bacteria [6]. Th17 cells differentiate in the presence of IL-6 and TGF- β , through the expression of retinoic-acid-receptor-related orphan receptor (ROR)- γ t. While TGF- β drives Smad signaling, IL-6 activates STAT3 pathway, which promotes the expression of ROR γ t [2]. ROR γ t, which is a transcription factor also expressed on DP (double positive) cells in the thymus and the LTi (lymphoid tissue inducer) cells, is a master regulator of Th17 differentiation. Transduction of ROR γ t to CD4⁺ T cells makes them produce large amount of IL-17A, while depletion of it in Th17 cells results in decreased production of IL-17A. In addition to ROR γ t, Th17 cells also express ROR α , which is upregulated by STAT3 pathway. ROR α -deficient mice can produce normal level of IL-17A, while ROR γ t-deficient mice show impaired production of IL-17A. However, IL-17A production of ROR γ t mice depends on the expression of ROR α , and thus ROR α and ROR γ t double-deficient mice cannot produce any IL-17A [7]. IL-21 is an important cytokine for the differentiation of Th17. IL-6 promotes the production of IL-21 from Th17 cells independent of ROR γ t, and then IL-21 upregulates the expression of ROR γ t through the activation of STAT3 pathway. This process forms a positive feedback in the Th17 differentiation and is called “amplification.” Although IL-21 is not essential for the Th17 polarization, it is necessary for the amplification of Th17 cells. In fact, IL-21-deficient mice show a reduction in the number of Th17 cells [8]. IL-23 is also important for the Th17 pathway. Although IL-23 was originally thought to be essential for Th17 differentiation, this hypothesis is not accurate. Indeed, IL-23 receptor (IL-23R) is not expressed on naïve T cells. TGF- β signal mediates IL-23R expression on Th17 cells, which makes them to be responsive to IL-23. IL-23 is essential to the maintenance of the Th17 phenotype in long-term cultures. Thus, these roles of IL-23 for Th17 are defined as “stabilization” [8].

IL-23 is highly expressed in mucosa of human ileum, and there are many Th17 cells in the human GALT. These Th17 cells play an important role for the protective immunity against intestinal pathogens. Intestinal microbiota is essential for the development of Th17, since mice in germ-free condition show decreased number of Th17 cells [9].

Treg

Regulatory T cells (Treg) suppress effector T cells and plays a crucial role in peripheral tolerance, preventing autoimmune disease and chronic inflammation. Treg is a CD4⁺ T-cell population specialized to regulate activated cells negatively, which include subgroups classified by some surface molecules and developmental background [10]. Treg can be divided to natural Treg (nTreg), which are generated during T-cell development in a thymus, and induced Treg (iTreg), which are differentiated from naïve T cells in the peripheral tissues during the immune response. nTreg are identified as CD4⁺CD25⁺ T cells, which develop in the thymus as auto-reactive T cells and have TCR specific for auto-antigens (Fig. 15.2). Although CD25 (IL-2R α chain) was thought to be a marker of activated effector T cells previously, CD25 on the nTreg is expressed in response to these auto-antigens. In addition to CD25, nTreg express GITR, CTLA-4 and Foxp3, which is thought to be the master regulator of nTreg. Genetic depletion of Foxp3 leads to various autoimmune disease and chronic intestinal inflammation similar to IBD. Furthermore, induction of Foxp3 to CD4⁺ T cells causes them to suppress effector T cells [10].

iTreg is derived from naïve T cells in the presence of TGF- β and the absence of IL-6 through the expression of Foxp3. Retinoic acid and IL-2 promote iTreg differentiation. In addition, IL-2 is an important survival factor of Treg. On the other hand, IL-6 prevents iTreg differentiation and promotes Th17 differentiation [4–6, 10]. In this way, IL-6 is an important cytokine, which regulates the balance between Treg and Th17. However, these findings are mostly proved by in vitro system and whether they can be applied in vivo is largely unknown.

Th1/Th2 in IBD

In the past, CD was thought to be a Th1-mediated disease, while UC was thought to be a Th2-mediated disease. Actually, both Th1 and Th2 cells can induce inflammatory bowel disease in animal models (Table 15.1).

Th1 Animal Models

CD4⁺CD62L⁺ T cells from T-bet-deficient mice cannot induce colitis [11], while overexpression of T-bet in CD4⁺CD62L⁺ T cells can lead to exacerbation of colitis [11]. CD4⁺CD45RB^{high} T cells from STAT-4, which plays an important role in the differentiation of Th1, deficient mice develop attenuated colitis [12]. Administration of blocking antibody against IFN- γ suppresses colitis of IL-10^{-/-} mice [13] and CD4⁺CD45RB^{high} T cells transfer colitis mice [14]. On the other hand, CD4⁺CD45RB^{high} T cells from IFN- γ -deficient mice develop colitis [12]. Furthermore, IL-10^{-/-} $\times$ IFN- γ ^{-/-} mice develop colitis equally as IL-10^{-/-} mice when they are infected with *H. hepaticus* [15]. These findings suggest that IFN- γ is not essential for the development of colitis in these models.

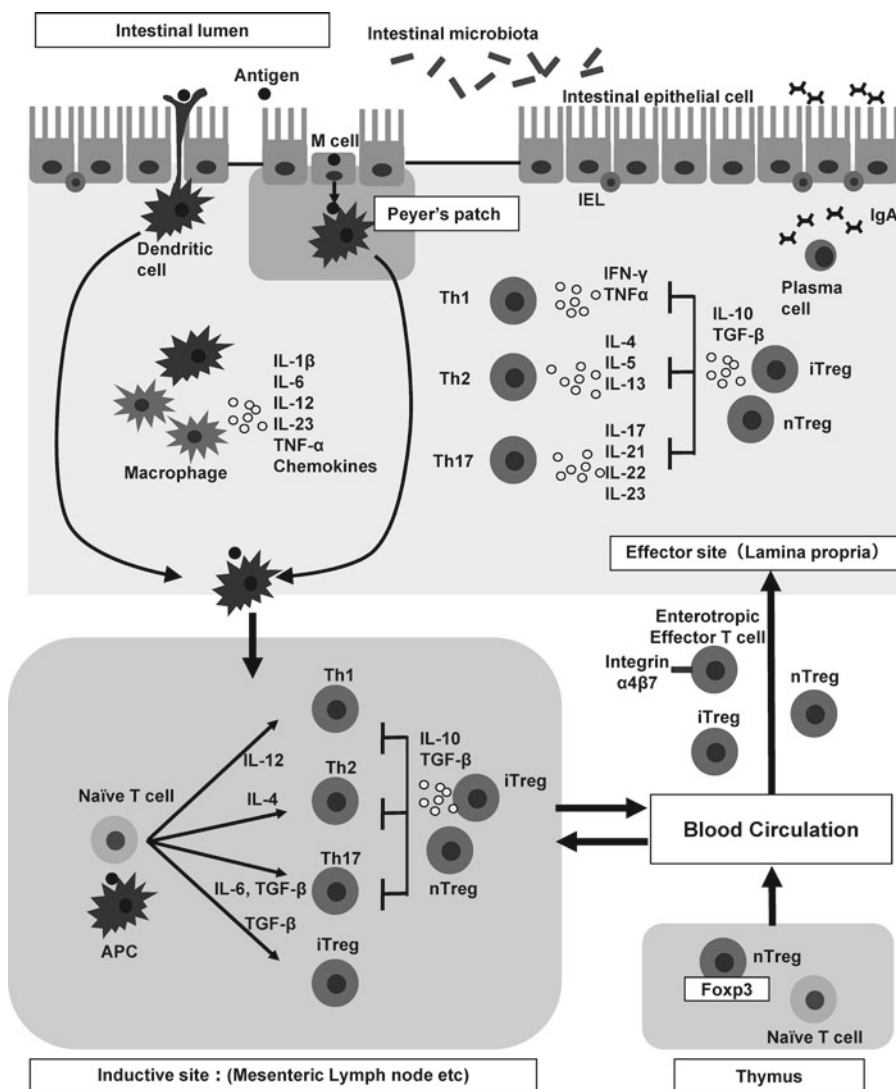


Fig. 15.2 Roles of effector T cells and regulatory T cells in the intestinal immune system. M cells in the peyer's patches uptake luminal antigens and transfer them to professional APC, such as DC or macrophages. DC can also capture luminal antigens directly, extending dendrites via the tight junctions of intestinal epithelial cells. Sensing antigens, DC and macrophages are activated and produce proinflammatory cytokines. After the development in the thymus, naïve T cells circulate in the bloodstream and the lymphatic system. When luminal antigens are presented to naïve T cells by APC in the inductive sites such as mesenteric lymph nodes or peyer's patches, naïve T cells differentiate to helper T cells. Effector T cells such as Th1, Th2, or Th17 migrate to effector site, such as intestinal lamina propria via blood circulation and secrete effector cytokines, which exert effector functions against various pathogens. Th1 cells play an important role in the cellular immunity against tumors or intracellular pathogens, whereas Th2 cells are important for humoral immunity and promote IgA production from plasma cells. Th17 cells play an important role for the protective immunity against the extracellular pathogens such as bacteria. nTreg are developed in the thymus, while iTreg are induced in the peripheral tissues. Treg suppress excess activation of effector T cells in the lymphoid organ and lamina propria, which contribute to the homeostasis of intestinal immune system

Table 15.1 Effector T cells involved in the pathogenesis of IBD mice models

Animal model	Type of Th	Mechanisms
Dysfunction of intestinal epithelial barrier		
DSS colitis	Th1, Th2, Th17	Direct damage to epithelial barrier
<i>Gαi2</i> ^{-/-} mice	Th1	Defect of intestinal epithelial barrier Defect of regulatory B cells
Dysregulation of innate immune system		
C3H/HeJBir mice	Th1, Th17	Dysfunction of sensitivity of TLR
Dysregulation of acquired immune system		
<i>TNFα</i> ^{ΔARE}	Th1	Overexpression of TNF-α Activation of effector T cells
<i>IL-7</i> -Tg mice	Th1	Overexpression of IL-7 Activation of effector T cells
<i>T bet</i> Tg mice	Th1	Activation of effector T cells
<i>STAT4</i> Tg mice	Th1	Activation of effector T cells
Bone marrow-reconstituted Tgε26 mice	Th1	Activation of effector T cells
<i>TCRα</i> ^{-/-}	Th2	Activation of effector T cells
Dysfunction or decrease of Treg		
CD4 ⁺ CD45RB ^{high} T cell transfer	Th1, Th17	Decrease of Treg Activation of effector T cells
<i>IL-10</i> ^{-/-}	Th1, Th17	Dysfunction of Treg
<i>TGFβ1</i> ^{-/-} , <i>TGFβR2</i> ^{-/-}	Th1	Decrease of Treg
<i>IL2</i> ^{-/-} , <i>IL2R</i> ^{-/-}	Th1	Decrease of Treg
<i>Smad3</i> ^{-/-}	Th1	Decrease of Treg
<i>WASP</i> ^{-/-}	Th2	Dysfunction of Treg
Other mechanisms		
TNBS colitis	Th1, Th2, Th17	Increase of activated effector T cells SJL/J mice=Th1, Th17/ Balb/c mice=Th2
Oxazolone colitis	Th2, NKT	Increase of activated effector T cells
<i>SAMP1/Yit</i> mutant mice	Th1, Th2	Dysfunction of intestinal epithelial cells Increase of activated effector T cells Increase of activated B cells

Th2 Animal Models

While Th1 plays important role in the pathogenesis of many kinds of animal models of IBD, there are few IBD models that are associated with Th2 [16]. Oxazolone-induced colitis mice, one of the colitis models induced by hapten, develop acute inflammation limited to colonic mucosa for 4–5 days [17]. CD4⁺ T cells in the intestinal mucosa of oxazolone-induced colitis mice produce large amounts of IL-4 and IL-5, which suggest that Th2 cells plays important role in this model [17], whereas those of TNBS-induced colitis mice, which are also one of the colitis models induced by hapten, produce mainly IL-2 and IFN-γ [18]. Actually, it is reported that neutralization of IL-4 attenuated the colitis in this model [17]. In the case of the more chronic colitis model, which is exposed to oxazolone prior to the rectal administration, IL-4 is increased in the early phase followed by IL-13 elevation in the chronic phase [18]. In the chronic model, the main sources of IL-13 are CD1d restricted invariant NKT cells [19]. In fact, depletion of NKT cells or administration of blocking antibody against CD1 before the administration of oxazolone prevents the development of colitis in this model [19].

CD Patients

A large number of CD4⁺ T cells that highly express T-bet and STAT-4 have been found in the intestinal mucosa of CD patients [11, 20]. CD4⁺ T cells in the intestinal lamina propria of CD patients produce large amount of IFN- γ and lower amount of IL-4 than that of healthy control [20]. Macrophages in the intestinal lamina propria of CD patients produce large amount of IL-12 [21]. Lymphocytes in the intestinal lamina propria of CD patients express elevated levels of IL-12R, and produce large amount of IFN- γ in response to IL-12 [22] (Table 15.2). Although treatment with blocking antibody against IFN- γ is not effective, treatment of monoclonal antibody against IL-12p40 is effective in CD patients [23].

UC Patients

When it was believed that helper T cells could be classified as Th1 or Th2, UC was thought as a “Th2-mediated disease,” because expression of IFN- γ in the colon of UC patients was found to be very low. Actually, CD4⁺ T cells in the intestinal lamina propria of UC patients produce higher amount of IL-5 and IL-13 than those of CD patients or healthy control [20, 24]. On the other hand, the most important Th2 cytokine, IL-4, expression in the intestine of UC is lower than that of CD patients or healthy control [20], which suggest that UC cannot be defined as merely a Th2-mediated disease. Similar to oxazolone colitis mice, the main sources of IL-13 in the colonic mucosa of UC patients are CD1d-restricted invariant NKT cells. In fact, there are many NKT cells in the inflamed mucosa of UC patients [24]. However, the role of IL-13 and NKT cells in the pathogenesis of UC is not clear and further investigations are needed.

Th17 in IBD

As mentioned above, although Th1 had been thought to play important roles in the pathogenesis of CD, recently it has been reported that Th17 is deeply involved in the pathogenesis of multiple animal models of IBD that were thought to be either Th1 or Th2 dominant previously. In addition, according to the results of GWAS (Genome Wide Association Study), there are highly significant associations between genomic regions of Th17/IL-23 pathway and IBD [25]. Accumulating evidences suggest that Th17 plays important roles in the pathogenesis of both CD and UC pathogenesis.

Th17 Animal Models

IL-23 forms a dimer made of p19 subunit and p40 subunit, which is shared with IL-12. It is difficult to decide whether IL-12/Th1 pathway or IL-23/Th17 pathway is important for the pathogenesis of animal models for which IL-12 has been previously thought to play an important role, because these experiments were usually proved by targeting IL-12p40. In fact, recently it is reported that Th17 cells are increased in the mice that have been thought as IL-12 dependent [5].

In addition to IFN- γ -producing cells, CD4⁺ T cells that produce large amount of IL-17 are increased in both IL-10-deficient mice and CD4⁺CD45RB^{high} T cells transfer colitis mice [26, 27], both of which have been thought as Th1 models. IL-23p19 transgenic mice develop colitis and

Table 15.2 Th subgroups involved in the pathogenesis of UC and CD patients

	CD		UC	
Th1				
T-bet	LP CD4 ⁺ T cells (mRNA)	↑	LP CD4 ⁺ T cells (mRNA)	→
IL-12	LPMC	↑	LPMC	→
	LP Macrophage	↑		
IL-12R	LPL	↑	Not clear	
TNF-α	Inflamed mucosa (mRNA)	↑	Inflamed mucosa (mRNA)	↑
	LP CD3 ⁺ T cells	↑	LP CD3 ⁺ T cells	↑
	LP Macrophage	↑	LP Macrophage	↑
IFN-γ	Inflamed mucosa	↑	Inflamed mucosa	→
	LP CD4 ⁺ T cells	↑	PB CD4 ⁺ T cells	→
	PB CD4 ⁺ T cells	↑		
IL-2	LP CD4 ⁺ T cells	↓	LP CD4 ⁺ T cells	→
	PB CD4 ⁺ T cells	↓		
IL-18	Intestinal mucosa	↑↑	Intestinal mucosa	↑
	LPMC	↑↑	LPMC	↑
Th2				
IL-4	LP CD4 ⁺ T cells	↓	LP CD4 ⁺ T cells	↓
IL-5	LP CD4 ⁺ T cells	↓	LP CD4 ⁺ T cells	↑
	PB CD4 ⁺ T cells	→	PB CD4 ⁺ T cells	→
IL-13	LPL	↑	LPL	↑↑
	PB CD4 ⁺ T cells	→	PB CD4 ⁺ T cells	→
Th17				
RORγt	LP CD4 ⁺ T cells (mRNA)	↑	LP CD4 ⁺ T cells (mRNA)	↑
IL-6	Inflamed mucosa (mRNA)	↑	Inflamed mucosa (mRNA)	↑
IL-17A	Serum	↑	Serum	↑
	LP CD3 ⁺ T cells	↑	LP CD3 ⁺ T cells	↑
	LP Macrophage	↑	LP Macrophage	↑
	PB CD4 ⁺ T cells	↑	PB CD4 ⁺ T cells	↑
IL-17F	Inflamed mucosa (mRNA)	↑	Intestinal mucosa (mRNA)	↑
IL-21	Inflamed mucosa	↑↑	Inflamed mucosa	↑
IL-22	LP CD4 ⁺ T cells	↑	LP CD4 ⁺ T cells	↑
IL-23	Inflamed mucosa (mRNA)	↑↑	Inflamed mucosa (mRNA)	↑
	LP MC	↑	LP MC	↑
IL-23R	LP CD4 ⁺ T cells (mRNA)	↑	LP CD4 ⁺ T cells (mRNA)	↑
Treg				
FOXP3	Intestinal mucosa (mRNA)	↑	Intestinal mucosa (mRNA)	↑
	LP CD4 ⁺	↓ ^a	LP CD4 ⁺	↓ ^a
	PB CD4 ⁺	↓	PB CD4 ⁺	↓
IL-10	LP CD3 ⁺ T cells (mRNA)	→	LP CD3 ⁺ T cells (mRNA)	↑
TGF-β	LP CD3 ⁺ T cells (mRNA)	→	LP CD3 ⁺ T cells (mRNA)	→

LP intestinal lamina propria, LPL intestinal lamina propria lymphocytes, LPMC intestinal lamina propria mononuclear cells, PB peripheral blood, PBMC peripheral blood mononuclear cells

^aCompared with other inflammatory disease such as diverticulitis

systemic autoimmune disease [28]. Administration of recombinant IL-23 exacerbates colitis in RAG-2^{-/-} mice transferred with CD4⁺ T cells from IL-10 deficient mice [27]. Genetic depletion or neutralization of IL-23p19 ameliorates colitis in both IL-10-deficient mice and CD4⁺CD45RB^{high} T cells transfer colitis mice [27, 29]. On the other hand, genetic depletion of IL-12p35 does not suppress colitis in IL-10-deficient mice [27], which suggest that Th17/IL-23 is more important than IL-12/Th1 in the pathogenesis of this model.

IL-17, which includes various subtypes such as IL-17A, IL-17F, is a main effector cytokine of Th17. The role of IL-17 in the pathogenesis of IBD is controversial, and there are different arguments that it exacerbates, ameliorates, or does not influence colitis. IL-17A-deficient mice developed more severe colitis than wild-type mice in the TNBS colitis model, and administration of antibody against IL-17A aggravates colitis induced by DSS [30]. In addition, although IL-23 is not dispensable for the development of colitis in CD4⁺CD45RB^{high} T cells transfer colitis mice, IL-17 is not essential [26]. IL-17A suppresses DSS colitis, while IL-17F accelerates colitis in this model [31]. Similarly, IL-17A suppresses colitis in CD4⁺CD45RB^{high} T cells transfer colitis mice, inhibiting the polarization of Th1 [32]. In summary, effects of IL-17 in colitis differ according to the difference of colitis models or experimental procedures such as genetic depletion or neutralization with blocking antibodies, and further investigations are needed.

IL-21 is important for the amplification of Th17, and IL-21 depletion ameliorates DSS colitis and TNBS colitis [33].

IL-22 is a suppressive cytokine unlike other Th17 cytokines. Genetic depletion or neutralization of IL-22 exacerbates colitis in CD4⁺CD45RB^{high} T cells transfer colitis mice and DSS colitis mice [34]. NK cells also produce IL-22 in addition to Th17. Recently, it is reported that IL-22-producing NK cells, ROR γ ⁺NKp46⁺ cells, play a critical role in the homeostasis of intestinal mucosal immune system. IL-22 not only suppresses excess activation of immune system, but it also induces anti-apoptotic molecules in the intestinal epithelial cells, which ameliorates epithelial disorder in colitis [35].

In the oxazolone colitis mice, a representative Th2 colitis model, depletion of IRF-4, which is an important factor for the Th2 differentiation, suppresses colitis [36]. However, IRF-4 relates to the differentiation of not only Th2 but also Th17, so the possibility that Th17 is more important than Th2 in the pathogenesis of this model cannot be excluded. In addition, it is known that depletion of IL-6, which is important factor for the differentiation of Th17, leads to suppression of colitis in this model, which suggests that Th17 plays an important role in the pathogenesis of this model.

IBD Patients

It is reported that IL-17A, IL-17F, IL-21, IL-22, IL-23, ROR γ ⁺, and IL-23R are highly expressed in the intestinal mucosa of CD patients [4, 37]. Furthermore, it is reported that a unique macrophage, which produce IL-23, TNF, and IL-6, are found to be increased in the intestinal mucosa of CD patients [38]. IL-17 and IL-23 in CD patients decrease after treatment with steroid or blocking antibody against IL-12p40 [39]. Similarly, IL-17A, IL-17F, IL-21, IL-22, IL-23, ROR γ ⁺, and IL-23R are highly expressed in the intestinal mucosa of UC patients [4, 37]. As mentioned above, several GWAS proved highly significant associations between genomic regions of Th17/IL-23 pathway and IBD [25]. Th17 is noticed as an important factor for the pathogenesis of IBD. However, unlike animal models, data in human patient are still not enough, and further studies are needed.

Regulatory T Cells

There are many animal models that suggest the importance of regulatory T cells for the homeostasis of intestinal mucosal immune system. Both CTLA4-deficient mice and Foxp3-deficient mice develop colitis and transfer of Treg suppresses colitis in multiple IBD model mice [10], which suggest that dysfunction or decrease of Treg may cause IBD.

Actually, the number of CD4⁺CD25⁺FOXP3⁺ T cells in the peripheral blood in both CD and UC patients are decreased as compared with that of the healthy control [40, 41]. On the other hand, the number of CD4⁺CD25⁺FOXP3⁺ T cells in intestinal mucosa of both CD and UC patients are increased [41, 42]. Furthermore these Treg from IBD patients can suppress effector T cells in vitro [42]. These findings that either decrease in the effector site or dysfunction of Treg is not observed in IBD patients may raise doubt to whether Tregs are involved in the pathogenesis of IBD. However, as compared with other intestinal inflammatory disease such as diverticulitis, intestinal Treg in IBD patients decrease relatively [40, 41], and it is thought that there exists an imbalance between Treg and effector T cells in IBD patients.

IL-10 is a suppressive cytokine that was produced by Treg. It is well known that IL-10-deficient mice develop colitis. Administration of recombinant IL-10 or transgene of IL-10 suppresses colitis in CD4⁺CD45RB^{high} T cells transfer colitis mice. In human, there is a significant association between genomic regions of IL-10 and UC [43]. Furthermore, it is reported that rare mutation of *IL10R* gene, which caused dysfunction of IL-10R, can lead to familial enterocolitis [44].

Conclusion

Previously, anti-inflammation and/or nonspecific immunosuppression played a central role in IBD treatment. However, Infliximab, anti-TNF monoclonal antibody, has made a revolution. Likewise, Th1/Th2 theory, which was the central dogma for the differentiation of helper T cells, has been replaced by new theory including Th17 and Treg. This section may be helpful for the understanding of future therapy in IBD, which seems to become more and more complex.

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Chapter 16

Preclinical Studies Using Mouse Models of Inflammatory Bowel Disease

Fridrik Karlsson, Iurii Kobozev, and Matthew B. Grisham

Keywords Animal models • Therapeutics • Biologics • Cytokines • T cells • Regulatory T cells

Introduction

No single year generated more excitement within the inflammatory bowel disease community than did 1993. In October of that year, the journal *Cell* published three different studies demonstrating that ablation of the T-cell receptor, IL-2, or IL-10 gene resulted in the development of chronic colitis in these genetically engineered mice [1–3]. One month later, *International Immunology* published a study by Powrie and coworkers who reported that adoptive transfer of antigen-inexperienced (naïve) CD4⁺CD45RB^{high} T cells into lymphopenic mice induced a chronic and unrelenting colitis in immune-deficient recipients [4]. These four studies initiated a frenzy of research activity to determine how genetic alterations or immunological manipulations of very different molecular and cellular pathways could converge to produce a common disease phenotype. Indeed, these original reports provided a much needed “jump-start” to what was, at that time, an active but relatively small research community investigating the underlying pathogenetic mechanisms responsible for the inflammatory bowel diseases (IBD; Crohn’s disease, ulcerative colitis). Virtually all experimental studies performed prior to 1993 used chemically induced, self-limiting models of colonic inflammation that were similar in concept to the animal models originally described by Kirsner and colleagues in the late 1950s [5–7]. The generation of genetically engineered and immune-manipulated mice that developed *chronic* gut inflammation attracted large numbers of investigators from diverse scientific backgrounds who began to employ these new models to identify the immunopathogenetic mechanisms involved in chronic intestinal inflammation as well as define the molecular and cellular

*Contributed equally to the preparation of the manuscript.

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F. Karlsson, PhD • I. Kobozev, BS • M.B. Grisham, PhD (✉)
Immunology and Inflammation Research Group, Department of Molecular and Cellular Physiology,
Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, USA
e-mail: mgrish@lsuhsc.edu

Table 16.1 Representative mouse models of small and large bowel inflammation

Acute/self-limiting inflammation			Chronic inflammation		
Chemical induction	Immunological induction	T-cell transfer	Genetic	Spontaneous	Bacterial
DSS	CD40 mAb	CD45RB ^{high} → RAG (SB + C)	IL-2 ^{-/-}	SAMP1/Yit (SB)	H. hepaticus
TNBS/ETOH		WT → CD3etg	IL-10 ^{-/-}	C3H/HeJBr	
Indomethacin (SB)			TCR ^{-/-}		
			Gαi2 ^{-/-}		
			Mdr1a ^{-/-}		
			TNF ^{FARE} (SB)		
			STAT3 ^{-/-}		
			STAT4 tg		
			CD40L tg		
			A20 ^{-/-}		
			TGFβ1 ^{-/-}		
			N-Cadherin DN		
			GPx 1 + 2 ^{-/-}		
			EP4 ^{-/-}		
			R59D-JAB tg (SOCS-1 tg)		
			WASP ^{-/-}		
			MUC2 ^{-/-}		
			IL10R2 ^{-/-} × TGFβR DN		
			IEC/IKK ^{-/-}		
			Tbet ^{-/-} × RAG ^{-/-} (TRUC)		
			CD4 ⁺ /TGFβRII DN		
			IL-7 tg		

Based on data from references [1–4, 8–14, 40, 73, 75, 77, 82, 83]

SB signifies mouse models expressing small bowel inflammation, whereas *SB + C* designates small bowel and colonic inflammation. All other are models of colitis. *tg* transgenic, *DN* dominant negative

mechanisms responsible for tolerance and immune regulation. The number of investigators and the amount of information that has been obtained using these models have grown unabated over the past 17 years.

Currently, there are several dozen mouse models of intestinal inflammation with the large majority of these mice expressing acute or chronic colitis (Table 16.1) [8–14]. A few of these mouse models have been characterized in sufficient detail that they are beginning to be used in preclinical studies to test the efficacy of new drug therapies targeted for IBD. Historically, biotechnology and pharmaceutical companies have not used disease-specific approaches for developing novel therapeutics to treat certain chronic inflammatory diseases choosing instead to develop anti-inflammatory medications that can be tested in a variety of preclinical animal models of autoimmune and chronic inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and IBD. Virtually all of the previous, and most of the current preclinical studies have relied heavily on the use of chemically induced models of IBD. Although a reasonable approach given the simplicity and inexpensive nature of these erosive/self-limiting models, it is becoming increasingly apparent that most, if not all of these models are self-limiting in nature and thus do not express the chronic immunopathologic characteristics observed in the human IBD. A quick survey of PubMed reveals that >1,000 studies have been published using the two most popular models of experimental colitis (e.g., dextran sulfate sodium and trinitrobenzene sulfonic acid models). Of these, more than 400 studies have reported

significant protective or anti-inflammatory activity of novel small molecules, biologics, immune manipulations or genetic alterations. Yet, few if any of these potential “targets” or therapeutic agents have been evaluated in animal models of chronic intestinal inflammation. This is an important point to note because when attempted, few studies have been able to translate the promising anti-inflammatory activities of novel therapeutics observed in chemically induced models to mouse models of chronic gut inflammation [15–19]. Furthermore, the failure to translate preclinical data into clinically effective medications is also becoming of increased concern. A good example of the disparity between preclinical studies and clinical efficacy can be found in the literature describing the development of the different leukotriene B₄ receptor antagonists and 5-lipoxygenase inhibitors for the treatment of various inflammatory disorders including IBD. Although these novel anti-inflammatory agents appeared to be quite promising in preclinical experiments using erosive models of colitis in rodents, investigators failed to demonstrate significant anti-inflammatory/protective effects in patients with Crohn’s disease (CD) or ulcerative colitis (UC) when evaluated in blinded, multicenter, placebo-controlled clinical studies [20, 21]. Compounding the problems associated with the predictive value of certain preclinical models, is the fact that CD and UC are complex, multifactorial diseases that may not respond to anti-inflammatory or immune-modifying agents that have proven to be efficacious in autoimmune diseases such as RA or MS. The well-established anti-inflammatory activity of the soluble tumor necrosis- α (TNF- α) receptor antagonist etanercept (Enbrel) in human RA but not in CD highlights these complexities and the different pathogenetic mechanisms involved in these two chronic inflammatory disorders [22].

It would seem clear that the “one size fits all” philosophy for drug development may not represent the best approach for facilitating the development of new therapeutic agents targeted for IBD. One potential strategy for improving our ability to discover new therapeutics that may have a reasonable chance of success in well-controlled clinical trials is to identify the most immunologically relevant mouse models of human CD or UC. This chapter presents a critical evaluation of the different mouse models of IBD and discusses their utility for use in preclinical studies.

Mouse Models of IBD

There are now more than 50 mouse models of IBD that may be grouped into two major groups including the acute/self-limiting and chronic models of small and/or large bowel inflammation (Table 16.1) [8–10, 12]. Because of the large number of mouse models expressing different types of inflammation in the small and/or large bowel, investigators are confronted with the daunting task of deciding which of these mouse models is/are appropriate to use for pharmacologic intervention studies. In reality, there is no single best model of IBD; however, the choice of an animal model should depend upon the specific questions being posed. Unfortunately, it appears that many of the pharmacological studies choose animal models based upon their simplicity, rapid onset of inflammation, and expense. Therefore, it is not surprising that the most popular models of intestinal inflammation are the acute/self-limiting models of colitis induced by rectal or oral administration of noxious chemicals.

Acute/Self-Limiting Models of Colonic Inflammation

The dextran sulfate sodium (DSS) model of colitis is by far, the most popular mouse model of IBD. Oral administration of this sulfated polysaccharide to rats, mice, hamsters, or guinea pigs induces an acute colitis that is reminiscent of UC. Animals develop bloody diarrhea and weight loss over a

period of several days depending upon the specific strain of animal [23]. Histopathological inspection of the colon reveals extensive infiltration of PMNs, monocytes, and macrophages as well as frank ulcerations, goblet cell loss, and occasional crypt abscesses [24–27]. It is clear that much of the acute inflammatory response induced by oral administration of DSS arises from the nonspecific disruption or injury of the colonic epithelium. Although enteric bacteria have been suggested to contribute to gut pathology in this model, other studies demonstrate that luminal bacteria play little role in DSS-induced inflammation and may actually play a protective role in this model [28–31]. Exactly how DSS “injures” the epithelium is not known; however, it has been demonstrated that only specific molecular weights of DSS are able to induce colitis. For example, 5,000 MW DSS induces cecal inflammation and proximal colitis, whereas the 40,000–50,000 MW polymers induces more distal bowel inflammation. Higher MW polymers (500,000 MW) do not induce colonic inflammation [32]. These data suggest that smaller polymers of DSS induce colonic inflammation by gaining access to the basolateral/intercellular space of the epithelium where they injure/disrupt the colonic epithelium. We propose, that the negatively charged sulfate residues may act to chelate or bind specific divalent cations (e.g., calcium and magnesium) that are required for maintaining normal epithelial cell tight junction integrity as well as for promoting epithelial cell-basement membrane attachment. The degree of sulfation of DSS is a critical determinant for inducing colonic inflammation [32]. Because of significant differences in responses to DSS among the various mouse strains [23], the concentrations and/or time of exposure of DSS must be determined for each strain. In general, ad libitum administration of 2–5% DSS-containing drinking water induces diarrhea, rectal bleeding, and weight loss within 4–8 days. Of note, DSS administration induces robust colitis in T- and B-cell-deficient SCID or recombinase activating gene-1 or -2 deficient (RAG^{-/-}) mice demonstrating that the adaptive immune system plays no role in the pathogenesis of the inflammation [33, 34]. Not surprisingly, macrophage- and PMN-derived IL-1 β , IL-6, and TNF- α are increased in the inflamed colons in this model. Another interesting observation that has been made using this model is that DSS may interfere with colonic epithelial cell proliferation and mucosal repair by creating an “M1” inflammatory response [35]. Infiltration and activation of macrophages with IFN- γ and/or LPS induces an M1 cytokine profile which is characterized by the production of large amounts of reactive oxygen and nitrogen metabolites as well as IL-1 β , TNF α , and IL-6. It has been suggested that one or more of these inflammatory mediators directly or indirectly suppresses colonic mucosal barrier repair [35].

A second model of acute/self-limiting colitis that has been used by a number of different laboratories is the trinitrobenzene sulfonic acid (TNBS) model. This chemically induced model was originally developed for use in rats [24]; however, it has received wide-spread use for inducing colitis in mice over the past 10–15 years [25]. Colonic inflammation is induced by the intrarectal administration of the hapten TNBS dissolved in 50% ethanol (EtOH). It has been proposed that EtOH is required to “break” the mucosal barrier thereby allowing TNBS to enter the colonic interstitium where it interacts with amino groups on colonic and possibly enteric bacterial proteins. This covalent modification or “haptization” of colonic proteins is thought to induce a localized, immunological response akin to the classical delayed-type hypersensitivity (DTH) reaction. It should also be noted, however, that only a small percentage of the published studies using the TNBS/EtOH method actually perform an initial pre-sensitization with the hapten (i.e., skin painting) that is normally required to induce a DTH reaction [36]. The immunological similarities and differences of colonic inflammation-induced in the absence or presence of prior sensitization have not been investigated. It should be noted that neutralized solutions of TNBS (pH 7.4) in the absence of EtOH are in fact quite toxic to intestinal epithelial cells [26, 35, 37]. In addition, we have found intrarectal administration of unbuffered TNBS dissolved in distilled water (pH < 2.0) is sufficient to injure the mucosa and induce colitis in rats suggesting that EtOH may not be required for induction of inflammation (unpublished data). Nevertheless, this model does induce a Th1 (and possibly Th2) inflammation characterized by production of significant amounts of IL-2, INF- γ , TNF- α , and IL-12 as well

as by the infiltration of PMNs, macrophages, and lymphocytes. A few studies have suggested that CD4⁺ T cells play an important role in the pathogenesis of TNBS-induced colitis [25, 38]. This model has been useful in identifying different cytokines and inflammatory mediators responsible for leukocyte infiltration as well as mediators that appear to interfere with epithelial repair [9, 39–41]. Furthermore, the inflammation induced by intrarectal administration of TNBS/EtOH is highly dependent upon mouse strain thereby requiring a substantial investment of time to optimize concentrations and volumes of TNBS/EtOH in order to reproducibly induce robust colitis without significant mortality [36].

When one is evaluating the suitability of the DSS or TNBS models for use in pharmacologic studies, it is important to consider a few of the basic principles that have emerged from studies using these mouse models of IBD. First, it must be appreciated that oral or rectal administration of a damaging agent to an immune-competent mouse induces a *self-limiting* inflammation via the non-specific injury to the colonic epithelium. The *magnitude* of this initial insult cannot be modulated unless the concentration and/or delivery of the noxious chemical have been inadvertently altered. This seemingly obvious, yet frequently overlooked, fact has important ramifications when interpreting data from pharmacologic studies where a drug or intervention alters water consumption and/or enhances colonic fluid/bicarbonate secretion. For example, any situation that alters the delivery or effective concentration of the damaging agent will alter the inflammatory response [36, 42]. If a drug inadvertently decreases water consumption in mice receiving DSS in their drinking water, the delivery/effective concentration of DSS will be reduced and thus the initial insult to the colonic mucosa will be decreased [42]. This will result in less inflammation thereby leading the investigator to the erroneous conclusion that the drug possesses significant anti-inflammatory activity. Likewise, any drug that enhances colonic fluid/bicarbonate secretion will dilute and/or neutralize lumenally administered acids (e.g., TNBS) and/or solvents (e.g., EtOH) thereby reducing the initial colonic injury and its subsequent inflammatory response. Therefore, it is critical to anticipate and account for these types of nonspecific effects.

Another important principle that has emerged from studies using these models is that certain pro-inflammatory mediators interfere with mucosal wound repair. It is becoming increasingly appreciated that PMN-, DC-, and/or macrophage-derived cytokines/mediators interfere with normal epithelial cell reparative processes [35, 39–41, 43–45]. Thus, any intervention that promotes epithelial cell restitution/proliferation and barrier repair or abrogates pathways that interfere with epithelial barrier repair will be observed as having “anti-inflammatory” activity in these erosive models [43–45]. Indeed, these are the most likely mechanisms involved in the protective effects observed when leukocytes are prevented from migrating to the colonic interstitium or when specific inflammatory mediators are blocked or neutralized. A third important principle related to most, if not all of the chemically induced models is that colonic inflammation does not require the adaptive immune system. There is no question that this is true for the DSS model [33, 34]; however, the role of the adaptive immune system in the *conventional* TNBS/EtOH model where a pre-sensitization protocol is not used, is somewhat more controversial. The evidence implicating CD4⁺ T cells as important mediators of disease pathogenesis in this TNBS model is largely indirect and derived from studies demonstrating that ex vivo-activated lamina propria T cells produce copious amounts of Th1 cytokines and that administration of mAbs directed toward IL-12, CD40L, or IFN- γ attenuate TNBS/EtOH-induced colitis [10, 46]. In addition, it has been reported that colitis can be induced in healthy wild-type mice by adoptive transfer of CD4⁺ T cells obtained from the colons of TNBS/EtOH-treated mice with active colitis [38]. Although compelling, these studies are difficult to interpret since the recipient animals were also exposed to luminal TNBS/EtOH. Furthermore, other laboratories have demonstrated that intrarectal administration of TNBS/EtOH to lymphopenic SCID or RAG^{-/-} mice induces colonic inflammation that is histopathologically indistinguishable from that induced in wild-type mice suggesting that lymphocytes are not required for the induction of colitis in this model [47]. In one of the few studies where mice were pre-sensitized to TNBS via skin painting

1 week prior to intrarectal administration of TNBS/EtOH, it was found that colonic inflammation was reduced by approximately 50% in RAG^{-/-} mice compared to wild-type animals suggesting that lymphocytes may be important for some but not all of the inflammation induced in this variation of the conventional TNBS model [48]. The reasons for these differences are not readily apparent but most probably reflect the inherent variability of this model as well as the different methods used for disease induction. Therefore, it is imperative that the investigator carefully consider and define mouse strain, concentrations of TNBS and/or EtOH, and method for induction of colitis. Taken together, the literature suggests that despite their limitations, both the DSS and TNBS models are quite useful for evaluating novel therapeutics designed to promote epithelial restitution and proliferation as well as mucosal wound repair in the presence of an acute inflammatory response. Their usefulness for evaluating the role that the adaptive immune system plays in disease pathogenesis and/or drug efficacy may be limited.

The chemically induced models have been promoted as reasonable models to study the role of the innate immune system in colonic inflammation. This is true for the most part; however, their requirement for a damaging agent to induce the colonic inflammation renders these models more susceptible to unanticipated effects of different drugs/therapeutics which may lead to a misinterpretation of data. One model of self-limiting colitis that does not require the use of noxious chemicals to induce an innate immune cell-mediated colitis is the CD40 mAb model (Table 16.1) [49]. Acute colitis can be induced in lymphopenic RAG^{-/-} mice by a single injection (*ip*) of agonistic CD40 mAb. The mice develop rapid weight loss and diarrhea within the first 4 days with splenomegaly, hepatopathy, and lymphadenomegaly of the mesenteric lymph nodes apparent by day 7. Histological inspection of the colons obtained from these mice at 7 days post mAb treatment reveals marked epithelial hyperplasia and extensive leukocyte (PMN, monocyte) infiltration within the lamina propria as well as goblet cell depletion, and epithelial cell destruction [49]. Colitis persists for 10 days and is completely resolved by 3 weeks following treatment with the CD40 mAb. It is proposed that CD40 ligation initiates an inflammatory cascade that begins with the activation of different myeloid cells including dendritic cells (DCs) and monocytes to produce IL-23 and IL-12. These cytokines then promote the release of TNF- α , IFN- γ , and IL-6 by DCs and macrophages as well as enhance the production of IFN- γ by natural killer cells [49]. Thus, this model offers investigators an alternative model for studying the pathogenesis of innate immune cell-mediated colitis induced by an immunological manipulation rather than by a noxious chemical.

Mouse Models of Chronic Colitis

A major limitation in understanding the immune-pathogenetic mechanisms responsible for the induction and perpetuation of chronic intestinal inflammation prior to the mid-1990s was the availability of rodent models that developed chronic intestinal inflammation. Although no single animal model completely recapitulates the clinical and histopathological characteristics of human IBD, a number of different mouse models of chronic small and/or large bowel inflammation have been developed over the past 15 years that have greatly advanced our understanding of disease pathogenesis (Table 16.1). Data obtained from several studies using a variety of chronic models have revealed several recurring themes. First, *chronic* gut inflammation is mediated by T cells. Second, commensal (nonpathogenic) enteric bacteria drive gut inflammation by providing continuous antigenic and adjuvant stimulation. Third, defective immune-regulation promotes chronic inflammation and finally, the genetic background of the animal modulates disease onset, severity and phenotype. Taken together, these studies suggest that chronic intestinal inflammation develops as a result of a dysregulated immune response to components of the normal enteric flora [50–52]. Thus, it is reasonable to propose that mouse models of chronic gut inflammation are in general, more relevant to human IBD than are acute/self-limiting models. Of the many models of chronic gut inflammation

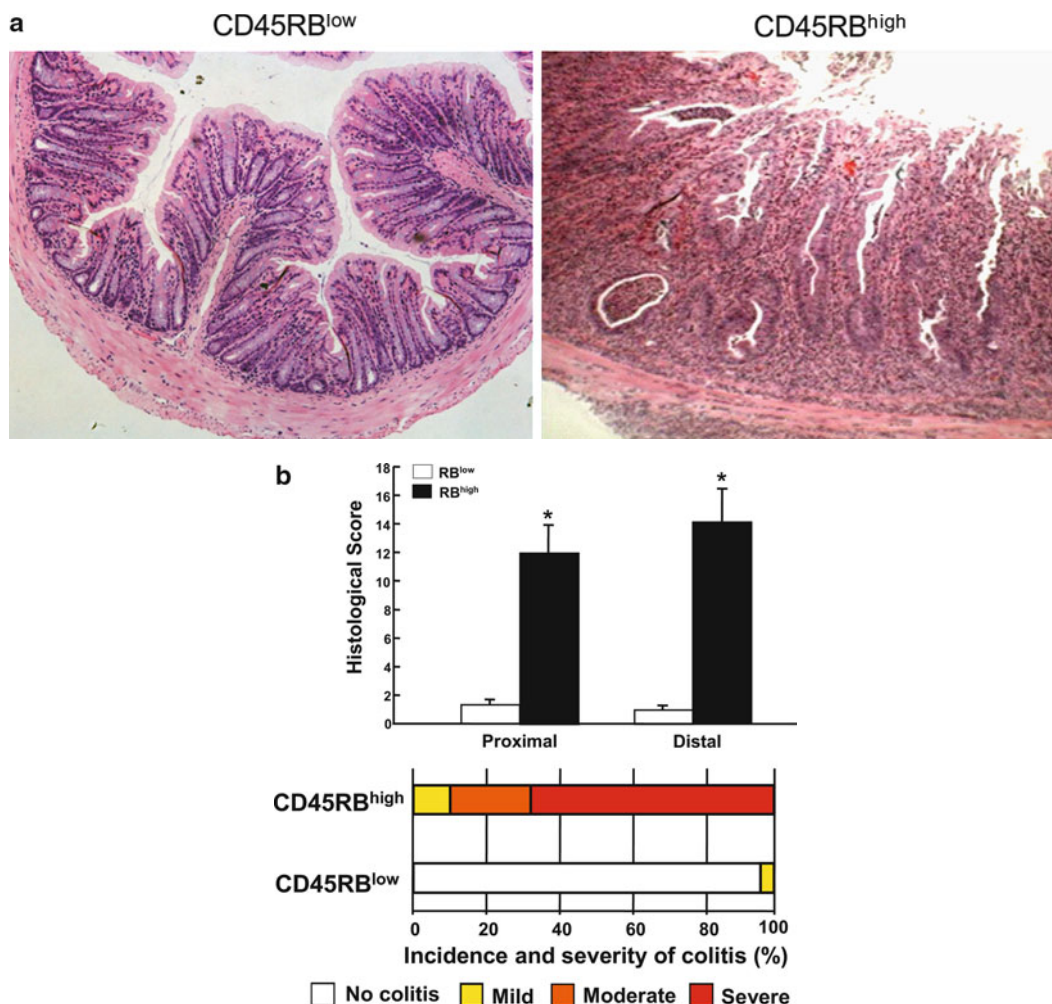


Fig. 16.1 Adoptive transfer of CD4⁺CD45RB^{high} T cells into RAG-1^{-/-} recipients induces chronic colitis. (a) Representative histopathology of RAG-1^{-/-} injected (*ip*) with 5×10^5 CD4⁺CD45RB^{low} (CD45RB^{low}) or CD4⁺CD45RB^{high} (CD45RB^{high}) T cells at 8 weeks following T-cell transfer. (b) Blinded histopathological analysis of colons obtained from RAG-1^{-/-} injected with CD45RB^{low} or CD45RB^{high} T cells. Colonic inflammation was quantified using our published scoring criteria [54]. [Reproduced from Ostanin et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G135-G146. With permission from Am. Phys. Soc.]

that have been developed, only a few of these models have been used by sufficient numbers of investigators to yield detailed information on the penetrance, severity, and pathogenesis of the inflammation. Three models of chronic colitis that have been well-characterized and are readily available to all investigators are the T-cell transfer, IL-10-deficient (IL-10^{-/-}), and multidrug resistance pump 1a-deficient (Mdr1a^{-/-}) models (Table 16.1).

The prototypical and best-characterized model of chronic colitis induced by disruption of T-cell homeostasis is the CD45RB^{high} T-cell transfer model (Table 16.1) [4, 53]. Adoptive transfer of antigen-inexperienced (i.e., naïve) CD4⁺CD45RB^{high} T cells from healthy wild-type mice into syngeneic, lymphopenic recipients induces a chronic and unrelenting pancolitis at 5–8 weeks following T-cell transfer (Fig. 16.1) [4, 53, 54]. There is good evidence to suggest that in the absence of regulatory T cells (Tregs; CD4⁺Foxp3⁺), naïve T cells undergo *enteric antigen*-driven priming, polarization,

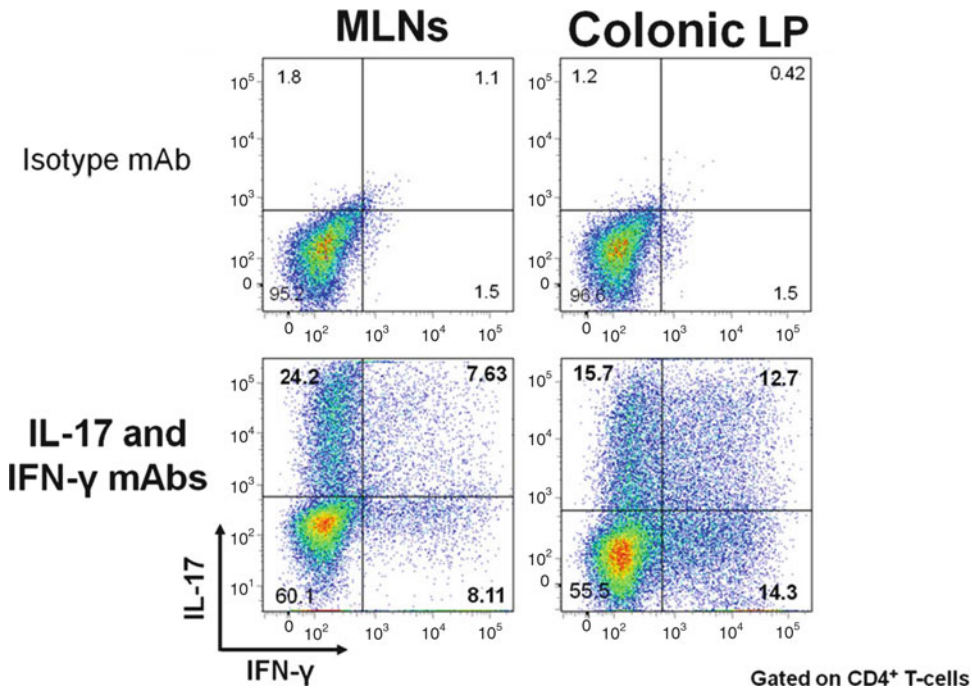


Fig. 16.2 Adoptive transfer of $CD4^+CD45RB^{high}$ T cells into $RAG-1^{-/-}$ recipients induces a mixed Th1/Th17 colitis. Mononuclear cells from MLNs and colonic lamina propria (LP) of colitic mice at 8 weeks post T-cell transfer were activated in vitro, permeabilized, and stained with isotype control or IL-17 and IFN- γ mAbs. Representative intracellular staining for the 2 cytokines within $CD4^+$ cells is shown. [Reproduced from Ostanin et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G135-G146. With permission from Am. Phys. Soc.]

and expansion to yield large numbers of colitogenic effector cells such as Th1 and Th17 cells within the gut-draining mesenteric lymph nodes (MLNs) and colon [4, 51, 53, 54]. Because naïve T cells will, by default, convert to disease-producing effector cells in the absence of Tregs, virtually any T-cell deficient recipient (e.g., $RAG-1^{-/-}$, $RAG-2^{-/-}$, nude, SCID, $CD3^{-/-}$, $TCR\beta^{-/-} \times TCR\delta^{-/-}$, etc.) can be used as recipients [51, 53, 55]. We have found that Balb/c SCID mice develop a slightly more rapid and severe form of colitis than do the C57Bl/6 $RAG-1^{-/-}$ mice. In addition, we have observed that the presence of B cells delays modestly the onset but not severity of colitis supporting the concept that B cells may act to suppress colonic inflammation in mice [54, 56, 57]. Histopathological analysis of colons obtained from mice with active disease reveals transmural inflammation, epithelial cell hyperplasia, PMN and mononuclear leukocyte infiltration, occasional crypt abscesses and epithelial cell erosions (Fig. 16.1). Depending upon the strain of the donor and recipient, reconstituted mice exhibit varying degrees of weight loss and diarrhea/loose stools. Historically, the T-cell transfer model has been described as a Th1 model of colitis because of the large increase in IFN- γ production within the inflamed tissue [54]. However, we and others have found that the inflammation induced in this model represents a mixed Th1/Th17 inflammation because $CD4^+$ T cells isolated from the spleen, mesenteric lymph nodes, and colonic lamina propria of colitic mice produce both cytokines (Fig. 16.2). The major advantages of this model are that one may examine the earliest immunological events associated with the induction of gut inflammation, as well as the time-dependent induction of disease. Furthermore, chronic colitis can be attenuated by treatment with a variety of different pharmacologic, immunologic, biologic, and antibiotic treatment protocols (Table 16.2) [9, 58–65]. In addition, this model has been proven to be very useful to study the role

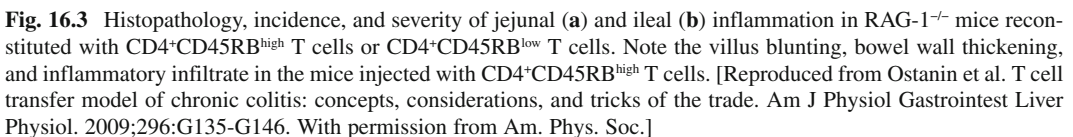
Table 16.2 Pharmacological studies using mouse models of chronic gut inflammation

Model	Therapeutic	Efficacy
IL-10 ^{-/-}	TNF/TNFR antisense	Yes
	IL-6/IL-6R mAb	Yes
	IFN γ /IFN γ R mAb	Depends on timing
	IL-12/IL-23 mAb	Yes
CD45RB ^{high} $\rightarrow$ SCID/RAG	OX40L mAb	Yes
	B7-H1 mAb	Yes
	CD70 mAb	Yes
	Anti-angiogenic peptide	Yes
	FTY720	Yes
	FasL mAb	No
	Dexamethasone/prednisolone	Yes
	Azathioprine	No
	Sulfasalazine/5-ASA	No
	Cyclosporine	No
	Tacrolimus	No
	TNF/TNFR mAb	Yes
	Integrins mAb (β 7,MAC-1,LFA-1, α 4)	Yes
	IL-6/IL-6R mAb	Yes
	IFN γ /IFN γ R mAb	Yes
	IL-12/IL-23 mAb	Yes
TNF ^{AAARE}	β 7 mAb	Yes
	TNF/TNFR mAb	Yes
SAMP1/Yitc	Dexamethasone	Yes
	TNF/TNFR mAb	Yes
	β 7,MAC-1,LFA-1, α 4 mAb	Yes
	IL-6/IL-6R mAb	Yes
	IFN γ /IFN γ R mAb	Yes
	IL-12/IL-23 mAb	Yes
	CCR9 mAb	Yes

Based on data from references [9, 15, 19, 34, 38, 47, 50, 60, 62, 63, 65, 66, 73, 79]
mAb monoclonal antibody

that Tregs play in suppressing or limiting the onset and/or severity of colonic inflammation [66–68]. Furthermore, Te Velde and coworkers have found, using genome-wide gene expression profiling of inflamed colons, that the pattern of gene expression in the CD45RB^{high} T-cell transfer model most closely reflects the altered expression profile observed in human IBD when compared to the other, chemically induced models of colitis [18]. Disadvantages of this model include the expense, the requirement for cell sorting and recognition that the inflammation induced in this model arises from the homeostatic proliferation of naïve T-cells.

The original reports describing this model suggested that the inflammation was largely confined to the colon; however, more recent data demonstrate that the transfer of naïve T-cells into RAG-1^{-/-} (or TCR β ^{-/-} $\times$ δ ^{-/-}) mice induces both colitis and small bowel inflammation (Fig. 16.3) [54, 69, 70]. We and others have found that the small intestine of these reconstituted RAG-1^{-/-} mice exhibited loss of Goblet and Paneth cells along the entire length of small intestine, which was most noticeable in the distal portion [54]. This model may prove useful for investigators who wish to ascertain whether the immunological mechanisms responsible for colitis are similar to those that induce inflammation of the small intestine. The reasons for the relative paucity of reports by other laboratories describing small intestine inflammation in this model are not known; however, most of the original studies used SCID recipients whereas we have used RAG-1^{-/-} mice. It is well-known that



SCID mice are “leaky” in that they can begin to develop B cells with age which may influence the development of intestinal inflammation. In addition to the small intestine, reconstitution of RAG-1^{-/-} mice with naïve T cells induces chronic liver and lung inflammation. We have observed extensive periportal and lobular lymphocytic inflammation, hepatocellular necrosis but no bile duct damage (Fig. 16.4a) [53]. In addition, we have found that virtually all colitic mice develop chronic bronchitis/pulmonary inflammation (Fig. 16.4b) [71, 72]. In many cases (>60%) we observed confluent inflammation merged together between small- and medium-sized bronchi. Varying degrees

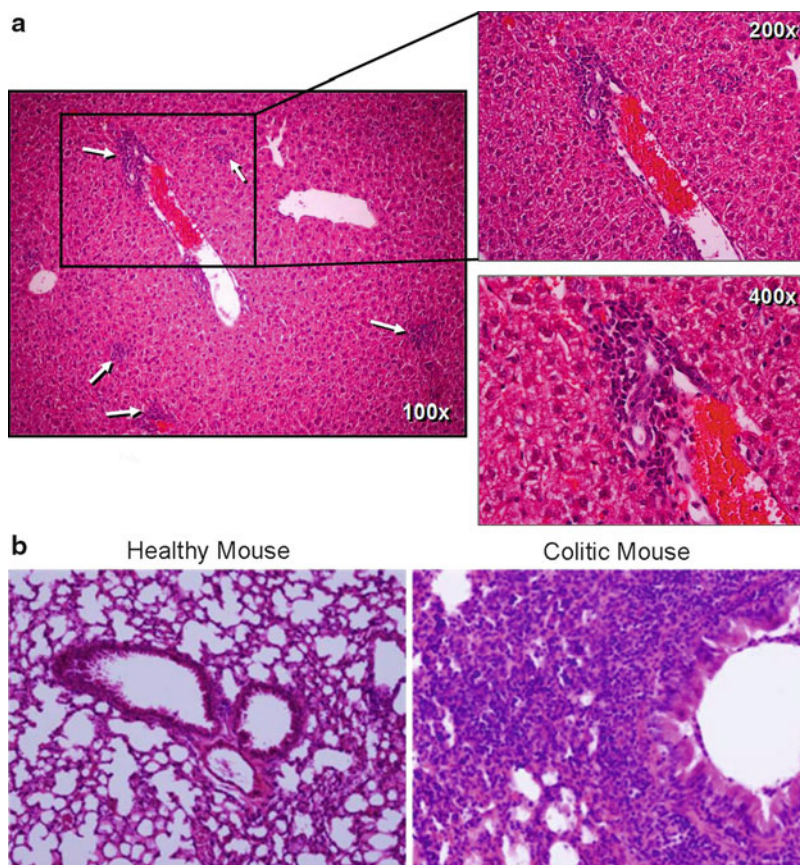


Fig. 16.4 Adoptive transfer of CD4⁺CD45RB^{high} T cells into RAG-1^{-/-} recipients induces chronic liver and lung inflammation. (a) Histopathology of the liver. Note the periportal and intralobular inflammatory infiltrate composed primarily of mononuclear cells. [Reproduced from Ostanin et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G135–G146. With permission from Am. Phys. Soc.]. (b) Histopathology of lungs obtained from healthy wild-type or RAG-1^{-/-} mice reconstituted with CD4⁺CD45RB^{high} T cells (8 weeks post transfer). Virtually all colitic mice presented with chronic bronchitis/pulmonary inflammation with the majority of these animals exhibiting confluent inflammation merged together between small and medium-sized bronchi [71, 72]. Varying degrees of perivascular lymphocytic cuffing were also observed in most of the mice; however, few PMNs were observed and vasculitis was absent

of perivascular lymphocytic cuffing were also observed in virtually all of the mice; however, few PMNs were observed and vasculitis was absent.

A second, widely used model of chronic colitis is the IL-10^{-/-} model. Mice with targeted disruption (i.e., deletion) of the IL-10 gene develop spontaneous pancolitis and cecal inflammation by 2–4 months of age [1, 73]. Histopathological analysis of colons obtained from mice with active disease show many of the same characteristics as those observed in human CD including transmural infiltration of mononuclear leukocytes, T-cells, and plasma cells. The genetic background of the mouse is a major modifier of disease with disease penetrance and severity occurring to a much greater extent in 129SvEv IL-10^{-/-} than in C57Bl/6 or Balb/c IL-10^{-/-} animals. The advantage of using the IL-10^{-/-} model is that it is a well-established Th1 model of transmural colitis and cecal inflammation that can be attenuated by administration of various pharmacologic, immunologic, biologic, antibiotic, and probiotic agents (Table 16.2) [9]. However, the onset and severity of disease is variable and in some cases requires many months to develop.

A third model of chronic colitis that is receiving increasing attention is the multidrug resistance pump 1a-deficient (*Mdr1a*^{-/-}) mouse [74]. *Mdr1a* is a P-glycoprotein expressed on the surface of several different cell populations including intestinal epithelial cells, T-cells, monocytes, macrophages, natural killer cells, dendritic cells, and brain microvascular endothelial cells. This transport protein pumps small amphiphilic and hydrophobic molecules across the cell membrane in an ATP-dependent manner. Interestingly, *Mdr1a*^{-/-} mice develop a moderate to severe colitis beginning at 12–15 weeks of age when housed under specific pathogen-free conditions [74]. The colonic inflammation can be significantly reduced by administration of antibiotics demonstrating yet another model of IBD where inflammation is dependent upon the presence of commensal bacteria. Histopathological analysis of colons obtained from these mice reveals an inflammation that is similar in many respects to human UC. There is substantial thickening of the mucosa, extensive infiltration of inflammatory cells into the lamina propria and occasional crypt abscesses and ulcerations. The mechanisms by which ablation of the *Mdr1a* gene induces chronic gut inflammation are not known at the present time; however, it is known that the induction of colitis in these mice is dependent upon defective *Mdr1a* activity on the intestinal epithelial cells and not the lymphocytes [75]. It may be that the lack of intestinal epithelial cell *Mdr1* results in increased accumulation of enteric antigens within these cells. Antigen-loaded cells could then process and present these microbial antigens to T cells immediately underlying the epithelium thereby priming the T cells to become “hyper-reactive” to the enteric microbiota and drive colonic inflammation [74, 75]. More recent studies have demonstrated a significant dysregulation of expression of cytokines and chemokines prior to onset of colitis in young *Mdr1a*^{-/-} mice. These data suggest that the intestinal immune system in these mice may be in a continuous state of immune activation which makes the mice particularly susceptible to induction of chronic gut inflammation [74]. Another interesting aspect of this model is that *mdr1a* gene is located on chromosomal region 7q21.1 which is one of the chromosomes that has been shown to be associated with both CD and UC susceptibility [74].

The large majority of mouse models displaying chronic colitis require the participation of the adaptive immune system for expression of disease. However, recent evidence suggests that innate immune cell activation alone is capable of inducing and perpetuating protracted intestinal inflammation [49, 76, 77]. For example, Maloy et al. have developed a mouse model of innate immune cell-mediated chronic colitis that is induced by associating lymphopenic *RAG*^{-/-} animals with the *Helicobacter hepaticus* (Hp) [49, 77]. Although Hp does not normally induce chronic gut inflammation in healthy/immunocompetent mice, introduction of this gram-negative, spiral bacterium into *RAG*^{-/-} animals establishes a life-long colonization that ultimately induces chronic cecal and colonic inflammation (typhlocolitis) [49, 76, 77]. This IL-23-driven inflammation is characterized by epithelial cell hyperplasia and extensive infiltration of PMNs and monocytes which is more severe in the cecum than in the colon [77, 78]. A recent variation of this model has been described in which chronic colitis can be induced in immunocompetent mice by combining the introduction of Hp with systemic administration of a mAb to IL10R [79–81]. Another, interesting model of innate immune cell-driven chronic colitis was recently described by Garrett and coworkers [76]. These investigators demonstrated that ablation of T-bet (a T-box transcription factor family member) in *RAG*^{-/-} mice results in the development of spontaneous and *communicable* colitis that is similar to human UC. The colonic inflammation in these *T-bet*^{-/-} × *RAG*-2^{-/-} ulcerative colitis (TRUC) mice is characterized by remarkable PMN and monocyte inflammation, crypt abscesses, bowel wall thickening, erosions, and ulcerations. These investigators also demonstrated colonic epithelial barrier disruption prior to the onset of active colitis. The fact that colitis is communicable and will develop in wild-type mice when housed with TRUC mice suggest the selection of a colitogenic microbiota that develops in response to immune defects [76]. This model may represent an important new addition to the toolbox of IBD investigators who wish to examine the relationship among the innate immune system, mucosal barrier function, and chronic gut inflammation.

Models of Chronic Small Bowel Inflammation

In addition to the well-characterized models of chronic colitis described above, two models of spontaneous small bowel inflammation are gaining increasing interest for studying the immunopathology of Crohn's ileitis as well as for evaluating new drug therapies. One model is the TNF-over-expressing $TNF^{\Delta ARE}$ mouse. These mice were developed by targeted deletion of a 69 base pair region of the AU-rich elements in the 3' UTR region of the TNF- α gene [82]. The deletion results in increased TNF- α mRNA stability and enhanced TNF- α protein production. Mice possessing the deletion in the homozygous state display severe and rapid wasting and die within 1–3 months of age. These mice are runted in appearance, exhibit alopecia-like lesions, and develop chronic and degenerative inflammatory arthritis. Interestingly, there appears to be a gene dosage effect as heterozygote littermates (HET ; $TNF^{\Delta ARE/+}$) develop a slower onset of disease and live at least 7–8 months. Histopathological analysis of the GI tract of both $TNF^{\Delta ARE}$ and their HET littermates at 2–3 weeks of age reveals inflammation within the terminal ileum. Lesions consist of villous blunting (and broadening) associated with the transmural infiltration of acute and chronic inflammatory cells, including mononuclear leukocytes, plasma cells, and PMNs. In general, severe ileitis is observed by 3 weeks of age in $TNF^{\Delta ARE}$ mice and 8 weeks in the HET s. This transmural inflammation extends deep into the muscular layers of the bowel wall, displaying characteristics of Crohn's ileitis. In fact, older mice (4–7 months of age) begin to display granulomatous lesions similar to what is seen in CD. The inflammation observed in this model can be treated with several biologics and immune-modifying drugs used in human medicine (Table 16.2). This model offers the unique opportunity to examine the immunopathological mechanisms underlying TNF-induced ileitis and arthritis with the possibility of identifying new therapeutic strategies to treat patients with CD.

The $SAMP1/Yit$ model of chronic ileitis is another model that is gaining increasing popularity and is readily available to interested investigators. As with $TNF^{\Delta ARE}$ mice, these animals are useful for studying the underlying mechanisms involved in the pathogenesis of Crohn's ileitis and preclinical evaluation of new drug therapies. This model of spontaneous ileitis was originally generated by >20 generations of brother–sister mating of a senescence-accelerated mouse line [83]. $SAMP1/Yit$ mice are unique in that 100% of these animals develop spontaneous ileitis at ~30 weeks of age without any genetic, immunological, or environmental manipulation. Histopathological analysis of these mice reveal robust and chronic ileitis that is similar in many respects to human CD. The segmental inflammation is localized to the terminal ileum, displays transmural involvement, and occasional granulomas. Chronic ileitis can be adoptively transferred by CD4⁺ T cells to SCID or RAG^{-/-} recipients suggesting that activated CD4⁺ T cells may home specifically to and recognize antigens within the terminal ileum [84]. Over the past few years, it was found that with further brother–sister matings for an additional 20 generations, a new phenotype emerged in which chronic ileitis developed by 10 weeks of age with the occurrence of perianal disease, ulceration, and fistula in a small subset of these mice. This substrain has been renamed $SAMP1/YitFc$ and has been propagated through continued brother–sister mating [85]. Studies using the $SAMP1/YitFc$ model have shown that immune-blockade of Th1- polarizing cytokines or certain T-cell adhesion molecules attenuate the severity of ileitis (Table 16.2). Thus, both the $TNF^{\Delta ARE/\Delta ARE}$ and the $SAMP1/YitFc$ mice provide the investigator with the unique ability to investigate the underlying immune-pathological mechanisms responsible for the development of ileal inflammation. An interesting yet perplexing observation that is common to virtually all mouse models of chronic small and/or large bowel inflammation is the lack of frank ulcerations with overt intestinal bleeding. The reasons for this are not known at the present time but are worthy of investigation. In summary, it is clear that at least 5 well-characterized mouse models of *chronic* small and/or large bowel inflammation are readily available for use in pharmacological studies.

Conclusions

Most therapeutic agents used in clinical practice were originally developed and tested in animal models. Retrospective analyses of preclinical intervention studies using different disease models demonstrate that few interventions/therapeutics reporting promising effects translate to clinical efficacy with only 10% of these therapeutic agents approved for use in patients [86, 87]. It is becoming increasingly appreciated that the failure to translate therapeutic efficacy from bench to bedside may be due, in part, to selection of an animal model that does not express the immunopathologic features of the human disease under investigation. This is especially true for preclinical investigations using mouse models of IBD. Thus, it is imperative to identify the most immunologically relevant mouse models of human CD or UC. It is hoped that by providing an objective evaluation of the different mouse models of IBD, investigators will be able to choose more appropriate models for their preclinical investigations.

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Part III

Diagnostic Approach

Chapter 17

Enhanced Endoscopy in Inflammatory Bowel Disease

Ralf Kiesslich

Keywords IBD • High-resolution endoscopy • High-definition endoscopy • Chromoendoscopy • Autofluorescence • NBI • i-scan • FICE • Endomicroscopy

Introduction

Patients with longstanding extensive ulcerative colitis as well as patients with Crohn's-associated colitis are at increased risk of developing colorectal cancer [1]. Colonoscopic surveillance is recommended in those patients to reduce associated mortality [2]. Surveillance relies on the detection of premalignant dysplastic tissue, and where multifocal dysplasia is detected, proctocolectomy remains the management of choice for colitis-associated neoplasia.

In general, the prognosis for patients with malignancies of the GI-tract is strictly dependent on early detection of premalignant and malignant lesions. However, small, flat, or depressed neoplastic lesions [like colitis-associated neoplasias] remain difficult to detect with conventional endoscopic technologies thereby limiting their value for polyp detection and cancer screening.

Thus, what should an ideal enhance imaging method to be able to accomplish? Three diagnostic steps are important: recognition, characterization, and confirmation (see Fig. 17.1).

Recognition of lesions in the gut can be improved using better scopes which have better imaging modalities like high resolution or high definition. Also auto fluorescence might help to recognize lesions better. Characterization of lesion type and surface architecture is important to predict histology, which can be eased with chromoendoscopy, digital chromoendoscopy, or new software filters.

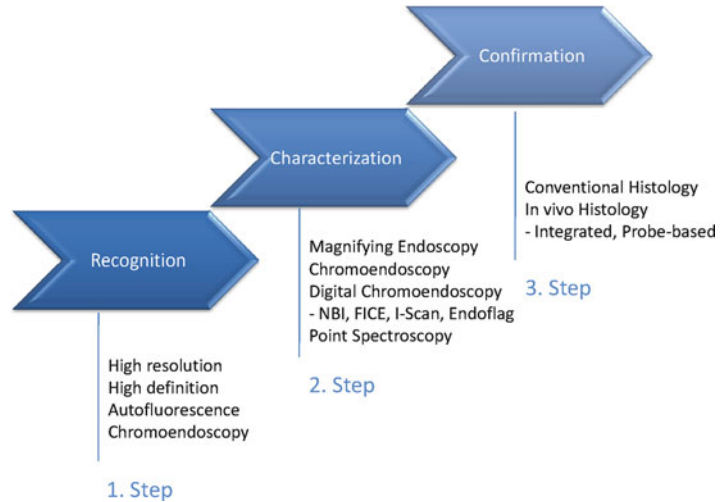
Here, a proposal for a consensus terminology for new imaging modalities is available and should be used [3].

Finally, histological confirmation is needed to define whether cancer is present or not. This can be accomplished by conventional or most recently with in vivo histology (confocal laser endomicroscopy (CLE)). Here, molecular imaging might open a new door of tailored and individualized diagnosis.

R. Kiesslich, MD, PhD (✉)

Endoscopy Department, I. Med. Klinik und Poliklinik, Universitätsmedizin Mainz,
Johannes Gutenberg Universität Mainz, Langenbeckstrasse 1, Mainz, Deutschland 55131, Germany
e-mail: kiesslich@uni-mainz.de

Fig. 17.1 Enhanced endoscopic technologies to improve the three essential steps to final diagnosis



High-Resolution, High-Definition, and Magnifying Endoscopy

High-resolution and magnification endoscopes offer image quality that is significantly better than that of first-generation video endoscopes or the older fiberoptic systems. The *resolution* of an endoscopic image is a different quality from the *magnification*, and is defined as the ability to distinguish between two points that are close together. High-resolution imaging improves the ability to discriminate details while magnification enlarges the image. In digital video imaging, resolution is a function of pixel density. By incorporating high-pixel density charged-coupled devices (CCD), high-resolution endoscopes provide slightly magnified views of the gastrointestinal tract with greater mucosal detail. Magnification endoscopy utilizes a movable lens controlled by the endoscopist to vary the degree of magnification, which ranges from $\times 1.5$ to $\times 150$. Newly designed magnification endoscopes provide high-resolution and magnification features [4].

Most recently high-definition endoscopes became broadly available. Here, CCDs convert light information into an electronic signal. This signal is processed in the video-processor into an image. The standard analogue broadcasting systems (PAL or NTSC) generate approximately 480–576 scanning lines on a screen. The new high-definition endoscopes can generate up to 1,080 scanning lines on a screen, which further increases the resolution. Surface analysis on distinct lesions can be done even before magnification (see Fig. 17.2). There are convincing data about screening colonoscopy emerging showing that high-definition endoscopy lead to an increased detection of patients having at least one adenoma [5].

Chromoendoscopy

Chromoendoscopy or tissue staining is a relatively “old” endoscopic technique that has been used for decades. It involves the topical application of stains or pigments to improve localization, characterization, or diagnosis of lesions [6]. It is a useful adjunct to endoscopy, the contrast between normally stained and abnormally stained epithelium enables the endoscopist to make a diagnosis and/or to direct biopsies based on a specific reaction or enhancement of surface morphology (see Fig. 17.2).

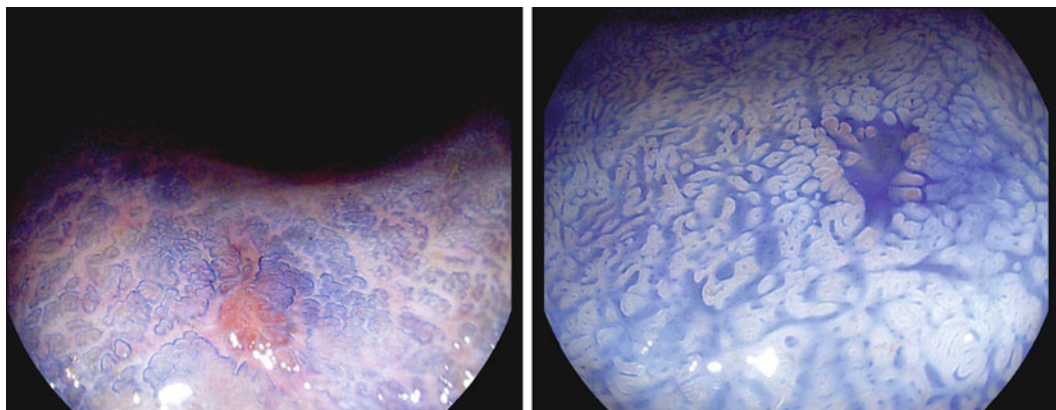


Fig. 17.2 Flat and depressed colitis-associated neoplasia diagnosed with chromoendoscopy [methylene blue]

The technique for staining is simple and easy to learn. Chromoendoscopy can be done in an untargted fashion of the whole segment (Panchromoendoscopy) or directed towards a specific lesion (targeted staining). While spraying dyes in the colon in an untargted fashion, the endoscopist needs to direct the endoscope and catheter tip towards the colorectal mucosa and use a combination of rotational clockwise–counter clockwise movements with simultaneous withdrawal of the endoscope tip.

Surface analysis of stained colorectal lesions was a new optical impression for the endoscopists in the 1990s. First, Kudo et al. described that some of the regular staining patterns are often seen in hyperplastic polyps or normal mucosa, whereas unstructured surface architecture was associated with malignancy. Also the kind of adenoma (tubular vs. villous) can be seen by detailed inspection. This experience has led to a categorization of the different staining patterns in the colon: the so-called pit-pattern classification [7] differentiated five types and several subtypes. Types 1 and 2 are staining patterns predicting non-neoplastic lesions, whereas types 3–5 are predicting neoplastic lesions. With the help of this classification the endoscopist may predict histology with good accuracy. Although the pit-pattern classification was developed with the help of magnifying endoscopes, the question arises whether an endoscopic differentiation of different staining patterns can also be done with the help of the more common high-resolution endoscopes.

Chromoendoscopy has shown significant advantages in the detection of flat colorectal neoplasia and in colitis-associated neoplasia [8].

Magnifying chromoendoscopy using either methylene blue or indigo carmine is a valid and proven tool for improving endoscopic detection of intraepithelial neoplasia in patients with long-standing ulcerative colitis. Chromoendoscopy increased the diagnostic yield of intraepithelial neoplasia as compared with conventional colonoscopy 3–4.5-fold. Differentiation of non-neoplastic from neoplastic lesions is possible with a high overall sensitivity and specificity. Chromoendoscopy is endorsed in multiple guidelines as a superior and recommended alternative to conventional colonoscopy with random biopsies [9].

Digital Chromoendoscopy

Conventional white light endoscopy uses the full visible wave length range to produce a red–green–blue image. In contrast, narrow band imaging, in combination with magnification endoscopy, illuminates the tissue surface using special filters that narrow the respective red–green–blue bands.

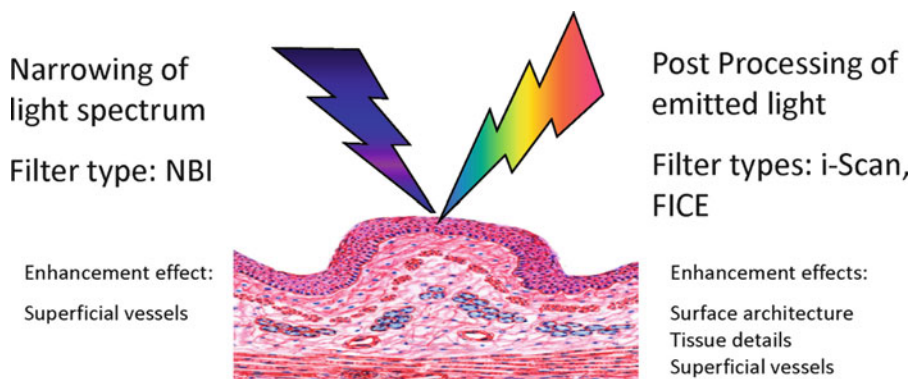


Fig. 17.3 Types and function of digital chromoendoscopy (filter-aided endoscopy)

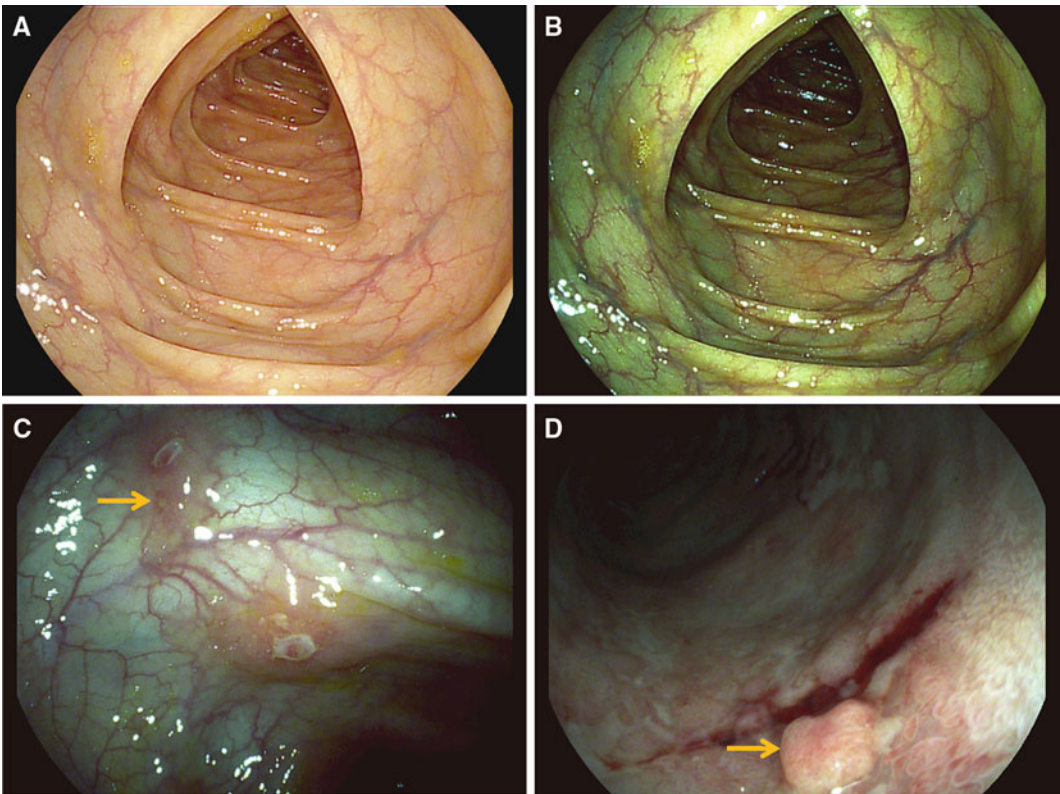


Fig. 17.4 Filter-aided colonoscopy in IBD. (a) Normal colonic mucosa with and (b) without i-scan imaging, (c) inflamed mucosa, and (d) neoplastic changes visualized with i-scan

This enhances the tissue microvasculature mainly as a result of the differential optical absorption of light by hemoglobin in the mucosa associated with initiation and progression of dysplasia, particularly in the blue range. To some extent, the resulting images look like “chromoendoscopy without dyes” (see Figs. 17.3 and 17.4).

Alternatively, the light which is reflected from the mucosal can be modified using post-processing computer algorithms (FICE, i-Scan). This can modulate different forms of enhancement, which

leads to an accentuation of the vasculature, the surface architecture, or the pattern visualization (see Figs. 17.3 and 17.4). However, none of these filters has proven so far any benefit diagnosing colitis-associated neoplasia [10, 11].

Autofluorescence Imaging

A new autofluorescence imaging (AFI) system has recently been developed that uses a video endoscope with two CCDs: one for high-resolution white light endoscopy and one for AFI. The autofluorescence image is a pseudo-color image composed from three integrated images: (1) the total autofluorescence after blue light excitation (395–475 nm), (2) the green reflectance (540–560 nm), and (3) the red reflectance (600–620 nm).

Most recently a new system has become available that incorporates high-resolution endoscopy, AFI, and NBI in one single system: endoscopic tri-modality imaging (ETMI). This system has a new autofluorescence algorithm in which the fluorescence image is composed of two integrated images instead of three images: total autofluorescence after blue light excitation (395–475 nm) and green reflectance. Autofluorescence in conjunction with NBI can help to unmask colitis-associated neoplasias [12].

Optical Coherence Tomography

Optical coherence tomography (OCT) is a high-resolution cross-sectional imaging technique. It is analogous to B-mode high-resolution endosonography but uses light waves instead of acoustic waves. As a result, OCT has a high resolution, up to 10 times higher than high-frequency ultrasound, enabling microstructural features of tissue to be identified, but it has a limited sampling depth of 1–2 mm [13]. OCT measures the intensity of back-scattered light from tissue at various depths using low-coherence interferometry.

OCT is a probe-based technique in which the probe is passed through the accessory channel of an endoscope. Unlike endosonography, OCT can be performed without a coupling media (e.g., water). The OCT catheter has to be positioned adjacent to the mucosa within the system's focal distance (1–5 mm), which may be difficult due to the movement of the esophagus by peristalsis and heartbeat. In addition, compression artifacts may be seen when the probe comes in contact with the mucosa. OCT can easily differentiate normal epithelium from abnormal tissue with high accuracy. However, the detection and grading of high-grade dysplasia and early cancer remain still challenging [14].

Future developments in OCT, such as ultra-high resolution OCT, spectroscopic OCT, Doppler OCT, and optical frequency-domain imaging, may enhance the accuracy of OCT for the detection of dysplasia and give rise to other applications.

Endocytoscopy

Endocytoscopy is based on the principle of light contact microscopy. The first studies using this system were performed in the field of otolaryngology. After application of methylene blue, the tip of a rigid endoscope was placed in direct contact with the surface. With this method cytological details can be directly visualized, making direct observation of living cells feasible [15]. The first endocytoscopy system was, however, a rigid instrument, which is not practical for use in the gastrointestinal tract. Therefore, a novel endocytoscope system has been developed, the Endocytoscope.

This system consists of two flexible endoscopes with a diameter of 3.4 mm that can easily pass through an accessory channel with a diameter of 3.7 mm: one low-magnification endocytoscope with a maximal magnification of 450 $\times$ and a high-magnification endocytoscope with a maximal magnification of 1,100 $\times$. This system has been recently evaluated for the detection of inflammatory lesions of the small intestine [16]. Most recently, the integration of the endocytoscope within an otherwise conventional endoscope could be achieved.

Endocytoscopy is used to identify cellular and nuclei architecture of the surface layer of the epithelium. Residual mucin and moving artifacts can alter its accuracy. However, the combination of macroscopy (white light endoscopy) and microscopy (endocytoscopy) has led to better understanding of the surface micro-architecture.

The current system, however, cannot visualize the deeper layers of the epithelium and is, therefore, not yet suited for evaluating early neoplasia with respect to depth of invasion. In addition, this system requires the use of 1% methylene blue which significantly decreases the brightness of the white light image and may increase DNA damage in the tissue after illumination.

Confocal Laser Endomicroscopy

CLE is a new imaging modality for gastrointestinal endoscopy. It offers in vivo imaging of the mucosal layer at cellular and even subcellular resolution. Thus, in vivo histology becomes possible during ongoing endoscopy. This new imaging modality provides more than conventional histology, because cellular interaction can be observed over time (physiology) and distinct changes can be identified (pathophysiology) [17].

Principle of Confocal Microscopy and Types of Endoscopic Endomicroscopes

Confocal microscopy allows a better spatial resolution compared with conventional fluorescence microscopy, because images are not contaminated by light scattering from other focal planes. A low-power laser is focused to a single point in a defined microscopic field of view and the same lens is used as both condenser and objective folding optical path. Thus, the point of illumination coincides with the point of detection within the specimen. Light emanating from that point is focused through a pinhole to a detector and light emanating from outside the illuminated spot is rejected from detection. Illumination and detection system are at the same focal plane and termed as “confocal.” All detected signals from the illuminated spot are captured and measured. The created grey scale image is an optical section representing one focal plane within the examined specimen. The image of a scanned region can now be constructed and digitized by measuring light returning to the detector from successive points.

The first publication about an integrated confocal fluorescence microscope into the distal tip of a conventional colonoscope (Pentax EC 3830FK, Tokyo, Japan) was made in 2004 [18] showing that in vivo microscopy at subcellular resolution (0.7 μ m) simultaneously displayed to white light endoscopy became possible and achieved high accuracy. This approach, designated CLE, permitted immediate diagnosis of colorectal intraepithelial neoplasias using fluorescein or acriflavine as contrast agents.

More recently a probe-based confocal endomicroscope was developed which can be passed over the working channel of standard endoscopes [19] (see Fig. 17.5).

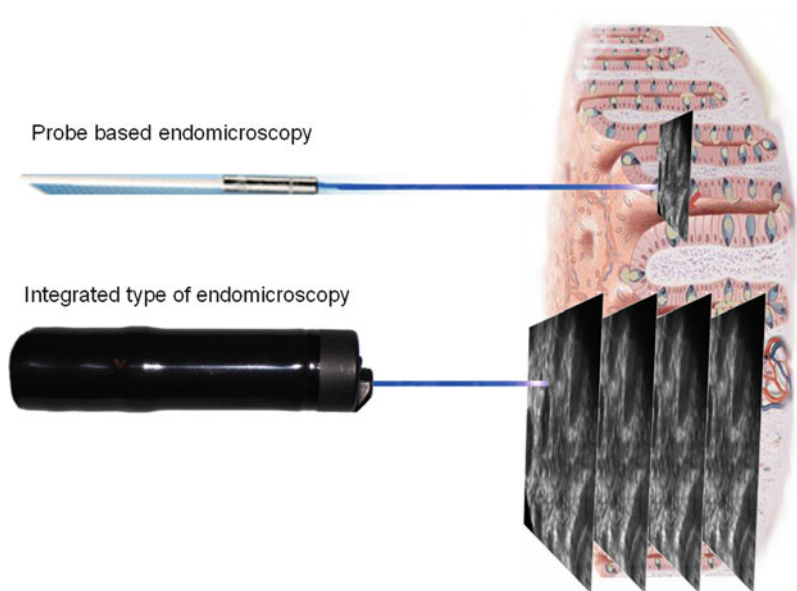


Fig. 17.5 Types of endomicroscopy

Contrast Agents

Fluorescence confocal imaging is only possible using exogenous fluorescence contrast agents. Potentially suitable agents are fluorescein, acriflavine, or cresyl violet [17]. The most common and safe contrast agent is fluorescein sodium (5–10 ml of a 10% solution; intravenous application) [20].

Clinical Data of Endomicroscopy

Endomicroscopy enables to predict and see histological changes with high accuracy based on a simple classification which includes vessel and cellular detail [18]. Endomicroscopy is more accurate than high-definition or filter-aided endoscopy [21] (see Fig. 17.6).

Endomicroscopy further expands the diagnostic possibilities of chromoendoscopy in patients with inflammatory bowel diseases. Chromoendoscopy is able to reveal circumscribed lesions, and confocal laser microscopy can be used to confirm intraepithelial neoplasias with a high degree of accuracy [22]. Biopsies can therefore be limited to targeted sampling of relevant lesions. In vivo histology with endomicroscopy may lead to significant improvements in the clinical management of patients with ulcerative colitis, with reduced numbers of biopsies being needed for confirmation of the condition and time being gained for immediate therapeutic intervention.

Future of Endomicroscopy

Endomicroscopy can not only be used to receive histology. The great potential of endomicroscopy is to display and observe physiologic and pathophysiologic changes during ongoing endoscopy. Furthermore, molecular imaging becomes possible.

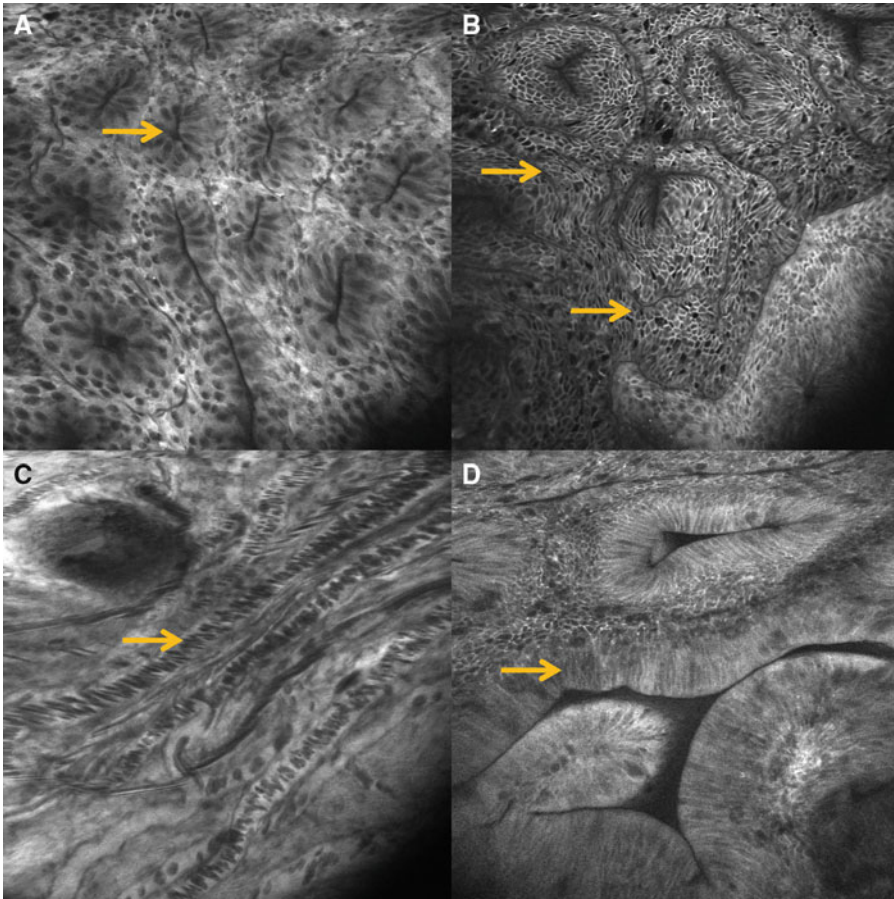


Fig. 17.6 Image examples of endomicroscopy. (a) Normal colonic crypts (*arrow*) with goblet cells, (b) hyperplastic changes (*arrow*), (c) vessels in inflamed mucosa with visible red blood cells (*arrow*), and (d) tubular changes with loss of goblet cells (low-grade intraepithelial neoplasia)

Cell shedding is a physiologic process. After cell shedding an epithelial gap occurs, this is sealed within seconds. Patients with inflammatory bowel disease show malfunction of gap closure which can lead to invasion of bacteria into the lamina propria. These changes can be observed with endomicroscopy and might define new options for treatment of inflammatory bowel diseases [23]. Endomicroscopy displays not only tissue but also bacterial interaction with the mucosal layer can be seen [24] (see Fig. 17.7).

Molecular imaging is already achieved [25]. Dysplastic colonic crypts could be selectively stained with heptapeptides which were linked with fluorescein. This approach will open the door for new clinical algorithms, which will be dependent on the endomicroscopic findings (e.g., prediction of the efficiency of chemotherapy with biological). Also distinct receptors can be visualized [26, 27] (see Fig. 17.7).

Conclusion

New imaging modalities of gastrointestinal endoscopy are rapidly evolving and they will greatly influence every day work in the very near future. Patients with IBD will substantially profit of these new developments because they face an increased cancer risk even at young age.

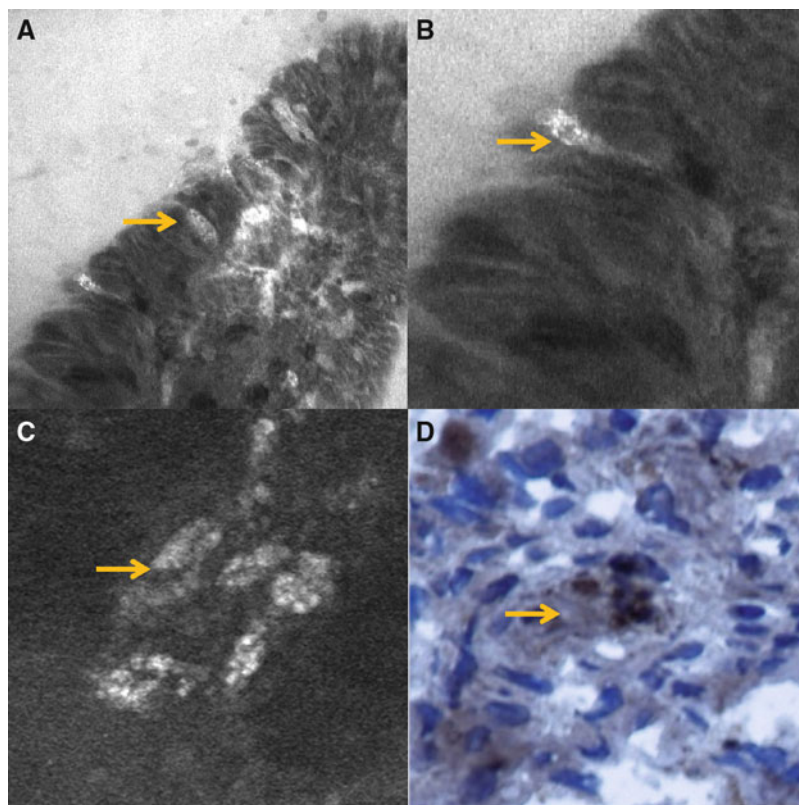


Fig. 17.7 Functional and molecular imaging using endomicroscopy. (a) Cell shedding with visible gap in the terminal ileum (epithelial cell layer) of a patient with Crohn's disease, (b) translocation of bacteria (*bright spots*) can be visualized. Fluorescein-labeled antibodies can visualize VEGF-Receptors (c) in vivo which correlates with ex vivo immune-histochemistry (d)

The progress of endoscopic techniques follows substantial innovations in information technology (IT) and multimedia. We are currently facing only the beginning of a new era where computer-assisted solutions and technical innovations will greatly influence our diagnostic strategies.

Main goal is the early detection of gastrointestinal cancer, because it is highly cost-effective and convincing. Evolving technologies are helping to improve our diagnosis from recognition over characterization to confirmation.

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Chapter 18

MRI in Inflammatory Bowel Disease

Patrik Rogalla and Tanya Chawla

Keywords MRI • Enteroclysis • Enterography • Multi-planar • Functional • Motility • Non-invasive • Radiation • Disease activity • Perianal

Introduction

Cross sectional imaging has largely replaced fluoroscopic and barium imaging due to its ability to optimally demonstrate the entire bowel as well as its depiction of extra-luminal pathology. Evolution in MR scanning techniques and increasing concern about radiation related sequelae has resulted in MR adopting a more prominent role in the radiological work-up and follow-up of patients with inflammatory bowel disease.

The estimated combined prevalence of ulcerative colitis (UC) and Crohn's disease (CD) is 1.4 million in the USA and 2.2 million in Europe and continues to rise with an approximate 31% increase since 1991 in the USA alone [1]. The diagnosis is most commonly made when patients are in their 20s and 30s, although up to 15% are diagnosed earlier in childhood. The clinical course of inflammatory bowel disease and in particular CD is characterized by a relapsing and remitting pattern. The most frequent site of disease involvement; the small bowel, is least amenable to endoscopic assessment. Small bowel involvement occurs in 70% of patients with CD and exclusive small bowel disease is seen in approximately 30% of cases. In the era of immune modulating drugs, there is a requirement to obtain frequent evaluations of disease severity and activity. In particular clinical and laboratory assessment of disease activity is unreliable.

Endoscopic methods are useful in the assessment of superficial mucosal disease and healing as well as in obtaining histological confirmation. They are less optimal in assessing extra-enteric complications. These include techniques such as double balloon enteroscopy, ileo-colonoscopy and wireless capsule endoscopy (CE). The presence of strictures may preclude endoscopic evaluation and limited availability and expertise may limit some of these techniques to tertiary referral centres.

P. Rogalla, MD

Department of Medical Imaging, Toronto General Hospital, University of Toronto, Toronto, ON, Canada

T. Chawla, MBBS, MRCP, FRCP, FRCPC (✉)

Abdominal Imaging Division, Joint Department of Medical Imaging, Mount Sinai Hospital,
University of Toronto, Room 567, 600 University Avenue, Toronto, ON, Canada M5G 1X5

e-mail: tchawla@mtsinai.on.ca

Thus there is increasing reliance on imaging. This has been tempered by growing concern with regard to cumulative radiation burden in this young population group [2]. There has been significant variance in reported doses from computed tomography (CT) examinations of the abdomen. Multiple studies have expressed concern and tried to quantify the effective dose delivered to this category of patients. Effective dose takes into account the absorbed dose of radiation and organ susceptibility to the radiation induced somatic or genetic effects and is measured in units of millisieverts (mSv). An analysis of a population based cohort of patients with inflammatory bowel disease found the radiation exposure in the cohort was similar to background radiation at around 3 mSv. The annual median effective dose for CD and UC was 3.1 and 1.2 mSv/year, respectively. CD patients however had a 2.46 times greater total effective dose as compared to those with UC [3]. This difference was largely contributed to by a difference in the numbers of CT scans between the two groups. As would be expected, another study looking specifically at patients with CD found higher doses in patients whose diagnosis was made at an early age, those who had penetrating disease or upper tract inflammation and who had required intravenous steroids, infliximab or had multiple surgical procedures [4].

Traditional mainstays of imaging including barium follow through and enteroclysis examinations, provide views of the bowel lumen and mucosal outline but fail to portray the full extent of extra-luminal involvement and can have limited sensitivity due to anatomical constraints of overlapping pelvic small bowel loops. Cross sectional imaging techniques such as CT and magnetic resonance imaging (MRI) have the unique ability to display the spectrum of macroscopic disease, extra-enteric disease and secondary complications.

MRI in particular with its capability for multi-planar imaging, high intrinsic soft tissue contrast as well as an absence of ionizing radiation is increasingly becoming the imaging gold reference standard for disease evaluation in this category of patients. Enhanced imaging with gadolinium-based sequences improves sensitivity for detection of areas of subtle inflammation and multi-phasic imaging can safely be performed following gadolinium without the risk of incremental radiation seen with multi-phasic CT. The safety profile of gadolinium-based contrast media is such that it may be administered when CT based contrast is contra-indicated. However, even in the absence of contrast material (for instance, if the patient has severe renal impairment, when there is concern regarding entities such as nephrogenic systemic fibrosis), non-enhanced MRI provides useful diagnostic images and depiction of pathology.

There are limitations to MRI however and access remains limited with economic constraints being another factor limiting the number of studies performed. Despite the improved robustness of new scanning techniques, MRI examinations are more time onerous than other modalities and suffer from lower spatial and temporal resolution as compared with CT. It may therefore be useful in this population sub-set to obtain baseline imaging with CT and perform follow-up with MRI as well as utilizing MRI as the primary modality when CT is contra-indicated, such as in pregnancy.

MR Enteroclysis vs. MR Enterography

When enteric contrast material is administered orally, the technique is called enterography. However, when it is directly given via an enteric tube, the procedure is called enteroclysis. Bowel distension is acknowledged as being superior via the enteroclysis route but is achieved by a greater degree of invasiveness and diminished patient tolerance [5].

Other than a technical benefit, MR enteroclysis (MRE) has been proven to have better diagnostic performance for depiction of mucosal abnormalities but similar results are obtained by both techniques when identifying stenoses and fistulas. Patient acceptance is greater when MR enterography is performed as compared with MRE. Finally, the additional cost and time entailed by having to perform and schedule a fluoroscopic tube insertion means many departments perform MR enterography in preference for both logistic and technical reasons. In a meta-analysis performed in 2008; however, the sensitivity of MR was lower ($p=0.16$) in establishing the diagnosis of

inflammatory bowel disease via the oral route (83.7%) as compared with enteroclysis (95.9%) [6]. Enteroclysis may be the preferred method of small bowel assessment in the setting of low-grade small bowel obstruction or in scenarios when the patient is unable to tolerate or ingest the oral contrast material.

MR Enteroclysis

Technique and Protocol

At a minimum we require a 4–6 h fast or preferably an overnight fast in this category of patients. Colonic loading with faecal matter is known to impair the transit of contrast material through the small bowel. In some institutions, the fasting is augmented by a low residue diet for between 3 and 5 days prior to the study. The rationale is also to minimize the number of false positives, which can be generated by stool particles masquerading as filling defects or polyps.

Small bowel distension can be achieved via nasojejunal intubation (enteroclysis) or orally (see below). X-ray fluoroscopic guidance is used to confirm catheter placement at or beyond the duodeno-jejunal flexure in the former technique. Baseline imaging is then obtained prior to instillation of 1.5–2 l of fluid at a rate of 100–120 ml/min using an infusion pump. The progress of the contrast and the degree of distension can be monitored via thick slabs or dynamic MR fluoroscopic sequences. These are typically true FISP (fast imaging with steady state precession), 5-mm section images aligned parallel to the longitudinal axis of the segment of concern. The images are acquired at between 0.5 and 2 frames per second. When the contrast reaches the ileocecal junction, we administer antispasmodics (see below).

The introduction of parallel imaging techniques as well as improvements in coil technology has contributed to the increased robustness of MRI as well as reduction in acquisition times. Individual protocols will be tailored depending on manufacturer and magnet strength, however, Table 18.1 gives a sample protocol used for both small and large bowel MRI. As a guideline, most sequences include a combination of T2-weighted and contrast enhanced gradient echo (GE) sequences. Half Fourier single shot turbo spin echo (HASTE) imaging is heavily T2-weighted and susceptible to both intra-luminal motion and flow artefacts. Fat suppression is imperative for improving visibility of subtle changes in signal intensity within the bowel wall and the adjacent peri-enteric fat.

Performance Characteristics

A meta-analysis (Table 18.2) conducted to assess the accuracy of four imaging modalities, specifically ultrasonography (US), MRI, CT and scintigraphy, found comparable accuracy for the diagnosis of inflammatory bowel disease [7]. Thirty-three studies were selected for inclusion in this meta-analysis. On a per patient basis, ultrasonography performed better in predicting the absence of IBD when both the small bowel and the colon were examined, nevertheless, this older meta-analysis may be less accurate in reflecting the current differences between CT enteroclysis and MRE or reflecting the rapid advances in technique seen in both modalities. On a segmental basis, CT was the least sensitive and specific for the diagnosis of IBD, whereas scintigraphy was slightly less specific on a per patient basis. MR imaging performed better via the enteroclysis technique than when contrast was administered orally. This was attributed to the inconsistent distension achieved via the oral route, which may mask segments of disease or alternately mimic diseased segments. This is contrary to the findings of another study which found no significant difference between MR enterography and MRE in a direct head to head comparison [8].

Table 18.1 Sample protocol for MR enteroclysis

Sequence	Plane	Number of sections	Section thickness (mm)	Field of view (mm)	Advantages	Limitations
HASTE/SSFSE	Coronal	1	50	512×512	Minimal susceptibility artefact	Prone to flow artefacts
True-FISP/ FIESTA	Coronal and axial	19–25	5/0	160–512× 160–400	Useful for extra-enteric visualization including mesentery and nodes when fat saturation absent	Susceptibility artefact
With and without fat suppression					In presence of fat suppression useful for enteric changes and subtle mural abnormality	
HASTE with fat saturation	Coronal and axial	19–25	2–4	As above		Mesenteric evaluation impaired due to k-space filtering
2D or 3D FLASH or VIBE with fat saturation	Dynamic coronal with axial delayed	52–64	1.5–2.5	288–400× 312–400	Mural assessment and evaluation of disease activity	3D better for spatial resolution and SNR but more prone to misregistration artefact

Table 18.2 Meta-analysis of four imaging modalities

Modality	Sensitivity (%)	Specificity (%)
US	89.7	95.6
MRI	93	92.8
Scintigraphy	87.8	84.5
CT	84.3	95.1

Mean sensitivity and specificity on a per-patient basis. With the exception of specificity for US and scintigraphy, there were no significant differences between all four imaging modalities for the diagnosis of IBD (based on data from [7])

A more recent study evaluated 33 patients with suspected active ileal CD that underwent CT enterography and ileo-colonoscopy, 30 of which subsequently underwent MR enterography. The sensitivities of MR enterography and CT enterography for detecting small bowel disease were very similar (90.5 versus 95.2%). In eight patients (24%) MR enterography and CT enterography identified inflammatory changes when endoscopy demonstrated normal ileal mucosa. In three cases, there was inability to intubate the ileum. This meant radiological input had an incremental benefit. The study also confirmed the complementary role of cross sectional imaging with endoscopy where mucosal assessment alone may underestimate the extent of transmural involvement. Image quality scores were high for both modalities but however lower than for CT enterography ($p=0.005$). MR studies were felt to be confounded by motion, artefacts and signal heterogeneities [9].

Prospective comparative studies of MRE and wireless CE have shown MRE to be less sensitive than CE although there was no statistically significant difference in diagnostic performance [10].

In patients with strong underlying suspicion and markers for CD, negative imaging should not deter assessment of mucosal involvement either by conventional endoscopy or conventional enteroclysis.

Characteristic Findings

The earliest changes of CD such as erythema, superficial aphthous ulcers and nodularity of the mucosa are difficult to appreciate on MR. An attempt to address this deficiency has been made by authors who have obtained high resolution thin section images (2–3 mm thick) aligned parallel to the bowel segment [11]. This allows a detailed depiction of the mucosa and provides in plane resolution of 1–2 mm.

There are several findings that are characteristic of inflammatory bowel disease and they are discussed in some detail below.

Wall, Fold and Mucosal Abnormality

A normal bowel wall should be no more than 3 mm in thickness if it is optimally distended. In CD, wall thickness ranges between 5 and 10 mm. The degree of mural thickening correlates well with the CD activity index (CAI) [12]. In the absence of mural oedema, the bowel wall is usually low to moderate in signal intensity on T2-weighted MR sequences. The black boundary artefact seen on true FISP sequences can confound assessment of wall thickness. HASTE sequences are relatively insensitive to this artefact and more accurate at estimating bowel thickness.

Fold abnormalities are more evident along the mesenteric border. They can manifest in several ways including diffuse fold thickening, ulceration of folds or in more severe disease as areas of cobblestoning. This is seen where there is a combination of longitudinal and horizontal ulcers. Adequate distension is the key to identifying these abnormalities as areas of luminal collapse may result in false negative or false positive evaluations.

Early areas of aphthous ulceration are typically seen as small focus of high signal intensity surrounded by a rim of oedema (seen as an area of moderate signal intensity). Deeper areas of penetrating ulcers are easier to appreciate than superficial ulcers. They are seen as areas of high signal intensity within areas of segmental wall thickening.

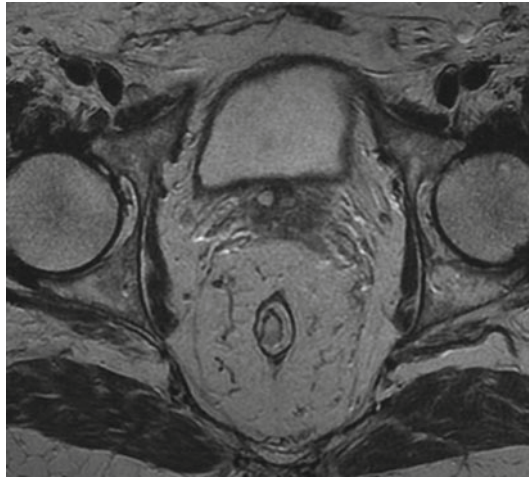
Strictures

These are seen as fixed, consistent areas of luminal narrowing. If there is upstream dilatation of bowel loops by greater than 3 cm, the stricture is considered to be functionally significant. If however there is no dilatation and a more than 10% narrowing in the lumen as compared with subjacent bowel, the stricture is most likely functionally insignificant.

Differentiating the chronicity of a stricture has important prognostic and management implications, and MRI can contribute significantly here. If there is acute mural oedema, it is seen as areas of high T2-weighted signal intensity within thickened loops of small bowel on fat saturated sequences. This is also an independent correlate of disease activity and is related to oedema in the mucosal or sub-mucosal layers [13, 14].

Fibrotic strictures are hypointense on T1- and T2-weighted sequences and enhance heterogeneously following contrast material injection. There is an absence of accompanying mesenteric

Fig. 18.1 Fatty replacement of the submucosal layer in long-standing Crohn's disease. Note the circular fat signal intensity within the bowel wall and proliferating perirectal fat



hyperaemia or inflammatory change. They may cause bowel obstruction and MR fluoroscopic sequences can be helpful in locating the level and degree of obstruction.

Fatty replacement of the sub-mucosa is also seen in long standing IBD and is seen as an area of high signal intensity (Fig. 18.1), which suppresses on fat saturated sequences.

Wall Enhancement

Mural hyper-enhancement in diseased segments is usually seen in comparison with adjacent normal loops of small bowel, which act as a reference standard. The pattern of enhancement is also useful in determining the level of activity that exists:

- *Stratified* enhancement refers to enhancement of the mucosa exceeding that of the poorly enhancing sub-mucosa. This is seen where there is active disease and sub-mucosal oedema. When combined with areas of serosal hyper-enhancement the bowel wall may adopt a target like appearance.
- *Diffuse* enhancement reflects transmural disease with uniform enhancement of the entire bowel wall.
- *Minimal* and somewhat heterogeneous enhancement is seen in areas where fibrosis predominates.

The absolute level of enhancement has been suggested as a surrogate marker of disease activity but its value is inconsistent and there may be considerable inter- and intra-observer variation [15]. In particular inadequate distension of segments of bowel may result in false positive assessment of hyper-enhancement. Areas of relative sparing along the anti-mesenteric border of the bowel may result in redundancy and pseudo-sacculation. In contra-distinction the diseased mesenteric wall is often shortened and may be fibrotic.

Extra-Enteric Manifestations

Mesenteric hyperaemia and vascular engorgement (vasa recta) are seen in conjunction with the *comb* sign. As its name implies it is seen as areas of parallel low signal intensity perpendicular to the long axis of the diseased segments of bowel on true FISP sequences. There is avid enhancement in these vessels following intravenous administration of contrast material. Mesenteric oedema like

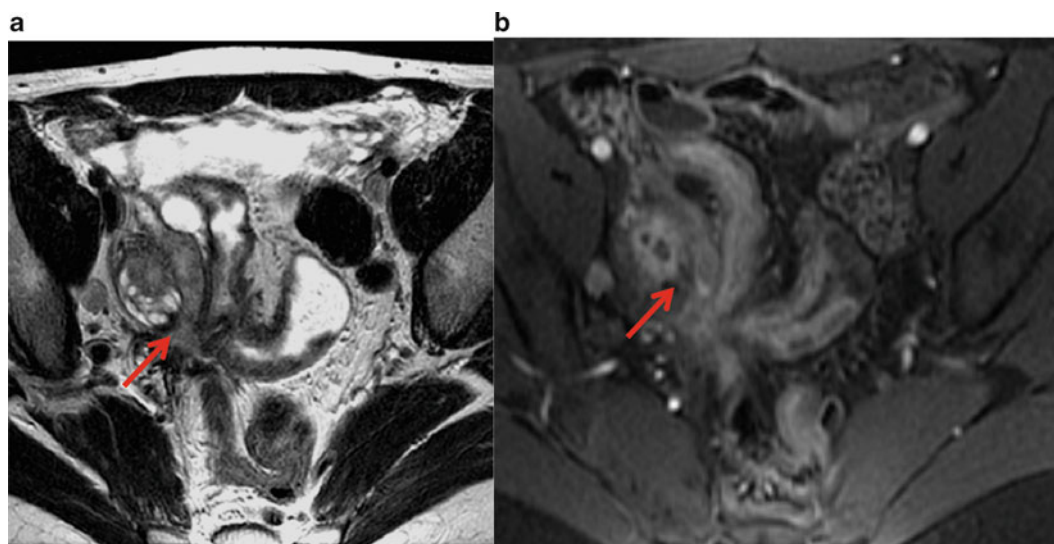


Fig. 18.2 Axial T2W (a) and post-gadolinium T1W axial (b) images through the pelvis in a young female with Crohn's disease who presented with symptoms suggestive of pelvic inflammatory disease. There is terminal ileal disease with a stellate fistula to the right ovary (*arrow*). The diseased segment is strictured

the comb sign may be an accompaniment of active disease and is seen as areas of high signal intensity on T2-weighted images tracking along a diseased segment [16].

Hypertrophy of the mesenteric fat also preferentially involves the mesenteric border and causes significant mass effect with separation of adjacent bowel as well as of the mesenteric vessels. It is commonly seen in the context of transmural disease.

Nodal enlargement and enhancement in a specific vascular territory may be seen in areas of active disease and is well displayed on fat saturated T2-weighted sequences. The degree of enhancement within a node relative to that of an adjacent vessel has been speculated as being predictive of disease activity [16].

Fistulas and Sinuses

Penetrating disease is a hallmark of CD. Deep areas of transmural ulceration may communicate with adjacent epithelial surfaces resulting in formation of a fistula (Fig. 18.2). The fluid containing tracts are seen as areas of high signal intensity on T2-weighted sequences and enhance following intravenous contrast material administration. If they are complex, they may have a more stellate appearance with multiple tracts emanating from a central point to adjacent bowel loops. Imaging has high sensitivity for depiction of these fistulae ranging from 71 to 100% for entero-enteric fistulae to those communicating with other viscera [16, 17].

Enterocutaneous fistulae may be harder to depict on MRI due to their superficial location, compression effects on prone imaging exacerbate (see section on technique below) this and supine imaging is recommended if this is a clinical concern.

Abscesses are seen as encapsulated fluid collections demonstrating peripheral enhancement. They may contain air but are often heterogeneous due to the presence of particulate content. Using negative enteric contrast agents may increase the conspicuity of smaller, subtle inter-loop abscesses. The presence of abscesses is an important contra-indication to the use of anti-tumour necrosis factor drugs and therefore, their detection on imaging is important.

Table 18.3 MRI score in assessing Crohn's disease activity

MRI findings	0	1	2
Wall thickness (mm)	<3	3.1–4	>4
WCE ^a (%)	<70	70–100	>100
Luminal stenosis (%)	<50	50–80 (mild)	>80 (severe)
Mucosal abnormalities ^b	Absent	Present	
Layered wall enhancement	Absent	Present	
Peristalsis	Present	Absent	
Distensibility	Present	Absent	
Mesenteric involvement ^c	Absent	Present	
Pathologic lymph nodes ($n > 3$) ^d	Absent	Present	
Sinus tracts-fistulas	Absent	Present	
Inflammatory masses	Absent	Present	

^aWCE Wall contrast enhancement; follows the equation $WSI_{\text{post-Gd}} - WSI_{\text{preGd}} / WSI_{\text{post-Gd}} \times 100 \times (SD_{\text{noise preGd}} / SD_{\text{noise post-Gd}})$ (WSI bowel wall signal intensity, SD standard deviation of the signal intensity of background, Gd gadolinium)

^bNodularity and/or ulcers and/or cobblestone appearance

^cComb sign and/or fibro fatty proliferation

^dShort axis diameter >10 mm

Table 18.4 MRI activity score for Crohn's disease

Score	Disease activity
0–1	No activity
2–6	Mild activity
>7	Moderate to severe activity

MR Scoring Systems

There are recognized (but imperfect) clinical scoring systems such as the CDAI or the Harvey–Bradshaw Index which are utilized in planning patient management and in monitoring the effects of therapy. Many imaging studies have attempted to correlate specific imaging parameters with disease activity. Few authors have however attempted to grade MRI findings into a generic score to reliably evaluate the extent of disease activity [18, 19].

A more recent imaging study graded MRI findings using ileo-colonoscopy with biopsy as a gold standard [20]. The patients were stratified into three main categories:

1. No disease activity, including patients without a final diagnosis of IBD or patients with chronic, quiescent disease.
2. Mild disease activity.
3. Moderate to severe disease activity.

The authors attempted to create a scoring system as a tool for standardized overall interpretation of the MRI findings in the small bowel as shown in Tables 18.3 and 18.4. Utilizing this activity scores the authors were able to achieve a sensitivity, specificity, PPV, NPV and overall accuracy in assessing disease activity of 93.1, 87.5, 93.1, 87.5 and 91.1%, respectively. This study confirms the validity of imaging criteria.

MR Enterography

Technique and Protocol

The majority of MR studies are performed as an enterography rather than an enteroclysis. The degree of distension as well as the mucosal detail is superior when there is direct intubation of the small bowel with a nasojejunal catheter but this has to be balanced against the higher invasiveness and lower patient tolerance of this technique [21]. Direct head-to-head comparisons between the two techniques have shown similar performance in the detection of active inflammation [22].

Oral Contrast

Luminal distension with oral contrast is vital for detailed assessment of the bowel wall. Additional benefits include the displacement of air from the lumen by oral contrast. This is necessary as air produces susceptibility artefact and degrades image quality.

Oral contrast utilization will be determined by several factors including local availability, patient tolerance, cost and the effectiveness of distension (Table 18.5). In addition to the choice of agent there is departmental variation in the volumes administered and the timing of image acquisition following ingestion of the contrast material. In some instances there may be rapid transit of contrast to the right colon but in the majority of individuals a delay of 40–60 min from the start of contrast ingestion is adequate to obtain small bowel distension.

Significant variance exists between the agents available and it is important to be cognizant of the differences. Agents can be stratified into several categories:

- *Negative contrast agents* are of low signal intensity on T1-weighted and T2-weighted imaging. These include superparamagnetic iron oxide agents such as ferumoxsil.
- *Positive contrast agents* are of high signal intensity on T1-weighted and T2-weighted imaging.
- *Biphasic agents* are of variable signal intensity depending on the sequence that is applied. In clinical practice, these are the most utilized and there is consequently a greater choice in this category.

Table 18.5 Overview of oral contrast agents

Agent category	Name	Benefits	Limitations	Practical issues
Positive (high T1 and high T2)	Gadolinium chelates, manganese, milk with high fat content, fruit juices (e.g. pineapple, blueberry)		Limit detection of subtle mucosal abnormality or wall enhancement	Cost, availability
Negative	SPIO, Ferumoxsil oral	Improve conspicuity of wall edema		Unpleasant taste, cost
Biphasic (low T1 and high T2)	Water, methylcellulose, Mannitol or mannitol with locust bean gum, PEG, VoLumen	High intrinsic contrast between wall and lumen allows assessment of subtle fold thickening	Distension with agents such as water inconsistent The hyperosmolar agents may promote diarrhoea	Absorption, taste, diarrhoea



Fig. 18.3 The benefits of a biphasic agent are seen well on these coronal images displaying optimal enteric distension uniformly throughout the small bowel. Note the thickness of the wall is less than 3 mm in the distended state in this normal subject (*arrows*)

The purpose of the oral contrast is to allow uniform distension of the small bowel loops. This allows ready assessment of diseased segments and ready identification of mucosal abnormality. On T1-weighted sequences post-gadolinium administration and after administration of a biphasic contrast agent, the low signal of the contrast improves the conspicuity of the hyper-enhancing bowel wall (Fig. 18.3).

In the reported literature as well as in our department we utilize a total ingested volume of between 1 and 2 l of contrast ingested in divided aliquots over a 45–60 min time period. Water is an undesirable biphasic contrast agent due to its rapid absorption and erratic distension of the distal small bowel. This can be problematic as the distal small bowel is most commonly involved in CD. There are many commercially available biphasic contrast agents. In our practice we have utilized both dilute barium 0.1% wt/vol solution with sorbitol as well as a bulk fibre laxative, which helps luminal expansion by retaining water. High osmotic agents such as mannitol and sorbitol can have undesirable side effects depending on the sugar alcohol concentration. They can cause diarrhoea, excessive gas and cramping. This can be improved by keeping the concentration below 2.5%.

An optional method for simultaneous large bowel assessment is administration of a rectal water enema. This allows the colonic wall to be examined for disease involvement. Patient positioning also varies. In many institutions these studies are performed prone to allow separation of pelvic small bowel loops. This position also allows maximum coverage on coronal images and decreases scanning volume. We prefer supine imaging for patient comfort in these patients, many of whom are slim and find prone imaging uncomfortable, in some cases due to prior surgical procedures.

Most institutions routinely administer an anti-peristaltic agent to minimize peristaltic artefact. This is of prime importance in maintaining image quality in sequences that are prone to flow artefacts, such as the HASTE or SSFSE (single shot fast spin echo sequences). We usually administer a divided dose of an anti-peristaltic with the second aliquot being given prior to the gadolinium enhanced sequences. Depending on local availability this can either be obtained with 10 mg of hyoscine butylbromide (Buscopan®) or 0.2 mg of glucagon given intravenously. Buscopan is not licensed for use in the USA. To promote gastric emptying whilst ingesting oral contrast, either erythromycin or metoclopramide (20 mg) can be given once the first 500 mg of contrast has been drunk.

Intravenous Contrast

Gadolinium derivatives are typically administered intravenously at a dose of 0.2 mmol/kg body weight. Peak bowel enhancement is thought to occur between 70 and 75 s following contrast material administration but can vary between individuals [23]. It is therefore standard practice to perform multi-phasic MRI commencing in the arterial phase at 25 s and obtaining at least two further acquisitions. In circumstances where gadolinium usage is relatively or absolutely contra-indicated, unenhanced sequences usually suffice to demonstrate areas of mural abnormality as well as mesenteric hyperaemia in diseased segments.

MR Motility Imaging

MRE allows not only the static display of morphology but can be combined with ultrafast imaging techniques (cine MRI) for analysis of bowel motility. Traditional X-ray fluoroscopic imaging confirms that involvement with Crohn's may result in motility disorders. Pathological changes in the bowel wall result in areas of aperistalsis or diminished motility. When used in conjunction with routine MRE it can improve lesion detection. In a study performed to assess if cine MRI improves additional lesion detection in patients with Crohn's, 40 patients with CD underwent cine MR enterography in addition to a standard MR protocol. Blinded reads were performed with and without cine MRI. Overall cine MRI detected more Crohn's specific findings than static MR enterography alone ($p=0.007$) and more patients with CD relevant MR findings ($p=0.03$) [24].

Cine MRI can be performed by single slice techniques, which are repeated at intervals of 0.5–1 s in the same plane but in a stepwise location from anterior to posterior. Anti-spasmodics are routinely administered to minimize peristaltic artefacts. The absence of radiation means that potential sites of disease can be repeatedly evaluated without incurring any risk. It must be emphasized that cine MRI does not have sufficient spatial resolution to be used in isolation when performing diagnostic MRI.

MR Colonography

Conventional colonoscopy remains the gold standard for mucosal evaluation and method for obtaining histological confirmation of a suspected diagnosis of inflammatory bowel disease. Entire colonic visualization and stepwise biopsies are considered mandatory and IBD is typically correctly classified in 80–90% of cases at the time of initial examination. In specific circumstances, however, imaging is a suitable alternative for the establishment of disease extent and severity. These include the presence of a fulminant colitis when the patient is at higher risk of colonic perforation. Similarly for both patient and technical reasons incomplete colonoscopy may preclude colonic assessment in between 5 and 20% of cases. As when assessing small bowel involvement, endoscopy alone may underestimate the full extent of transmural disease and extra-enteric involvement. Imaging is also invaluable for assessment of colonic anastomoses [25, 26]. The assessment of an anastomosis can be challenging on endoscopy and is the most common site of disease recurrence. Moreover in assessing a patient with an indeterminate colitis MRI can be useful as a discriminator between CD and UC. The additional benefit to assess or exclude the presence of small bowel disease as a one stop imaging can add weight to a suspected diagnosis.

Table 18.6 Sample imaging protocol for MR Colonography

Imaging sequence	Imaging plane	Section thickness (mm)	Number of sections	Field of view (mm)	Imaging time per volume (s)	Imaging time per section (ms)
True FISP/FIESTA	Coronal	5	40	400×400	24	N/A
Dual echo	Axial	5	70	300×300	N/A	20
GE T1W						
VIBE (FLASH)	Coronal	1.4	128	440×440	25	N/A
TIW with fat saturation	Axial	5	70	400×400	N/A	22
Half Fourier (e.g. RARE)	Coronal	5	40	450×450	N/A	20
Fat saturated Half Fourier	Axial	5	70	300×300	N/A	17

MRC Technique

Routine bowel cleansing is performed as for optical colonoscopy. Replacing standard cleansing with faecal tagging is not advocated in this patient sub-group as compared with those undergoing screening examinations for colorectal cancer [27]. Adequate colonic distension is a requisite in order to minimize false positive or false negative studies. MR colonography (MRC) can also be performed with positive, negative or biphasic agents. A warm tap water enema has the ideal characteristics of a biphasic agent being of low signal intensity on T1-weighted and high signal on T2-weighted sequences.

A volume between 1.5 and 2.5 l is administered via a rectal catheter once the patient is on the table. Routine utilization of an anti-peristaltic agent (Hyoscine 20 mg or Glucagon 1 mg) produces reflex atonia that helps in retention of the water enema. In addition, it minimizes peristaltic artefacts as well as achieving more optimal colonic distension. A full discussion of the protocol is beyond the scope of this chapter, however a combination of the following sequences is usually utilized (Table 18.6):

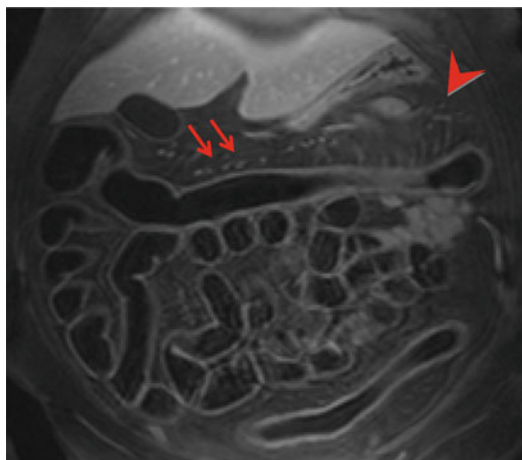
- A true FISP allows initial evaluation to see if there is adequate colonic distension. On this dark lumen technique the colonic wall is well seen with minimal peristaltic artefact due to short image acquisition time.
- A heavily T2-weighted imaging sequence (RARE, SSFSE) provides information regarding oedema in the bowel wall, peri-enteric inflammatory changes and fluid collections within the adjacent mesentery. When combined with fat suppression techniques, this sequence is invaluable in distinguishing oedema from fatty infiltration within the colonic wall. This sequence is however particularly prone to flow artefacts and is less helpful in depicting the mesenteric and nodal changes that accompany the colonic pathology.
- Finally, a post-gadolinium 3D sequence (e.g. VIBE, LAVA or similar) is performed following fat suppression and an injection of 0.2 mmol/kg of contrast media. This enables assessment of degree of mural enhancement in pathologically involved colonic wall. Images are typically acquired approximately 75 s following contrast material administration.

Imaging Findings

Ulcerative Colitis

Classic findings on imaging include involvement of the rectosigmoid colon which extends proximally to involve the entire colon. Less commonly there is a sub-total colitis (usually right sided) and

Fig. 18.4 This dark lumen MRC image shows the classic findings of UC with a featureless colon (*arrows*) as well as engorgement of the vasa recta in proximity to diseased segments (*arrowhead*)



in 15% of cases there may be a backwash ileitis. Uniform thickening of the wall is a hallmark feature and is seen on both T1W and T2W sequences. The degree of thickening is usually less marked in UC than in CD with mean values of 7–8 mm as compared with 13 mm, respectively.

In more active disease however mural thickness can exceed 10 mm. Mural stratification is observed in 60% of patients with UC versus 8% of patients with CD [28]. This is due to the presence of fat or oedema in the sub-mucosal layer as described in the small bowel above.

The outer contour of the colonic wall is typically smooth and regular in patients with UC, whereas serosal and outer mural irregularity is seen in 80% of patients with CD [29].

As in Crohn's there is engorgement of the vasa recta and hyper-enhancement as well as enlargement of regional peri-colonic nodes. The presence of small traces of peri-colonic fluid is usually a marker of focal serosal involvement and typically indicates the presence of severe disease.

In long standing disease there will be wall thickening, an absence of haustra and associated luminal narrowing (Fig. 18.4). This is thought to be due to hypertrophy of the muscularis mucosa. Mesenteric fatty proliferation in UC is typically confined to the peri-rectal space.

Crohn's Disease

Involvement of the colon can be seen in more than 60% of patients with CD and is exclusively limited to the colon in approximately 15–25% of cases. The rectum may be involved in approximately 50% of patients [30].

As in the small bowel, colonic wall thickening is present and post-contrast enhancement is a good correlate of disease severity. The inner profile of the colonic wall may have an undulating configuration on T2-weighted and T1-weighted sequences. This is thought to represent mucosal oedema and there may also be a relative absence of haustration for the same reason.

In severe disease a conglomeration of longitudinal and transverse ulcers encompass much of the colonic circumference and result in the cobblestone appearance also seen in the small bowel. Other imaging findings that overlap with those in the small bowel include the presence of fistulae and sinus tracts. These tracts are typically of high signal intensity on T2-weighted imaging sequences and enhance avidly following gadolinium based contrast material.

Long standing quiescent disease may depict no abnormality on imaging. If however there is transmural fibrosis there is shortening of the bowel with haustration. This results in a relatively fixed and tubular configuration to the bowel. On post-gadolinium sequences there is homogenous enhancement and absence of wall stratification.

Diagnostic Accuracy

A study by Ajaj et al. [26] assessed 23 patients with IBD on MRC. The imaging findings (MRC score) were correlated with histopathological specimens obtained on endoscopy. In this study group, MRC correctly identified 68 of 73 segments found to reveal IBD changes on pathology. The imaging score utilized four quantifiable criteria including colonic wall thickness, colonic wall enhancement, number of haustral folds and number of peri-enteric nodes. There were no false positive findings. MRC detected and characterized clinically relevant IBD of the large bowel with sensitivity and specificity values of 87 and 100%.

Rottgen et al. correlated bowel enhancement on MRC with inflammatory activity of CD at colonoscopy in 42 patients and found a significant correlation between colonoscopic inflammatory activity and changes in signal intensity within the bowel wall. These relatively modest patient numbers need to be replicated in larger cohorts; however, the data suggest that MRC is a credible alternate to conventional colonoscopy in both monitoring activity as well as evaluating therapeutic response [31].

A study to assess the accuracy of dark lumen MR for the assessment of recurrence at the colonic anastomosis following end-to-end anastomosis, revealed an overall sensitivity and specificity of 84 and 100%, respectively [32]. Fifteen of the 39 patients assessed had a history of inflammatory bowel disease. The remainder comprised a heterogeneous group who had undergone surgical resection for entities such as colorectal malignancy and diverticulitis. This contrasts favourably with the sensitivity values of 73–98% obtained by CT for assessment of anastomotic pathology [33]. Another study looking at recurrence after ileo-colic resection also demonstrated a total agreement of 95.1% (kappa 0.84) between MRI and the endoscopic Rutgeerts score in a population of 30 patients with suspected recurrence of CD [34].

Role of Diffusion Weighted Imaging

DWI relies on differences in the motion of water molecules between tissues to provide image contrast. Several studies have aimed to assess the value of DWI in patients with IBD but in many instances there was no endoscopic gold standard. A more recent study however attempted to address this deficiency by comparing the results of DWI in evaluation of the large bowel with conventional endoscopy as well as to correlate the imaging findings with clinical and biological IBD activity indices. The study assessed patients with Crohn's and also UC. Moreover the imaging was performed without the benefit of any cleansing, or any oral/rectal preparation. Colonoscopy was performed within 48 h of the imaging study in 68 of 96 patients with IBD. DWI (with a B -value of 0 and 600 s/mm) was incorporated into a routine MRC protocol including multi-phase post-gadolinium sequences.

In patients with UC the MR score detected endoscopic inflammation with a sensitivity and specificity of 89.47 and 88.67%, respectively. The performance in the CD group was less optimal at a sensitivity and specificity of 58.33 and 84.48%, respectively.

The accuracy of DWI hyperintensity for detecting colonic inflammation was greater in UC than CD. The authors speculated that this might be related to the contiguous involvement seen in UC rather than the segmental changes seen in Crohn's. To support this theory bowel wall thickening on imaging was a better correlate of endoscopic changes in Crohn's ($p < 0.0001$) as compared with DWI as it reflects the true transmural nature of this disease process [35]. In the future, if DWI is proven to be of benefit in larger patient groups, it may circumvent the need for gadolinium and potentially avoid issues related to cost and patient safety with this intravenous contrast media. This early trial suggests it may have benefit as a non-invasive method for evaluation of colonic inflammation particularly in patients with UC.

MR Imaging of Perianal Fistulas

Around 40% of patient with CD will develop a perianal fistula and this proportion increases in those with underlying anal strictures. Up to 36% of patients with CD present with this entity as their presenting symptom. Adequate evaluation of the full extent of disease including its anatomic relations and degree of inflammation is vital to make management decisions. MRI is recognized as being the reference standard for the anatomical depiction of disease extent. Increased enhancement on T1-weighted sequences following gadolinium is considered a good correlate of active inflammation [36]. Prior to commencing treatment with immunosuppressive and anti-tumour necrosis antibodies it is imperative to exclude an abscess. Recurrence following surgery is a common concern in circumstances where pre-operative imaging has not been performed.

There may be irreversible functional consequences if a fistula is inappropriately managed. MRI is excellent at depiction of perianal fistulising disease and in particular can be used to track the relationship to the sphincter complex as well as to the remainder of the pelvic floor.

Classification

A surgically based classification system by Parks utilizes the external anal sphincter as a reference point. There are also imaging (MR) based classification systems [36]. In order to strategize patient management, fistulas can be sub-divided into two main categories, simple or complex:

- A *simple fistula* is superficial, inter-sphincteric or low trans-sphincteric with only one opening, not associated with an abscess and showing no communication with an adjacent structure such as the vagina or bladder.

In contrast, a *complex fistula* has greater involvement of the anal sphincter (high trans-sphincteric, extra-sphincteric or supra-sphincteric). It has multiple openings or a complex course (horseshoe), is associated with a perianal abscess and/or communicates with an adjacent structure such as the bladder or vagina (Fig. 18.5).

Technique

Perianal MRI can be performed with an endoanal coil, or a phased array external coil. Wider availability, a non-invasive procedure and consequently better patient acceptance mean that the phased array coil is more commonly used in perianal imaging. In addition, the larger field of view (FOV) allows accurate assessment of fistulas that may extend beyond the sphincter complex (Fig. 18.6a, b).

The higher spatial resolution of the endoanal coil does however improve visualization of the internal opening and smaller tracts particularly in the case of recto-vaginal fistulas. The average diameter of 12–19 mm for an endoluminal coil may preclude placement in patients with local pain or in the presence of anal strictures. In comparison with the phased array technique there is a limited FOV and it is more prone to motion artefact.

At our institution, high resolution T2-weighted images are acquired in the axial and coronal planes after aligning with the plane of the anal canal. In addition, fat suppressed sequences are routinely performed and are useful for depiction of smaller tracts. A T1-weighted post-gadolinium sequence helps in distinguishing between fluid and granulation tissue.

MRI allows not just precise anatomical localization of disease extent but also permits an assessment of disease chronicity. On T2-weighted sequences, active tracts containing particulate material,

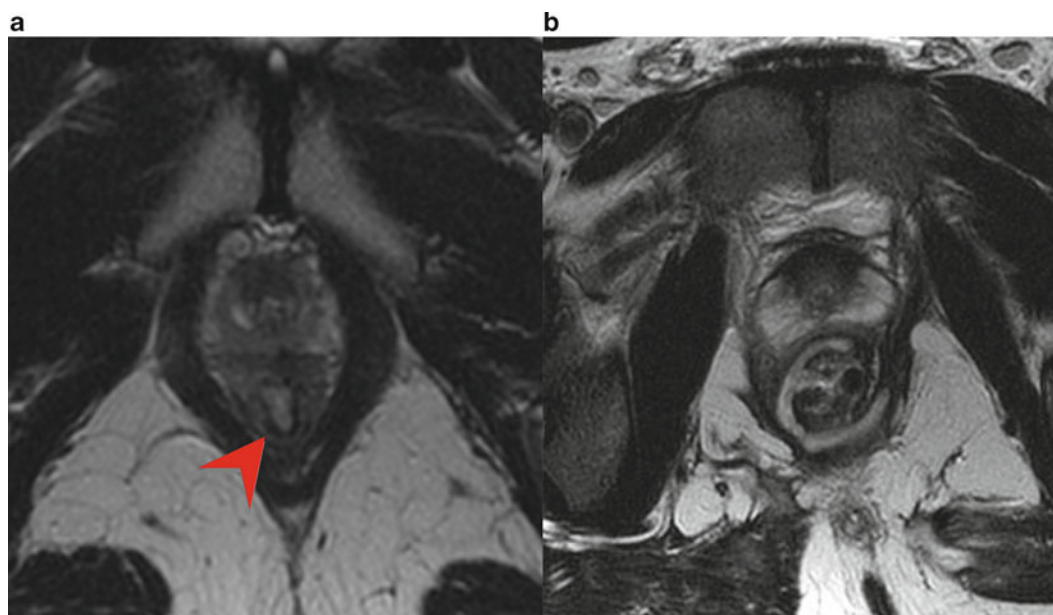


Fig. 18.5 (a) T2W High resolution images show examples of a simple inter-sphincteric fistula (*arrow head*) at 6 o'clock. Image (b) illustrates a more complex horseshoe fistula with multiple ramifications

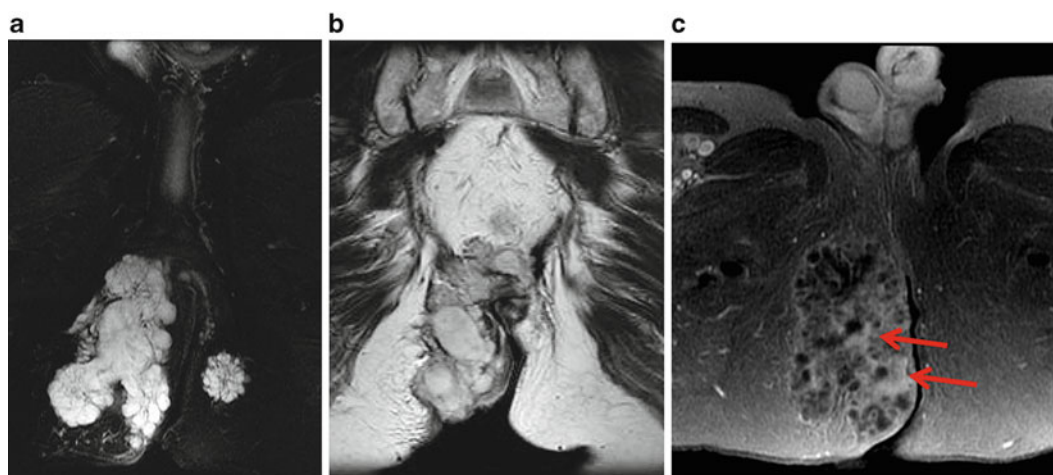


Fig. 18.6 Axial T2W images with fat saturation (a) and coronal T2W high resolution images at the level of the perineum in a patient with chronic perianal disease demonstrate a lobular high signal intensity mass. (b) The internal architecture of the lesion is more readily appreciated in the fat saturated sequences. The T2W images display the characteristic lobulated appearance of a mucinous malignancy. Another patient (c) shows the typical post-contrast features of malignancy with irregular walls and delayed enhancement (*arrows*)

pus or debris are hyperintense, whereas more fibrotic tracts show low signal intensity. Post-gadolinium sequences show fluid as being non-enhancing, whereas areas of fibrosis or granulation tissue show delayed enhancement. A study by Horsthuis et al. [37] demonstrated good correlation between maximum enhancement and the patient's CRP. In their study of 33 patients with perianal CD larger numbers of rapidly enhancing pixels were seen in patients who required changes in medication or developed new abscesses during follow-up.

Efficacy

Multiple studies using both endoscopic ultrasound and MRI have demonstrated persistent fistula activity long after the fistula has ceased draining. Ng et al. [38] assessed 26 patients treated with either infliximab or adalimumab. Complete cessation of drainage occurred in 46% of patients, whereas MRI demonstrated that there had been complete healing in only 28%. As there is such disparity between the clinical “picture” and the imaging findings there is a compelling argument for obtaining imaging at baseline as well as to assess and monitor healing response.

Studies comparing examination under anaesthesia (EUA) with pelvic MRI or endoluminal ultrasonography (EUS) also suggest that in 10% of cases EUA incorrectly classifies the disease. This leads to inappropriate management decisions or additional surgical procedures [39]. Another study showed MRI as adding additional information to EUA and led to additional surgery in 21% of 56 patients with fistulising disease [40].

Radiological input is also vital in providing prognostic information. Patients who show signs of persistent disease at a 10-week imaging evaluation were more likely to recur [41]. In the study by Ng, MRI was used to decide how long to continue with anti-TNF therapy [38]. This need for imaging has been recognized by the American Gastroenterological Association and the European Crohn’s and Colitis Organization who have issued guidelines recommending imaging for perianal disease.

Cancer in Perianal Disease

Patients with CD have a 4- to 20-fold increase in the risk of colorectal carcinoma [42]. The incidence of colorectal cancer is 3.7% and patients with perianal disease have a 0.7% incidence of carcinoma [43]. This complication poses both a clinical and imaging challenge. Early diagnosis is imperative to improve patient outcome and facilitate appropriate management.

A retrospective review of six patients with anorectal carcinoma and perianal CD at our institution was used to describe the MRI features of cancer [44]. Four of the patients had a mucinous adenocarcinoma, whereas the remainder had squamous malignancy. Typical features of malignancy included irregular wall contours, double layer enhancement and delayed internal tissue enhancement. The mucinous carcinomas displayed a lobulated pattern with fluid filled cavities again showing delayed internal enhancement (Fig. 18.6c). This stresses the importance of post-gadolinium imaging in perianal disease not merely to assess activity of tracts but to exclude concomitant malignant transformation.

Summary

MRI is an ideal imaging modality for assessment of inflammatory bowel disease. The absence of ionizing radiation, its multi-planar capability and superior soft tissue contrast resolution confer inherent advantages as compared with traditional fluoroscopic imaging and even multi-detector CT.

Continuing improvements in field strength, parallel imaging techniques and sub-second acquisitions aimed to minimize motion and peristaltic artefact ensure that previous concerns regarding spatial resolution and length of examination time are being continually improved on. Coupled with its ability to provide morphological and functional information, MRI serves as the imaging reference standard for determining disease activity at baseline and subsequent surveillance. Local availability and expertise will influence how this is disseminated at non-academic centres. Additional perfusion and diffusion techniques are being established in IBD and show promise in disease evaluation.

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Chapter 19

CT in Inflammatory Bowel Disease

Joel G. Fletcher

Keywords CT • Small bowel • CT enterography • Crohn's disease • Radiation risk

CT enterography (CTE) is a high spatial resolution CT exam of the abdomen and pelvis, following the administration of a large volume of enteric contrast agent that distends the small bowel. It provides exquisite images of the small bowel wall, lumen, and perienteric tissues. Over the past decade, CTE has become a widely available test interpreted with local expertise, and is complementary to other imaging modalities, providing unique information that impacts clinical management of patients with inflammatory bowel disease (IBD).

Role of CTE in IBD

CTE is used to image the small bowel to detect IBD and alternative diagnoses (such as pancreatic disease, sprue, and small bowel tumors). For known patients with Crohn's disease, in particular, CTE is used to detect the location(s) and severity of enteric inflammation; the presence, location, and type of penetrating disease (fistula, abscess, or phlegmon); and the location and degree of obstruction caused by inflammatory and fibrostenotic strictures. In ulcerative colitis, CTE is used to gauge the extent of colonic inflammation, usually used in conjunction with ileocolonoscopy, and to exclude small bowel involvement prior to colectomy. Both Crohn's disease and ulcerative colitis also cause a variety of extraenteric complications, such as neoplasia, mesenteric thromboses, nephrolithiasis, cholelithiasis, sacroiliitis, etc., that can be visualized by CT. CTE also detects complications from surgical and medical therapies, such as avascular necrosis, leak, pancreatitis, etc.

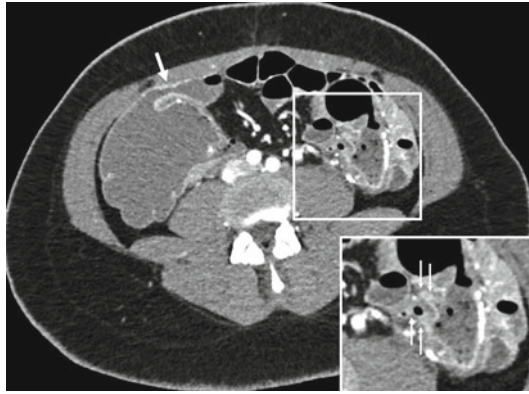
Patient Preparation and Image Acquisition

Patients generally are requested to fast 4 h prior to CTE so that ingested materials are not in the stomach or small bowel. An enteric contrast agent is then administered in multiple aliquots over 45–60 min

J.G. Fletcher, MD (✉)

Department of Radiology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
e-mail: Fletcher.joel@mayo.edu

Fig. 19.1 Example of dose reduction at lower kV in 43-year-old female with prior right hemicolectomy. CTDIvol lowered from 24 mGy (routine protocol) to 10 mGy after kV selection (80 kV). Note active ileitis in neoterminal ileum (*arrow*) and small fistulous tracts between descending colon and adjacent small bowel (*inset, arrows*)



prior to CT examination. Enteric contrast agents generally contain sugar alcohols or osmotic laxatives to prevent resorption of water along the course of the small bowel, thereby improving ileal distention [1, 2]. Commonly used enteric contrast agents include low-contrast barium solution (which contains sorbitol, Volumen®, Bracco Diagnostics, Princeton, NJ), polyethylene glycol electrolyte solution, or methylcellulose solution. Side effects from oral contrast are minimal but can include nausea, time-limited diarrhea, and excess gas [1]. Neutral enteric agents distend the lumen better than water and provide a long temporal window over which imaging can occur and still provide adequate luminal distention. In the hospital setting, water is typically given as an oral contrast agent, as patients typically have an underlying degree of bowel obstruction, and a predisposition to nausea is increased. Glucagon or buscopan can be given to limit peristalsis but has not been proven to improve test performance.

Intravenous iodinated contrast is given at a rapid rate (e.g., ≥ 4 cc/s) to enhance the bowel wall, with CT imaging typically initiated during an enteric phase of contrast enhancement, when small bowel enhancement is maximal, typically occurring 50 s after initiation of the IV injection of contrast [3]. Alternatively, imaging can be performed in the portal or hepatic phase, when the radiologic imaging of solid organs is optimal, without decrement in the identification of mural inflammation [4].

CT parameters governing spatial resolution and imaging speed are chosen so that the entire abdomen and pelvis can be imaged in 20 s or less (i.e., single breathhold) and so that spatial resolution in non-axial planes is optimized. With multi-detector CT with 16 detector rows or more, the minimal detector configuration less than 1 mm is chosen so that isotropic coronal images can be obtained. Generally, narrow slice thickness of 3 mm or less is reconstructed in the axial and coronal plane for image review. Imaging is typically performed at a tube voltage of 120 kV, which facilitates excellent image quality across the entire gamut of patient sizes and can generally be performed with a CT dose index of <18 mGy (or an effective dose of 12 mSv) [5], owing to the high contrast of the enhancing bowel. Further, reduction in radiation dose is easily obtained through reduction in tube current (i.e., mAs) by employing automatic exposure control and/or kV selection [6, 7]. Automatic exposure control is used on most modern multi-detector CT scanners to modulate the tube current as the X-ray tube travels around the patient and as the patient travels through the scanner, generally reducing dose on the order of 30% [8]. Selection of kV can lower the tube energy in smaller patients and simultaneously increases the iodine contrast, to permit further dose reductions (Fig. 19.1), but requires technique charts or vendor-supported software. Alternatively, lower kV (80 or 100 kV) imaging may be chosen to improve iodine signal and contrast to compensate for reduced injection rate or amount of intravenous iodinated contrast (Fig. 19.2).

Multiple practices have demonstrated that there is no reduction in the performance of CTE using lower radiation doses, as the increase in image noise is offset by the high contrast of enhancing bowel loops and penetrating disease [9, 10]. Noise reduction techniques, which decrease image noise, may make further dose reductions possible, and improve image quality by decreasing image noise so that low-dose images resemble routine-dose images (Fig. 19.3) [11, 12].

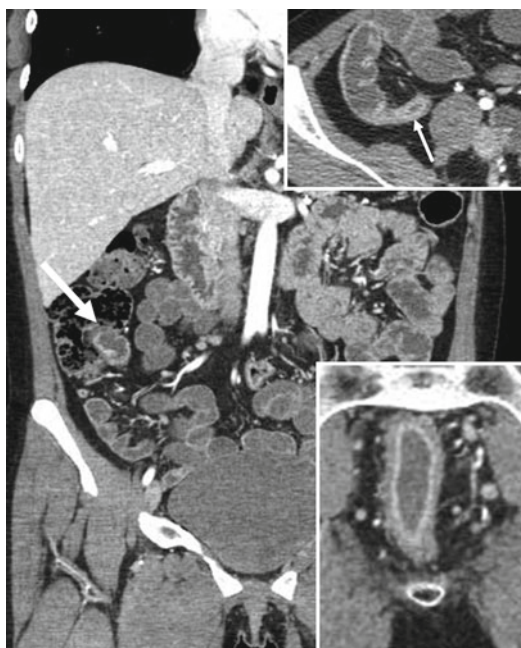


Fig. 19.2 CT enterography performed in a 32-year-old person with indeterminate proctitis and poor intravenous access. To compensate, tube voltage was lowered to 100 kV with initiation of scanning delayed until 70 s. Large coronal image shows mild asymmetric hyperenhancement at terminal ileum reflecting mild inflammation (*large white arrow*), while insets show stratification and enhancement of the appendiceal tip (*upper inset, arrow*), and active proctitis with mural stratification, hyperenhancement, and reactive lymphadenopathy (*lower inset*)

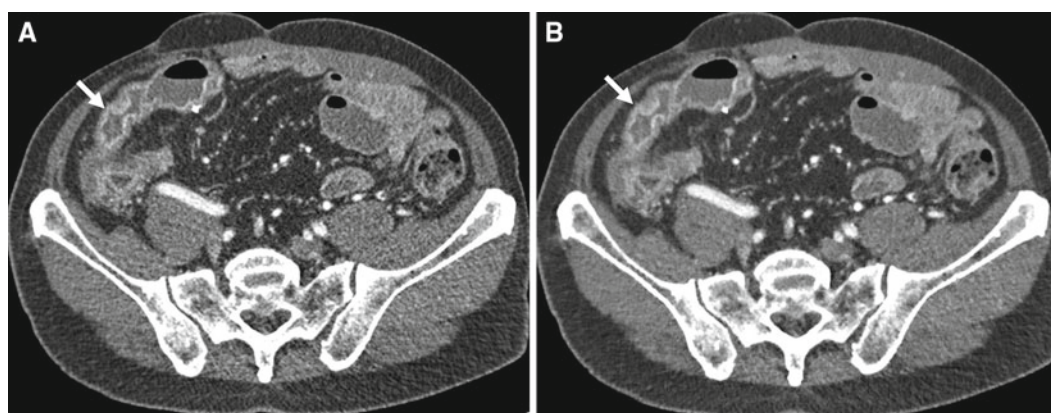


Fig. 19.3 Lower-dose CT enterography in 63-year-old male showing twofold reduction in image noise from routinely reconstructed image (**a**) to image reconstructed using image noise reduction techniques (**b**) Note that mural stratification and hyperenhancement indicating active ileitis in the terminal ileum (*arrow*) between ileoileostomy and ileocecal valve can be detected before image quality improvement with noise reduction

Imaging Findings

Underlying Mural Inflammation

Mural hyperenhancement refers to segmentally increased attenuation of bowel loops compared with adjacent loops, and this correlates histologically with areas of active inflammation [13].

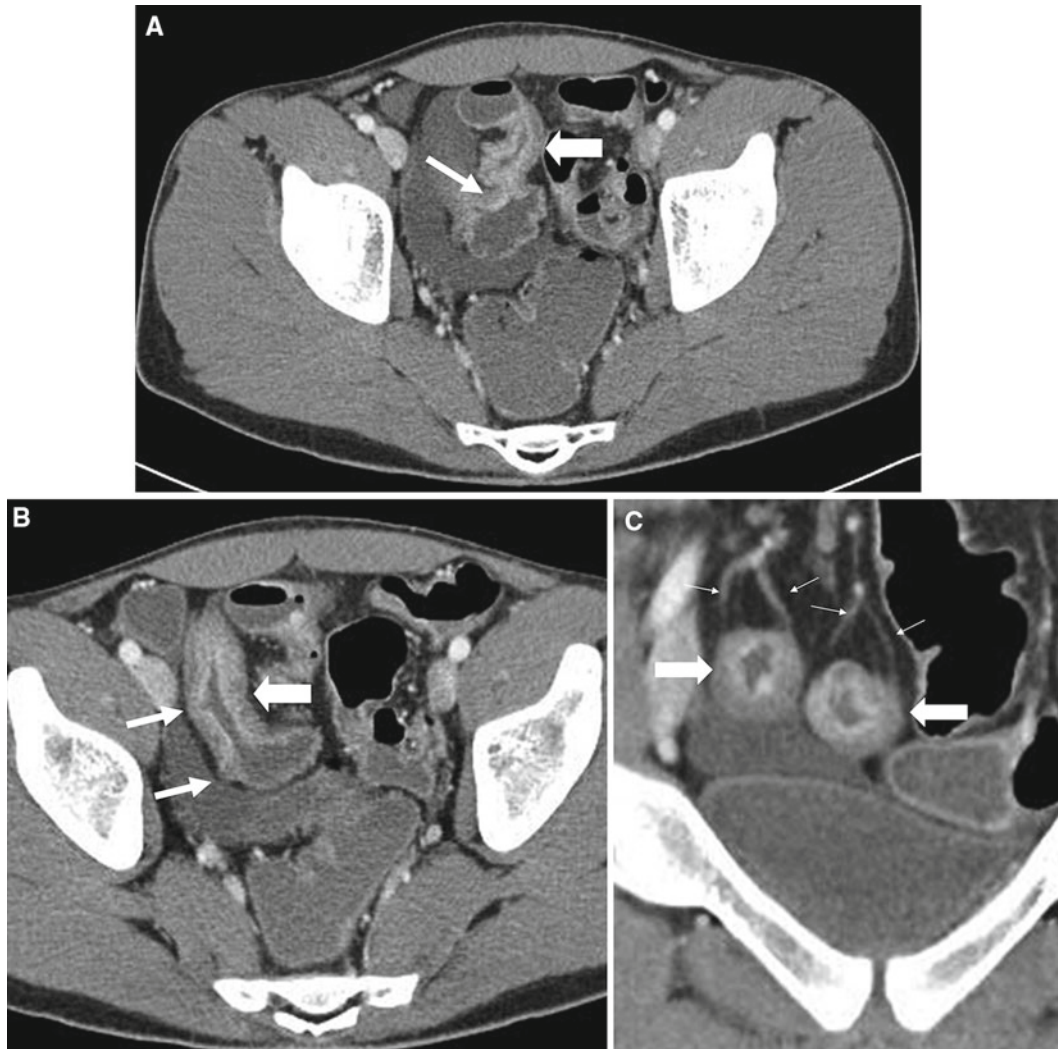
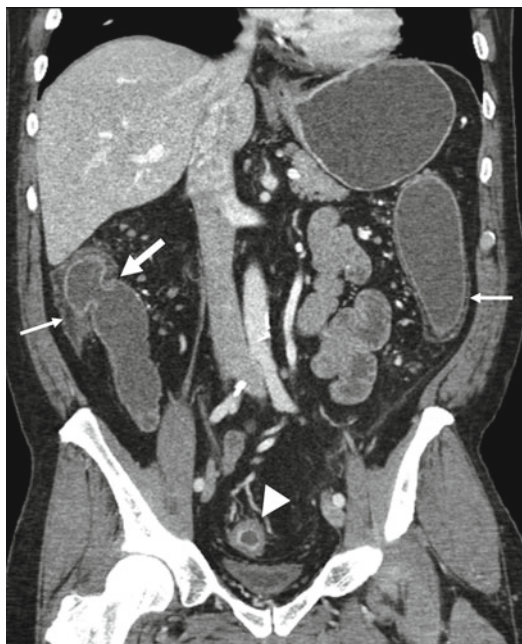


Fig. 19.4 Transverse CT enterography images in a 19-year-old Crohn's patient with "back pain." Images demonstrate mural thickening and stratification (*large arrows, a–c*), with a bilaminar appearance to the bowel wall. Note that mural thickening and luminal hyperenhancement are asymmetric (*a, c*), with one loop demonstrating mesenteric border thickening and hyperenhancement and antimesenteric border pseudosacculation (*small arrow, a*). One inflamed loop demonstrates small penetrating ulcers (*small arrow, b*), whereas another has prominent vasa recta, or "comb sign" (*c, small arrows*)

Mural hyperenhancement is the most sensitive sign of bowel inflammation, and it can be an isolated finding in mild inflammation. Hyperenhancement alone is a non-specific finding in the small bowel, but asymmetric and patchy hyperenhancement, particularly along the mesenteric border, is indicative of Crohn's disease (Figs. 19.1–19.4).

Mural hyperenhancement is often accompanied by wall thickening, which in the small bowel is greater than 3 mm in thickness in a bowel loop that is distended with luminal contrast. Wall thickening in Crohn's disease is often asymmetric and more prominent along the mesenteric border as well. As mural inflammation increases, wall thickening is often accompanied by mural stratification, which refers to a bi- and trilaminar appearance to the bowel wall (Fig. 19.4). The presence of both

Fig. 19.5 Fifty-eight-year-old person with a 4-month history of altered bowel pattern. Coronal CT enterography images show findings of chronic ulcerative colitis, including moderate sigmoid inflammation (*arrow head*) and mild chronic proximal colitis in the descending colon and cecum (*small arrows*), as manifested by intramural fat, loss of haustration, and prominent pericolic vessels with reactive lymphadenopathy. The ileocecal valve is widely patulous (*large arrow*)



segmental mural hyperenhancement and wall thickening yields the best imaging criteria for mural inflammation in Crohn's disease [5, 14].

Penetrating ulcers can also be seen in inflamed bowel loops, and they may appear as filling defects in the inflamed bowel wall; however, they are more frequently seen at MR enterography. The "comb sign" refers to engorged vasa recta, which supply inflamed bowel loops and penetrates the gut wall perpendicular to the lumen, resembling the shape of a comb [15]. The comb sign is associated with increased serum C-reactive protein, length of hospitalization, and response to anti-inflammatory treatment (Fig. 19.4) [16–18].

CTE can also display findings of chronic inflammation. Intramural fat can be seen in actively and uninflamed small bowel loops in the colon, indicating chronic inflammation, but intramural fat in the terminal ileum is a normal finding. Alternatively, pseudopolyps are frequently seen throughout the colon after healing of acute inflammation [19]. Fibrofatty proliferation is seen as proliferation of fat, usually allowing the mesenteric aspect of the bowel loop, displacing nearby abdominal loops, and in the rectum it can resemble pelvic lipomatosis, except that there are prominent perirectal vessels and reactive lymphadenopathy.

Findings of intestinal inflammation in ulcerative colitis are similar to Crohn's. The pattern of inflammation in ulcerative colitis that is most typical is continuous inflammation from the rectum proximally. A patulous ileocecal valve is often seen when inflammation extends to the cecum (Fig. 19.5), as well as backwash ileitis, which involves the terminal ileum symmetrically without penetration. Forshortening of the colon it is also seen with chronic ulcerative colitis. As inflammation becomes chronic, intramural fat is deposited throughout the colon, but this finding is not unique to ulcerative colitis.

Perienteric Inflammation

The most subtle findings of perienteric inflammation in Crohn's disease are perienteric fat stranding, which correlates with serum C-reactive protein [16]. Fistulas appear as hyperenhancing extraenteric

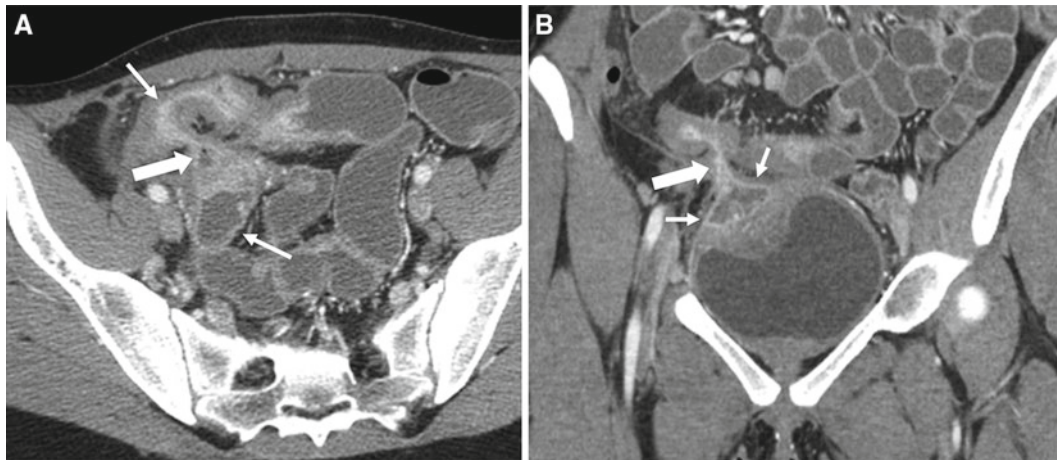


Fig. 19.6 CTE in a 21-year-old female demonstrating a complex fistula (*large arrow, a, b*) connecting two loops of ileum (*small arrows, transverse image, a*) in the right lower quadrant, with an inferior arm extending to the dome of the bladder, where there is a 3.2-cm abscess within the bladder wall (*small arrows, coronal image, b*). (b is reprinted from *Gastroenterology* 2011;140:1795–1806)

tracts, which may or may not contain air and fluid [20]. Fistulas frequently arise from inflamed bowel loops and cause tethering of the involved loops, frequently forming asterisk-shaped fistula complexes (Fig. 19.6). Fistulas that arise from surgical anastomoses and perianal fistulas are often not hyperenhancing due to their chronic nature. As fistulas extend into the mesentery, they frequently cause mesenteric thromboses, which are associated with Crohn's related inflammation (Fig. 19.7), and collateralization via the gut arcade is often seen.

Obstruction

Mural inflammation causes luminal narrowing, but luminal narrowing at enterography does not imply lack of distensibility or stricturing disease. Distensibility can be observed fluoroscopically, for example using peroral pneumocolon. At CTE assessment, luminal narrowing indicating lack of distensibility is only indicated by obstruction significant enough to cause dilation of proximal bowel loops. Most Crohn's strictures demonstrate a spectrum of inflammatory and fibrotic changes [21], and most Crohn's strictures will demonstrate some degree of hyperenhancement at imaging, so enhancement alone is not a good indicator of potential response to treatment for a stenotic small bowel segment demonstrating hyperenhancement.

CTE Performance and Correlation with Clinical Symptoms

CTE has an estimated sensitivity for detecting ileal inflammation of approximately 75–90% using mucosal inspection and biopsy as a reference standard [13, 14, 22, 23]. When clinical assessments additionally including surgery, serum, and clinical follow-up are integrated, the sensitivity of enterography for detecting ileal and inflammation improves to 90–95% [5, 24, 25], as studies utilizing ileoscopic reference standards necessarily exclude patients with stenotic ileocecal valves and misclassify patients with proximal ileal disease. The performance of enterography for identifying active jejunal inflammation is likely decreased due to the greater enhancement of jejunal loops and

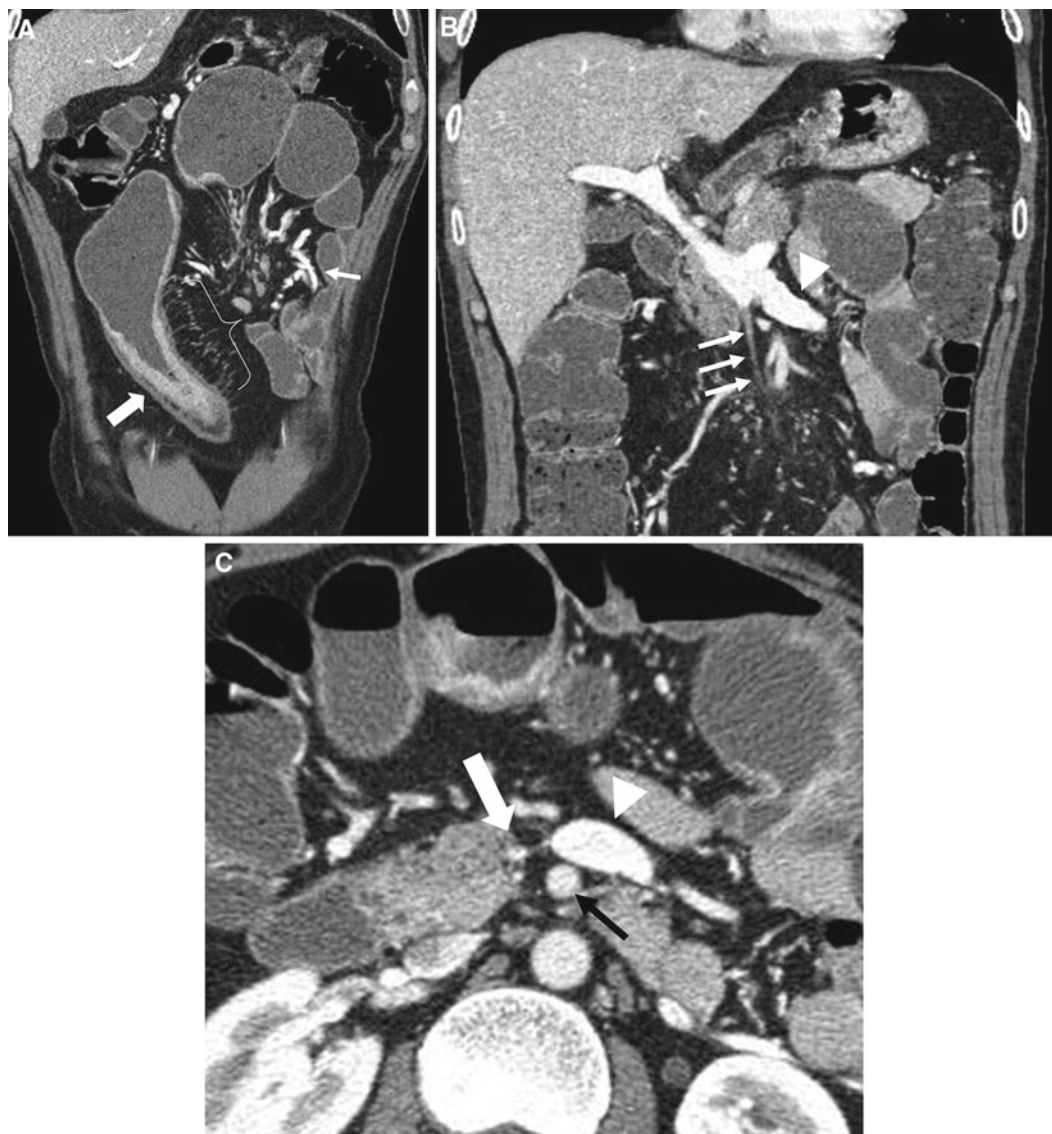


Fig. 19.7 Coronal CTE image in 46-year-old Crohn's patient with obstructive symptoms demonstrates long segment inflammation (*large arrow, a*) with mural stratification, wall thickening, and comb sign (*brackets, a*), with multiple loops of dilated proximal small bowel having prominent mesenteric veins (*small white arrow, a*). Coronal image slightly posteriorly (*b*) with corresponding transverse image (*c*) shows chronic occlusion and narrowing of the superior mesenteric vein (*small arrows, b*; *large arrow, c*) and enlarged inferior mesenteric vein (*arrow head, b, c*). (SMA=*black arrow, c*)

the complexity of jejunal folds [22], and capsule endoscopy is complementary to CTE for detecting more proximal inflammation [23]. Like other objective measures of inflammation in Crohn's disease, enterography findings often do not correlate with symptomatology. In a retrospective study, Higgins et al. found that enterography added unique information to clinical assessment and changed perception of steroid benefit in nearly two-thirds of patients [26]. In a prospective study of 270 patients at Mayo Clinic, Bruining et al. recorded management decisions before and after

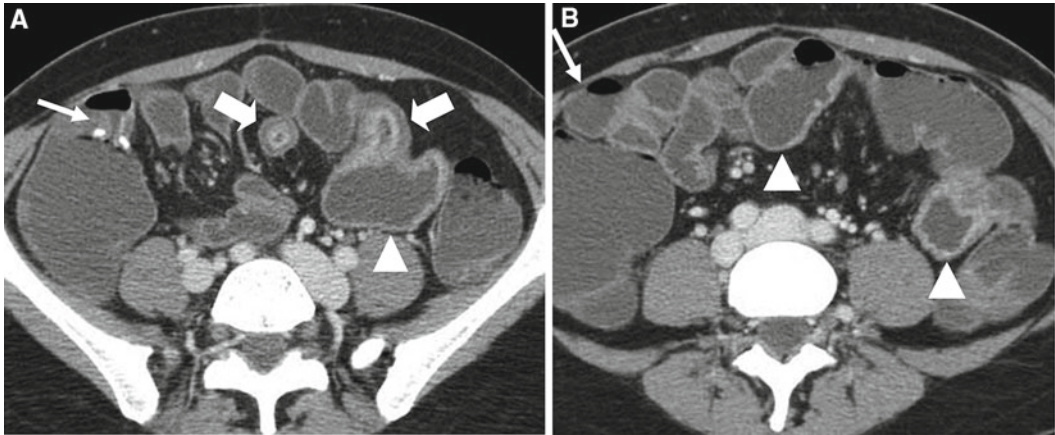


Fig. 19.8 Forty-six-year-old female with prior right hemicolectomy and frequent diarrhea thought to be without obstructive symptoms. CT enterography demonstrates two regions of luminal narrowing (*large arrows, a*) with proximally dilated loops (*arrow heads, a, b*). Luminal narrowing at anastomosis (*small arrow, a*) does not cause proximal dilation of neoterminal ileum (*small arrow, b*). At operative assessment the two strictures in the mid small bowel underwent strictureplasty, but ileocolic anastomosis was distensible

CTE, and found that CT imaging changed management decisions in about half of Crohn's patients, and a similar portion of those with suspected Crohn's disease [18].

CTE has a high accuracy for identifying penetrating disease, which is often unsuspected [20, 27, 28]. It has an estimated accuracy for identifying enteric fistulas of 86%, with a per-patient sensitivity of 94% and 97–100% in detecting patients with fistulas and phlegmon/abscesses, respectively [27]. Booya et al. found that nearly half the patients with penetrating disease had either no clinical suspicion or remote clinical suspicion of penetrating disease at pre-imaging clinical assessment [20].

Although CTE has a high sensitivity for detecting obstructive strictures in surgical series [27], its performance is suboptimal in evaluating low-grade obstruction. Nevertheless, many Crohn's patients with partial small bowel obstruction at CTE do not have obstructive symptoms. The fact that capsule endoscopy retention rates are highest in this cohort underscores the frequency of asymptomatic stenotic lesions in Crohn's disease [29]. In one prospective study, 17% of patients without obstructive symptoms had an inflammatory stricture causing partial small bowel obstruction at CTE (Fig. 19.8) [23]. When CTE does not reveal partial obstruction associated with luminal narrowing, capsule endoscopy maybe useful in identifying additional enteric inflammation [23, 30].

CTE and Multimodality Imaging Assessment

Ileocolonoscopy assessment is generally considered complementary to enterography, as a colonoscopy is more sensitive for identifying mucosal inflammation, particularly in the colon, provides cancer surveillance not provided by CTE, and is usually performed with random biopsies, which also assist with risk stratification for cancer [23, 31]. While CTE is more sensitive than fluoroscopic analysis, particularly in identifying penetrating complications of Crohn's disease [25], fluoroscopy can provide functional information relating to luminal distensibility and partial obstruction that cannot be ascertained from volumetric CT imaging at a single time point. Capsule endoscopy is more sensitive at identifying mucosal inflammation than enterography (particularly in the jejunum), but is less specific and cannot identify perienteric complications, and usually requires pre-imaging assessment or use of a patency capsule [23, 32, 33]

MR enterography has an estimated performance for the detection of mural inflammation similar to CTE [24, 25], but is not available at all centers, requires much more imaging time, and is likely more prone to interobserver variability [34]. While the American College of Radiology recommends CTE as the most appropriate imaging test for patients with suspected Crohn's disease, or known Crohn's patients with fevers and abdominal pain [35], MR enterography is likely more appropriate for the follow-up of patients with known Crohn's disease for monitoring therapeutic response or those with obstructive symptoms [36]. MR is also able to stage perianal fistulas when they are present. Because of the improved spatial and temporal resolution of CT compared to MR, CTE can be used as a subsequent test when MR enterography demonstrates complex penetrating disease that may require surgical or interventional treatment.

Benefits and Risks of CT Imaging

The principal benefits of CT imaging in patients with Crohn's disease is to identify suspected and occult mural inflammation and penetrating complications that will change medical or interventional treatment. Protean patient symptomatology and the poor relationship between symptoms and biologic activity in Crohn's disease result in substantial benefit to patients from imaging [18]. However, CT utilizes low levels of ionizing radiation. While the low risk estimates of radiation-induced malignancy are uncertain enough that the absence of risk cannot be excluded on a scientific basis for a single CT exam [37, 38], a small risk should be assumed to protect patients [39, 40], particularly for younger patients who are at greatest risk [41]. Ameliorating conditions which make CT a more appropriate test compared with imaging alternatives include patient symptomatology, absence of prior imaging, contemplated surgical intervention, higher age, and contraindications to MRI. A principal advantage of CTE is its widespread availability at most medical institutions, being performed and interpreted with a high degree of local expertise.

Summary

CTE has become an accepted adjunctive test in the evaluation of patients with IBD. Its role is to detect mural inflammation and penetrating complications of Crohn's disease which are clinically occult, and its routine use results in substantial impact on patient management decisions. It is complementary with other imaging tests, and is providing new insights into Crohn's disease and newer medical therapies in IBD.

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Chapter 20

Ultrasound in Inflammatory Bowel Disease

C. Agné and M. Gebel

Keywords Transabdominal B-mode ultrasound • Color Doppler ultrasound • Power Doppler ultrasound • Small bowel contrast ultrasound • SICUS • Endoanal ultrasound • EAUS • Perianal ultrasound • PAUS • Contrast enhanced ultrasound • CEUS

Crohn's Disease

Typically Crohn's disease (CD) is diagnosed in young patients and takes a chronically relapsing course. The risk of exposure to cumulative ionizing radiation should be taken into account when choosing the diagnostic means in the management of these patients [1].

The exposure to diagnostic radiation of Crohn's patients increased from 12 mSv (1992–1997) to 32 mSv (2002–2007). An exposure of 75 mSv was calculated for up to 16% of the patients which theoretically induces a lifetime cancer risk of 7.3% [2].

Therefore, the avoidance of diagnostic radiation should be the intention of the gastroenterologist treating CD patients. Apart from MRT imaging only ultrasound fulfills the pretension to be radiation free. Besides ultrasound is a real-time imaging technique that is noninvasive, risk free, and highly accepted in everyday practice by CD patients.

A recent meta-analysis [3] indicated that there were no significant differences in diagnostic accuracy among CT, MR imaging, and US. Martinez et al. even showed slight advantages of ultrasound in diagnostic sensitivity and specificity in a recent study compared to MRI (US 91% vs. MRI 83%, US 80% vs. MRI 72%, respectively) [4].

The commonly accepted ultrasound features of activity in CD, bowel wall thickness and mesenteric lymph node enlargement, showed a good interobserver reproducibility [5]. Additionally, loss of stratification and hyperemia of the wall correlated well with severity and course of the disease [6–9].

The updated German S3 Guideline regarding the diagnosis of CD (implementation of radiological modalities) underlines the importance of ultrasound as one of the backbones in the diagnostic management of CD [10].

C. Agné, MD • M. Gebel, MD, PhD (✉)

Departments of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School,
Carl-Neuberg-Str. 1, Hannover 30625, Germany
e-mail: gebel.michael@mh-hannover.de

Initial Diagnosis of Crohn's Disease by Transabdominal B-Mode Ultrasound

For a long time ultrasound was neglected as a diagnostic tool in patients with CD. The gaseous content and the delicate anatomical structures of the bowel were believed to make a reproducible diagnostic evaluation by ultrasound difficult. One of the first publications leading the way in this field hinted to the hidden possibilities associated with this technique [11]. Modern high frequency transducers make a differentiation of five layers of the bowel wall possible [12] (Figs. 20.1–20.3):

Fig. 20.1 Longitudinal ultrasound scan: normal colon (empty) [A=colon lumen, B=colon wall, C=muscular parietal wall]

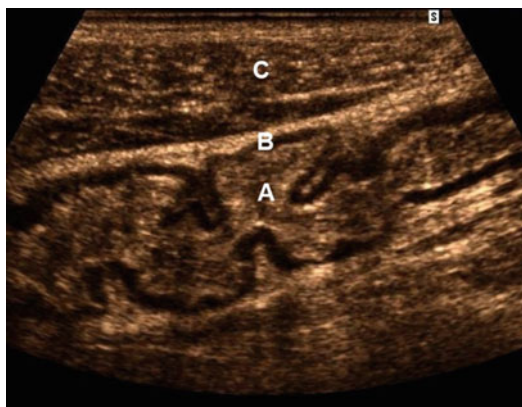


Fig. 20.2 Longitudinal ultrasound scan: normal colon (full) [A=colon lumen, B=anterior colon wall with semilunar folds]

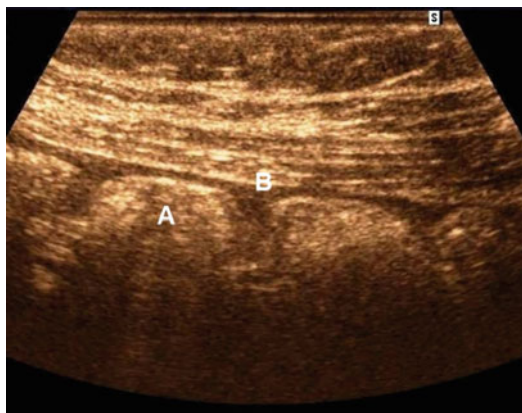
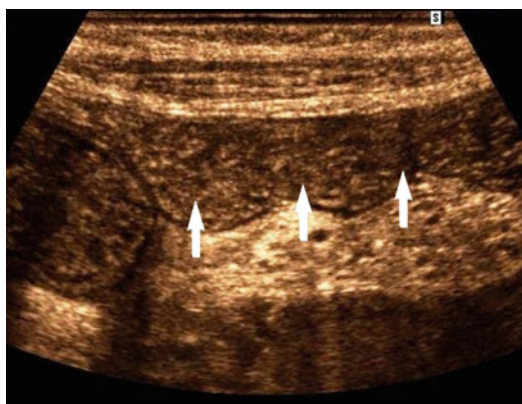


Fig. 20.3 Longitudinal ultrasound scan: normal ileum. Smooth external wall with valvulae conniventes in constant peristalsis and no visible bowel wall lumen (arrows)



1st layer:	Hyperechoic, interface between the lumen and superficial mucosa
2nd layer:	Hypoechoic, interface between the deep and superficial mucosa
3rd layer:	Hyperechoic, interface between submucosa and the muscularis propria
4th layer:	Hypoechoic, muscularis propria
5th layer:	Hyperechoic, the superficial interface of the perivisceral serosa

Typical and constant features of CD revealed by B-mode ultrasound are the presence of thickened and stiff bowel wall, modification or disappearance of echo stratification of the bowel wall, loss of peristalsis in the small bowel, and loss of haustra in the colon [13] (Figs. 20.4 and 20.5). Disease activity can be accompanied by mesenteric fat hypertrophy and enlargement of surrounding lymph nodes.

Fig. 20.4 Active Crohn’s disease with areas of retained and lost bowel wall stratification [A=bowel wall thickening with retrained bowel wall stratification, B=loss of wall layer stratification, arrows=irregular wall delineation]

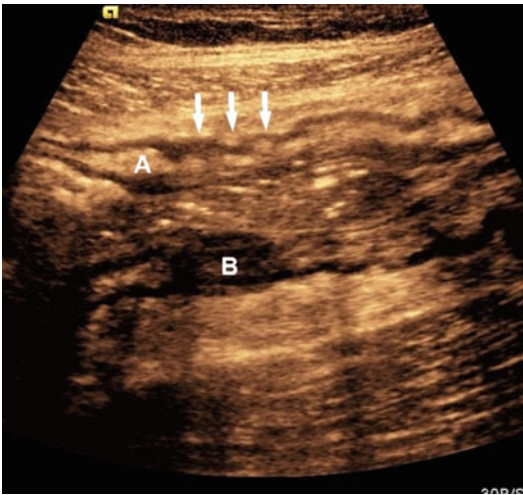
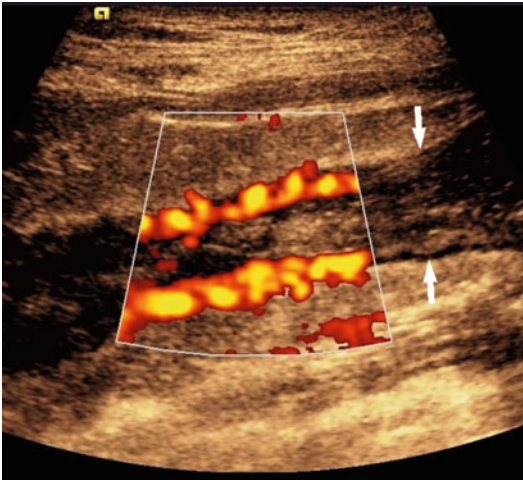


Fig. 20.5 Longitudinal ultrasound scan: inflamed small bowel wall (arrows) – hyperemia (grade 3) visualized by power Doppler ultrasound



B-mode ultrasound revealed a high sensitivity and specificity in the detection of CD (73–96% and 90–100%, respectively) compared with other methods such as endoscopy and/or radiological imaging [14]. Many studies differed in terms of study design, population characteristics, and reference standard, as did the accuracy of ultrasound as a diagnostic tool in CD. Fraquelli et al. demonstrated in a recent study that a raise of the selected threshold of bowel wall thickness from 3 to 4 mm increased the specificity (93–97%) at the extent of the sensitivity (88–75%) [15].

Assessment of Disease Activity

Several sonomorphological findings of disease activity in CD are commonly used in daily practice: degree of bowel wall thickening, echo pattern, luminal narrowing, fibro-fatty proliferation, and mesentery lymphadenopathy.

A Japanese study established a strong correlation not only between the maximum bowel wall thickness and the histological findings of surgical specimen but also between the echo pattern and the histopathological disease activity. A loss of stratification (hypoechoic echo pattern) predicted the prevalence of severe inflammation [16]. Another study described a blurred wall layer more frequently in active than in inactive disease (62.0 vs. 5%, $p < 0.05$) [14].

One of the first attempts to create a sonographic score to describe disease activity in CD was published by Futagami [17]. The sonographic score showed a good correlation to endoscopic findings but the implemented sonomorphological features – bowel wall thickness and wall stratification – correlated weakly with CDAI or biological indices of inflammation (C-reactive protein, erythrocyte sedimentation rate).

Many studies showed this just marginal correlation between disease activity measured by CDAI and sonographic findings. As a reference standard the CDAI is widely used even if it mirrors the subjective complaints of the patient without necessarily reflecting sonomorphological abnormalities or endoscopic or histological findings [18].

Assessment of Disease Location

Several studies have compared the accuracy of bowel ultrasound to various radiologic techniques in defining the anatomical location of CD [19–22]. One of the largest studies ($n = 296$) demonstrated a sensitivity and specificity according to the location of the affected bowel segment (overall: sensitivity 93%, specificity 97%). The highest accuracy was achieved in the examination of the terminal ileum (sensitivity 95%). The upper small bowel was much more difficult to assess correctly (jejunum 72%). Especially the detection of lesions in the pelvic area by transabdominal ultrasound remained poor. Proctitis was diagnosed correctly in only 15% of patients [19].

Alternative ultrasound techniques such as small bowel contrast ultrasound (SICUS) and perianal ultrasound (PAUS) enriched the noninvasive arsenal in evaluating the upper small bowel or the pelvic region. These examination techniques will be discussed later.

Detection of Complication

CD patients are affected by complications such as bowel strictures, fistulae, and abscesses in approximately 50–70% of the cases during the course of their disease [23]. Complications of CD that effect the further treatment can often be found even in asymptomatic patients. In a study by

Hirche et al. [24] routine ultrasound detected transmural inflammation in 17/255 patients without clinical signs of active disease (CD activity index <150). Eleven patients out of this group had interenteric, enteromesenteric, or perirectal fistula, whereas six patients revealed a transmural mesenteric inflammation reaction without fistula. In another series of 100 consecutive patients without active disease, fistulae were detected in 4% of the cases, mostly interenteric [14].

Detection of Abscess

An abscess develops in 15–20% of the Crohn's patients in the course of their illness. The identification of this complication is essential for the right choice of management. Abscesses require surgical or percutaneous drainage [13] and are seen as a contraindication for numerous modern medical approaches.

Abscesses appear as hypoechoic lesions with an irregular wall in transabdominal ultrasound. Internal echoes are a sign of debris and a posterior echo enhancement can hint at liquid compartments of the structure (Figs. 20.6 and 20.7). The detection of vascular signs in a lesion is a sign of inflammatory masses and offers the possibility to differentiate this lesion from an abscess [25, 26].

Fig. 20.6 Transverse ultrasound scan: terminal ileum – an intra-abdominal abscess appearing as hypo-echoic lesions with irregular walls. [A=lumen, B=thickened bowel wall, arrows=abscess]

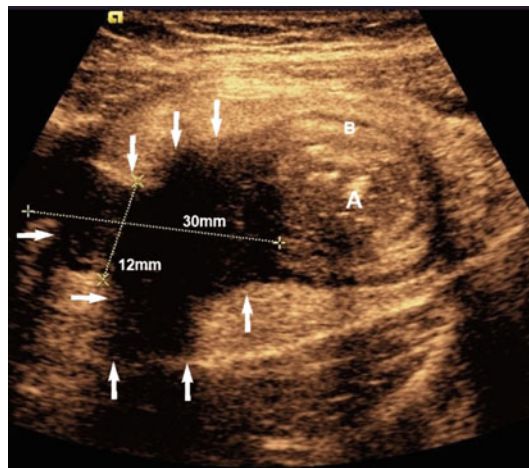
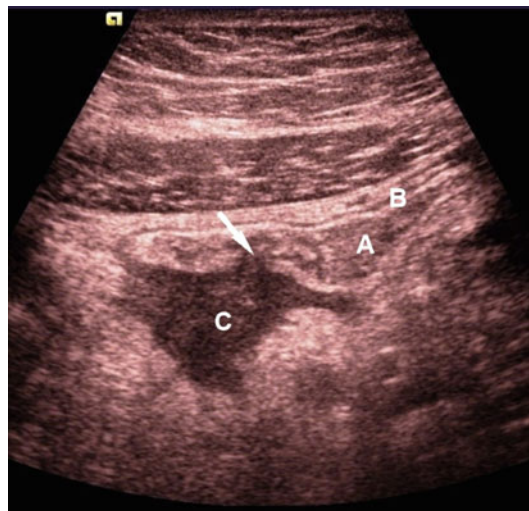


Fig. 20.7 Longitudinal ultrasound scan: loop abscess fed by transmural fistula (arrow) [A=lumen, B=bowel wall, C=abscess]



Most of the studies evaluating the accuracy of ultrasound in the detection of abscesses used computed tomography or surgico-pathological findings as the gold standard. The sensitivity and specificity ranged between 90 and 100% [27–30].

In a head-to-head comparison of ultrasound and CT scan, using only surgical findings as reference, no significant difference between the two approaches in matters of sensitivity was detected (US 91%, CT 86%)[29]. Ultrasound lacks accuracy in the detection of abdominal abscesses deep in the pelvis or of retroperitoneal collections obscured by overlying bowel gas. An advantage of ultrasound is seen in the detection of small intraparietal or para-intestinal abscesses, which may be missed or misinterpreted as short fistulous tracts or hypoechoic lymph nodes by CT scan [31].

Detection of Fistula

Fistulae connect the gut with abdominal organs (e.g., enterovesical, enterovaginal, interenteric), reach the cutaneous surface (enterocutaneous), or end blindly in the mesentery (enteromesenteric). The most common fistulae are enteroenteric (50%) which connect two bowel loops and develop often in the presence of stenosis (Fig. 20.8).

Fistulae appear as hypoechoic ducts or hypoechoic areas arising from a thickened bowel wall. Fistulae can show signs of air, debris, or intestinal material in the form of stationary or moving echoic spots [27, 28] (Fig. 20.9).

The gold standard in fistulae detection has to be considered the surgical specimen. CT scan misses fistulae in up to 40%. Therefore, CT scan cannot be recommended as reference standard in clinical trials [32]. Sensitivity and specificity of ultrasound in the detection of surgically proven fistulae varies between 70–87% and 90–96%, respectively [27, 29].

Fistulae carrying bowel segments often show a greater wall thickness and the wall stratification appears blurred with a loss of the layer architecture [14].

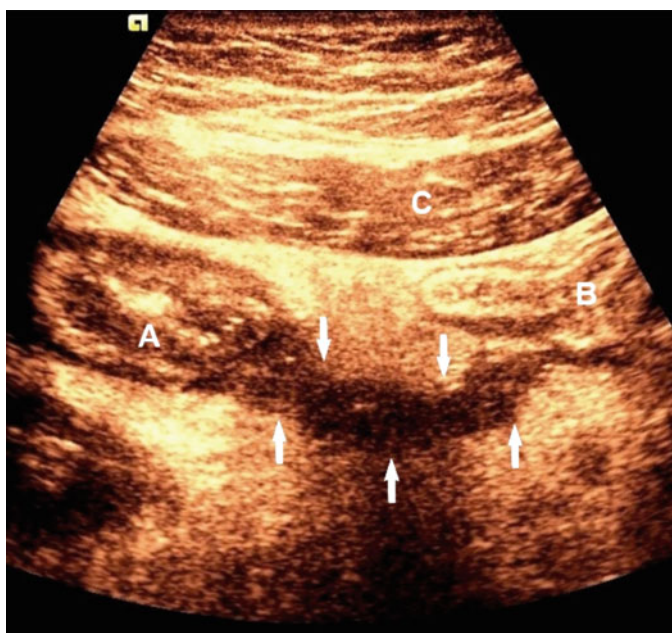


Fig. 20.8 Transverse ultrasound scan: enteroenteric fistula (*arrows*) revealed as a hypoechoic duct between two intestinal loops [A and B], [C=muscular parietal wall]

Fig. 20.9 Visualization of a fistula: forced fluid movement in the fistula (*arrows*) by mechanical pressure with the ultrasound transducer visualized by Doppler ultrasound [A = abscess, B = bowel lumen]

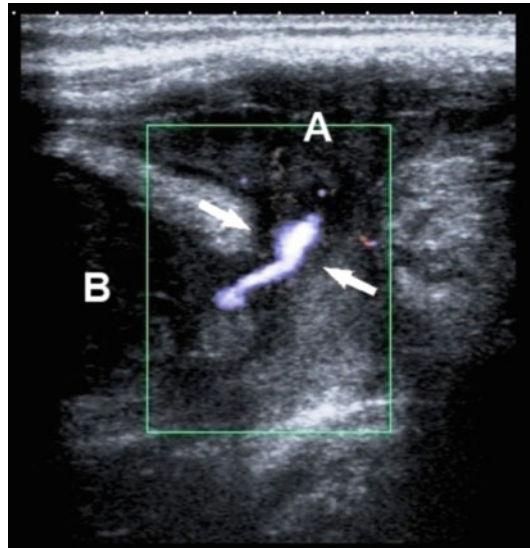
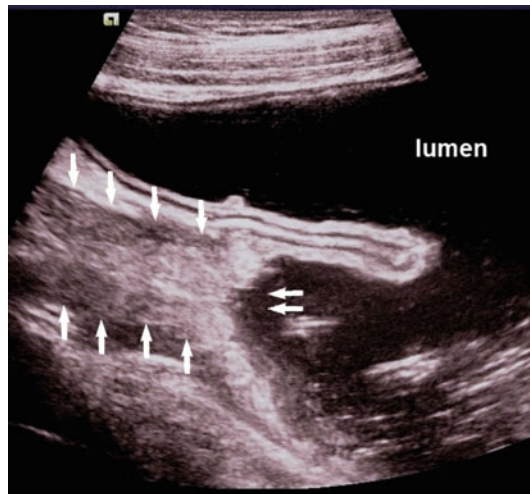


Fig. 20.10 Longitudinal ultrasound scan: high grade intestinal stenosis (*arrows*) with prestenotic dilatation [double arrow = direction of intestinal peristalsis]



Detection of Stricture

Strictures are a common cause of surgery in CD and develop in 21% of patients with ileal disease and 8% of those with ileocolic disease [33].

Significant strictures are marked by prestenotic bowel dilatation above 3 cm. The stricture itself shows a thickened bowel wall with an associated narrowed lumen (Fig. 20.10). The peristaltic of the prestenotic bowel loop is often increased [34].

The accuracy of the detection of bowel wall strictures by transabdominal ultrasound varies between 74–91% sensitivity and 93–100% specificity compared to radiological or surgical findings [22, 27, 28, 30, 35].

The differentiation between inflammatory and fibrotic strictures is of major interest in clinical practice. Inflammatory changes can be targeted by anti-inflammatory agents, whereas fibrotic strictures are treated surgically or by endoscopic intervention. A length of >30 mm of the stricture increases the probability of an inflammatory nature [36]. The loss of stratification of the affected

Fig. 20.11 Longitudinal ultrasound scan: inflammatory stenosis (*arrows*) with prestenotic dilatation [A=thickened bowel wall with loss of wall layer stratification, B=prestenotic distention of the bowel lumen]

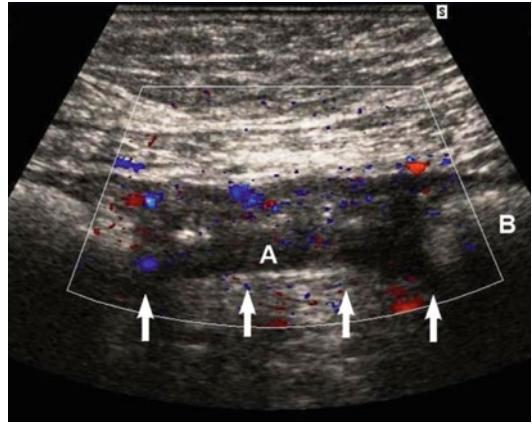
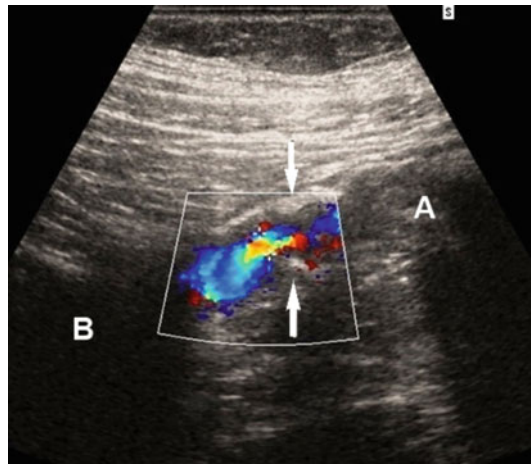


Fig. 20.12 Longitudinal ultrasound scan: fibrotic stenosis (*arrows*). There is no significant bowel wall thickening and no color Doppler signal in the bowel wall. The lumen of the stenosis (2 mm) is visualized by the color Doppler ultrasound signal when intraluminal material is pressed through the stenosis and induces a Doppler jet [A=prestenotic bowel lumen, B=poststenotic bowel lumen]



bowel wall has also been associated with an inflammatory stenosis. The hypoechoic echo pattern is due to hyperemia and neovascularization related to the inflammatory response [37] (Fig. 20.11).

On the other side preserved bowel wall layers, especially with a pronounced third layer (submucosa), hint at a fibrotic nature of the stricture (Fig. 20.12). The increase in bowel wall thickness of the third layer seems to represent an increased collagen deposition in the submucosa [38].

Extraluminal Findings

Extraluminal findings of CD by ultrasound are ascites, lymphadenopathy, and mesenteric fat hypertrophy.

Enlarged lymph nodes and free fluid are a common sighting in active CD but a correlation to disease activity could not have been shown in the past [14].

Abnormalities of fat in the mesentery including adipose tissue hypertrophy and fat wrapping have been long recognized on surgical specimens as characteristic features of Crohn's disease. Mesenteric fat hypertrophy appears in transabdominal ultrasound as a hyperechoic area surrounding the bowel wall, predominantly along the mesenteric side of the bowel [39, 40].

Different studies showed that mesenteric fatty alterations, as evaluated by CT scan or MRI, correlate with biological activity [41–43].

One of the few studies concerning the finding of mesenteric fatty alterations by ultrasound showed a significant correlation to biochemical/clinical activity of CD, the presence of internal fistulas, and increased bowel wall thickness [44]. A prediction of clinical relapse according to the presence of mesenteric fat hypertrophy was not successful.

There are few studies about extraluminal findings of CD by ultrasound. The clinical significance and implication of these findings in daily practice remain to be worked upon in the future.

Detection of Postsurgical Recurrence

Postoperative recurrence after ileo-colonic resection is a common feature of CD. The recurrence rate evaluated by endoscopy after one and three years is 73% and 90%, respectively [45–48]. The diagnostic gold standard for the detection of early signs of recurrence is conventional ileocolonoscopy with the severity of the lesions graded according to the Rutgeert's score [48].

Noninvasive techniques for the detection of postoperative recurrence in CD have been evaluated in numerous studies. One approach is the measurement of the bowel wall thickness of the anastomosis after bowel resection by ultrasound. It could have been shown that an increase in bowel wall thickness was not due to physiological healing of the anastomosis, because cancer patients did not show any increase in bowel wall thickness of the anastomosis [49]. Similar to the ultrasound findings in active CD an increase of bowel wall thickness (>3–5 mm) can be interpreted as a recurrence of the disease. The sensitivity and specificity of ultrasound in the detection of postsurgical recurrence of CD is about 80% and 85–100%, respectively [49, 50]. A more recent study by Rispo et al. [51] used a cutoff level in bowel wall thickness of 5 mm for the differentiation between mild and severe disease recurrence. A bowel wall thickness above 5 mm predicted a severe postsurgical recurrence with a sensitivity and specificity of 94% and 100%, respectively. The author concluded that by these means a clinical significant postsurgical recurrence that would need specific treatment can be detected by ultrasound with sufficient accuracy.

Different studies already stressed the value of ultrasound in the prediction of postsurgical recurrence in CD. In a study of 127 consecutive patients the group with an unchanged or worsened bowel wall thickness in month 12 after the operation measured by transabdominal ultrasound developed clinical recurrence in 90% of the cases in the next 5 years [52]. Only 33% of the patients with improved bowel wall thickness relapsed in this course of time. Similar data was shown a few years earlier by Maconi et al. evaluating the bowel wall thickness before and 6 month after bowel resection [53].

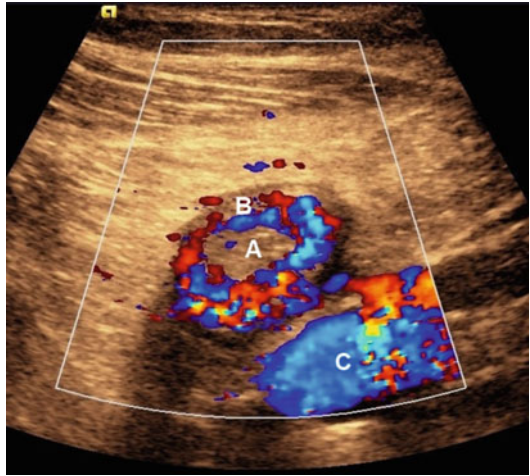
Color/Power Doppler Ultrasound

Additional information on CD can be gained using color/power Doppler ultrasound. Especially the disease activity has been studied by visualizing the extent of the bowel wall vascularity. Examination techniques of color/power Doppler ultrasound are:

- Semiquantitative documentation of the intensity of color signals and/or the analysis of Doppler curves obtained from the vessels detected within the bowel wall
- Quantitative measurement of flow parameters of the superior and inferior mesenteric arteries

Studies correlating clinical (CDAI) or biochemical (e.g., CRP/ESR) parameters with color/power Doppler ultrasound findings did not produce conclusive results. Even if an increased bowel wall vascularity was described more often in active than in quiescent CD in numerous studies, these results rarely reached clinical significance [54–56].

Fig. 20.13 Transverse ultrasound scan; inflammatory activity visualized by color Doppler ultrasound (hyperaemia grade 3) [A=bowel lumen, B=bowel wall, C=iliac vessels]



On the other hand, recent studies showed a significant correlation between endoscopic and color/power Doppler ultrasound activity scores in CD [57, 58]. A prognostic significance of color/power Doppler ultrasound findings was demonstrated by Ripolles et al. [59]. Patients in clinical remission after treatment with residual hyperemia on sonographic examination (week 4) had an unfavorable clinical course compared with patients with no or barely visible residual hyperemia.

In order to achieve optimal results in daily practice it is essential to use the right color Doppler ultrasound settings. The settings should be optimized for slow flow detection (pulse repetition frequencies 800–1,500 Hz, wall filter of 40–50 Hz, maximal color signal gain immediately below the noise threshold). Color Doppler flow is considered present when color pixels persist throughout the examination. The blood flow can be confirmed by visualization of an arterial or venous flow pattern at the location of the color pixel on spectral analysis. Vascularity is graded subjectively as absent (grade 0), barely visible (grade 1), or marked (grade 3) (Fig. 20.13).

Another access to disease activity by color/power Doppler ultrasound is the analysis of quantitative parameters. In active disease the end-diastolic blood flow in bowel wall vessels is increased over proportion, which leads to a drop in the resistance index. The decreased resistance index in active CD was described several times in the literature [54, 56, 60] (Fig. 20.14a, b).

CD affects mainly the bowel segments being supplied by the superior mesenteric artery (SMA). In active CD, the SMA flow volume was found to be greater than 500 ml/min and the resistance index was significantly lower than in the reference group with quiescent disease [61]. The pulsatility index of the SMA can also be of value in the prediction of relapse in CD when it is repeatedly evaluated [62, 63]. The decrease in the pulsatility index of the SMA is associated with remission in CD.

The measurement of quantitative pulsed Doppler indices remains to be time consuming and highly demanding. Therefore, it still is not established in daily practice.

Special Ultrasound Method in Crohn's Disease

Small Bowel Contrast Ultrasound

The extent of Crohn's lesions especially in the upper small bowel is sometimes underestimated by transabdominal ultrasound because of the insufficient distention of the bowel lumen. A new technique of SICUS was introduced by Pallotta et al. [64] in order to optimize visualization of the small

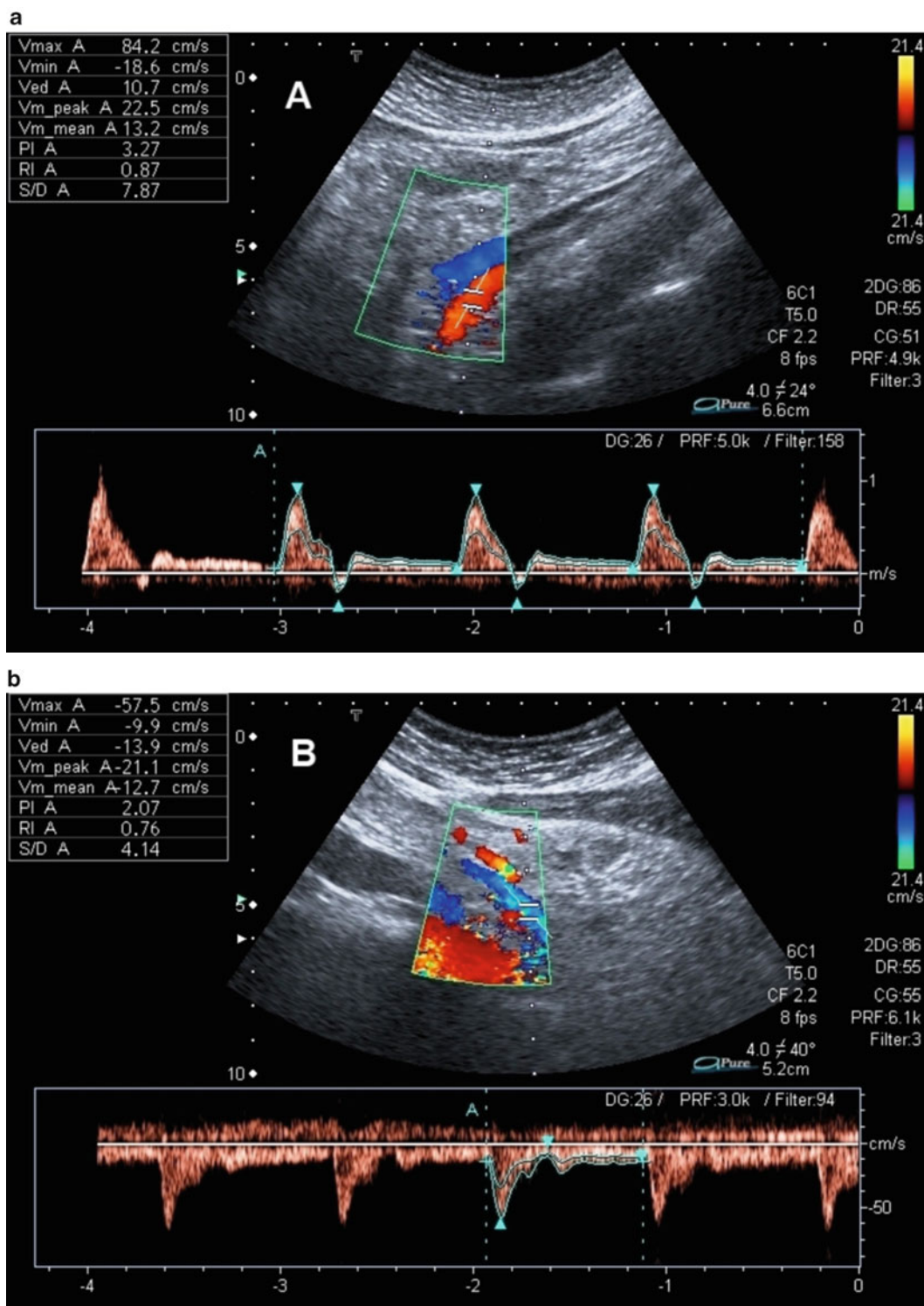
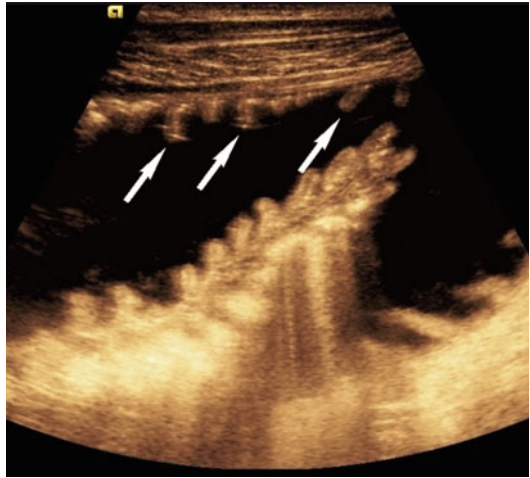


Fig. 20.14 Longitudinal ultrasound scan: proximal (a) and distal (b) superior mesenteric artery – fasting measurement of pulsatility (PI) and resistance index (RI). Blood flow volume is increased due to inflammation. This is shown by increased peak systolic velocity and the significant diastolic flow in the supplying blood vessel (decreased RI, especially in the distal superior mesenteric artery)

Fig. 20.15 Small bowel contrast ultrasound (SICUS): optimal distension of the bowel walls. Note the preserved valvulae conniventes (*arrows*), without signs of edema or inflammation



bowel in CD patients. An isosmolar solution containing a nondigestable, nonabsorbable, and non-fermentable hydrophilic macro molecule, such as polyethylene glycol (PEG) was used to distend the small bowel walls, which allowed a more detailed assessment of the wall thickness and lumen diameter. A follow-up study showed that the entire small intestine could be visualized on ultrasonography about 45 min after the ingestion of 600 ml or less of contrast solution without any significant side effects [65]. In daily practice, 300–500 ml of PEG are sufficient to achieve an optimal distention of the small bowel walls (Fig. 20.15). If stenosis is absent the examination will be completed in 10–20 min.

In comparison to small bowel follow through, SICUS reached a sensitivity of 72–100% and specificity of 97–100% in the detection of small bowel pathologies [66, 67]. False negative findings in the study by Cittadini et al. were mainly due to lymphoid hyperplasia, which is a feature of unknown significance in adults.

As expected the advantage of SICUS over conventional ultrasound could be shown especially in the detection of pathologies in the upper small bowel (jejunum: conventional ultrasound 80% vs. SICUS 100% detection rate) [68]. The sensitivity of identifying multiple strictures increased from 55% to 78% using SICUS [69] (Fig. 20.16a, b).

Other convincing features of SICUS are a good interobserver agreement [68] and easy access by inexperienced ultrasound users that even reached higher accuracy rates in comparison to an experienced examiner using conventional ultrasound [69].

SICUS was the topic of several studies evaluating the accuracy in the prediction of postsurgical recurrence [70–72]. A sensitivity and specificity of 77% and 94% for transabdominal ultrasound and 82% and 94% for SICUS was shown in a study on 40 patients with CD with previous bowel resection [70].

Endoanal and Perianal Ultrasound

Perianal disease is a common manifestation in complicated CD occurring in 20–40% of patients in the course of their disease [73]. Pelvic magnetic resonance imaging (MRI) and endoanal ultrasound are the established methods for the assessment of perianal inflammatory lesions in patients with CD. MRI and EAUS are especially recommended by German S3 Guidelines of CD in patients with

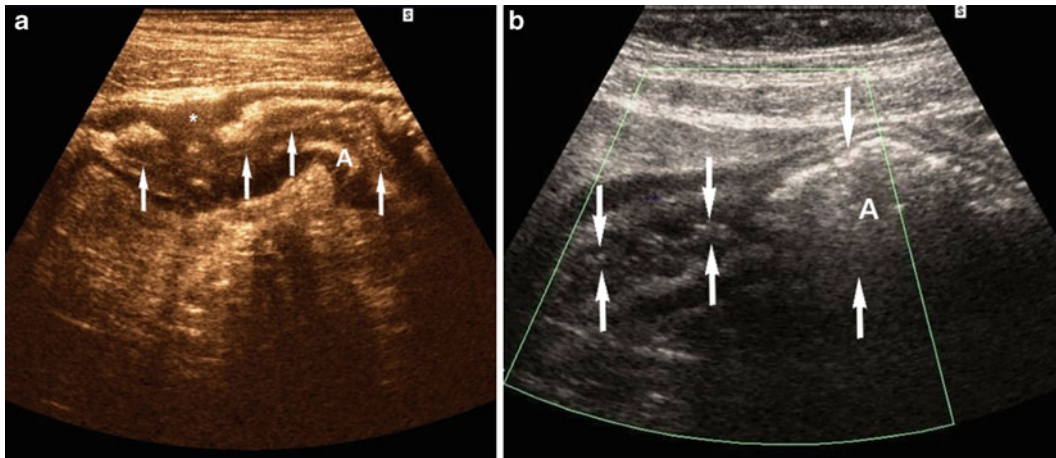


Fig. 20.16 (a) Longitudinal ultrasound scan: inflammatory stenosis. No prestenotic dilatation can be detected in B-mode ultrasound [A=thickened bowel wall, arrow=lumen, *=loss of bowel wall stratification] (b) Small bowel contrast ultrasound (SICUS): inflammatory stenosis. Demarcation of prestenotic bowel dilatation by anechoic contrast flow [arrows=lumen, A=liquid and air in the dilated lumen]

perianal disease [10]. The combination of MRI and EAUS is capable of detecting perianal fistulae with a sensitivity of 100% [74, 75]. These methods require specialized and fairly expensive equipment and experienced investigators. Furthermore, MRI is not applicable in patients with metallic clips or suffering from claustrophobia. EAUS can be painful or impossible to perform in patients with anal stenosis. Alternatively PAUS was introduced into clinical practice. It can be performed using regular ultrasound probes without special patient preparation. Further advantages are the unproblematic use in patients with anal stenosis and the examination of the gluteal region which is a restriction to EAUS. PAUS is comparable in sensitivity and specificity to MRI and EAUS in the detection of perianal fistulae and/or abscesses [76–79].

Contrast Enhanced Ultrasound

Contrast enhanced ultrasound (CEUS) is the next logical step after color Doppler ultrasound in the assessment of bowel wall vascularization. The accuracy of power Doppler ultrasound is limited by tissue motion artifacts and a possible transmural vessel perfusion below the detection threshold [80].

CEUS combines second-generation US contrast-enhancing agents with low mechanical index real-time harmonic US. A real-time visualization of the small vessels in the bowel wall with image contrast similar to that with computed tomography and MRI becomes available [81].

The absence of established CEUS parameter has led to the development of different semi-quantitative and quantitative approaches to predict disease activity in CD.

Highly recognized by the medical society was a study by Migaleddu et al. [82] defining three major enhancement patterns: submucosal enhancement or transmural enhancement with an outward or inward flow direction. Using the endoscopic and histologic findings as reference standards, CEUS showed higher performance than conventional ultrasound or color Doppler ultrasound in the detection of active disease (93.5% sensitivity, 93.7% specificity, and 93.6% overall accuracy). Despite the impact of the study, the enhancement pattern remained a semiquantitative approach that is highly influenced by the subjective assessment of the ultrasound examiner.

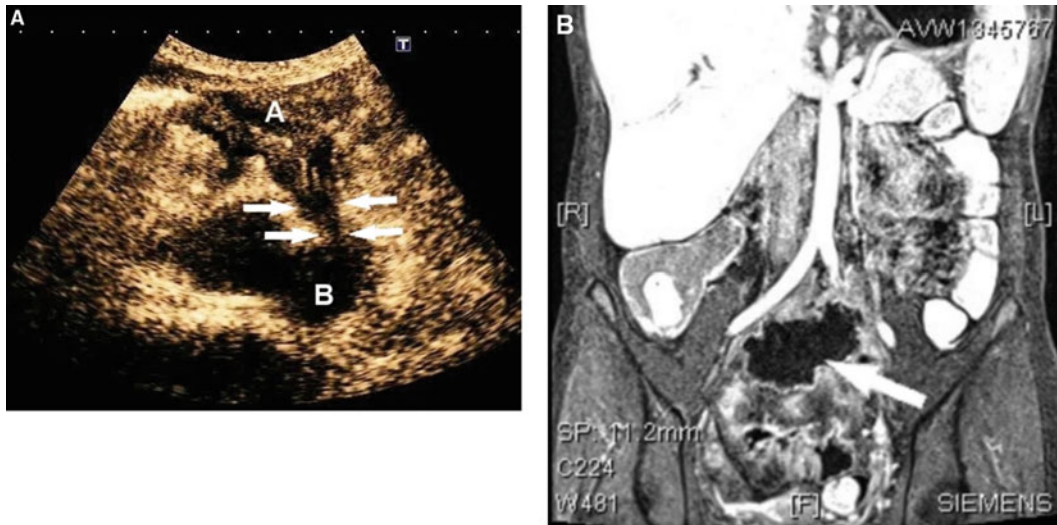


Fig. 20.17 (a) Contrast enhanced ultrasound (CEUS): bowel and abscess wall enhancement after second-generation contrast agent injection. [A=longitudinal section of the terminal ileum, B=abscess, arrows=fistula] (b) MRI: abscess (arrow)

The use of the increase of bowel wall enhancement after contrast application in relation to the baseline enhancement did show promising results. In a population of 61 patients, the sensitivity and specificity to predict moderate and severe disease was 96% and 73%, respectively [7].

Two studies indicated that the time to peak in CEUS examinations could be a parameter worth measuring to evaluate disease activity. An increase of time to peak was connected to a decrease in clinical activity [8]. Furthermore, a negative correlation between a histopathological score, according to Bataille et al. [83], and the time to peak was found [84].

The differentiation of fibrotic and inflammatory strictures could be one of the beneficial applications in clinical practice but there is still no established approach to solve this puzzle by CEUS. Furthermore, the use of CEUS in the detection and localization of fistulae and abscesses need further studies (Fig. 20.17a, b).

Ulcerative Colitis

The regions of interest in patients with ulcerative colitis (UC) are far more accessible by endoscopy than in patients with CD. A continuous inflammation from the anus that can extend up to the cecum is found in most of the cases. Rectal sparing and backwash ileitis are phenomenon that can be detected in rare cases of UC.

In early and mild cases of UC, unspecific signs of an inner hypoechoic layer can be found. It was postulated that the hypoechoic layer represents the endoscopic findings of the swollen mucosa. In more severe to fulminant cases, a transmural bowel wall thickening similar to CD has been described. Normally the bowel wall stratification is preserved [14]. In active UC, the thickened mucosa can be explained by the round cell infiltration in the lamina propria, whereas the submucosa swells with edema development.

Unfortunately, no correlation between sonographic activity parameters of UC (wall thickness, symmetry of thickness, transmural reaction, and extraluminal findings, e.g., more than two lymph

nodes) and endoscopic disease activity (Colitis activity index, CAI) has been found [14]. Two recent studies from Parente et al. [85, 86] contradict these common findings. These studies suggest that an ultrasound score based on bowel wall thickness and intramural blood flow, graded by color Doppler ultrasound can be used as a surrogate of colonoscopy in assessing a short-time response of severe forms of UC to therapy. Furthermore, a prediction of the outcome at 15 month after the steroid treatment was possible based on the ultrasound findings at month 3. This is very interesting data which has to be reproduced in further studies before transabdominal ultrasound can be recommended more strongly in the management of UC patients.

The extent of the inflammation in UC measured by ultrasound was evaluated in two studies. The sensitivity varied according to the bowel segment. The best results were achieved in the left colon (>95%) [87, 88].

A quantitative assessment of power Doppler indices can help to distinguish between active and inactive disease or can be used as a parameter to evaluate the response to treatment. The mean blood flow volume, mean peak systolic and end diastolic velocity in the inferior mesenteric artery were significantly higher in active UC than in inactive patients [89].

Detection of Primary Sclerosing Disease by Ultrasound

Primary sclerosing cholangitis (PSC) is a chronic liver disease that is characterized by inflammation, fibrosis, and the destruction of intra- and extrahepatic bile ducts.

PSC is the most common form of chronic inflammatory liver disease in IBD patients, with a prevalence of up to 8% [90, 91]. The vast majority of PSC patients (70–90%) had, or subsequently will develop, an inflammatory bowel disease [92, 93].

The detection of enlarged perihilar lymph nodes was identified as a highly predictive indicator for the presence of PSC [94, 95]. It was proposed to use ultrasound as a screening tool for PSC in IBD patients. In combination with serological makers ultrasound allowed a better selection of patients for invasive procedures as ERC.

Perspective

The transmural aspect of CD in imaging is only marginally understood. This kind of “extra” information is just sufficiently delivered by ultrasound and MRI and should have an impact on daily treatment decisions. A lot of questions have to be unraveled, e.g., which bowel wall findings predict Crohn’s complications or response to treatment? A cooperation of experts in gastroenterology radiology and pathology will hopefully enlighten this aspect in the future.

Quantification of ultrasound findings will be an important step in the development of ultrasound techniques in inflammatory bowel disease. Subjective estimations will be replaced by hard numbers. At the moment we see the first big leaps forward in software development for quantitative CEUS. The clinical and prognostic value will be evaluated in the future.

Elastography techniques (Fibroscan – ARFI acoustic radiation force imaging) are established tools in hepatic fibrosis measurement. The first data about the impact on the management of patients with inflammatory bowel disease is expected. The quantification of intestinal inflammatory or fibrotic changes by ARFI is one of the goals we pursue in our unit at the moment. The first promising results will be published soon.

Conclusion

Ultrasound is the backbone in the detection and management of patients with CD. It is the only noninvasive, risk free, and real-time method to assess the important features in CD patients from intestinal activity to postsurgical recurrence. In the hands of an experienced investigator, ultrasound is a powerful technique to guide the treatment in CD directly from the patient's bedside.

Modern high frequency transducers offer the highest local resolution in bowel imaging today. Ultrasound is capable to assess extraluminal findings and complications in CD that can be missed by endoscopy or other imaging techniques. Limitations of the normal transabdominal B-mode ultrasound can be overcome by the use of extended ultrasound features (e.g., color Doppler ultrasound) or special ultrasound methods (e.g., CEUS or SICUS). Intestinal inflammatory activity can be visualized by color Doppler ultrasound or CEUS. Abscess demarcation can also be supported by these ultrasound techniques. SICUS is a useful tool in the detection of pathologies in the upper small bowel region. Besides SICUS offers a real-time assessment and grading of intestinal stenosis. Endoanal and PAUS have a comparable accuracy to MRI in the detection of perianal inflammatory pathologies in CD.

So far mucosal healing has been defined endoscopically as the absence of ulcers. Ultrasound findings include trans- and extramural events indicating the state of the disease independent of clinical markers. If some kind of healing beyond superficial markers is regarded, ultrasound methods seem to be indispensable for the development and evaluation of actual and future effective treatment regimens.

In order to support the use of modern ultrasound techniques in clinical practice and to sustain the quality of the examinations, the ultrasound simulator was introduced into teaching routine. The training on specific patient models (e.g., CD) is helpful in the acquisition of ultrasound skills [96, 97].

In order to achieve the best quality in intestinal ultrasound, the investigator should be taught in these specific ultrasound techniques.

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Chapter 21

Laboratory Evaluation Including Novel Diagnostic Markers of Inflammatory Bowel Disease

Iris Dotan

Keywords IBD diagnosis • Activity • Stool markers • CRP • ESR • Anti-glycan antibodies

Introduction

Laboratory markers in inflammatory bowel diseases (IBD) may be used for several reasons. Their evaluation may be performed in order to diagnose IBD, to substratify it to ulcerative colitis (UC) or Crohn's disease (CD), and to determine prognosis (including response to therapy). In addition, disease activity needs to be constantly re-evaluated. When using a laboratory marker in IBD one should recognize that no laboratory marker can serve all functions, and that they are adjunct bricks, not sole determinants, in determining diagnosis, prognosis, activity, etc. Their importance is usually in being accessible, easy to perform, not invasive, and relatively cheap compared with the endoscopic and imaging studies used for the same tasks. This review focuses on diagnostic and prognostic markers in IBD, as well as on modern indices of disease activity. The purpose is not to perform an exhaustive description of every laboratory marker studied in IBD, but to mention the ones that the author believes are most relevant currently and for the next 5 years.

Of note, pharmacogenetic studies, emerging as laboratory markers that will be increasingly used, such as TPMT evaluation, are beyond the scope of this review.

IBD Diagnosis

Using clinical, endoscopic, histologic, and imaging studies may be straightforward. However, in ~10–15% of patients with disease limited to the colon, a firm diagnosis of UC or CD cannot be established, a condition designated IBD-undetermined (IBD-U). In this situation, as well as in the pediatric population where IBD-U is diagnosed more frequently [1], additional measures such as serologic markers may be needed for disease diagnosis and differentiation. Serologic markers also

I. Dotan, MD (✉)

Department of Gastroenterology and Liver Disease, IBD Center, Tel Aviv Sourasky Medical Center,
6 Weizmann Street, Tel Aviv 64239, Israel
e-mail: irisd@tasmc.health.gov.il

Table 21.1 Serologic markers, presumed antigens/targets, and their immunologic effects

Serologic marker	Presumed antigen/antigen source	Antibody isotype, presumed immunologic effects	Reference
ANCA antineutrophil cytoplasmic (auto) antibodies	Antigens in azurophilic granules of polymorphonuclear leukocytes <i>Microbial component</i>		[2, 3, 26, 27]
ASCA anti- <i>Saccharomyces cerevisiae</i> antibodies gASCA	Mannan, <i>Saccharomyces cerevisiae</i> strain <i>Su 1</i> Covalently bound mannan	IgA, IgG	[4–6]
OmpC outer membrane porin C	<i>E. coli</i>	IgG	[3]
I2	<i>P. aeruginosa</i>	IgG2a T cell superantigen bioactivity	[7, 8]
CBir1-flagellin	Flagellin of commensal bacteria	Activate innate immunity via TLR5, adoptively transferred flagellin-specific CD4+T cells induce murine colitis	[9]
ALCA anti-laminaribioside carbohydrate antibodies	Laminaribioside, a component of laminarin, b-1-3-glucan family cell walls of fungi and yeast, food (oats) and algae	IgG. Activation of innate immune responses, activation of dectin-1-cytokine secretion from macrophages and T cell proliferation	[10–12]
ACCA anti-chitobioside carbohydrate antibodies	Chitobioside, a component of chitin, N-acetyl-b-D-glucosamine insect cuticle, cell walls of bacteria and yeast	IgA chitinase 3-like-1 that can bind chitin is expressed by lamina propria lymphocytes, and intestinal epithelial cells, and is upregulated in intestinal inflammation	[13]
AMCA anti-mannobioside carbohydrate antibodies	Mannobioside	IgG	
Anti-L	Laminarin	IgA	[14]
Anti-C	Chitin	IgA	[14]

enable disease stratification into high-risk groups, thus potentially contributing to disease management. Thus, it seems reasonable to assess serologic markers at diagnosis, serving diagnostic and prognostic needs. As separation between diagnostic and prognostic needs seems artificial, the characteristics of each serologic marker would be reviewed in one section, including both diagnostic and prognostic features.

As a group, serologic markers (Table 21.1) are directed against microbial antigens – usually proteoglycans – and their existence, especially in high titers, and when there is response against more than one antigen is usually associated with a more aggressive disease phenotype (Table 21.2). The exact link between innate and adaptive immune responses that result in the formation of these antibodies is still unclear.

Serologic Markers

ANCA (anti-neutrophil cytoplasmic antibodies): the ANCA family consists of several groups of antibodies distinguished by their characteristic pattern as detected by immunofluorescence of alcohol-fixed neutrophils, where pANCA is characterized by a perinuclear and cANCA by a diffuse cytoplasmic staining pattern. pANCA is a heterogeneous group targeting different antigens such as

Table 21.2 Serologic markers and their clinical associations

Serologic marker	Association with IBD	Disease location	Disease behavior	Reference
ANCA	UC		In UC: treatment-resistant left-sided disease, early surgery, development of pouchitis	[15–17]
	CD	LB	In CD: UC-like behavior. Older age at onset	
ASCA	CD	SB	Earlier age of disease onset, perforating, stricturing	[16–18]
gASCA	CD	SB	Early surgery, more aggressive pediatric disease. Need for surgery	[19]
OmpC	CD	SB	Perforating, stricturing, small bowel surgery, rapid disease progression in pediatric population, higher titers associated with NOD2 carriage, better response to antibiotic treatment	[6, 15, 20–22]
I2	CD	SB	Perforating, stricturing, small bowel surgery, rapid disease progression in pediatric population, higher titers associated with NOD2 carriage, better response to antibiotic treatment	[6, 15, 20–22]
CBir1-flagellin	CD	SB	Small bowel disease, perforating, stricturing, higher titers associated with NOD2 carriage	[21–23]
ALCA	CD	SB	In combination with ACCA and gASCA – small bowel and penetrating or stricturing disease	[24, 25]
ACCA	CD		Need for surgery	[25]
AMCA	CD		Need for surgery	[25]
Anti-L	CD	SB, LB CD	Addition of anti-L to ANCA and ASCA differentiated UC and colonic CD	[14]
Anti-C	CD	Penetrating perianal		[14]

myeloperoxidase (MPO), proteins within azurophilic granules of neutrophils (β -glucuronidase) and intracytoplasmatic proteins that are not contained within granules (catalase, α -enolase, lactoferrin, elastase, and cathepsin G). Moreover, pANCA may lose the perinuclear pattern if neutrophils are fixed with formalin. DNase digestion increases the specificity of the pANCA group to UC. The antigen targeted by the IBD-specific pANCA may be mycobacterial histone 1 [26]. This, as well as additional observations [3, 27], raised the notion that pANCA, in similarity with the other serologic markers to be discussed, actually targets microbial antigens.

The relatively low sensitivity of ANCA [28, 29], the difference in testing, and its existence in several other immune-mediated diseases makes its use as a single serologic marker suboptimal. pANCA is detected in ~50–85% of patients with UC [30–32], but also in ~5–28% of CD [33], and control (non-IBD gastrointestinal disorders or healthy) patients [34, 35].

In order to increase the specificity for UC diagnosis, the combination pANCA and ASCA may be used with sensitivities of 44–57 and 97% specificity [30, 35].

ANCA may contribute to differentiating IBD-U into UC or CD, a differentiation that becomes increasingly important as surgical intervention is considered. In addition, pANCA may contribute to stratification of CD where patients positive for pANCA tend to have a more “UC-like” disease, with a more benign disease course [18].

ASCA (anti-*Saccharomyces cerevisiae* antibodies): This antibody, described more than 30 years ago [36], targets mannan epitopes of the yeast *Saccharomyces cerevisiae*, specifically strain Su1 [4]. Detected in ~48–80% of patients with CD [37, 38], ASCA may also exist in ~10–15% of patients with UC and HC [38, 39]. It is highly specific for CD (80–95%) with variable sensitivity [39]. Recently ASCA was detected in patients with celiac disease as well [40, 41], suggesting that mannan residues may evoke small intestinal immune response that is not specific only to patients with CD. This notion makes antibodies to tissue transglutaminase a necessary component of the serologic diagnosis of IBD.

ASCA positivity or high ASCA titers were associated with small intestinal CD and with a more aggressive phenotype such as stricturing or penetrating and the need for surgery [15, 42, 43].

Interestingly, ASCA was detected in up to 20% of apparently healthy family relatives of patients with CD [44–46] suggesting that antibodies to microorganisms in CD may represent a common predisposition, rather than merely small intestinal inflammation and leaky mucosal barrier. It may also identify patients with CD years before diagnosis, as shown by Israeli et al. [47]. Thus, intervention may be offered to these populations at risk, if additional prospective follow-up studies will demonstrate that ASCA positivity is associated with later disease development.

OmpC: Anti-*Escherichia coli* outer membrane porin c, directed at antigens from commensal *E. coli*, exist in 37–55% of patients with CD and 2–11% of patients with UC [3, 42]. OmpC positivity was associated with more aggressive disease phenotypes such as the need for surgery or internal perforating disease [15] as well as more rapid progression toward a more aggressive (i.e., internal perforating/stricturing) disease phenotype in children [20]. Of note, similarly to several serologic markers, OmpC positivity was demonstrated in unaffected family members of patients with CD [48] suggesting that they reflect genetic/familial risk factor for CD.

I2: directed against the *Pseudomonas fluorescens* sequence I2, exist in 54% of patients with CD [7, 8] as well as control (especially inflammatory) patients [49]. Antibodies against I2 are associated with small bowel CD, and with better response to fecal diversion in patients with CD [50].

CBir1 Flagellin: Directed at flagellin of commensal bacteria, these antibodies exist in ~50% of CD, but not in patients with UC or OGD [9]. CBir1 was independently associated with small bowel CD and the more complicated (fibrostenotic and internal perforating) phenotypes [23]. Flagellin is a ligand for TLR5 [51] and their ligation activates NFκB, thus resulting in the expression and secretion of various proinflammatory mediators [52].

The Novel Anti-glycan Antibodies

A group of antibodies directed at glycan epitopes found on microorganisms. ALCA, anti-laminaribioside carbohydrate antibodies (ALCA, IgG), directed against laminaribioside, exists in approximately a third of patients with CD [24]. ACCA, anti-chitobioside carbohydrate antibodies (ACCA, IgA), directed at chitobioside, a glycan found in microorganisms, crustacea, insects, is less prevalent than ALCA, but similarly, is associated with more aggressive disease phenotypes [53].

AMCA, anti-mannobioside carbohydrate antibodies (AMCA), directed at manobioside, a building block of mannan is different from the phosphopeptidomannan epitopes targeted by ASCA [14, 24].

Importantly, 44% of ASCA-negative patients with CD were ALCA or ACCA positive, suggesting that the novel anti-glycan antibodies may further improve the serologic diagnosis of CD. ALCA positivity and higher titers were associated with small bowel CD as well as with a more aggressive disease phenotype, i.e., stricturing or penetrating or the need for surgery [24].

Anti-L and Anti-C: Anti-laminarin (Glc(β 1,3))₃ n (Glc(β 1,6))_n carbohydrate IgA antibody (anti-L) and anti-chitin (GlcNAc(β 1,4) n carbohydrate IgA antibody (anti-C) were described in CD by Seow et al. [14]. The rationale was to investigate serologic responses to glycans that were associated with the originally described ALCA and ACCA but will be directed against the larger glycans, laminarin, and chitin, instead of laminaribioside and chitobioside, respectively. The main significance of anti-L and anti-C according to the Seow study was that adding anti-L and anti-C to gASCA IgG and pANCA improved discrimination between CD and UC ($p < 0.001$). Importantly, adding anti-L to gASCA and pANCA contributed to better differentiation of colonic CD and UC ($p = 0.02$). Generally combinations and panels of antibodies may increase the diagnostic specificity of IBD (specifically CD) [5, 25, 54] as well, as serve as prognostic factors, associated with more aggressive disease phenotypes [15, 24, 35].

Disease Activity

White blood cell count increases as part of the acute phase response. Thus, it is not a specific feature of IBD and may be seen in other inflammatory conditions and stressful events. Glucocorticoids may increase and azathioprine/6-mercaptopurine may decrease white blood cell count. Platelet count will also increase and is therefore an indication of, without being a specific marker of, inflammation. Given the wide range of normal values for platelet count, it has been less useful. Albumin is a typical example of a negative acute phase reactant and decreased levels may be found during inflammation. However, other conditions prevalent in IBD such as malnutrition and malabsorption may also cause hypoalbuminemia.

Erythrocyte sedimentation rate (ESR) depends on the plasma concentration and the number and size of the erythrocytes, thus it is influenced by anemia, commonly observed in patients with IBD, as well as by polycythemia, and thalassemia.

Older studies showed a good correlation between ESR and clinical activity [55]; however, this depended on disease location, and correlation with proctitis or proximal CD was low [55, 56]. Both CRP and ESR correlated well with disease activity but the correlation was better for CRP [57]. Good correlation existed between CRP and endoscopic and histological activity in CD. For UC, this correlation was less strong [57, 58].

C reactive protein (CRP) is among the most important acute phase proteins in humans [59]. During an acute phase response such as inflammation, its production by hepatocytes significantly increases under the influence of interleukin (IL-6), tumor necrosis factor α (TNF- α), and IL-1 β . It has a short half-life (19 h) compared with other acute phase proteins and will therefore rise early after the onset of inflammation and rapidly decrease after its resolution. CRP levels in severe active inflammation or bacterial infection are typically 50–200 mg/L, and levels of 200–250 mg/L may be seen in more extreme conditions such as burns [60, 61]. CRP and ESR were the best predictors of CD relapse in patients with medically induced remission, compared with other laboratory markers such as blood count, α 1 antitrypsin, and orosomucoid [62]. Remarkable heterogeneity in the CRP response between CD and UC was reported [60]. Although CD was associated with a strong CRP response, UC had only a modest-to-absent CRP response. The introduction of hs-CRP assays to clinical practice enabled evaluation of CRP concentrations that were previously thought to be within normal limits when studied with less sensitive assays. Despite the fact that patients with CD generally present higher hs-CRP concentrations, hs-CRP concentration and IBD disease activity correlated, and were similar for both UC and CD [63]. Importantly, increased CRP levels are associated with better response rates to treatment, and normal CRP levels predict high placebo response rates in clinical trials with biological markers [64].

Fecal Markers: Calprotectin

Stools are easily accessible in patients with IBD and may have a higher specificity for IBD in the absence of gastrointestinal infection, compared with serum indices that may be influenced by the acute phase. Fecal markers may also better represent the situation in the intestinal mucosa, thus potentially avoiding invasive/imaging studies.

Calprotectin, a neutrophil-derived protein, is a 36-kDa calcium- and zinc-binding protein. Its presence correlates with neutrophil presence in the gastrointestinal tract [65]. A cut off of 30 mg/g had 100% sensitivity in discriminating active CD from IBS. Using a higher cut off of 50 mg/L, IBD relapse within a year after remission could be predicted with sensitivity and specificity of 90 and 83%, respectively [66]. This cut off was associated with a 13-fold increased risk of relapse. These values were similar when patients with CD or UC were examined separately. Fecal calprotectin also correlates well with endoscopic and histological activity in patients with UC [67] and in CD [68] and increased calprotectin levels normalize once the inflammation is resolved. Of note, in addition to IBD, calprotectin may increase in neoplasia, polyps, infections, and the use of NSAIDs [69].

Lactoferrin: Lactoferrin (LF) is an iron-binding glycoprotein secreted by most mucosal membranes and a major component of secondary granules of neutrophils [70, 71]. Elevated LF characterizes active IBD, may be used to differentiate between IBD and IBS and to assess disease activity in the pediatric as well as the adult population [72–76]. Importantly, in a study that included 465 patients and controls, Sidhu et al. also showed that LF levels were significantly higher in patients with inactive IBD than in patients with diarrhea predominant IBS, a feature that may contribute to the differential diagnosis of IBS symptoms in an inactive patient with IBD [77].

Conclusions

Laboratory markers in the serum and feces may be used for better diagnosis of IBD, and for discriminating between quiescent IBD and IBS. Serologic responses against microorganisms contribute to disease diagnosis as well as for differentiation between CD and UC. However, their major significance may be the possibility to use them at diagnosis for prognostic reasons. Panels and combinations of serologic markers, especially when high titers are observed, seem to be more contributory regarding diagnosis, prognosis, and prediction of complication.

Of all the serum laboratory markers assessing activity, CRP is the most studied and has been shown to have the best overall performance as a marker of inflammation, disease activity, specifically in CD.

Fecal markers are attractive laboratory aids because of their ability to directly represent intestinal inflammation as well as their easy accessibility, especially in patients with IBD where diarrhea is a prominent symptom. The possibility that these markers may serve as an alternative to the sophisticated endoscopic and imaging examinations used for the same purposes still needs to be examined. Better characterization of disease phenotypes, associated with the different laboratory markers may have significant implications in optimizing IBD management.

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Chapter 22

Histology and Pathology of Inflammatory Bowel Disease

Nora E. Joseph and Christopher R. Weber

Keywords Inflammatory bowel disease • Ulcerative colitis • Crohn's disease • Dysplasia • Infectious colitis • Microscopic colitis • Ischemia • Inflammatory polyp

Introduction

The diagnosis of inflammatory bowel disease (IBD) depends as much on pathologic findings as it does on clinical presentation and endoscopic appearance. This chapter focuses on the gross and microscopic features used to diagnose IBD. As the clinical course for many IBD patients is often complicated by surgical and medical interventions it is important to relay diagnostically important information between team members, particularly the pathologist and gastroenterologist. Emphasis has been placed on the written pathology report to provide a framework for future communication. Starting with a discussion on general features of IBD, subsequent sections will focus on unique criteria required to differentiate Crohn's disease (CD) from ulcerative colitis (UC). A major hurdle in rendering a diagnosis of IBD can be excluding other chronic disease processes. Therefore, the final segment of this chapter delineates the differential diagnosis of IBD in detail. Relevant aspects of disease etiology, pathogenesis, epidemiology, and management are only briefly addressed when pertinent to the diagnostic process, as these aspects of IBD are extensively reviewed elsewhere in this book.

General Features of IBD

IBD is a disease characterized by relapsing and remitting episodes of diarrhea, which can be further subdivided into CD and UC based on unique clinical, endoscopic, and pathologic features. Prior to rendering a diagnosis of IBD, careful inspection of macroscopic and microscopic features is required to rule out other disease processes. To that end, three key histologic features must be closely evaluated: chronic injury, disease distribution, and disease activity.

N.E. Joseph, MD • C.R. Weber, MD, PhD (✉)

Department of Pathology, The University of Chicago, 5841 South Maryland, MC 1089, Chicago, IL 60637, USA

e-mail: christopher.weber@uchospitals.edu

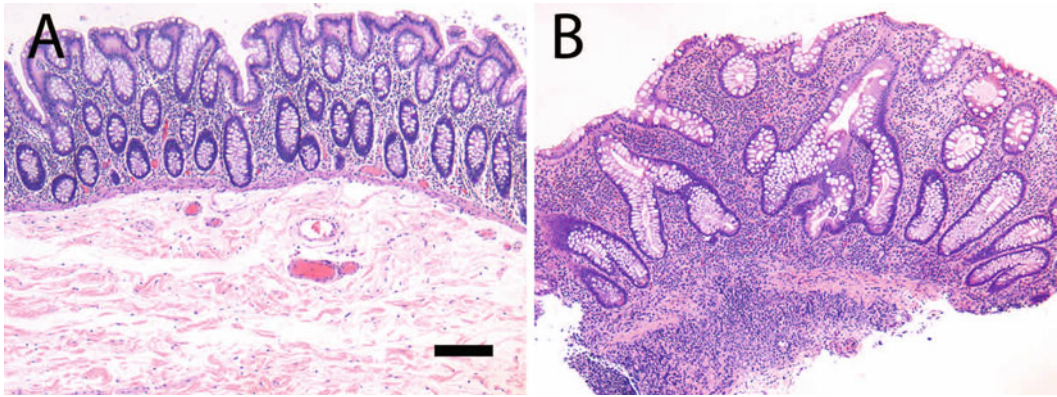


Fig. 22.1 Architectural distortion is a feature of chronic injury. (a) Normal colonic mucosa displays evenly spaced nonbranched crypts that line up along the muscularis mucosa. The lamina propria contains a sparse infiltrate of inflammatory cells. (b) Crypt branching and a dense, basal, lymphoplasmacytic infiltrate are features of chronic injury in CD

Chronic Injury

Chronic injury is not synonymous with IBD, as other chronic intestinal diseases may show similar features of cyclical injury and regeneration. However, once histologic evidence of chronic injury is identified, self-limited processes, such as most forms of infectious colitis, can essentially be ruled out. Mimics of IBD are discussed in detail in the last section of this chapter. Chronic mucosal injury occurs during periods of regeneration following repeated episodes of crypt destruction and is defined by three hallmark histologic features: architectural distortion, basal lymphoid hyperplasia, and metaplastic epithelial changes.

Architectural distortion. Crypts are irregularly spaced, branched, and/or reduced in number. Crypts in normal mucosa extend to the muscularis mucosae (Fig. 22.1a); however in IBD, crypts are of variable lengths (Fig. 22.1b).

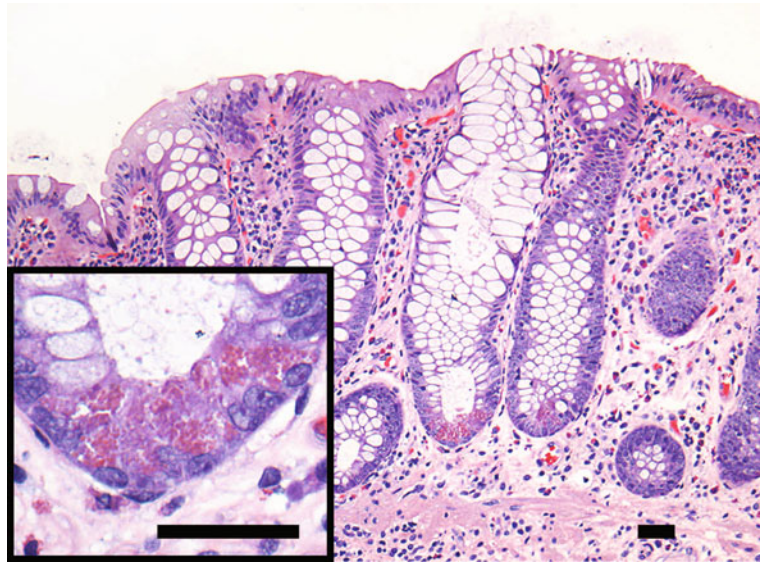
Metaplasia. Defined as “a reversible change in which one differentiated cell type (epithelial or mesenchymal) is replaced by another cell type” [1]. In intestinal chronic injury there are two common types of metaplasia: paneth cell (Fig. 22.2) and pyloric cell. While paneth cells normally reside throughout the small intestine and in colonic mucosa close to the ileocecal valve, the mucosa distal to the splenic flexure should have none [2]. Similarly, the presence of pyloric glands, normally restricted to gastric epithelium, signifies chronic injury when observed in ileal or colonic mucosa (e.g., as in Fig. 22.8 inset).

Basal lymphoid hyperplasia. (Fig. 22.1b) In IBD, an increase in lymphocytes and plasma cells is characteristically seen in the basal portion of the lamina propria where they form a band-like infiltrate. Increased eosinophils and mast cells may also be occasionally observed in this location; however, an abundance of neutrophils should raise the suspicion of active inflammation.

Disease Distribution

In the pathology report, descriptive words such as “patchy,” “focal,” and “diffuse” are used to describe distribution of chronic injury. These terms are avoided in descriptions of active inflammation as specific patterns of chronic, but not active, injury have diagnostic implications in IBD. To

Fig. 22.2 Metaplasia is a feature of chronic injury. Paneth cells are normally present in the small intestine and variably in the right colon. When identified in the left colon, as in this biopsy, paneth cells are considered to result from a metaplastic process secondary to chronic injury. (scale bars: 40 μ m)



accurately assess distribution of disease the pathologist must rely on the endoscopist to sample endoscopically normal and abnormal areas of mucosa as well as, submit the biopsies in separate containers clearly labeled with anatomic location. A thorough treatment history should accompany the endoscopy report as therapeutic interventions can alter disease distribution and create diagnostic confusion, as detailed later [3].

Disease Activity

Disease activity refers to the presence of neutrophils within the epithelium. By definition, IBD occurs on a background of chronic injury; however, activity can occur without evidence of chronic injury. Therefore, a diagnosis of “chronic active colitis” describes the histologic findings of intraepithelial neutrophils superimposed on features of chronic injury. This diagnosis differs from “active colitis,” which is present in acute self-limited inflammatory processes devoid of chronic injury. No system for grading of disease activity is universally accepted since studies show poor correlation between microscopic appearance, endoscopic impression, and clinical symptomatology [4–7]. However, knowledge of disease severity is important clinically and many pathologists use scales such as the one below to quantify activity (Fig. 22.3).

1. Quiescent: Features of chronic injury are present, but intraepithelial neutrophils are not observed (Fig. 22.3a).
2. Mildly active: Scattered neutrophils are seen within the epithelium (Fig. 22.3b).
3. Moderately active: Neutrophils have migrated across the epithelium to collect within crypts and form microabscesses referred to as “crypt abscesses” (Fig. 22.3c). Crypt rupture and destruction can also be observed.
4. Severely active: Crypt abscesses have evolved into erosions and/or ulcerations (Fig. 22.3d).

Mucin depletion, decreased numbers of goblet cells, and increased mitotic activity are additional regenerative features that are commonly present in IBD but these are not diagnostic, as they may also be present in active disease without chronic features. Although UC and CD share many of these key aspects of injury, unique details define each entity, which are elucidated in the subsequent sections.

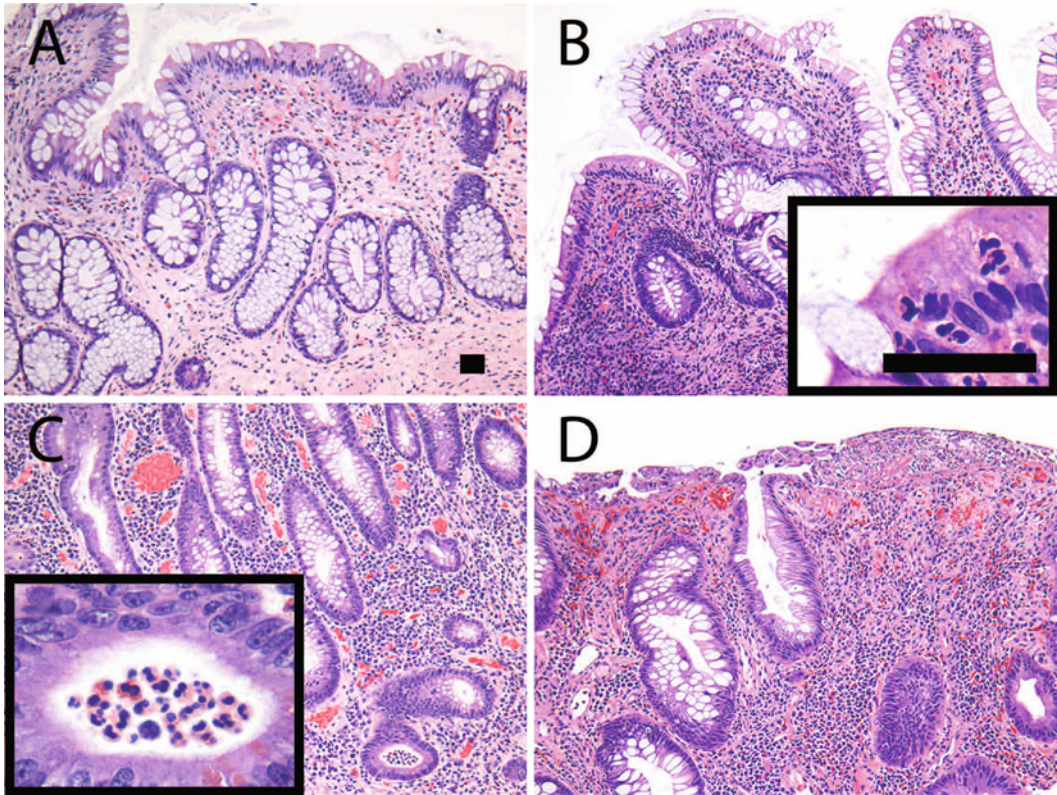


Fig. 22.3 The acute, neutrophilic component of inflammation is graded as activity. (a) Quiescent IBD displays chronic injury, but no intraepithelial neutrophils. (b) The presence of intraepithelial neutrophils indicates mild active inflammation. (c) Crypt abscesses indicate moderate activity. (d) Ulcerations or erosions are a feature of severely active IBD. (a–d: scale bars: 40 μ m)

Ulcerative Colitis

UC is characterized by diffuse chronic injury starting in the rectum and extending proximally to involve sequential segments of colon. Ulcerative proctitis refers to disease limited to the rectum, whereas ulcerative pancolitis describes disease involving the entire colon and rectum. In addition to a diffuse pattern of distribution, untreated UC inflammation is limited to the mucosa and submucosa in most cases.

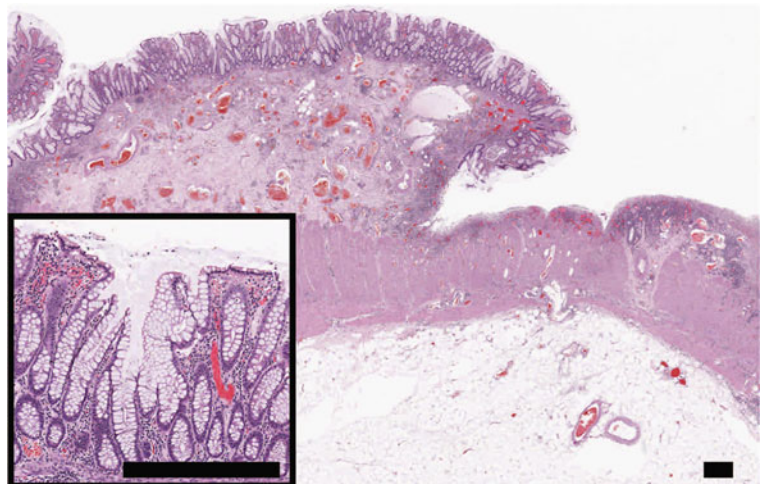
Gross Appearance

Key aspects defining UC as a distinct entity are observed macroscopically. Typically, the diffusely affected UC resection specimen lies flat when opened on the pathologist's bench, in contrast to the rigid CD specimen (Fig. 22.4). Further inspection reveals a smooth external surface (serosa) and bowel wall of normal thickness, devoid of strictures and fistulas. These simple observations reflect two characteristic pathologic features of UC: diffuse superficial mucosal inflammation and absence

Fig. 22.4 Features of UC are evident on gross examination. In this total colectomy specimen, the entire mucosa from distal rectum to the cecum is hemorrhagic and friable. The specimen lies flat because of the absence of full thickness inflammation. There is a sharp transition to normal tan colored small intestinal mucosa at the ileocecal valve. (IC; dashed line) (scale bar: 5 cm)



Fig. 22.5 Inflammation in UC is limited to the mucosa and superficial submucosa. In this case of severely active disease, a broad based area of ulceration is present in the right portion of the image. Inflammation does not extend deeply into the muscularis propria, there is no fibrosis, and there are no granulomas present. In an area of epithelium away from the ulcer, architectural distortion is present and is indicative of chronic injury. (scale bars 0.6 mm)



of fibrosis. Inflamed mucosal surfaces appear red, granular and friable with areas of hemorrhage and ulceration in severe cases. The following histologic correlates provide explanation for these macroscopic features.

Microscopic Appearance

Diffuse chronic injury. Histologic features of chronic injury described earlier are seen diffusely throughout the affected colon and rectum. Caveats are discussed at the end of this section.

Depth of disease. In differentiating UC from CD, it is important to consider depth of disease. Active UC shows varying degrees of neutrophilic inflammation; usually limited to the mucosa and superficial submucosa, superimposed on chronic injury (Fig. 22.5). Significant mural fibrosis is not usually present. However, in severe cases with extensive ulceration (i.e., severely active disease), inflammation may extend to the muscularis propria or even the subserosal tissues.

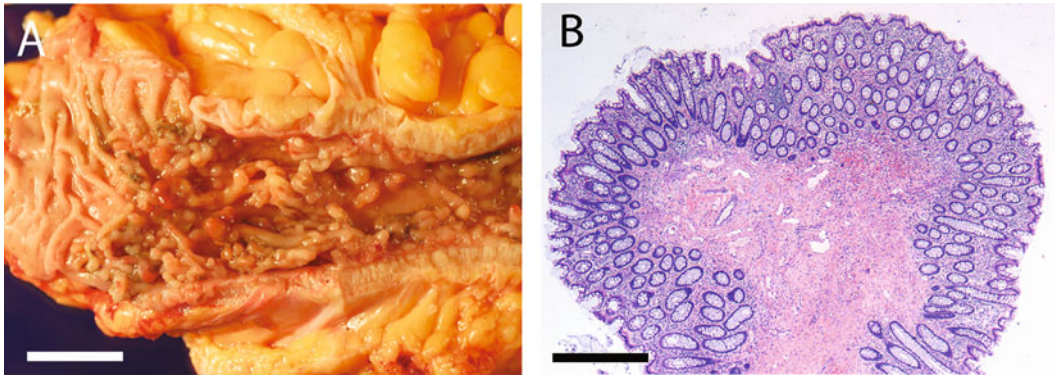


Fig. 22.6 Inflammatory polyps may be numerous. (a) In this total colectomy specimen, numerous filiform polyps are present in the descending colon and rectum (scale bar: 2.5 cm) (b) Histologic examination of one inflammatory polyp with no evidence of dysplasia. (scale bar: 1 mm)

Type of ulcer formation. UC ulcers are morphologically distinct from those observed in CD. Broad based superficial ulcers (Fig. 22.5), typical of UC, markedly contrast with the deep knife-like ulcerations associated with CD (below). These different types of ulcers reflect the superficial and transmural extent of active disease in UC and CD, respectively.

Inflammatory polyps. Also known as pseudopolyps, inflammatory polyps are believed to form during repeated cycles of ulceration and regeneration in both UC and CD. Pseudopolyps are not associated with an increased risk for neoplastic transformation; however, they may be extensive (Fig. 22.6) and are frequently biopsied to rule out polypoid dysplasia. Although not a premalignant lesion, the presence of pseudopolyps, in general, indicates long duration of severe disease, a factor which increases overall risk for dysplasia or frank malignancy anywhere in the affected colorectum [8].

Microscopic Features of UC That Can Mimic CD

Continuous distribution and superficial extent of disease are hallmarks of UC; however, overlapping features with CD may arise and are discussed in this section.

Backwash ileitis [9, 10]: Activity and even low grade chronic injury may extend into the distal ileum, particularly in severe UC pan-colitis. In contrast to Crohn's ileitis, which typically shows a patchy pattern throughout the ileum, involvement in UC is distal, diffuse, and predominately limited to the ileocecal valve. As ileal involvement by UC resolves following totally colectomy, the inflammation is believed to be caused by proximal extension of colonic disease through an incompetent ileocecal valve. Thus, the clever name “backwash ileitis” is a diagnosis which should be only made if cecal disease is severe. It can be a challenge to make a definitive diagnosis of backwash ileitis and completely rule out CD, therefore a detailed diagnostic comment should be added to the pathology report to facilitate clear communication amongst the clinical team.

Cecal red patch [11–13]. Disease localized to the appendix and periappendiceal cecal mucosa, or “cecal red patch,” and can be seen in up to one-third of patients with UC. Histologically, these biopsies resemble mildly active UC (i.e., evidence of chronic injury and intraepithelial neutrophils) and should not be interpreted as patchy CD-like distribution.

Transitional areas. Biopsies from the immediate transition between normal and the most proximal extent of diseased mucosa may create a patchy, CD-like, appearance of disease distribution to the

pathologist. Therefore, if possible, the endoscopist should focus on sampling, and submitting in separate containers, representative regions of diseased and normal mucosa to provide an accurate map for the pathologist.

True rectal sparing. Although rectal involvement is one of the key features of UC, pediatric patients may present with rectal sparing [14, 15]. This unconventional distribution of disease is considered a normal variant in the pediatric UC population.

False rectal sparing. UC patients receiving long-term therapeutic enemas may completely resolve rectal disease but still show proximal involvement. The pathologist will be able to explain the significance of normal rectal biopsies in this clinical context. However, if the treatment history is withheld, an incorrect diagnosis of CD is possible.

Granuloma formation. Presence or absence of granulomas are common histologic features used to differentiate UC from CD. Well or poorly formed granulomas, characteristic of CD, appear as pale compact aggregates of histiocytes surrounded by lymphocytes, discussed in detail later. Diagnostic confusion may arise when similar features are seen in UC patients. Although well-formed granulomas are not associated with UC pathogenesis, loose aggregates of histiocytes, or macrophages, may be seen in UC patients. Histiocytes function to clear cellular debris and foreign material following injury. During episodes of active inflammation, crypts may rupture, releasing mucin into the lamina propria. The extracellular mucin signals histiocytes to the affected area, and results in formation of so-called mucin granulomas. Additionally, multinucleated histiocytes, known as “foreign body type giant cells” can cluster near areas of ulceration and mimic granuloma formation. Thus, it is essential to carefully inspect the quality of the histiocytic reaction with respect to the associated background features to determine the significance of granuloma formation in IBD patients.

Crohn’s Disease

The first microscopic description of what is now known as CD was made by Crohn, Ginzburg, and Oppenheimer in 1932, although autopsy and clinical case reports of patients with CD-like symptoms have existed for centuries (e.g., Louis XIII of France) [16]. In the description by Crohn et al., the term “regional ileitis” was used to describe full-thickness inflammation of the bowel wall, fibrosis, stricture formation, well-formed granulomas, and a tendency to form fistula tracts [17]. Today, we recognize a similar spectrum of findings anywhere in the gastrointestinal tract as CD.

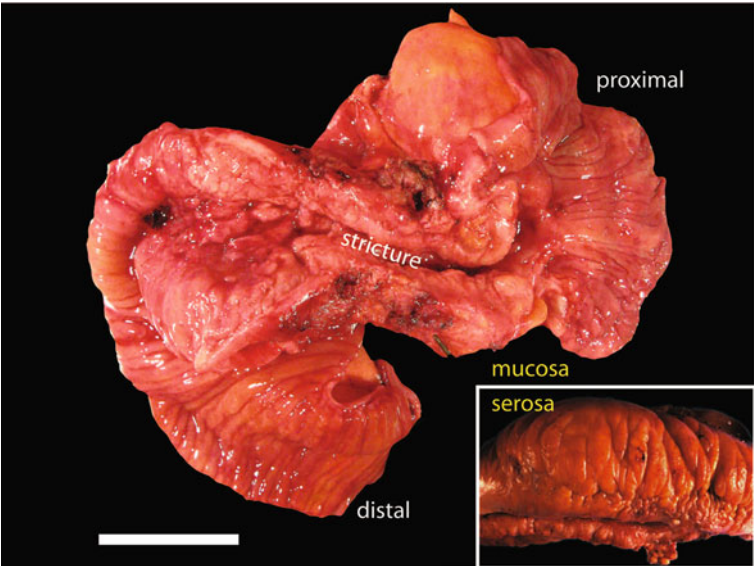
Gross Appearance

Unique gross and microscopic features of CD and UC are given in Table 22.1. CD occurs in a patchy or segmental distribution anywhere in the gastrointestinal tract in contrast to UC which is diffuse and limited to the colon and rectum. Segmental distribution is shown in Fig. 22.7. Here the involved external serosa reveals “creeping fat” or “fat wrapping” due to fibrous adhesions and increased fat deposition (Fig. 22.7 inset). The affected area in this resection specimen is characteristically narrowed from stricture formation and flanked by discrete regions of normal appearing intestine. Most notably, the opened specimen does not lie flat (compare Fig. 22.7 CD specimen to Fig. 22.4 UC specimen), a result of extensive fibrosis and transmural disease. On closer inspection, a markedly thickened bowel wall and strikingly narrowed luminal diameter can be appreciated in the strictured segment. The mucosa takes on a cobblestone appearance due to alternating areas of ulcerated and preserved mucosa. Deep, knife-like ulcerations, which give rise to fistula tracts, are

Table 22.1 Key gross and microscopic features of UC and CD

	Ulcerative colitis	Crohn’s disease
Gross features		
Distribution	Continuous extending proximally from rectum	Segmental, involving any part of the G.I. tract
Thickness	Superficial, limited to mucosa and submucosa	Full thickness, often extending through muscularis propria
Microscopic features		
Ulcerations	Broad and shallow	Knife like and fissuring
Granulomas	Associated with ruptured crypts and areas of ulceration	Poorly or tightly formed in any layer of the bowel wall
Distribution	Diffuse throughout biopsy	Patchy, alternating areas of normal and injured mucosa in single biopsy

Fig. 22.7 Features of Crohn’s colitis are evident on gross examination. In this segmental resection of colon, the resection margins have a normal pink tan appearance. The lumen in the center portion of the specimen is narrowed, and the wall is thickened. In contrast to UC, the specimen will not lay flat. The serosal surface (inset) displays evidence of fat wrapping or creeping fat. (scale bar: 5.0 cm)



common but not easily appreciated in this image. When examining a severely active CD specimen such as this, it is not difficult to understand how stricture and fistula formation carry a high risk of perforation and abscess formation, both indicators for segmental resection. Pseudopolyps (e.g., Fig. 22.6), created by cycles of regeneration and injury, are identical to those observed in UC (see above discussion).

Microscopic Appearance

As CD may involve any part of the gastrointestinal tract classic histologic features can be observed in the oral cavity (Fig. 22.8a), esophagus, stomach (Fig. 22.8b), and small (Fig. 22.8c) and large intestines (Fig. 22.8d). Along with distribution and depth of disease, several additional histologic features define CD: type of ulcer, fibrosis, granuloma formation, and neuronal hyperplasia.

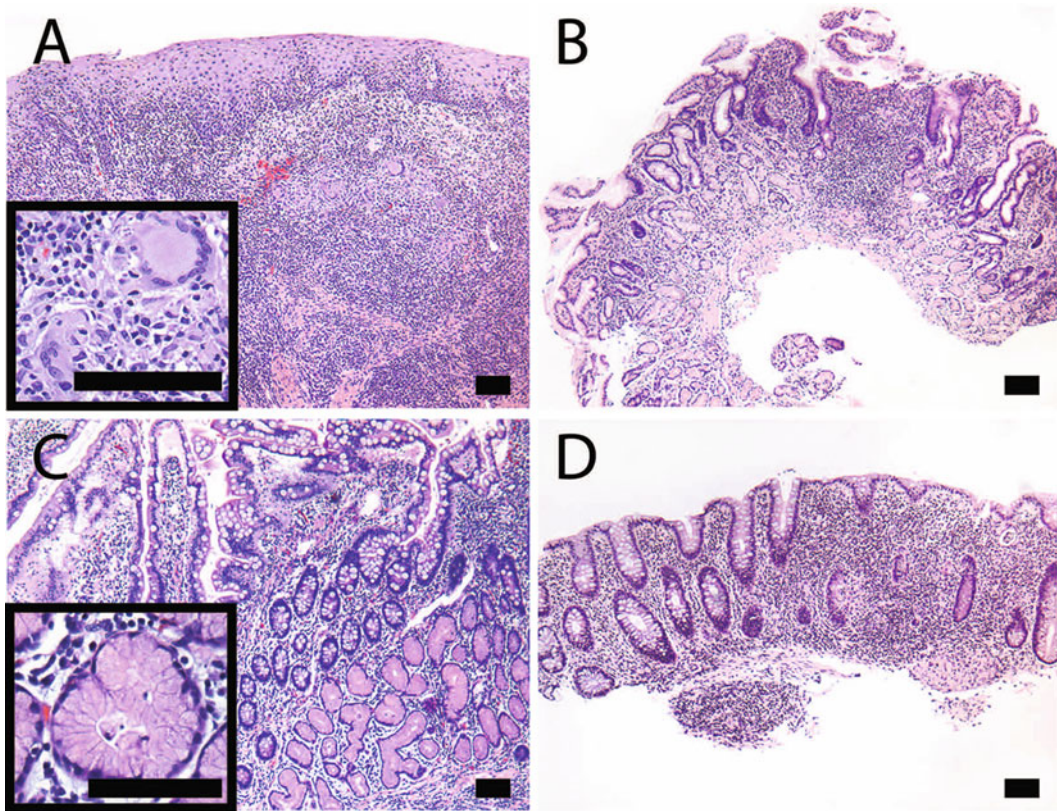


Fig. 22.8 CD may occur anywhere in the gastrointestinal tract. (a) Squamous mucosa demonstrates intraepithelial lymphocytes and numerous inflammatory cells in the lamina propria. Epithelial cells are reactive, and mild basal cell layer hyperplasia is present. A granuloma is present in the lamina propria (*inset*). (b) Antral mucosa in the left portion of the image appears normal with clear mucous and bicarbonate secreting foveolar cells. Focal active CD with ulceration is present in the right portion of image. The regenerating foveolar epithelial cells appear mucin depleted and the lamina propria has a dense lymphoplasmacytic infiltrate. Antral glands have been destroyed in this area. *Helicobacter pylori* organisms were not present. (c) Ileal villus architectural distortion and pyloric metaplasia (*inset*) are present in this patch of ileal mucosa involved by CD. (d) Focal chronic active colitis is present in this biopsy from a patient with CD. (all scale bars: 60 μ m)

Patchy chronic injury. Analogous to the gross morphologic features described in the previous discussion, histologic discontinuous disease distribution is illustrated by distinct areas of normal and diseased mucosa within a single biopsy (Fig. 22.8d), as well as from sequential segments of small and large intestine. Please refer to the UC section on caveats to disease distribution.

Depth of disease. Similar to UC, active neutrophilic inflammation ranging from quiescent to severely active is superimposed on chronic injury (Fig. 22.4). However, in CD, inflammation may extend transmurally to form strictures and fistula tracts, features not present in UC. Limited superficial mucosal inflammation without full thickness involvement can occur and should not rule out a diagnosis of CD.

Type of ulcer. Small aphthous ulcers and deep, knife-like, fissuring ulcers are characteristic of CD. Aphthous ulcers seen endoscopically correlate with small collections of intraepithelial neutrophils overlying lymphoid aggregates histologically (Fig. 22.9). These are believed to coalesce, resulting in deep ulceration eventually extending into the muscularis propria. Fistula formation likely

Fig. 22.9 Aphthous ulcerations are early lesions of CD. Collections of intraepithelial neutrophils are present over a lymphoid follicle. (*inset*) (scale bars: 40 μ m)

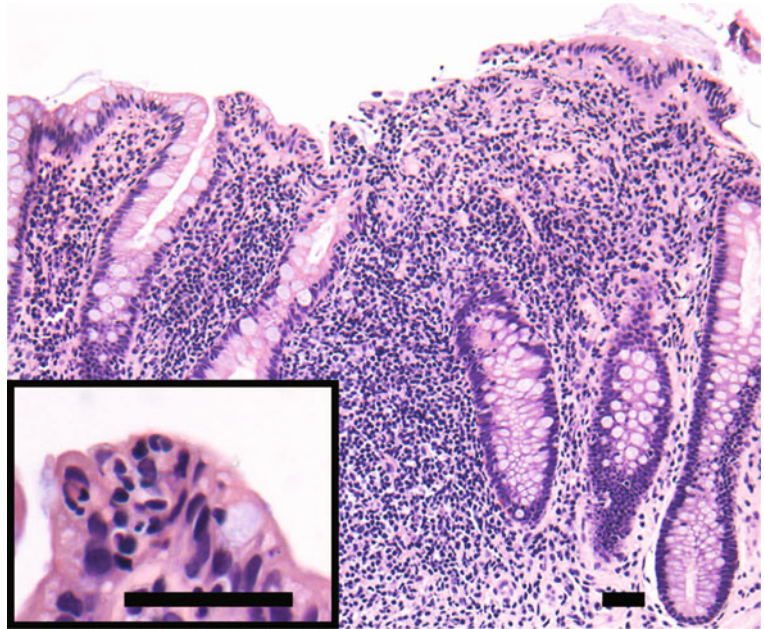
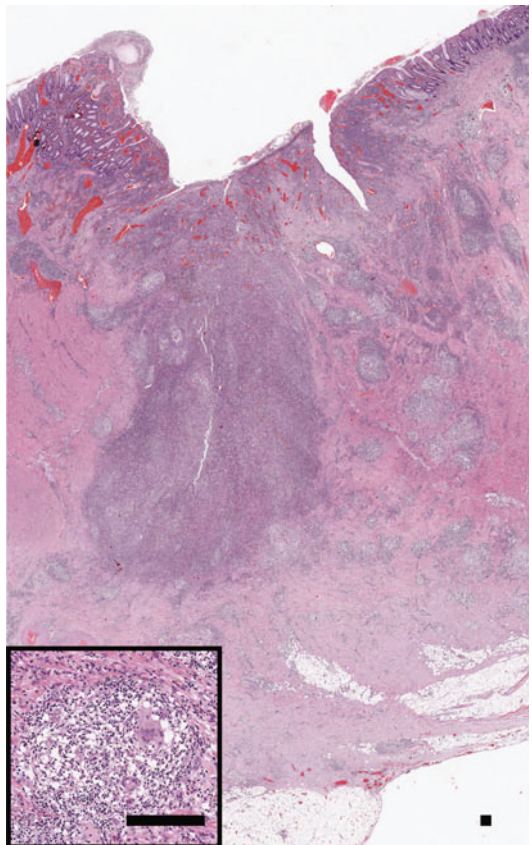


Fig. 22.10 Inflammation in CD may be transmural. In this case of severely active colonic disease, a knife-like fissuring ulcer is present, in association with submucosal and serosal fibrosis and numerous granulomas. (scale bars 0.4 mm)



develops from these large deep, knife-like, ulcers extending through the serosa and into pericolic fibroadipose tissue (Fig. 22.10).

Fibrosis. In CD, transmural lymphoid aggregates along with diffuse lymphoplasmacytic and variable neutrophilic inflammatory infiltrates are observed within a markedly thickened, fibrotic stroma. Proliferation of inflammatory cells, fibroblasts, and myofibroblasts along with increased collagen deposition can extend into the serosa and pericolic fibroadipose tissues culminating in stricture formation.

Granulomas. The granulomas of CD may be poorly or tightly formed and can be found in any layer in the bowel wall. As described in the UC section, mucin granulomas and foreign body type reactions secondary to epithelial injury must be differentiated from well-formed granulomas diagnostic of CD. Sarcoidosis and tuberculosis are other granuloma forming processes to be considered and ruled out prior to rendering a diagnosis of CD.

Neuronal hyperplasia. Parasympathetic ganglia in the submucosa (Meissner's plexus) and muscularis propria (myenteric, or Auerbach's, plexus) can become hypertrophic in CD, displaying irregularly shaped nerve bundles and increased ganglion cells.

Although many cases of CD will display some or all of the above morphologic features, there is often significant histologic and clinical overlap between CD and UC. Therefore, it is important to include detailed descriptions in the pathology report to properly assess future biopsy material in order to render a definitive diagnosis.

Complications of IBD

Management of the IBD patient is complicated by the natural evolution of the disease process as well as the therapeutic interventions used to halt disease progression. This next section will highlight clinically relevant pathologic consequences of prolonged manifestations of IBD.

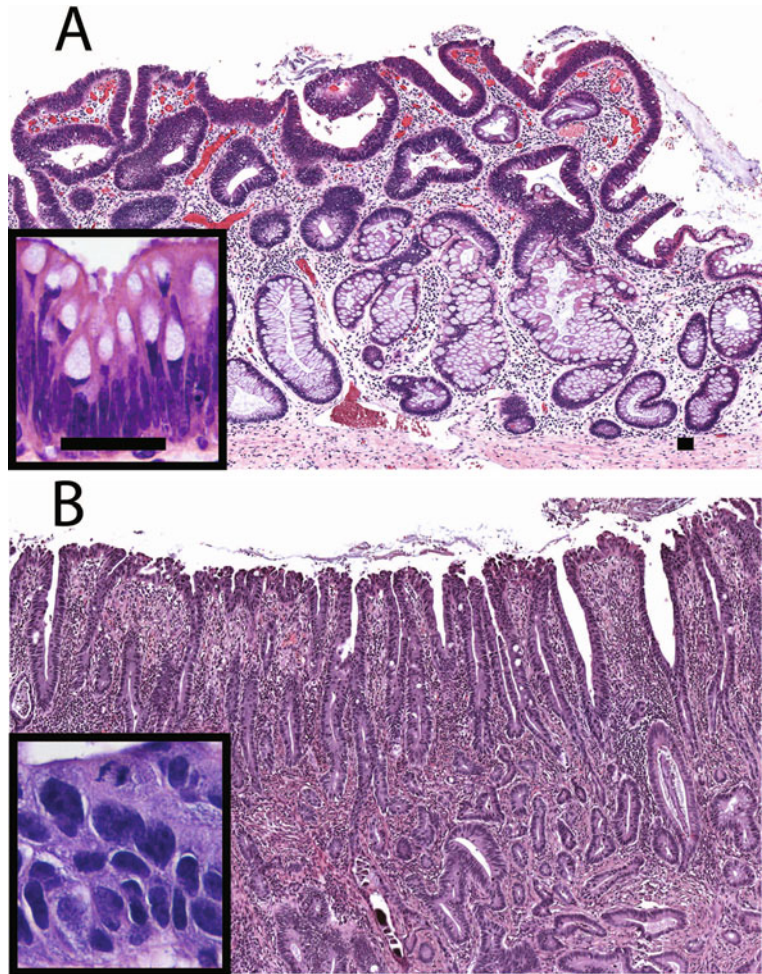
Complications of Severe IBD

Obstruction, fissures, and fistulas. As discussed earlier, CD is associated with stricture and fistula formation necessitating segmental resection. Strictures seen in UC patients can also be associated with obstruction from diverticular disease, but usually represent an advanced adenocarcinoma.

IBD-associated dysplasia. IBD is linked to an increased risk of colorectal carcinoma. IBD-associated dysplasia, a precursor lesion, and carcinoma occur at a younger age than the general population [18]. Endoscopic screening allows for the detection of dysplastic lesions and early carcinomas, thereby decreasing the risk of developing invasive and metastatic disease, respectively [19]. Guidelines for screening and management are discussed in Chap. 55.

IBD-associated dysplasia may present with an endoscopically apparent lesion or mass (dysplasia-associated lesion/mass; DALM), or may be flat, which can be difficult to detect. Therefore, random biopsies of both suspicious and normal appearing mucosa are critical in the surveillance of dysplasia in IBD patients. To complicate the scenario further, sporadic adenomas occur in IBD patients with the same frequency as non-IBD patients. Treatment for sporadic adenomas is simple polypectomy whereas IBD associated dysplasia necessitates aggressive follow up and often resection [19]. Differentiating the two is a difficult task since many histologic features overlap [20]. The key morphologic feature in diagnosing IBD associated dysplasia is the presence of definitive chronic injury

Fig. 22.11 Patients with inflammatory bowel disease have an increased risk of developing premalignant, dysplastic epithelial lesions. **(a)** Low grade dysplasia is characterized by the presence of hyperchromatic, enlarged nuclei, with some stratification (*inset*). All dysplastic cells are similar in shape and oriented in the same direction. **(b)** High grade dysplastic cells display loss of polarity evidenced by markedly pleomorphic nuclei with haphazard orientation and cribriforming, back to back, glands. A focus of invasion, highlighted by a desmoplastic (fibrotic) response is seen in the lower right corner of the image. (scale bar 40 μ m)



in the mucosa surrounding the lesion. Essential clinical features to consider are age and duration of disease. For instance, a patient under the age of 40 rarely develops sporadic adenomas. Similarly, dysplasia arising in a patient with new-onset IBD would be unusual.

Histologically, dysplasia can be low or high grade. Prior to rendering a diagnosis of dysplasia, reactive atypia must be excluded. Intense inflammation causing regeneration of the epithelium can cause bizarre cytologic atypia; however, maturation towards the surface is a key feature in ruling out true dysplasia. Histologically, dysplasia can be low or high grade. Low grade dysplasia is characterized by tall epithelial cells with elongated, pseudo-stratified, basally oriented hyperchromatic nuclei (Fig. 22.11a). In contrast, high-grade dysplasia displays severe cytologic and architectural abnormalities (Fig. 22.11b). Multiple layers of pleomorphic nuclei, abnormal mitotic figures, and back to back, or cribriform, glands advance a diagnosis from low grade to high grade dysplasia. A third category of “indefinite for dysplasia” may be used sparingly to communicate to the clinician that a firm diagnosis cannot be made on the biopsy material sent for review. In most situations this diagnosis is given when the biopsy is limited or poorly preserved. Therefore, it is critical to write a comment in the pathology report detailing the reasoning for why a diagnosis could not be rendered. For example, if the mucosa is ulcerated leaving few epithelial available to examine, the clinician may decide to treat first and then rebiopsy after the mucosa has had time to regenerate. On the other

hand, if the tissue was inadequate or damaged, then the clinician may decide to rebiopsy immediately, so as to address a possible diagnosis of dysplasia in a timely fashion.

Toxic Megacolon. Several inflammatory conditions, most commonly UC, can involve rapid dilation and loss of contractility within a colonic segment. Inflammation and or stretching causes increased wall tension and subsequent damage to the muscularis propria and myenteric plexus. Nonfunctional muscle and nerve results in extreme bowel wall thinning that, when subjected to increased luminal pressure, is at high risk for perforation. This process, known as “toxic megacolon” is associated with a high mortality rate and is an indication for immediate colectomy.

Complications of Therapy

Diversion Colitis. Segments of bowel surgically isolated from the normal fecal stream can develop extensive mucosal follicular hyperplasia, a condition known as diversion colitis (Fig. 22.12). Inflammation can also extend more deeply and resemble CD. Knowledge of the surgical anatomy is critically important in differentiating diversion colitis from CD.

Pouchitis. Total abdominal colectomy with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for uncontrolled UC. An ileal pouch is created to serve as a fecal reservoir. Since the small bowel is not adapted to perform colonic functions, the mucosa can become irritated and inflamed (Fig. 22.13). Histologically, inflammation in the pouch may resemble recurrent IBD, raising the possibility of a missed initial diagnosis of CD. Features favoring a diagnosis of pouchitis include:

1. Prior tissue specimens that do not display macroscopic or microscopic features of CD (Table 22.1).
2. Absence of histologic evidence of IBD in the ascending loop (proximal ileum). (Fig. 22.13a vs. b).
3. Absence of clinical and radiographic evidence of CD elsewhere in the body.

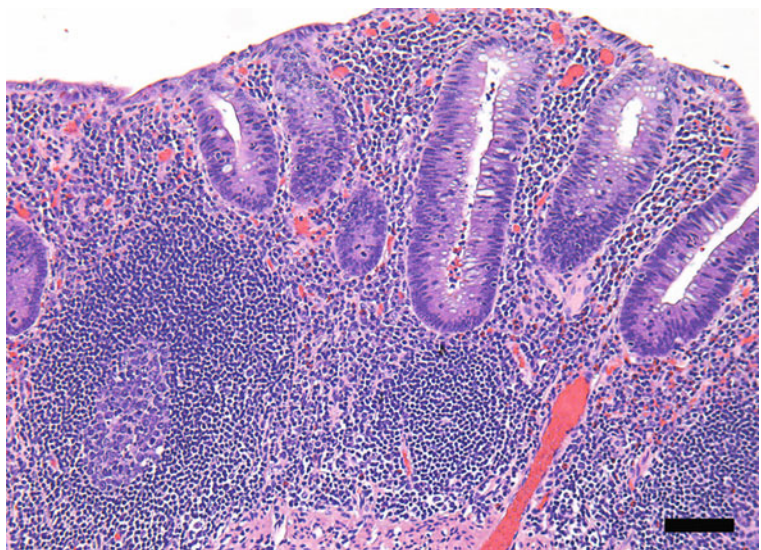


Fig. 22.12 Histologic features of diversion colitis are seen in segments of bowel removed from the normal intestinal flow. In this biopsy, typical diversion colitis features (i.e., lymphoid hyperplasia) are present

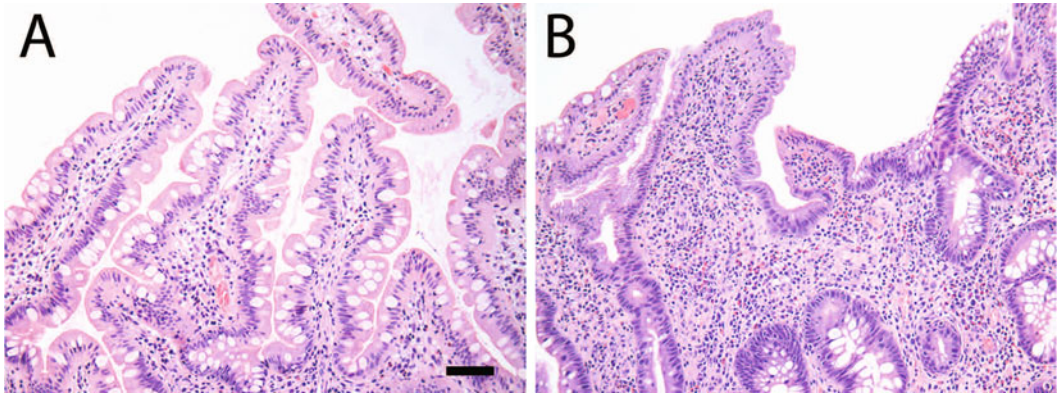


Fig. 22.13 Pouchitis may be associated with features of chronic injury. (a) The prepouch biopsy shows relatively normal small intestinal architecture. (b) Diffuse active inflammation and villous architectural distortion are seen in this pouch biopsy. These features could represent either pouchitis or CD; however, normal prepouch biopsies favor a diagnosis of pouchitis

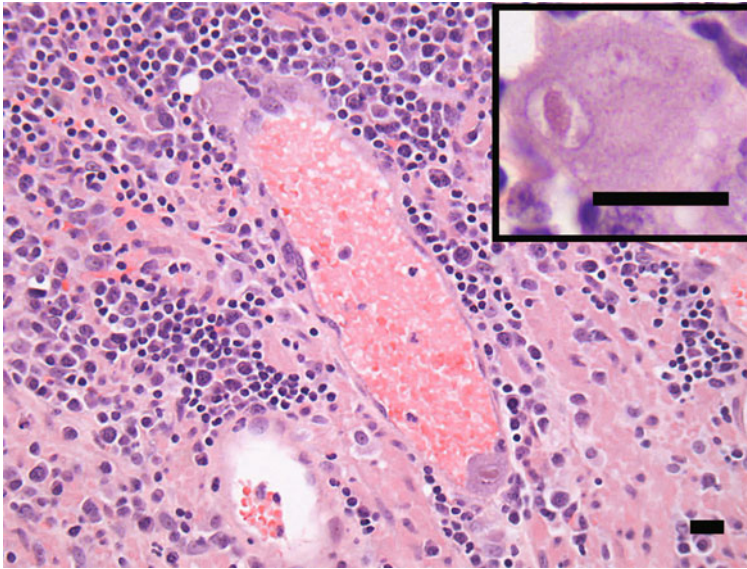
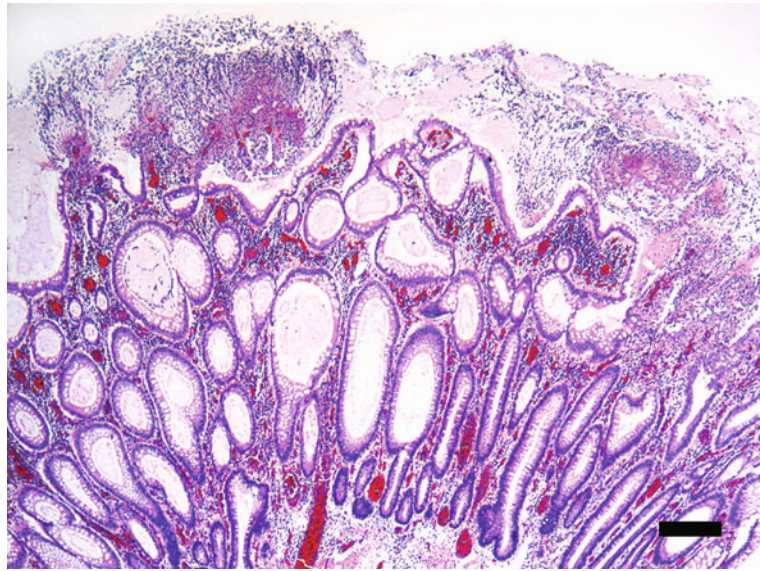


Fig. 22.14 Cytomegalovirus superinfection is a complication that should not be overlooked in any immunosuppressed patient. Endothelial cells contain intranuclear viral inclusions (scale bar 20 μ m)

Cytomegalovirus infection. IBD patients are considered at baseline to be immunocompromised compared to the general population. With the standard use of immunosuppressive agents for therapy, treated patients will become even further immunosuppressed. As such, they are at increased risk for many infections, including cytomegalovirus (CMV). Unsuspected CMV infection during treatment is of particular importance because it can mimic therapeutic failure and worsen symptoms. The classic eosinophilic cytoplasmic and intranuclear inclusions (Fig. 22.14) are present in enlarged endothelial and epithelial cells. When surrounded by a clear halo, the nuclear inclusions are referred to as “owl’s eyes.” These inclusions can be difficult to find, thus, multiple H&E levels must be examined. When clinical suspicion is high, immunohistochemical stains can be utilized to highlight viral proteins.

Fig. 22.15 Pseudomembranous colitis may coexist with IBD. A pseudomembrane composed of fibrin and inflammatory cells is seen on top of the superficial epithelium. Several “volcano lesions” are notable in the upper left portion of the image. (scale bar: 300 μ m)



Pseudomembranous Colitis. After antibiotic therapy, infection with *Clostridium difficile* or other toxigenic bacteria may lead to severe diarrhea in IBD and non-IBD patients. White to yellow pseudomembranes seen on endoscopy correlate with supraepithelial lamellated layers of fibrin, mucin, neutrophils, and necrotic cells histologically. Eruptions of the purulent mucin streaming out of crypts are referred to as “volcano lesions.” When the active inflammatory component is not obvious, epithelial withering and other features of ischemia can provide clues to the diagnosis (Fig. 22.15).

Extraintestinal Manifestations of IBD

IBD manifests in other organs besides the gastrointestinal tract, including the pancreas, skin, and the musculoskeletal system. Detailed discussions of these entities are beyond the scope of this chapter (see Chaps. 49 and 50), but a few clinically important entities will be briefly addressed here.

Primary Sclerosing Cholangitis. Primary Sclerosing Cholangitis (PSC) is a chronic cholestatic disease characterized by inflammation, fibrosis, and strictures of extra and intrahepatic bile ducts ultimately leading to cirrhosis. PSC is strongly associated with UC and affected patients carry a threefold increased risk of developing colonic dysplastic lesions [21]. The hallmark histologic features in liver specimens include “onion-skinning” fibrosis and lymphocytic inflammation surrounding large, mainly extrahepatic, bile ducts (Fig. 22.16).

Mucocutaneous lesions. Skin and oral lesions have been reported in up to 20% of IBD patients [22]. These lesions may be direct manifestations of the primary inflammatory process or secondary to nutritional deficiency.

Pyoderma gangrenosum. [23] More common in UC than CD, pyoderma gangrenosum is seen exclusively in the IBD patient population. These lesions occur during times of active disease but can also herald a diagnosis of IBD. Clinically, the lesions appear most commonly in the pretibial region, as ulcers with dark necrotic centers surrounded by bright erythematous skin. Histologic features of necrosis, vasculitis, thrombosis, and dense neutrophilic infiltrates are identified but are nonspecific.

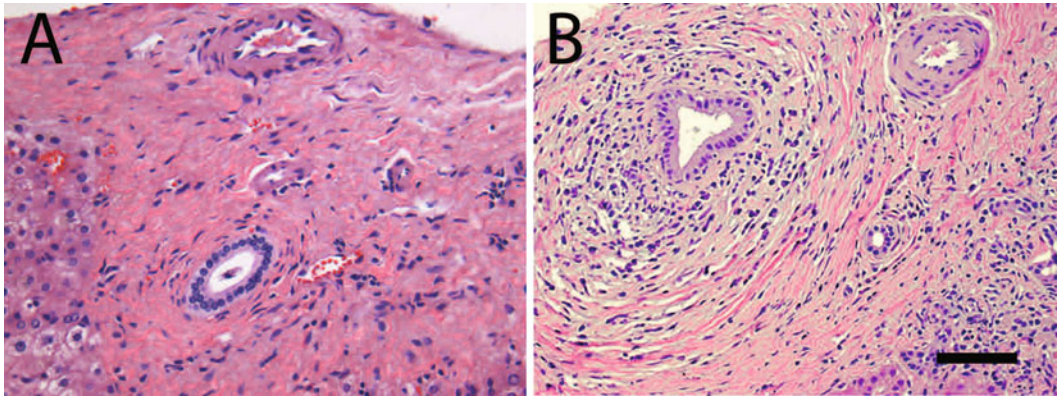


Fig. 22.16 Primary Sclerosing Cholangitis affects extrahepatic and larger intrahepatic bile ducts. (a) A normal portal tract demonstrates an uninjured bile duct lined by simple cuboidal epithelium. (b) The bile duct from a patient with primary sclerosing cholangitis is surrounded by “onion-skinning” fibrosis and a prominent periductal lymphocytic infiltrate. (scale bars: 150 μ m)

These lesions become important clinically when ulcerations extend into the bone resulting in osteomyelitis, an infection requiring intensive medical treatment.

Cutaneous Crohn’s Disease. Inflammation can extend directly from perianal and ostomy sites to the skin, and rarely can involve areas of skin without direct spread from the gastrointestinal tract. Lesions can be raised or flat, with or without ulcerations [24]. Histologically, noncaseating granulomatous inflammation is observed predominantly in the dermis. Since several other inflammatory and infectious processes result in granulomatous inflammation in the skin, it is important to be aware of cutaneous manifestations of CD.

Oral lesions. Recurrent oral aphthous ulcers are the most common extraintestinal manifestation of IBD. These well-defined, “punched-out” superficial ulcerations may indicate primary IBD or result from nutritional deficiencies secondary to gastrointestinal pathology. Occurring most often during active disease, aphthous ulcers are often associated with other extraintestinal demonstrations of IBD.

Eye lesions. A variety of eye lesions involving not only the conjunctiva and retina but also peri-orbital skin and muscle are observed in approximately 10% of IBD patients.

Mimics of IBD

Many diseases characterized by ongoing active inflammation for an extended period of time are included in the clinical differential diagnosis of IBD. Therefore, entities lacking features of chronic injury such as acute self-limited infectious colitis, lymphocytic colitis, and collagenous colitis are frequently biopsied, but are easily differentiated from IBD under the microscope. However, in disease processes associated with a longer duration of injury, such as radiation, ischemia, mucosal prolapse, diverticular disease, autoimmune enteritis and medication toxicity, knowledge of unique histologic features is required.

Infectious colitis. Infectious colitis due to bacterial infections, such as *Yersinia*, *Campylobacter*, *Salmonella*, and *Shigella*, are self-limited, and have a similar microscopic appearance. Inflammation is predominantly neutrophilic, similar to active IBD, however chronic injury is absent (Fig. 22.17). Interestingly, some infectious organisms can mimic the histologic appearance of IBD [25]. For instance, some bacterial species create granulomatous inflammation, a characteristic feature not to

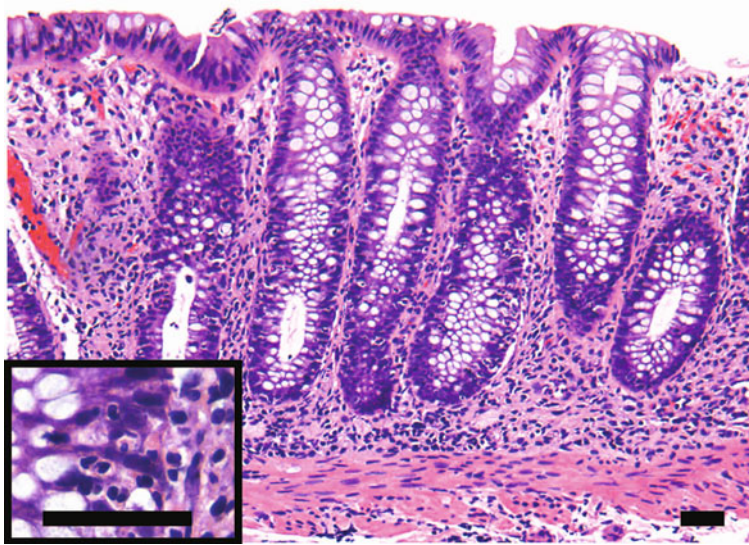


Fig. 22.17 Self-limiting infectious colitis is associated with active inflammation, but not chronic injury. Mild to moderately active inflammation is present within this colonic biopsy (*inset*). There is no evidence of crypt architectural distortion. (scale bar: 80 μ m)

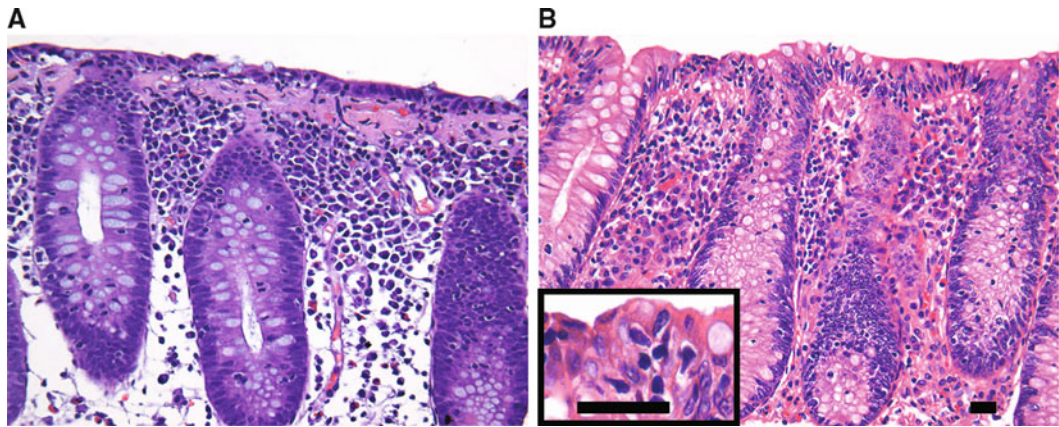


Fig. 22.18 Microscopic findings in two patients with watery diarrhea and normal endoscopy. (a) Features of collagenous colitis include increased intraepithelial lymphocytes and a thickened, irregular subepithelial fibrous band. (b) Intraepithelial lymphocytes are numerous in this biopsy from a patient with lymphocytic colitis. Absence of a thickened collagen band differentiates lymphocytic from collagenous colitis. Overall crypt architectural distortion is preserved in panel A and B. (scale bars: 20 μ m)

be confused with CD. Parasitic infections, such as *Entamoeba histolytica* produce deep, CD-like, ulceration. Although, uncommon in most Western nations, infections such as these must be considered in the differential diagnosis of IBD.

Microscopic colitis. Microscopic colitis is defined by chronic, watery diarrhea and endoscopically normal mucosa. The term microscopic colitis actually describes two main pathologic entities: collagenous colitis (Fig. 22.18a) and lymphocytic colitis (Fig. 22.18b). Both collagenous and lymphocytic colitis demonstrate increased intraepithelial lymphocytes. Mild active inflammation may be present, but is not a principal histologic feature. Increased numbers of mononuclear cells are observed in the lamina propria; however, this tends to be more superficial and not the characteristic

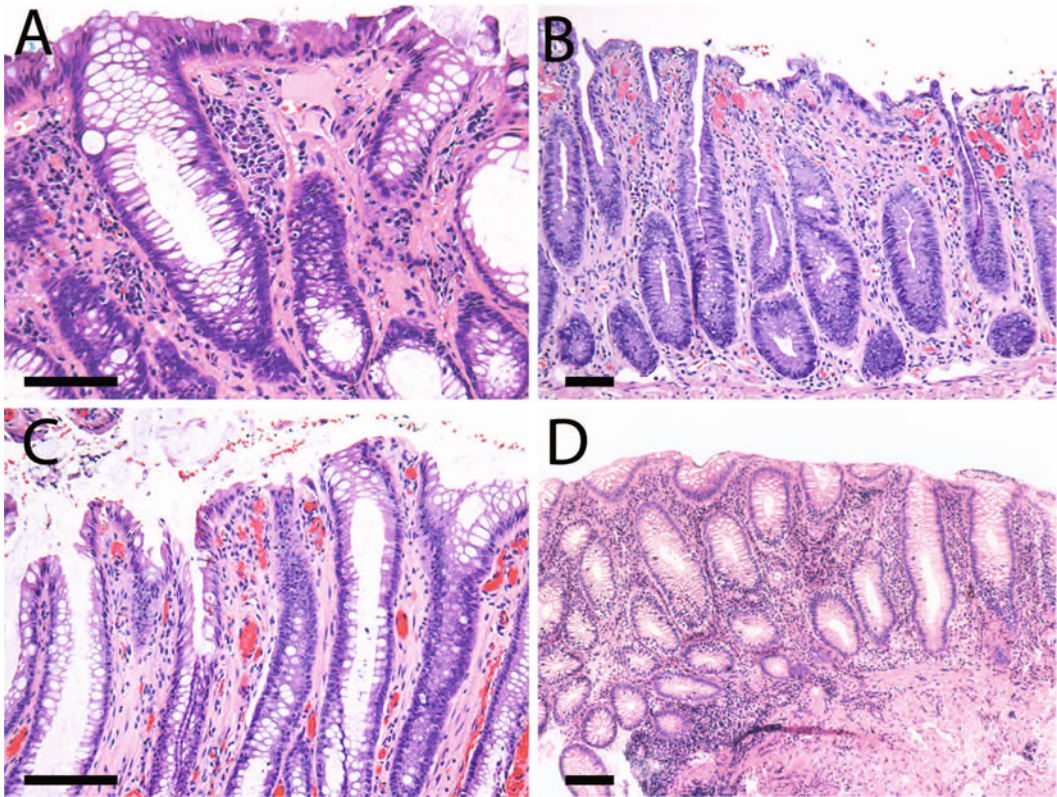


Fig. 22.19 Features of chronic injury are seen in disorders other than inflammatory bowel disease, and clinicopathological correlations are essential. (a) Chronic colonic injury may occur secondary to radiation therapy. Fibrosis of the basement membrane and surface cytological atypia are features consistent with a known history of radiation injury. (b) Ischemia is associated with withering of surface epithelial cells and mild fibrosis of the lamina propria. Basally located crypt epithelial cells appear relatively well preserved. (c) In solitary rectal ulcer syndrome smooth muscle fibers extend upward from into the lamina propria. (d) Diverticular-associated colitis may closely resemble IBD, displaying active inflammation on a background of chronic injury. (all scale bars: 80 μ m)

basal, band-like infiltrate of IBD. Some chronic regenerative features such as paneth cell metaplasia and mild crypt architectural distortion can also be seen, but [26] severe architectural irregularities should raise the possibility of IBD. Collagenous colitis is differentiated from lymphocytic colitis by the presence of a thickened, eosinophilic, collagenous table located directly beneath the epithelial layer, classically entrapping small capillaries and cellular debris.

Chronic Radiation Colitis. When provided with proper clinical history, radiation damage is usually not a diagnostic challenge; however, chronic mucosal injury sustained from radiation may remain clinically silent for years [27]. Biopsies reveal marked architectural distortion along with regenerative cytological atypia (Fig. 22.19a). Extreme cellular and nuclear enlargement of bizarre, flattened epithelial and endothelial cells and fibroblasts are clues to previous radiation therapy. Additionally, extensive hyalinization can be seen in connective tissue and within blood vessel walls. The damaged vessels result in vascular occlusion and superimposed ischemic injury (detailed in the next section).

Ischemia. Causes of ischemic injury may be primarily vascular or hematologic. Etiologies include intrinsic intestinal obstruction causing vascular compression, infection, and drug toxicity [28]. The characteristic features of ischemia are a withered or attenuated surface epithelium in association with relatively preserved crypt epithelium. This appearance reflects low luminal oxygen tension

while crypt epithelial cells remain in close proximity to the lamina propria vasculature (Fig. 22.19b). In severe cases the entire mucosa displays intense active inflammation with crypt abscess formation, ulceration or complete mucosal necrosis. The lamina propria is hemorrhagic and edematous early in the disease process, becoming increasingly fibrotic after repeated ischemic episodes (Fig. 22.19c). As ischemia is dictated by vascular supply, injury is usually focal or segmental, a pattern of distribution similar to CD. Careful examination of submucosal vessels may reveal diagnostic clues such as vessels with fibrin thrombi or amyloid deposition; however, the etiology of ischemia is often unknown.

Solitary Rectal Ulcer/Mucosal Prolapse. Solitary rectal ulcer syndrome typically occurs in the anterior rectal wall, and, ironically, may be neither solitary nor ulcerated. The histological features reflect injury due to prolapse of mucosa into the fecal stream, including epithelial hyperplasia and mucosal ischemic changes. Prolapsed strands of smooth muscle are seen in a hyalinized lamina propria running perpendicular to the muscularis mucosa (Fig. 22.19c). Depending on severity and duration of prolapse, the mucosa can resemble IBD, with marked inflammation, architectural distortion and fibrosis.

Diverticular disease [29–31]. Biopsies taken from the immediate area of an inflamed diverticulum may display similar features of IBD: crypt architectural distortion, crypt abscesses, and foreign body type giant cell reactions. Furthermore, IBD patients can manifest disease within segments of colon containing diverticula, most often the sigmoid colon. This is termed diverticular disease-associated colitis (Fig. 22.19d) and is not limited to patients with IBD [29]. Thus, knowledge of diverticular disease is essential information to relay to the pathologist.

Drugs and Toxins. Chemotherapeutic agents and NSAIDs cause a variety of nonspecific active and chronic injury throughout the GI tract that can appear identical to IBD. Therefore, knowledge of medication history is extremely helpful for the pathologist.

Behçet's disease [25, 32]. Although rare in Europe and North America, Behçet's disease is a systemic vasculitis that should be considered in individuals with a history of iritis and oral and genital ulcers. Gastrointestinal involvement manifests preferentially in the ileocecal mucosa as transmural, patchy Crohn's-like, inflammation. CD and Behçet's disease have several overlapping features; however, Behçet's disease is defined by a prominent vasculitis involving veins in varying stages of activity. When present, vascular lesions are usually only a minor component of CD histopathology.

Summary

As an exhaustive review of IBD pathology would not have been possible in this short chapter, emphasis has been intentionally placed on diagnostically and clinically relevant morphologic features of UC and CD. Classic features of UC or CD are not present in every patient, therefore, establishing a definitive diagnosis of UC or CD can be difficult. Furthermore, the majority of IBD patients have an extensive treatment history creating additional complexity to the diagnostic process. Thus, strong communication between the pathologist and the clinician is essential in providing optimal care for the IBD patient.

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Chapter 23

Activity Indices-Based Assessment of Disease Activity in Inflammatory Bowel Disease

Edouard Louis, Catherine van Kemseke, and Catherine Reenaers

Keywords Crohn's disease • Ulcerative colitis • Disease activity • Endoscopic activity • Mucosal healing • Crohn's disease activity index • Harvey–Bradshaw index • Crohn's disease endoscopic index of severity • Simple endoscopic score for Crohn's disease • Mayo score

Introduction

Inflammatory bowel diseases (IBD) are characterized by a chronic relapsing inflammation of the gastrointestinal tract. This inflammation may lead to complications, including intestinal strictures and fistulas in Crohn's disease (CD) and fulminant colitis, microrectum and microcolon in ulcerative colitis (UC). Assessment and quantification of disease activity by indices is important to distinguish remission from flares and to determine different degrees of disease activity and severity. These indices are particularly important in clinical trials where an objective and reproducible quantification of disease activity is mandatory to clearly establish the effect of a new drug or to compare treatment strategies. They also tend to be more frequently used than in the past in routine practice. This is linked to the availability of new treatment options, which have been associated with an upgrading of treatment objectives and the expanding use of treatment optimization in incomplete responders to a given therapy. The currently available indices assess either clinical activity or endoscopic activity of the disease. In UC, the importance of mucosal healing and its good correlation to clinical activity of the disease has been recognized for a long time [1, 2], and endoscopic assessment has been integrated in several composite scores of activity. In CD, the correlation between clinical and endoscopic activity, albeit significant, was usually weak [3]. The clinical activity of the disease has long been considered as the only relevant end point of treatments [4, 5]. It is only recently that the significance of the persistence of endoscopic lesions in patients with clinical remission has been questioned. Particularly, the disappearance of significant endoscopic lesions, also called “mucosal healing,” has been associated with a better outcome in a few clinical trials, cohort or population-based studies [6, 7]. The purpose of this chapter is to review major indices of clinical and endoscopic activity in CD and UC and to discuss their strength, limitations and potential usefulness for clinical trials and routine practice.

E. Louis, MD, PhD (✉) • C. van Kemseke, MD, PhD • C. Reenaers, MD, PhD
Department of Gastroenterology, CHU Liège and GIGA Research, University of Liège,
service de gastroentérologie, CHU de Liège, Domaine du Sart Tilman, Liège 4000, Belgium
e-mail: edouard.louis@ulg.ac.be

Activity Indices in Ulcerative Colitis

UC activity indices have been recently reviewed by the task force of the International Organization of Inflammatory Bowel Disease (IOIBD) [8]. Truelove and Witts first attempted to quantify disease activity and defined mild, moderate and severe colitis in 1955 [9]. In 1978, Powell-Tuck transformed these definitions into a continuous point scale based on 10 clinical variables (the Powell-Tuck Index also called St Mark’s Index) [10]. A variation of this score also included endoscopic assessment. Other authors tried to create simplified versions. Schroeder et al. developed the Mayo disease activity index and Sutherland et al. developed the ulcerative colitis disease activity index (UCDAI), both of which have four components and include endoscopy [11, 12]. Seo et al. proposed an index only based on symptoms and laboratory variables [13]. Walmsley and colleagues developed the simple clinical colitis activity index (SCCAI), a survey of six questions about symptoms [14]. Rachmilewitz et al. reported in 1989 a score based on six clinical variables and one laboratory finding named Clinical Activity Index (CAI) [15]. In 1990, Lichtiger et al. modified the Truelove and Witts severity index and described a Lichtiger Index based on eight clinical items [16]. In 2002, Levine et al. described another composite score based on five clinical items and endoscopic evaluation while Feagan et al. developed in 2005 the Ulcerative Colitis Clinical Score (UCSS), a modification of the Mayo Score [17, 18]. Finally, Hanauer et al. described two arbitrarily designed, multicomponent measure of disease activity using the physician’s opinion: the Physician Global Assessment and the Investigator Global Evaluation [19, 20].

Truelove and Witts Severity Index

In 1955, in a placebo-controlled trial of oral cortisone in ulcerative colitis, Truelove and Witts first described an instrument to measure disease activity: the Truelove and Witts Severity Index (Table 23.1) [9]. This index is based on the six following criteria: number of stools per day, blood in stools, temperature, pulse, hemoglobin, and erythrocyte sedimentation rate (ESR). Mild, moderate, and severe diseases were defined according to fixed threshold for the different criteria. Clinical remission was defined as one or two stools a day without blood, absence of fever and tachycardia, a normal hemoglobin or “returning towards normal,” ESR normal or “returning towards normal.” No change or worse was described as self-explanatory. All intermediate cases were defined as “improved.” The main drawback of this score is that it is not quantitative (i.e. no disease severity score is generated). Furthermore, it has not been validated.

Table 23.1 Truelove and Witts Severity Index

Criteria	Mild disease	Severe disease
Stools (number per day)	≤4	≥6
Blood in stool	Intermittent ^a	Frequent
Temperature (°C)	Normal	>37.5° ^b
Pulse (beats/minute)	Normal	>90
Hemoglobin	Normal	<75% of normal value ^c
Erythrocyte sedimentation rate (mm/h)	≤30	>30

Patients are classified as having severe disease only when all six items in the classification are fulfilled.

Moderately severe disease=intermediate between severe and mild

^aIntermittent=no more than small amounts of macroscopic blood in stool

^bMean evening temperature of 37.5° (99.5°F) or a temperature of 37.8° (100°F) or more on at least 2 days out of four

^cAllowance made for recent transfusion

Lichtiger Index (Also Called Modified Truelove and Witts Severity Index, MTWSI)

In 1990, Lichtiger et al. described a modified Truelove and Witts Severity Index (MTWSI), also named Lichtiger Index, in a pilot trial of intravenous cyclosporine for the treatment of severely active steroid-refractory UC [16]. Eight variables composed the Lichtiger Index: diarrhea (number of daily stools), nocturnal stools, visible blood in stools (% of movements), fecal incontinence, abdominal pain or cramping, general well-being, abdominal tenderness and need for antidiarrheal drugs. Total score ranges from 0 to 21 points. Clinical response was initially defined as score reduction $\geq 50\%$ from baseline. A score of less than 10 on two consecutive days was considered to indicate a clinical response in a subsequent study [21].

Neither the Lichtiger Index nor the definitions of clinic response have been validated.

Activity Index (Also Called Seo Index)

In 1992, Seo et al. collected prospectively 18 clinical, laboratory and endoscopic parameters from 72 patients during 85 clinical relapses. A multiple regression analysis was done according to Truelove and Witts' classification with disease severity (mild, moderate, severe) as dependent variable and 18 clinical, laboratory and endoscopic parameters as independent variables [13]. Results showed that disease severity was significantly influenced by five factors: bloody stool (0=little or none; 1=present), bowel movements per day (1 if ≤ 4 ; 2 if 5–7; 3 if ≥ 8), erythrocyte sedimentation rate (ESR) (mm/h), hemoglobin (Hb) (g/dl), and serum albumin (g/dl). The Activity Index (AI) was expressed as follows: $AI = 60 \times \text{bloody stool} + 13 \times \text{bowel movements} + 0.5 \times ESR - 4 \times Hb - 15 \times \text{albumin} + 200$. Activity index scores < 150 points correspond to mild disease, 150–200 points to moderate disease, and > 200 points to severely active disease as classified by the Truelove and Witts Severity Index. In two subsequent study performed by Seo in patients with active ulcerative colitis, AI was able to predict remission or colectomy: an AI score < 180 points after 2 weeks of intravenous corticosteroids predicted remission [22] and, on the contrary, a score > 200 predicted colectomy [23]. In a randomized controlled trial on infliximab as rescue therapy in severe and moderately severe ulcerative colitis, AI significantly predicted response to medical therapy or need for colectomy [24]. The AI also correlated significantly with endoscopic findings [2].

Powell-Tuck Index (Also Called St. Mark's Index)

In 1978, Powell-Tuck et al. reported a disease activity measure (the Powell-Tuck Index) in a randomized controlled trial on oral prednisolone given as single or multiple daily doses for the treatment of active UC [10]. This disease activity index included 10 clinical variables: general health ("well-being," range 0–3), abdominal pain (range 0–2), bowel frequency (range 0–2), stool consistency (range 0–2), bleeding (range 0–2), anorexia (range 0–2), nausea or vomiting (range 0–1), abdominal tenderness (range 0–2), extraintestinal complications (eye, mouth, joint, skin) (range 0–2) and temperature (range 0–2). The Powell-Tuck index ranges from 0 to 20 points. One variation of the Powell-Tuck Index includes sigmoidoscopic appearance (0–2 points) increasing the total maximum score to 22 points. This endoscopic evaluation was mainly based on bleeding. "Remission" was defined as a symptom score of zero, "improvement" as a reduction in the baseline score by 2 or more points, "no change" as a fluctuation of the total score by one point or less and

“worse” as an increase of 2 or more. Neither the Powell-Tuck Index nor the definitions of remission and improvement have been validated.

Each of the 10 clinical variables was correlated with the sigmoidoscopic appearance in a 74 patients “cohort” [25]. The sigmoidoscopic appearance contributes little to the variation of the index score (only 2 points of the maximum 22 points). This was confirmed by Higgins et al. who suggested that clinical practice of treating patients based on reported symptoms without endoscopic evaluation might be appropriate [26].

Simple Clinical Colitis Activity Index

In 1998, Walmsley et al. evaluated 57 patients during 63 assessments of disease activity. They collected all items required to calculate the Powell-Tuck index including sigmoidoscopic assessment and added two additional clinical criteria: nocturnal bowel movement and urgency of defecation [14]. The general well-being score from the Harvey-Bradshaw simple index of Crohn’s disease activity was substituted for the general health question in the Powell-Tuck Index [27]. The category of extracolonic features incorporated arthritis, pyoderma gangrenosum, erythema gangrenosum, and uveitis. A multiple regression analysis was done according to Powell-Tuck Index as dependent variable and authors developed an equation with six variables which best predicted this index: bowel frequency (day) (range 0–3), urgency of defecation (range 0–3), blood in stool (range 0–3), general well-being (range 0–4) and extracolonic manifestations (score 1 per manifestation). Total score ranges from 0 to 19 points. This new index was further evaluated in a different group of patients and it showed a highly significant correlation with the Powell-Tuck index as well as with laboratory data (albumin, hemoglobin, platelet count, hematocrit, ESR). Walmsley also showed that SCCAI correlated well with the Seo Index and IBDQ. Clinical remission and response criteria were not defined in the initial study. Nevertheless, a cut-off value below 2.5 points has been shown to correlate with patient-defined remission and a decrease of >1.5 points from baseline correlated with patient-defined significant improvement [28].

Mayo Score (Also Called Mayo Clinic Score or the Disease Activity Index-DAI)

Schroeder et al. developed an instrument to measure disease activity (the Mayo Score) in a placebo-controlled trial on 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis [11]. This score is a composite of clinical and endoscopic parameters. Mayo Score consists of four items: stool frequency (range 0–3), rectal bleeding (range 0–3), findings of flexible proctosigmoidoscopy (range 0–3), and physician’s global assessment (range 0–3) (Table 23.2). Total score ranges from 0 to 12 points. In addition, a patient’s functional assessment representing a general sense of well-being was also measured and participated to evaluation of physician’s general assessment. It was not included in the 12-point index calculation. A complete response (remission) was defined as complete resolution of all symptoms (all assessment scores were zero). A partial response was defined as substantial but incomplete improvement in the assessment scores. If any assessment score was noted to worsen, despite improvement in other scores, the patients were excluded from this category and considered to have had a treatment failure. Neither the Mayo Score nor the definitions of complete or partial responses have been validated.

Table 23.2 Mayo score (Mayo Clinic Score or disease activity index – DAI)

Variables	Score 0	Score 1	Score 2	Score 3
Stool frequency ^a	Normal number of stools for this patient	1–2 stools more than normal	3–4 stools more than normal	5 or more stools more than normal
Rectal bleeding ^b	No blood seen	Streaks of blood with stool less than half the time	Obvious blood with stool most of the time	Blood alone passed
Findings of flexible proctosigmoidoscopy	Normal or inactive disease	Mild disease (erythema, decreased vascular pattern, mild friability)	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	Severe disease (spontaneous bleeding, ulceration)
Physician's global assessment ^c	Normal	Mild disease	Moderate disease	Severe disease

^aEach patient served as his or her own control to establish the degree of abnormality of the stool frequency

^bThe daily bleeding score represented the most severe bleeding of the day

^cThe physician's global assessment acknowledged the three other criteria, the patient's daily record of abdominal discomfort and general sense of well-being (patient's functional assessment [Patient's functional assessment was not included in the 12-point index calculation but represented a general sense of well-being. It was also measured (score 0=generally well, score 1=fair, score 2=poor, score 3=terrible) and participated to evaluation of physician's general assessment]) and other observations such as physical findings and the patient's performance status. Score of 0 meant there were no symptom of colitis, the patient felt well and the flexible proctosigmoidoscopy score was 0. A score of 1 indicated mild symptoms and proctoscopic findings that were mildly abnormal. A score of 2 reflected more serious abnormalities and proctosigmoidoscopic and symptom scores of 1 to 2. A score of 3 indicated that proctosigmoidoscopic and symptom scores were 2 to 3 and the patient probably required corticosteroid therapy and possibly hospitalization

Subsequent studies have modified the definitions of remission or clinical response. A remission has been defined in the majority of the studies by a global score of zero, while clinical response has been defined different ways. The definition of response ranged from an improvement in the baseline physician's global assessment score and improvement in at least one other clinical assessment without worsening in any other clinical assessment [29] to a decrease in the disease activity index of at least three points [30] or two points [31]. More recently, the definition of remission has been extended to a score of 1 for either stool frequency or global physician's assessment while the endoscopic and bleeding subscore had to be zero [32, 33] or a total score no greater than 2 with a maximum of 1 for each subscore [34]. In this last study, a mucosal healing was defined by an endoscopic subscore not greater than 1. Despite the lack of formal validation, the clinical relevance of these last definitions was demonstrated by correlation between these definitions and significant improvement in quality of life as measured by the Inflammatory Bowel Disease Questionnaire (IBDQ) and by the Short Form-36 (SF-36) questionnaire [35–37].

Sutherland Index (Also Called Disease Activity Index and the UC Disease Activity Index-UCDAI)

Sutherland et al. described an instrument to measure disease activity named the Sutherland Index (also called Disease Activity Index-DAI and the UC Disease Activity Index-UCDAI) in a placebo-controlled trial of mesalamine enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis [12]. This index is very close to the Mayo score. It consists of four items: stool frequency

(range 0–3), rectal bleeding (range 0–3), mucosal appearance (range 0–3), and physician’s rating of disease activity (range 0–3). Total score ranges from 0 to 12 points. Efficacy was defined as a statistically significant reduction in the Sutherland Index Score and a significant reduction in the individual subscores (both calculated individually for each patient). Neither the Sutherland Score nor the definitions of complete or partial responses have been validated. Subsequent studies have modified the definitions of remission and clinical improvement. Remission was defined as Sutherland score of ≤ 1 point with a score of 0 for rectal bleeding and stool frequency, while clinical improvement was defined as a reduction in the Sutherland score ≥ 3 points from baseline [38, 39]. Despite the lack of formal validation, the Sutherland Index has been shown to correlate with an index based on patient’s opinion developed by Higgins et al. [28].

Ulcerative Colitis Clinical Score

In 2005, Feagan et al. described the Ulcerative Colitis Clinical Score in a double-blind placebo-controlled trial on a humanized $\alpha 4\beta 7$ integrin-antibody for the treatment of ulcerative colitis [18]. This instrument was a modification of the Mayo Score and consisted on four items which were scored from 0 (normal) to 3 (severe): stool frequency (referring to the usual normal number of stools per day when the patient was in remission), rectal bleeding, functional assessment by the patient, and global assessment by the physician. To define clinical remission and response it was used with a modified version of a previously described endoscopic index: the Baron score [40]. This score consisted of a 4-point scale mainly based on the severity of bleeding and not including ulceration. The modified Baron score was based on a 5-point scale (0–4). Clinical remission was defined as a UCCS score of 0 or 1 without rectal bleeding and with a normal mucosa or only granular mucosa with an abnormal vascular pattern on sigmoidoscopy. Clinical response was defined as an improvement of three points or more on the UCCS and endoscopic response (defined by a Baron score of zero or a decrease of at least two points). Neither the UCSS nor the definitions of clinical remission and clinical response have been validated.

Clinical Activity Index (Also Called Rachmilewitz Index)

In 1988, Rachmilewitz et al. reported an instrument subsequently named the Clinical Activity Index (CAI) in a randomized trial comparing coated mesalazine versus sulphasalazine in the treatment of active ulcerative colitis [15]. This index was composed of seven variables: number of stools (range 0–3), blood in stools (range 0, 2, or 4), investigator’s global assessment of symptomatic state (range 0–3), abdominal pain or cramps (range 0–3), temperature due to colitis (range 0 or 3), extraintestinal manifestations (range 0, 3, 6, or 9), and laboratory findings (range 0, 1, 2, or 4). Total score ranges from 0 to 29 points. It has been validated in one study in which remission was defined as a CAI score ≤ 4 points [41]. Rachmilewitz also developed an endoscopic index [15]. This index was based on four items, each scored on a 4-point scale (0–3): granulation scattering reflected light, vascular pattern, vulnerability of mucosa, and mucosal damage. It has not been validated.

Improvement Based on Individual Symptom Scores

In 2002, Levine et al. reported an instrument to measure disease activity in a randomized, double-blind controlled trial on balsalazide versus mesalamine treatment in active mild to moderate ulcerative

colitis [17]. This instrument was based on individual symptom scores including rectal bleeding, patient functional assessment, stool frequency, abdominal pain, sigmoidoscopic grade, and physician global assessment. The scores of each item were graded from 0 to 3 points (0=normal, 1=mild, 2=moderate, 3=severe). Bowel event was strictly defined as an event during which stool, stool with blood, or blood alone passed. Sigmoidoscopic evaluation was done at 10 cm of the anal verge or at the area of most severe involvement and was evaluated as follows: 0=normal; 1=mild/edema, loss of vascular pattern, fine granularity without ulceration; 2=moderate/friability, petechiae, coarse granularity with pinpoint ulceration; 3=severe/visible ulcers, spontaneous bleeding. Improvement was defined as a reduction from baseline of ≥ 1 point in rectal bleeding and at least one of the other individual symptoms. This instrument has not been validated.

Physician Global Assessment and Investigator Global Evaluation

In 1993 and 1998, respectively, Hanauer et al. described two arbitrarily designed, multicomponent measure of disease activity using the physician's assessment of disease activity and evolution from baseline: the Physician Global Assessment (PGA) and the Investigator Global Evaluation [19, 20]. Scores range from one to six points for PGA (1=complete relief of symptoms; 2=marked improvement; 3=moderate improvement; 4=slight improvement; 5=no change; 6=worsening) and from zero to four points for Investigator Global Assessment (0=normal or in remission; 1=mild; 2=moderate; 3=severe; 4=incapacitating). These scores have not been validated.

Sigmoidoscopic Index and Sigmoidoscopic Inflammation Grade Score

These scores are only endoscopic scores. They were described alongside different clinical trials. The sigmoidoscopic index is composed of five variables: erythema, friability, granularity/ulceration, mucus, and disappearance of vascular pattern [19]. Each variable was assigned a value from 0 to 3 and a sigmoidoscopic remission was defined by a score 0–4. It has not been validated. The sigmoidoscopic inflammation grade score is a 4-point scale (0–3) ranging from normal mucosa (0) to spontaneous hemorrhage and visible ulcers [3, 20, 42]. Remission was not defined and the score has not been validated.

Clinical Scores in Crohn's Disease

Assessment of Crohn's disease (CD) activity and monitoring for treatment response require the establishment of reliable and reproducible tools. The development of instruments that would accurately reflect the activity of CD was more complex than in ulcerative colitis (UC) due to the heterogeneity of the disease.

Crohn's Disease Activity Index

In 1970, the National Cooperative Crohn's Disease Study (NCCDC) was initiated to test the efficacy of prednisone, sulphasalazine, and azathioprine in clinical controlled trials. The Crohn's Disease Activity Index (CDAI) was developed to investigate the efficacy of these drugs [43]. It was

published in 1976 and, after nearly 30 years, remains the most suitable instrument to evaluate CD activity in clinical trials [44–49]. CDAI was initially developed from 112 patients and then later re-analyzed on a larger group with the same conclusions. Eighteen variables were first tested and eight were finally retained for the regression equation, out of which seven variables are purely clinical and one is biological (hematocrit). Moreover, four of them (liquid stools, well-being, use of loperamide, and abdominal pain) should be determined prospectively by keeping a 7-day patient diary and CDAI is therefore considered as a prospective index. In order to establish threshold cut-off value that indicates the disease activity, the global assessment of physicians was confronted to CDAI score. A value less than 150 was defined as remission and over 450 as a severe active disease. The clinical response to a treatment has generally been defined as a reduction of 70 or 100 points of the CDAI. The use of 100 points in CDAI decrease instead of 70 has been promoted to have more stringent criteria to differentiate between active drugs and placebo in clinical trials. However, the superiority of these judgment criteria has not been fully demonstrated [50]. CDAI has a good inter-observer reproducibility. This index remains the score of choice in CD clinical trials and was used to assess the efficacy of azathioprine, corticosteroid, methotrexate, cyclosporine, and more recently the anti-TNF therapies [46–49]. It has also been demonstrated recently that a retrospectively assisted evaluation of the CDAI was as accurate as the traditional prospective evaluation [51]. Although CDAI is considered as the gold standard for the evaluation of CD activity, it may not reflect all the aspects of the disease and has some limitations. For instance, CDAI is not suitable for pediatric patients whose disease presentation differs from that of adults. Therefore, a Pediatric Crohn's Disease Activity Index (PCDAI) has been developed [52]. Moreover, CDAI does not take into account symptoms linked to predominant perianal CD and refers to unsuitable parameters in case of previous surgery or stoma, like the use of loperamide or stool frequency, which can be affected by biliary salt malabsorption or short bowel syndrome. Therefore, CDAI is not applicable for the assessment of perianal or operated CD. Studies comparing CDAI and serum or stool biomarkers, mainly CRP and fecal calprotectin, have demonstrated only weak correlations [3, 53]. Poor correlation between CDAI and endoscopic examination has also been demonstrated [3, 54, 55].

Harvey–Bradshaw Index

Because the CDAI has been reported to be difficult to use in daily clinical practice, other scores have been developed. The more practical one is the Harvey–Bradshaw Index (HBI), also known as the Simple Index or the Modified CDAI (Table 23.3) [27]. The HBI is based on subjective and clinical factors. It reduced the original eight items of the CDAI to five, removing the use of anti-diarrheal drugs, the hematocrit and the body weight. It looks only at symptoms over the preceding 24 h which makes the measurement of severity easy and quick at an outpatient visit. However, it could be less reliable and its value could significantly change from one day to another. The original prospective study of 112 patients demonstrated a good correlation ($r=0.93$) between HBI and CDAI. More recently, one study compared the CDAI and HBI in the assessment of CD activity in two large clinical trials, PRECISE 1 and PRECISE 2 and confirmed a good correlation between the two scores [56]. This suggests that HBI might permit simpler CD activity assessment not only in routine practice but also in long-term clinical trials.

Van Hees Index

In order to overcome the disadvantages and the potential subjectivity of the CDAI, Van Hees et al. elaborated in 1980 an activity index, the Van Hees Index (VHI), based on entirely objective variables

Table 23.3 Harvey–Bradshaw index

Patient's general well-being (for the previous day)	0=well 1=slightly below par 2=poor 3=very poor 4=terrible
Abdominal pain (for the previous day)	0=none 1=mild 2=moderate 3=severe
Number of liquid stools per day (for the previous day; score 1 per movement)	
Abdominal mass	0=none 1=dubious 2=definite 3=definite and tender
Complications (score 1 per item)	Arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissure, new fistula, abscess
Harvey–Bradshaw index score: remission <5; mild disease 5–7; moderate disease 8–16, severe disease >16	

of which albumin serum level contributed the most [57]. A score of less than 100 is considered as clinical remission and over 210 as a severe disease. Its correlation with the CDAI is poor, which is due to an absence of consideration of clinical symptoms. This score calculation is more complex and has not supplanted the CDAI in clinical practice.

Crohn's Disease Endoscopic Index of Severity

The lesions of the gastrointestinal tract are partly responsible for the symptoms of CD. Other factors such as previous intestinal resection or functional disorders may also be involved. These lesions of the gastrointestinal tract also play a key role in the development of the complications of the disease, including strictures and fistulas. They are thus the main objective manifestation of the disease. Therefore, the quantification of these lesions rapidly came as a priority in the assessment of disease activity. In the late 1980s, the GETAID (groupe d'études thérapeutiques sur les affections inflammatoires digestives) developed and validated a Crohn's disease endoscopic index of severity (CDEIS). The GETAID first prospectively recorded the data of colonoscopies performed in a large number of patients [58]. All the lesions including pseudopolyp, healed ulceration, frank erythema, frankly swollen mucosa, aphthoid ulceration, superficial or shallow ulceration, deep ulceration, nonulcerated stenosis and ulcerated stenosis were recorded in all the segments explored (maximum of five: ileum, right colon, transverse colon, left colon, sigmoid and rectum). In parallel, the endoscopists had to score the percentage of affected mucosa as well as the percentage of ulcerated mucosa per segment explored. They finally had to assess the global severity of the lesions, as indicated on a 10 cm linear analogue scale. Multiple regression methods were then used to define the correlation between the nine mucosal lesions and the global severity score. Four mucosal lesions (superficial ulcer, deep ulcer, ulcerated stenosis, and nonulcerated stenosis) were finally selected together with the percentage of affected mucosa and of ulcerated mucosa, to establish the formula of CDEIS. With this score, inter-observer agreement was very good ($P<0.001$) as well as the correlation between CDEIS and the global severity score ($r=0.81\text{--}0.83$; $p<0.001$). The CDEIS was further evaluated in several other studies, where it was correlated to the clinical index CDAI as well as to inflammatory

Table 23.5 Scoring system for recurrent lesions of Crohn's disease in the neoterminal ileum

i0	No lesions
i1	<5 aphthous lesions
i2	>5 aphthous lesions with normal mucosa between the lesions or skip areas of larger lesions or lesions confined to the ileocolonic anastomosis
i3	Diffuse aphthous ileitis with diffusely inflamed mucosa
i4	Diffuse inflammation with already larger ulcers, nodules and/or narrowing

advantages over CDEIS for routine practice, particularly its potentially better reproducibility and its easier use. Nevertheless, the semi-quantitative assessment of affected and ulcerated surfaces may result in a lower sensitivity to detect changes following therapy. This may have some importance, particularly for clinical trials.

Endoscopic Postoperative Recurrence Score

Curative surgery in CD is a particular situation where the patient has undergone a resection (mainly ileo-cecal) and has no remaining active lesion in the bowel. Despite this, cohort studies have clearly showed a high rate of surgical, clinical, and particularly endoscopic recurrence over time [61]. One year after surgery, the endoscopic recurrence is around 85% at the anastomosis and/or neoterminal ileum. Clinical and surgical recurrences are around 50 and 30%, respectively, over 8 years. Very importantly, the Leuven group has showed that the severity of the early (6–12 months after surgery) endoscopic recurrence was predictive of further clinical recurrence [62]. A scoring system could even be proposed, including five grades (i0–i4) (Table 23.5). Patients having no recurrent lesions or less than five aphthous ulcers lesions (i0–i1) had a risk of clinical recurrence around 10% over 8 years, while patients harboring larger ulcers in the neo-terminal ileum, diffuse aphthous ileitis, diffusely inflamed mucosa or narrowing (i3–i4) relapsed in almost all case. This score tends now to be routinely used after a first resection surgery to tailor the preventive treatment to the patient.

Conclusions

Several clinical and endoscopic indices of activity have been developed in IBD. Globally, they have mainly been used in clinical trials where they have allowed to demonstrating efficacy or inefficacy of currently available drugs.

In UC, the correlation between clinical and endoscopic indices has usually been good and simple sigmoidoscopic scores have been integrated to several global activity indices. However, the reproducibility of these simple endoscopic score has been questioned and the use of a large number of different scores across the UC clinical trials has led to some confusion. An optimal scoring instrument for UC is still to be developed and will require validation before extensive use in clinical trials or routine practice can be promoted.

In CD, the correlation between clinical activity and endoscopic activity indices has been weak reflecting a significant problem in the patient evaluation. Although still broadly used in clinical trials, CDAI has been extensively criticized as being too influenced by subjective aspects, while on the other hand it was not even a proper assessment of disease-related quality of life. Therefore in most recent trials, and although CDAI remained the main judgment criterion, health-related quality of life questionnaires were developed and used, and endoscopic scores were developed to assess tissue healing following treatment. Despite their more objective assessment of disease activity, these

endoscopic scores are still flawed with drawbacks: they require an invasive extensive endoscopic exploration, the most relevant level of tissue healing to be obtained following treatment has not been clearly defined and finally, they only assess the mucosal part of the disease while CD is a transmural disease. Therefore, the added value of biomarkers and cross-sectional imaging is currently actively studied. Furthermore, CD is a progressive disease leading to complications and surgeries in the majority of the patients. For this reason, the impact of specific treatment strategies may be best evaluated by scoring systems focusing on cumulated tissue damage instead of disease activity. Such score is also currently being developed.

The usefulness of all these UC and CD scores in routine practice, particularly their superiority over a simple qualitative assessment made by the clinician, has not been demonstrated. Nevertheless, the increase in the number of drugs available to treat these illnesses and the increasing complexity of therapeutic strategies that are developed may require in a near future some quantitative assessment also in routine practice to allow optimal patients management.

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Chapter 24

Endoscopic Assessment of Disease Activity and Mucosal Healing in Inflammatory Bowel Disease

Haruhiko Ogata and Toshifumi Hibi

Keywords Inflammatory bowel disease • Ulcerative colitis • Crohn's disease • Endoscopy • Mucosal healing • Activity indices • Medical therapy • Surgery

Introduction

The management of ulcerative colitis (UC) and Crohn's disease (CD), the two major forms of inflammatory bowel diseases (IBD) has dramatically changed over the last decade. Progress has been supported by the increasing evidences of the therapeutic strategies, the introduction of biologics providing more alternative options in patients with severe diseases, and new concepts as to how and when treatments should be used [1, 2]. Immunomodulators and biologics are classically used following a step-up approach in patients with refractory disease, who are unresponsive to conventional therapies or are steroid dependent. Beyond their high efficacy in induction and maintenance of remission, it has been demonstrated that anti-tumor necrosis factor (TNF) therapies can close fistulae and heal mucosal lesions, and reduce rates of hospitalization and surgery [3]. Several recent studies suggest that early intervention with combination therapy may modify the long-term course of CD [4–6]. Meanwhile studies performed with regard to prediction of the disease activity have mainly focused on clinical and biological parameters, and endoscopy has been rarely investigated as a predictor of the clinical course of IBD. However, there is now growing evidence that morphological examination, including endoscopy, may help to identify among IBD patients those who should be treated with more intensive treatments. Furthermore, as demonstrated in a recent study assessing early intervention with combination of infliximab and azathioprine in CD, endoscopy may help select patients who will get the best results upon early intervention [4].

Endoscopic assessment of mucosal healing is usually assessed by colonoscopy in patients with ulcerative colitis and by ileocolonoscopy in patients with CD, and recently balloon-assisted small bowel enteroscopy has also contributed to the evaluation of disease activity of small bowel CD. For UC, mucosal inflammation manifests initially as vascular congestion, erythema, edema and granularity [7].

H. Ogata, MD, PhD

Center for Diagnostic and Therapeutic Endoscopy, Keio University School of Medicine,
35 Shinanomachi, Shinjuku-ku, Tokyo 160-0016, Japan

T. Hibi, MD, PhD (✉)

Department of Internal Medicine, Keio University School of Medicine,
35 Shinanomachi, Shinjuku-ku, Tokyo 160-0016, Japan
e-mail: thibi@sc.itc.keio.ac.jp

With more severe mucosal inflammation, friability, spontaneous bleeding, macroscopic ulceration, and small and large ulcers may arise [7]. The pattern of CD viewed on endoscopy has been characterized and is associated with a number of mucosal characteristics, such as erythema, swelling, nodularity, strictures, aphthoid ulcerations and ulcers of variable size and depth [11]. Although there is no validated definition of mucosal or endoscopic remission in patients with IBD [8, 9], the severity of mucosal lesions directly reflects the activity of the disease and may thus be considered as an indication of an aggressive behavior of the disease. In this chapter, the important aspects of assessment of endoscopic severity in UC and CD are described and their role in predicting the course of these diseases and their impact on decisions regarding medical strategies is discussed.

Endoscopic Disease Activity Indices of UC

There are ten different scores proposed to measure endoscopic severity in UC (Table 24.1). Truelove and Witts were the first to report on the sigmoidoscopic appearance of the gut mucosa during a placebo-controlled trial of cortisone for the treatment of active disease [10]. Endoscopic lesions were classified as normal or near normal, improved, or no change or worse. Thereafter, several endoscopic scoring systems were developed using different items and definitions of mucosal healing. The Baron score [11] (Table 24.2) and the Powell-tuck sigmoidoscopic assessment [12] focus on the assessment of severity of bleeding without considering ulcers or other mucosal changes. The seven remaining endoscopic scores for ulcerative colitis, including the Mayo endoscopic subscore [13] (Table 24.3) and the Sutherland index [14] are listed in Table 24.1 [15–19]. These scores

Table 24.1 Endoscopic disease activity indices for ulcerative colitis

Endoscopic scores	Variables	Strength of scale	Notes
Mayo endoscopic subscore [13]	Erythema, vascular pattern, friability, bleeding, erosions, ulcerations	0–3; 4-point scale	
Truelove and Witts sigmoidoscopic assessment [10]	Overall appearance of the mucosa	No description	Interobserver variability
Baron score [11]	Bleeding	0–3; 4-point scale	Good interobserver correlation
Modified Baron score [15]	Friability, vascular pattern	0–4; 5-point scale	
Powell-Tuck sigmoidoscopic assessment [12]	Bleeding	0–2; 3-point scale	
Rachmilewitz endoscopic index [18]	Granulation, vascular pattern, vulnerability of mucosa, mucosal damage	0–3; 4-point scale with 4 items, total of 12 points	Complex
Sigmoidoscopic index [16]	Erythema, friability, ulceration, mucous, vascular pattern	0–3; 4-point scale with 5 items, total of 16 points	Complex
Sigmoidoscopic inflammation grade score [17]	Edema, vascular pattern	Granularity, friability, bleeding, ulcers	4-point scale
Sutherland mucosal appearance assessment [14]	Friability, exudation, bleeding	0–3; 4-point scale	
Endoscopic activity index [19]	Ulcers (size and depth), erythema, bleeding, edema, mucous	0–3; 4-point scale	Complex

Table 24.2 The Baron score

Score 0	Normal mucosa, ramifying vascular pattern clearly visible throughout, no spontaneous bleeding, and no bleeding to light touch
Score 1	Abnormal but not hemorrhagic: appearances between “0” and “2”
Score 2	Moderately hemorrhagic: bleeding to light touch, but no spontaneous bleeding seen ahead of instrument on initial inspection
Score 3	Severely hemorrhagic: spontaneous bleeding seen ahead of instrument at initial inspection, and bleeds to light touch

[Adapted by permission from BMJ Publishing Group Limited. Br. Med. J. Baron JH, Connell AM, Lennard-Jones JE. Variation between observers in describing mucosal appearances in proctocolitis; 1: 89–92.©1964]

Table 24.3 Mayo endoscopic subscore (proctosigmoidoscopy assessment)

Score 0	Normal or inactive disease
Score 1	Mild disease (erythema, decreased vascular pattern, mild friability)
Score 2	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
Score 3	Sever disease (spontaneous bleeding, ulceration)

[Reprinted from Schroeder KW, Tremaine WJ, Ilstrup, DM; Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N. Engl. J. Med. 1987; 317: 1625–1629. With permission from The Publishing Division of the Massachusetts Medical Society. ©1987]

include, variably, endoscopic assessment of vascular pattern, granularity, friability, bleeding and ulcers [9]. Overall, these ten different scores have not been fully validated, and are subject to inter-observer variations [20]. The development of a validated ulcerative colitis index of severity (UCEIS) is ongoing [21].

Feasibility of Endoscopy in Active UC

Carbonnel et al. [22] demonstrated that total colonoscopy is feasible in 86% of cases in severe UC (73/85). In this study, endoscopy accurately identified severe endoscopic lesions (extensive deep ulcerations). Eighty-five consecutive patients with attacks of UC were reviewed. Extensive deep colonic ulcerations were diagnosed in 46 of them. No complication related to colonoscopy occurred except for one colonic dilatation. Forty-three of the 46 patients with severe endoscopic colitis underwent surgery. Extensive ulcerations reaching at least the circular muscle layer were found on pathological examination and were confirmed in 42/43 [22]. Because of the potential risks of complications, some rules have to be applied when performing colonoscopy in patients presenting severe attacks of UC, including pre-radiological examination to exclude megacolon, minimal insufflations, and when severe lesions are detected, the examination can be stopped as further examination has no additional prognostic value.

Mucosal Healing Evaluated by Endoscopy Contributes to a Better Outcome in UC

To date, there is no consensus on the definition of mucosal healing in UC [1]. The International Organization of IBD proposed the following definition: absence of friability, blood, erosions and ulcers in all visualized segments of the gut mucosa. According to this definition, disappearance of the normal vascular pattern is compatible with mucosal healing. Mucosal healing may be obtained

with several types of drugs [1, 23]. It has been shown that it can be obtained with 5-aminosalicylates (5-ASA), steroids, azathioprine or methotrexate, and infliximab. Mucosal healing has been assessed in recent trials with different formulations of 5-ASA. In the ASCEND studies, evaluating different dosages of a delayed-released oral mesalazine in patients with mild or moderate UC, complete remission (including endoscopic remission) ranged between 18% and 25% at week 6 [24, 25]. Truelove et al. [26] demonstrated in 1954 that mucosal healing can be obtained with a high dose of oral steroids in 30% of the patients at week 6 compared with 10% in patients who received placebo ($P=0.02$). In a recent review, it was considered that corticosteroids induce mucosal healing in 12–41% of the patients with UC, depending on the method of administration and the medication [1]. Some data suggest that mucosal healing may also be obtained with azathioprine or methotrexate [27, 28]. Anti-TNF agents probably induce mucosal healing more rapidly. In ACT 1 and ACT 2, patients with refractory moderate-to-severe UC received placebo or infliximab intravenously [29]. Induction therapy with infliximab resulted in mucosal healing at week 8 in 61% of the patients (148/242) compared with 32% (79/244) in the placebo groups ($P<0.001$) [29]. At week 54 (ACT 1), scheduled maintenance therapy with infliximab resulted in mucosal healing in 45.5% (55/121) of the patients compared with 18.2% (22/121) in the placebo group ($P<0.001$). Data from several studies suggest that mucosal healing may be associated with a better outcome in UC, more specifically a decreased risk of relapse. Reduced relapse rates have been demonstrated in UC patients who achieved mucosal healing with steroids. In a study published in 1966, Wright et al. [30] found that 40% of the patients who achieved mucosal healing with oral and rectal steroids did not relapse during 1 year of follow up as compared to 18% of those who still had lesions. In the ACT1 and 2 studies on infliximab maintenance in patients with moderately to severely active UC, 48.3% of the patients who achieved mucosal healing at week 8 were in remission at week 30 as compared to only 9.5% of those who did not achieve mucosal healing [28]. Mucosal healing may also be associated with reduced risk of surgery in UC. In the IBSEN population-based study, UC patients who achieved mucosal healing at 1 year (whatever the treatment) had a decreased risk of colectomy at 5 years (2% vs. 7%, $P=0.02$) [31]. A study performed in the Leuven cohort of UC patients treated with infliximab showed that colectomy was more frequent in patients who did not achieve mucosal healing at week 4 or 10 (Mayo endoscopic subscore greater than 1) [32]. In ACT1 and 2, it was shown that patients treated with infliximab were less likely to undergo colectomy through 54 weeks than those receiving placebo [33]. However, data on the relationship between mucosal healing and risk of colectomy are not available in these studies. Finally, there is a clear relationship between the grade and chronicity of inflammation in the colon and the risk of colorectal cancer. Better control of inflammation, as demonstrated with mucosal healing, may be associated with decreased risk of colorectal cancer.

Endoscopic Observation of Severe Lesions of UC Contributes to an Increased Risk of Colectomy

Among patients hospitalized for a severe attack of UC, the presence of extensive and deep ulcerations at colonoscopy is associated with an increased risk of colectomy on that admission [22]. In their study performed in the prebiologic era, Carbonnel et al. [22] showed that colectomy was performed in 43 of the 46 patients who presented severe endoscopic lesions (93%) as compared to 10/39 (26%) of those without such lesions (OR 41). In another study performed in severe UC patients, severe endoscopic lesions at colonoscopy were significantly more frequent in non-responders to medical treatment (91%) compared with responders (34%) (OR>20) [34]. The colonoscopies performed during severe attacks of UC have also an impact on the long-term outcome, with an increased rate of surgery in the long term in patients who exhibit extensive and deep

ulcerations at index colonoscopy [35]. Namely, although intravenous cyclosporine treatment could exert high initial efficacy for severe attacks of UC, 50% of the patients who had relapse require a colectomy, especially, mucosal healing evaluated by a novel endoscopic activity index [19] at day 14 after cyclosporine injection was associated with the 1-year colectomy rate [36].

Endoscopic Disease Activity Indices of CD

Currently there are three major endoscopic indices for evaluating CD mucosal activities (Table 24.4). The Crohn’s disease endoscopic index of severity (CDEIS) [37] (Table 24.5) is a prospectively developed instrument that has been constructed to detect changes in disease activity on the basis of characteristics of the gut mucosa. Although this score is often considered to be the gold standard for classifying severity of disease, the CDEIS is regarded as complex to use and requires users to have training and experience, especially because the estimation of ulcerated or diseased mucosal surfaces and the distinction between deep and superficial ulcerations can be difficult. The CDEIS has been validated as a reproducible index and has good correlation with the Crohn’s disease activity index (CDAI) [38]. But data on CDEIS, mucosal healing and long-term outcome are lacking. A simplified index, the simple endoscopic score for Crohn’s disease (SES-CD) (Table 24.6), has also been developed and correlates well with the CDEIS [39]. For both indices, there is no agreement regarding cut-off values for defining endoscopic response to treatment, endoscopic remission or mucosal healing [8]. The Rutgeerts score (Table 24.7) has been proposed to determine the presence and severity of endoscopic disease recurrence in the neoterminal ileum after ileal or ileocolonic resection [40, 41]. Despite the absence of perfect validation, the popularity of this score comes from the demonstration

Table 24.4 Endoscopic disease activity indices for Crohn’s disease

Endoscopic scores	Variables	Strength of scale	Notes
CDEIS [38]	Deep ulceration, superficial ulceration, inflammation	Gold standard for the endoscopic assessment, reproducible	Complex, many variables range from 0 to 44
SES-CD [40]	Ulcers, inflammation, stenosis	Simplified score, correlated to CDEIS	Validated in 1 study range from 0 to 60
Rutgeerts score [41]	Aphthoid lesions, ulcers, inflammation, nodules, stenosis	Gold standard for the assessment of post-operative recurrence	Not for end point in clinical trials

CDEIS Crohn’s disease endoscopic index of severity, SES-CD simple endoscopic score for Crohn’s disease

Table 24.5 The Crohn’s disease endoscopic index of severity (CDEIS)

Endoscopic variables	Score (range 0–44)
Deep ulcerations	0 if absent or 12 if present
Superficial ulcerations	0 if absent or 6 if present
Length of ulcerated mucosa (0–10 cm)	0–10 according to length in cm
Length of diseased mucosa (0–10 cm)	0–10 according to length in cm

Four variables are scored for each of the following locations: rectum; sigmoid and left colon; transverse colon; right colon; and ileum. Total score is divided by the number of locations explored (1–5). An additional 3 points are given if ulcerated stenosis is present, and a further 3 points are given if non-ulcerated stenosis is present [Adapted from Gastrointest. Endosc;63. Rutgeerts P, et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn’s disease. pp. 433–442. ©2006, with permission from Elsevier]

Table 24.6 The simple endoscopic score for Crohn’s disease (SES-CD)

Variable	Score			
	0	1	2	3
Size of ulcers (cm)	None	Aphthous ulcers Ø0.1 – 0.5	Large ulcers Ø0.5 – 0.2	Very large ulcers Ø> 2
Ulcerated surface (%)	None	<10	10–30	>30
Affected surface (%)	Unaffected segment	<50	50–75	>75
Presence of narrowing	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Total SES-CD: sum of the values of the 4 variables for the 5 bowel segments. Values are given to each variable and for every examined bowel segment (for example, rectum, left colon, transverse colon, right colon and ileum)
[This table was adapted from *Gastroenterology*; 99. Rutgeerts P, et al. Predictability of the postoperative course of Crohn’s disease: pp. 956–963. ©1990]

Table 24.7 The Rutgeerts score

Grade 0	No lesions in the distal ileum
Grade 1	≤5 aphthous lesions
Grade 2	>5 aphthous lesions with normal mucosa between the lesions, or skip areas of larger lesions or lesions confined to ileocolonic anastomosis (<1 cm in length)
Grade 3	Diffuse aphthous ileitis with diffusely inflamed mucosa
Grade 4	Diffuse inflammation with already larger ulcers, nodules, and/or stenosis

[Adapted by permission from BMJ Publishing Group Limited. *Gut*. Rutgeerts P, et al. Natural history of recurrent Crohn’s disease at the ileocolonic anastomosis after curative surgery; 25: 665–672. ©1984]

that the severity of endoscopic disease recurrence measured by the score is predictive of clinical recurrence [40]. When an ileocolonoscopy is performed in asymptomatic patients in the year following ileocolonic resection, patients with a Rutgeerts score of grade 0 or 1 have a very low risk of clinical recurrence compared with those who have a score of grade 3 or 4 [40]. Grade 2 is associated with an intermediate risk of clinical recurrence, but the definition of grade 2 is more subjective than the definitions of the other grades and is exposed to variability. A 2002 expert consensus stated that both the CDEIS and Rutgeerts score are the gold standards for assessing mucosal healing in patients with CD, even though the CDEIS should be reserved for use in clinical trials only because of its complexity [8]. In the small bowel, capsule endoscopy is increasingly used to assess the severity of CD, but very few studies have examined capsule endoscopy and mucosal healing [42, 43]. A GETAID (Groupe d’Eetudes Thérapeutiques des Affections Inflammatoires Digestives) study to build an endoscopic index of severity of small bowel CD that includes the assessment of mucosal healing using capsule endoscopy is ongoing.

Severe Mucosal Lesions of Colonic CD Evaluated by Endoscopy

Severity of colonic lesions in CD relies on the extent in depth and in surface of the mucosal damage. A previous interobserver variation study targeted on evaluation of ileocolonoscopy lesions in CD [44] has shown that deep ulcerations and estimation of ulcerated surface were among the most reproducible endoscopic items. Such lesions were also selected by multivariate analysis for the construction of the CDEIS [45]. Nahon et al. [46] demonstrated that colonoscopy accurately predicts the anatomical severity of colonic CD attacks. In this retrospective study of 78 patients operated for colonic CD resistant to medical treatment, criteria of severity in colectomy specimens were

defined as either deep ulcerations eroding the muscle layer, or mucosal detachments, or ulcerations limited to the submucosa but extending to more than one-third of a defined colonic segment (right, transverse, left colon). Three endoscopic criteria of severity were defined: (a) deep ulcerations eroding the muscle layer; (b) deep ulcerations not eroding the muscle layer but involving more than one-third of the mucosal area and (c) mucosal detachment at the edge of ulcerations. Evaluation of endoscopic severity correlated well with findings on colectomy specimens. At least one of these criteria was found in 95% of the patients with severe anatomic lesions on colectomy specimens. The extent of ulcerations at colonoscopy was correlated to the results of colectomy specimen examination ($P < 0.001$). This study further demonstrates that colonoscopy can accurately assess anatomical severity of colonic CD.

Endoscopic severity may have an impact on the long term course of the disease. Allez et al. [47] showed in a retrospective study that patients with CD exhibiting deep and extensive ulcerations at colonoscopy have a more aggressive clinical course with an increased rate of penetrating complications and surgery. Among the 102 patients included, 53 had severe endoscopic lesions at index colonoscopy, defined as extensive and deep ulcerations covering more than 10% of the mucosal area of at least one segment of the colon. During the follow-up (median 52 mo), 37 patients underwent colonic resection. Furthermore, patients with severe endoscopic lesions needed significantly more colonic resections than patients without severe lesions [47]. These data suggest that a subset of CD patients have a more aggressive disease, characterized by severe endoscopic lesions in the ileocolon during symptomatic phases and a higher risk of surgery [47].

Mucosal Healing Evaluated by Endoscopy After Medical Treatment Against CD

Therapeutic effect in clinical trials against CD is usually assessed by improvement defined by a decrease of the CDAI. Assessment of endoscopic improvement was not usually performed until recently in clinical trials assessing the efficacy of drugs in CD. The main reason for this was that steroid-induced clinical remission is not associated with mucosal healing in two-thirds of CD patients. However, there is growing evidence that mucosal healing during therapy is a sign of a good efficacy of a drug [1, 48]. Data from the IBSEN cohort strongly suggest that mucosal healing predicts a generally favorable outcome of disease based on all types of treatment strategies and is related to treatment efficacy, reduced frequency of surgery and hospitalizations [31]. Moreover, it is now clearly demonstrated that mucosal healing can be achieved with azathioprine and anti-TNF [49–53]. Rates of mucosal healing under azathioprine vary among studies, probably due to differences in the timing of endoscopy and the population analyzed. In a randomized controlled trial performed in steroid-dependent CD patients, Mantzaris et al. [54] have recently shown that azathioprine was superior to budesonide in inducing mucosal healing at 1 year; complete or near complete healing was achieved in 83% of azathioprine-treated patients compared with only 24% of budesonide-treated patients ($P < 0.0001$). In a GETAID study, long lasting remission (≥ 42 months) maintained with azathioprine was associated with a complete mucosal healing (CDEIS=0) in only 36% of CD patients [55]. In the SONIC study, which concerned CD patients naïve to immunomodulators and biologics, only 15.6% of the patients treated with azathioprine achieved mucosal healing at week 26 [4]. In the endoscopic substudy of the ACCENT I study, patients treated with scheduled maintenance therapy with infliximab had superior rates of mucosal healing, and those who maintained complete mucosal healing over 1 year had a lower rate of hospitalizations and surgeries [56, 57]. A study of mucosal healing in a cohort of CD patients under long-term treatment with infliximab was recently reported [58]. In this study from the Leuven group, 214 patients had a colonoscopy before and a second one within months after starting infliximab. Mucosal healing was

observed in 68% of the 183 initial responders. Mucosal healing was associated with a significantly lower need for major abdominal surgery during long-term follow-up (14.1% major surgeries in patients with mucosal healing vs. 38.4% in patients without mucosal healing, $P < 0.0001$). Several studies suggest that immunomodulators and anti-TNF therapy may be more effective when given early in the course of the disease. Recently there are two studies of “top-down” therapy performed in CD patients naïve to immunomodulators and biologics, which refers to early introduction of immunosuppressive or biologic therapies. In the SONIC study, infliximab therapy was superior to azathioprine in inducing mucosal healing at week 26 (30.1% vs. 15.6%), but inferior to infliximab plus azathioprine combination therapy [4]. D’Haens et al. [5] compared a top-down strategy to a more classical step-up strategy. Top-down strategy, which consisted of early induction with infliximab and maintenance with azathioprine, resulted in mucosal healing in 19/26 of the patients (73%) at week 104. In the other arm (step-up strategy), mucosal healing was significantly less frequent (7/23 patients, 30%). Additionally, when mucosal healing was achieved at 2 years (SES-CD score at 0), 70% of the patients (17/24) were in stable clinical remission during the following 2 years as compared to only 6 of the 22 (27%) who had mucosal lesions (SES-CD score above 0) [6]. Fifteen of the 17 patients with mucosal healing at year 2 maintained in remission without further infliximab infusions during the following 2 years. Furthermore, mucosal healing obtained with immunomodulators or anti-TNF agents was also associated with a decreased risk of surgery in the long term. Altogether these data would suggest checking endoscopic response in patients treated with immunosuppressants or anti-TNF. In a placebo-controlled study of the GETAID, presence of ulcerations at ileocolonoscopy before withdrawal of azathioprine was not predictive of the risk of relapse [55]. A recent study from the GETAID assessed the risk of relapse after infliximab discontinuation in patients in remission on combined maintenance therapy, who continued the immunosuppressant (azathioprine or methotrexate). Mucosal healing was among the factors strongly associated with a decreased risk of relapse [59].

Endoscopic Assessment Contributes in Predicting Relapses of CD After Surgery

It is generally accepted that CD patients who have ileal resection and ileocolonic anastomosis are exposed to a high risk of postoperative recurrence [60]. Rutgeerts et al. [61] demonstrated that ileocolonoscopy performed within 1 year of surgery may predict the risk of clinical recurrence. Eighty-nine patients treated by ileal resection for CD were included in this prospective cohort follow-up to study the natural course of early postoperative lesions. Within 1 year of surgery, ileocolonoscopy detected recurrent lesions in the neo-terminal ileum in 73% of the patients, although only 20% had a clinical relapse. The rate of clinical relapse was 34% at 3 years. A score was devised to assess the severity of recurrent endoscopic lesions. The course of the disease was best predicted by the severity of the early postoperative lesions, as observed at ileocolonoscopy, on the anastomosis and/or on the neo-terminal ileum. Indeed, patients with less severe endoscopic lesions according to Rutgeerts’ score (less than 5 aphthoid ulcers at anastomosis site), have a lower risk of clinical recurrence risk at 9% compared with 100% risk at 4 years for patients with more severe endoscopic recurrence (Rutgeerts’ score i2 or greater). This score is widely used in clinical practice, and ECCO guidelines state that ileocolonoscopy should be the gold standard in the diagnosis of postoperative recurrence by defining the presence and severity of morphologic recurrence and predicting the clinical course. Ileocolonoscopy is recommended within the first year after surgery where decisions of postoperative treatment may be affected [62]. Furthermore, recently Regueiro et al. [63] showed that administration of infliximab soon after intestinal resection was effective at preventing endoscopic recurrence of CD in randomly assigned 24 CD patients who had undergone ileocolonic resection to

receive intravenous infliximab, administered within 4 weeks of surgery and continued for 1 year, or placebo. The rate of endoscopic recurrence at 1 year was significantly lower in the infliximab group (1 of 11 patients; 9.1%) compared with the placebo group (11 of 13 patients; 84.6%) ($P=0.0006$).

Conclusion

In patients with active IBD, endoscopy may help to select patients who should receive early and active therapies. One reason is that severe endoscopic lesions may predict a poor outcome with increased risk of colectomy and complications. Next, patients with no lesions gain no benefit in receiving active treatments with potential risks. In treated IBD patients, mucosal healing is associated with a better outcome, with decreased risks of relapse and major surgery. Assessment of mucosal healing may help to characterize the response to treatments and in decisions of optimal strategies. Finally, endoscopy, which allows a direct assessment of severity and extent of mucosal lesions, may thus help in the management of IBD.

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Chapter 25

Evaluation of Quality of Life in Inflammatory Bowel Disease

Pia Munkholm and Natalia Pedersen

Keywords Inflammatory bowel disease • Quality of life assessment • Inflammatory bowel disease questionnaire • Short Health Scale • Social functioning • Health-related quality of life • Rating Form of IBD Patient Concern • Short form-36 • SF-12 • Psychological general well-being index

What Is Health-Related Quality of Life?

*We should not worry about how long we live but rather how satisfactorily;
For in order to live a long time, you only require fate,
But living satisfactorily requires understanding. Life is long if it is full.*

Lucius Annaeus Seneca (4 BC–65 AD)

History

While quality of life (QoL) has long been an explicit or implicit policy goal in the treatment of inflammatory bowel disease (IBD), adequate definition and measurement have been elusive. The term *quality of life* is used to evaluate the general well-being of individuals in the field of health care. QoL should not be confused with the concept of standard of living, which is based primarily on income. Instead, standard indicators of the QoL include not only wealth and employment, but also the built environment, physical and mental health, education, recreation and leisure time, and social belonging. The young age of onset and the morbidity associated with ulcerative colitis (UC) and Crohn's disease (CD) and their therapies affect patients, not only physically but also through limitations in social, educational, professional, and emotional activities. It is well documented that IBD patients have an impaired perception of their health-related quality of life (HRQoL) compared with an age- and sex-matched population [1–10].

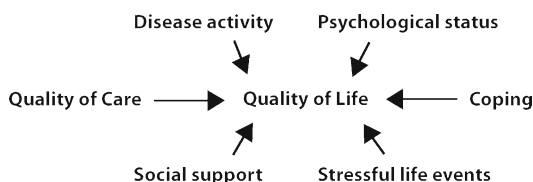
P. Munkholm, MD, DMSci. (✉)

Department of Gastroenterology, Herlev Hospital, University of Copenhagen,
Herlev Ringvej 75, Herlev, Copenhagen 2730, Denmark
e-mail: pia_munkholm@mail.dk

N. Pedersen

Herlev University Hospital, Copenhagen, Denmark

Fig. 25.1 Conceptual model of variables influencing QoL in IBD (reprinted from [12] with permission from John Wiley & Sons, Inc.)



The relationships between QoL and HRQoL have been investigated. It is well recognized that disease activity [11], psychological status, Quality of Care [12, 13], coping, social support, and stressful life events are factors influencing QoL in IBD (Fig. 25.1).

Clinicians and policymakers are recognizing the importance of measuring HRQoL to inform about patient management and policy decisions [14]. In the EMA and FDA guideline [15, 16] validated QoL measurement, for example, inflammatory bowel disease questionnaire (IBDQ) is being part of the secondary endpoint section among other claims such as steroid sparing, treatment of abscess, and endoscopic remission; treatment of obstruction however should not form a part of the indication, but may be included in other relevant section(s) of the prescribing information.

Quality of Life Index and Questionnaire

QoL questionnaires are being assessed as “disease specific” and “generic” entities (Table 25.1).

Disease Specific

In 1989, a group of doctors together with sociologist Guyatt at McMaster University in Hamilton in Canada developed and validated the disease-specific IBDQ [17]. To date, the questionnaire has been translated into 37 languages by forward and backward translation method. There is a fee per use of the IBDQ which can be purchased at Hamilton University <http://www.mcmaster.flintbox.com>.

The questionnaire instrument comprises 32 items, which measure the following broad domains: physical health, psychological health, social relationships, and environment. Responses are scored on a 7-point Likert scale, in which 7 corresponds to the highest level of functioning. Cumulative scoring above 170 points represents a good QoL with ranges from 32 to 224. The s-IBDQ is a shorter version of the original instrument that may be more convenient for use in the office, in large research studies, or clinical trials [18]. This has recently been applied to Web-based treatment validation of QoL assessment in self-initiated treatment with 5-ASA in UC [19]. This is a validated ten-question version of the McMaster IBD questionnaire covering all four dimensions and can be used to quickly assess a patient’s status. The IBDQ is not applicable to patients with stoma.

When compared with the general population, IBD patients have impaired QoL in all four categories. The most frequent concerns of UC patients are having an ostomy bag, developing cancer, side effects, the uncertain nature of disease, and the need for surgery. The most frequent concerns of CD patients are the uncertain nature of the disease, impaired energy level, side effects of medication, the need for surgery, and having an ostomy bag [2].

The s-IBDQ comprises ten items where good QoL is accepted with a scoring above 50 points (range 10–70).

Surgical quality of life with IBD has not been well examined. In 1992, a questionnaire to determine the subjective health status for patients with IBD has been developed and verified. To examine

Table 25.1 Overview of disease-specific quality of life and generic rating systems in IBD

Quality of life	Items	Domains	Points good QoL: level (range)
Disease-specific			
IBDQ [17]	32	4	Above 150 good (32–224)
	10	Bowel symptoms	
	5	Systemic symptoms	
	5	Social function	
	12	Emotional function	
Short IBDQ [18]	10	4	Above 50 good (10–70)
IBDQ outpatients [3]	36	5	(36–252)
		1. Systemic symptoms	
		2. Bowel symptoms	
		3. Functional impairment	
		4. Social impairment	
		5. Emotional function	
SHS [20, 21]	100-mm visual analog scale	4	Less than 25 mm (0–400 mm)
		1. Symptoms	
		2. Functional status	
		3. Worry	
		4. General well-being	
RFIPC [22]	100 mm VAS	25 items	0 mm represents “not at all”
Generic			
SF-36 [23]	36	8	(0, worst–100, best)
		1. Physical function (ten items)	Likert 1–3
		2. Social function (two items)	1–5
		3. Role limitations due to physical problems (four items)	1–2
		4. Role limitations due to emotional problems (three items)	1–2
		5. Energy/vitality (four items)	1–6
		6. Mental health (five items)	1–6
		7. Bodily pain (two items)	1–5/6
		8. General health perception (five items)	1–5
SF-12 [24]	12	Likert (0–3)	(0, worst–12, best)
PGWB [25]	22 Well-being	Likert scale (1–6)	Lower values – negative response (22–232)
	Anxiety (5)		
	Depressed mood (3)		
	Positive well- being (4)		
	Self-control (3)		
	General Health (3)		
	Vitality (4)		
	Distress items		

the QoL in a group of “well” outpatients with IBD, the group from Edmonton, Canada developed a self-administered form of this questionnaire containing 36 questions identifiable to five dimensions: systemic symptoms, bowel symptoms, functional impairment, social impairment, and emotional function. Scoring ranging between 36 and 252 points is obtainable but points above 180 are indicative of good QoL [3].

Table 25.2 Short Health Scale (SHS) with the four dimensions and four questions presented as 100 mm in VAS scale

1. How severe symptoms do you suffer from your bowel disease?	
No symptoms _____ Very severe symptoms	
2. Does your bowel disease interfere with your activities in daily life?	
Not at all _____ Interferes to a very high degree	
3. How much worry does your bowel disease cause?	
No worry _____ Constant worry	
4. What is your general sense of well-being?	
Very well _____ Dreadful	

Based on data from [20] and [21]

Short Health Scale

The Short Health Scale (SHS) is a 4-item self-administered questionnaire. Each item deals with one of the following subjective health dimensions: symptoms, functional status, worry, and general well-being (Table 25.1). Responses are graded on a 100-mm visual analog scale. The results, presented as an individual score for each of the four items, form a profile. The SHS has been validated in UC [20] and in CD [21] and correlates with the IBDQ (see Table 25.2).

Rating Form of IBD Patient Concern

The Rating Form of IBD Patient Concern (RFIPC), a 25-item disease-specific questionnaire, rates worries and concerns of patients with IBD on a 100-mm VAS scale (range 0–100 mm) [22]. 0 mm represents “not at all” and 100 mm “a great deal.” The sum score is the mean of the 25 items. The RFIPC is not applicable to patients with stoma.

Generic Questionnaires

Short Form-36

The Short Form-36 (SF-36) is a generic questionnaire containing 36 items [23]. Thirty-five of the items are grouped into eight multi-item scales. The eight domains of the SF-36 are as follows: physical function (ten items); social function (two items); role limitations due to physical problems (four items); role limitations due to emotional problems (three items); energy/vitality (four items); mental health (five items); bodily pain (two items); and general health perception (five items). In addition, it contains a one-item measure of self-evaluated change in health status (health transition) over the previous year. For each question, the raw score was coded and transformed into a percentage on a scale of 0–100, with 0 indicating the least favorable possible health status and 100 indicating the most favorable.

The 12-Item Short Form Health Survey (SF-12) was developed for the Medical Outcomes Study, a multiyear study of patients with chronic conditions [24]. The resulting short-form survey instrument provides a solution to the problem faced by many investigators who must restrict survey length. The instrument was designed to reduce respondent burden while achieving minimum standards of precision for purposes of group comparisons involving multiple health dimensions.

Psychological General Well-Being

The Psychological General Well-Being Index (PGWB) is a generic 22-item questionnaire measuring subjective feelings of well-being and distress [25]. Responses are graded on a 6-point Likert scale. Lower values correspond to more negative responses and higher values to more positive responses. The overall score is the summation of the item responses, ranging from 22 to 132.

In a more recent comprehensive analysis of 22 studies of QoL in CD [26], HRQoL was better in healthy controls and UC patients (except pre colectomy) than in CD patients. HRQoL was similar to or worse than that in many other chronic medical conditions. HRQoL was directly correlated with CD activity and was worse in active disease than in remission. HRQoL improved for a short time after surgical resection [27]. In addition, active IBD had a negative impact on labor force participation [28] and relationship and sexual health [29]. Thus, physicians should strive for the most important goal of medical therapy to make the patient well [30, 31] and not necessarily by avoiding surgery. Simple QoL scales can be used in the assessment of measuring the efficacy of medical therapy.

Evaluation of QoL in Cohorts

The role of extrinsic factors in HRQoL is well studied in IBD. Extrinsic factors are characteristics of the social surroundings (i.e., family, social network, quality of care, social security, infrastructure, occupation, cultural, and religious values). The significance of family and social support has been elucidated in some studies [32–34]. As referred to earlier in the European Community study of IBD (EC-IBD), it has been shown that QoL is being influenced by many factors, among others quality of care in the community setting [12]. A moderate correlation between patient-reported IBD-related concerns and adverse national parameters has been found [35]. Ethnic and religious differences have also been put forward as predictors of HRQoL outcome in IBD [36, 37].

Intrinsic factors are characteristics of the respondent in mental and physical aspects dependent on, respectively, (1) gender, age, personality, preference, life goals, coping strategy, experience, knowledge, education, psychiatric disorder and (2) comorbidity, disease severity and duration, complication, general physical health status. Female gender has in the majority of studies been associated with worse HRQoL in CD, in other diseases, and in the general population [38–40].

The elderly have reduced generic HRQoL in general population health surveys, particularly as regards physical domains [38]. In several studies on HRQoL in IBD, however, age had a minor or no effect [6, 41, 42]. Personality traits, coping strategies, and mental illness have been found influential on HRQoL in IBD [43–47]. The individual ability to cope with ill health is not necessarily unchanged over time. The impairment of health status may elicit a process of accommodation in which the internal standards, values, and conceptualization of subjective health evaluation are changed. This phenomenon – termed “response” – has been acknowledged as an important factor for HRQoL over time and an important factor for HRQoL outcome, possibly explaining the improvements of HRQoL over time and counterintuitive findings of enhanced HRQoL in patients with severe disease [48]. Despite this phenomenon and the generally described mitigating long-term disease course [49–51], HRQoL seems not to be affected by disease duration in CD [5, 42, 47, 52, 53]. A higher educational level has been associated with better IBD-related HRQoL [22, 42], but specific IBD education programs have not improved HRQoL [54, 55]. Low socioeconomic status and smoking have also been linked to a poorer HRQoL in CD [56, 57]. Disease activity and severity

in many studies have been the strongest predictors of HRQoL in IBD [45, 58, 59], even though the disease phenotype (as classified by age at diagnosis, location, and behavior) had no effect on HRQoL in a study by Casellas [8]. Finally, comorbidity has been detrimental on HRQoL in both physical and mental aspects, also when measuring with IBD-specific instruments [6, 52].

Conclusion

HRQoL is a useful instrument to characterize efficacy of medication in trials and as valid as disease activity scoring systems. Short forms of disease-specific and generic HRQoL are recommended in clinical everyday of which the recent published SHS is the absolutely shortest but correlates to the IBDQ. Short forms of generic HRQoL SF-12 correlates with the SF-36.

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Part IV

Therapy

Chapter 26

The Natural History of Inflammatory Bowel Disease

Charles N. Bernstein

Keywords Crohn's disease • Ulcerative colitis • Cohort studies • Surgery rates • Hospitalization rates • Phenotype • Biological therapy

Introduction

It is a difficult task to describe the natural history of IBD considering there is little information on the true “natural history” of these diseases. True natural history studies would necessitate having a cohort of patients that was followed without intervention once diagnosed to determine how untreated disease evolves. Some information in this regard can be gleaned from older IBD literature. Lennard-Jones reported in 1968 that “some patients with Crohn's disease seem to improve with rest” and that “some patients with gross X-ray changes of disease (but who are symptom free) and who are in excellent health require no treatment (sic)” [1]. These were among the earliest observations that one potential outcome of the natural history of Crohn's disease was untreated remission. Lennard-Jones also identified some diagnostic issues that could impact on comparing data from that era with current disease outcome studies. For instance, in the 1960s (and earlier) there was uncertainty in identifying isolated colonic Crohn's disease from ulcerative colitis (UC); that disease confined to the colon was mostly diagnosed in the early days of IBD as UC. Hence defining outcomes of Crohn's colitis would be flawed by underestimates in the prevalence of that condition and defining outcomes of UC would be biased by inclusion of Crohn's colitis cases. While Lennard-Jones was suggesting that persons with imaging evidence of disease but no symptoms did not require treatment, 40 years later the issue of treating mucosal disease in an asymptomatic patient has become hotly debated. In other words, intervening to impact on the natural history of the disease before it evolves, or advancing therapy to impact on already treated disease has become an important clinical consideration.

In UC there are also some older studies that could shed light on the “natural history” of that disease prior to modern therapy. Edwards and Truelove reported on 624 UC patients treated at

C.N. Bernstein, MD (✉)

Department of Internal Medicine, University of Manitoba IBD Clinical and Research Centre,
Bingham Chair in Gastroenterology, Health Sciences Centre, University of Manitoba,
804F-715 McDermot Avenue, Winnipeg, MB, Canada R3E3P4
e-mail: cbernst@cc.umanitoba.ca

Oxford 1938–1962 [2]. Amongst those presenting with first attacks of disease, the mortality rate was approximately 22% in 1938–1947, it fell to 12% in 1948–1952, and then to 2% in 1953–1957 and back up to 6% in 1958–1962. Amongst those presenting with relapse, the mortality rate from 1938 to 1957 was 6–12% with a rate of 4% in 1958–1962. While mortality rates dropped toward the 1960s mortality rates of 4–6% were still formidable. Mortality rates were as high as 38% in those presenting with a severe first attack and 26% presenting with a severe relapse (as opposed to those presenting with mild or moderate disease). Mortality rates were also higher in those older than 60 years of age at presentation. In 1938–1952, 25% of attacks were considered severe but in 1953–1962 only 12% were considered severe. Corticosteroids were introduced as therapy after 1952 and this helped to improve remission and perhaps reduce mortality rates. It is possible that other supportive therapy and possibly the willingness to pursue colectomies also may have improved outcomes. It was identified in 1960 and 1961 that elective colectomy had a mortality rate of 3–4% while emergency surgery had a mortality rate of 30% [3, 4]. In another study of 465 patients (1,324 patient-years) with UC evaluated in the UK 1952–1963, 55% of the time was spent in remission [5]. Of 658 patient-years accrued from those presenting with a first attack of UC, 84% achieved remission compared to 80% of 714 patient-years among patients presenting in relapse. The outcome of the disease was not impacted upon by extent of disease at diagnosis but was when presenting with a relapse. Notably, staging disease extent was limited to sigmoidoscopy plus barium enema as opposed to current approaches of biopsies obtained throughout the colon. The mortality rates were not as high in this study but were also impacted by age. For patients under age 60 the mortality rate was <1%/year but for those presenting over age 60 it was closer to 5%/year (even though the elderly have lesser disease extent). The elderly have other comorbidities and less reserve than younger patients when stricken with a disease like UC.

There is insufficient available information for this chapter to be a review of the true “natural history” beyond the early studies already described prior to 1970. Hence this chapter will review the history of Crohn’s disease and the history of UC after diagnosis and once treatment has been initiated. The pattern of disease activity, the incidence of hospitalization and surgery, and the evolution of disease by location and in Crohn’s disease by activity pattern will be discussed. The impact of some therapies on Crohn’s disease progression will also be discussed.

Crohn’s Disease

For Crohn’s disease relevant outcomes over time include disease activity (or remission), location of disease, and expression of disease behavior (inflammatory, stricturing or penetrating disease). It also includes important milestones of disease activity such as hospitalizations or surgeries.

Disease Activity

In a Danish inception cohort of 373 patients diagnosed between 1962 and 1987, in the first year after diagnosis, 80% had high disease activity, 15% had low activity, and 5% were in remission. After the first year annually, only 30% had high disease activity, and 55% were in remission [6]. The relapse rate within the year of diagnosis and over the following 2 years correlated positively with relapse rate over the ensuing 5 years ($p=0.00001$). Over years 3–7 after diagnosis, 25% had active disease every year, 22% were in remission, and 53% had fluctuating courses. This was independent of the nature of treatment (i.e., corticosteroids, 5-ASA or surgery – but it is unknown how these data might change in the era of immunosuppressives and biological therapy). The probability

of a relapse-free course decreased from 22% over 5 years to 12% over 10 years. Only 4% at 5 years and 1% at 10 years had continuously active disease and only 13% had a relapse free course. So having some fluctuation of disease activity occurred in the majority. Approximately half of patients with active disease could expect to be in full remission by 3 years. These are not very promising data but they are also from an era with limited use of effective maintenance therapies. Even during that era of limited therapy, only 20% of subjects in the first 7 years had active disease every year while 67% of subjects had a fluctuating disease course. However, having active disease in 1 year predicted that 70–80% would have active disease the next year, while being in remission in 1 year predicted that 80% would be in remission the next year. Whether or not a person was in remission or had active disease was independent of age, sex, and location of disease.

The Norwegian IBSEN cohort provides some insight into patients diagnosed between 1990 and 1994 and followed for a median of 10 years. The cumulative relapse rates were 53%, 85%, and 90% at 1, 5, and 10 years, respectively [7]. Sex, smoking status, disease location, and disease behavior at diagnosis did not predict relapse rates. In the second 5 years of follow up, 25% did not use any medications and this was not significantly impacted on by age, disease location, or disease behavior at diagnosis. As many as 44% were in clinical remission during the second 5-year follow-up period and the majority of them did not use any immunosuppressive therapy. Age at diagnosis over 40 years was associated with a greater likelihood of remission in years 6–10. Surgery for active Crohn's disease within the first 5 years from diagnosis did not influence the proportion in remission in the second 5 years.

In the European Inception Cohort (EC-IBD) of 358 subjects enrolled in 1991–1993, at a median of 10 years follow up, 27% had no recurrence [8]. The cumulative recurrence rates at 1, 5, and 10 years, respectively, were 34%, 69%, and 77%, respectively, all somewhat lower than in the Norwegian study of the same era. In the EC-IBD study ($n=358$), 27% experienced no recurrence after diagnosis and 18% had only 1 recurrence [8], although these may be underestimates since the study allowed for subjects to self-treat and not report if a mild flare of disease occurred. First recurrence rates occurred in 34% by 1 year and 78% by 10 years. For those who had surgery, recurrence rates were 6% at 1 year and 63% by 10 years. Within the first 5 years from diagnosis, 25% had undergone surgery. Of those who had surgery within 10 years from diagnosis, it occurred in the first year in 25%. Of those who had surgery, second surgeries occurred in 18%, 32%, and 54% by 1, 5, and 10 years, respectively. For each of first, second, and third recurrences, the time interval between recurrences progressively shortened. Hence, relapse rates can positively influence further relapse rates. People who quit smoking were protected from first recurrence rates ($p=0.01$). Patients with upper gastrointestinal disease had an excess risk of disease recurrence (hazard ratio, $HR=1.54$, 95% confidence interval, $CI\ 1.13-2.1$) and nearly all of those patients had nonpenetrating disease. Subjects with isolated colonic disease had a reduced risk for surgical resection ($HR=0.38$, 95% $CI\ 0.21-0.69$). Age greater than 40 years at diagnosis was protective against relapse of disease ($HR=0.82$, $0.7-0.97$).

A Markov model using population-based data from Olmsted County suggested that a representative patient spent 24% of the lifetime of disease in medical remission, 27% with mild disease, 1% with severe drug responsive disease, 4% with severe drug dependent disease, 2% with severe drug refractory disease, 1% in surgery, and 41% in postsurgical remission [9]. These data underscored the long-lasting benefit surgery can sometimes bring. A systematic review of population-based North American studies suggested that at any point in time after the first year of disease, approximately 10% of patients have high disease activity, 25% have low activity, and 65% are in remission. Approximately 13% have chronically active disease, 73% have fluctuating disease, and 10% are in longstanding remission [10].

It is difficult to summarize all of these population-based studies in a uniform statement. Follow up are available to about 2004. Through the 1970s to the 1990s, it could be estimated that 15% of patients remain in long-lasting remission, while 50% have mild disease, or rare or occasional

relapse. At least two thirds of patients have easily managed disease from an era of no biological drugs and little use of immunosuppressives. It was only 10% or so that had chronically active or difficult to manage disease. The remaining 25% had fluctuating disease with substantive relapses interspersed with periods of remission. It is this one third of patients that may be most impacted by more aggressive therapy.

Hospitalization

In the era of 1962–1987 in Denmark, 83% of patients were admitted at least once within the first year after diagnosis and after the first year the admission rate was 20% per year [6].

In a population-based study from Canada in the years 1994–2001, approximately 25% of subjects with Crohn's disease were admitted annually. Only 4% and 1% were readmitted two and three times, respectively, within 1 year. The hospitalization rate declined significantly from 29.2/100,000 to 26.9/100,000 over the 7 years of the study. Nearly half of hospitalizations for Crohn's disease involved major surgery [11]. In a population-based cohort of 201 newly diagnosed persons with Crohn's disease in 1987 followed for 15 years, 66% were admitted at least once to hospital for an IBD-specific diagnosis and of these 56% got readmitted at some time for a second time [12]. Fifty-two percent of patients had their admissions within the first 5 years.

In Olmsted County in the years 1970–1999, 69% of 211 subjects were hospitalized at least once and in 39% surgery was undertaken. The cumulative risk of hospitalization was 32% at 1 year, 52% at 5 years, and 62% at 10 years from diagnosis. There was a median of 2 hospitalizations per subject and re-hospitalization occurred in 60% within 5 years [13]. More recent US data from the Northern California Kaiser Permanente suggested that hospitalization rates between 1998 and 2005 decreased for Crohn's disease by 33% [14]. This marked a reduction in hospitalization would need to be corroborated in other cohorts to determine if this was a trend perhaps representative of an era of more aggressive medical therapy.

In general, at least from North America, there seems to be a recent trend toward reduced hospitalization rates. This needs to be further corroborated, however the past 15 years has seen an increase in use of more aggressive therapy to maintain disease remission hence it will be of great interest to determine if this translates into further reductions in hospitalizations (and surgeries).

Medical Therapy

It has been stated that when there is a full year of remission it will be followed by an 80% chance of remission in the following year, whereas a recent flare has only a 30% chance of remission in the following year [15]. Disease activity can be impacted upon greatly by both drugs and surgery. Despite the success of a number of drugs used in treatment, it has been suggested that little hard evidence exists that drugs will alter the ultimate outcomes (perhaps the need for surgery or development of complications such as fistulas) [16].

Corticosteroids

Corticosteroids have been a mainstay of therapy for acutely active disease. In a population-based cohort of 196 Danish patients diagnosed between 1979 and 1987, 56% had received at least one

course of corticosteroids by a median of 3.4 years, most of whom received their first course within the first year from diagnosis [17]. Among those who received corticosteroids, 48% achieved remission and 20% did not respond. At the end of follow up, 44% had a prolonged response, 36% were steroid dependent, and 20% had steroid resistance. A total of 21% required surgery including 59% of those with primary nonresponse to corticosteroids. So in this prebiologic era (and preaggressive immunosuppressive therapy era) at least half of patients who received corticosteroids required other therapy and for those who did not achieve remission with corticosteroids a high percentage came to surgery. These type of data have informed clinicians that if one does not initiate immunosuppressive therapy early when prescribing corticosteroids there is little rationale to wait if response to corticosteroids is not clearly evident. Similar data were reported from Olmsted County where among 171 patients diagnosed with Crohn's disease 43% received corticosteroids (of whom 58% achieved complete remission, 26% achieved partial remission, and 16% had no response) and 1 year after treatment, 32% were off corticosteroids with a prolonged response, 28% were steroid dependent, and 38% had undergone surgery [18]. Corticosteroids were first administered at a mean disease duration of 1 year.

In the IBSEN cohort, the need for corticosteroids for treating the first flare increased the risk of chronic continuous symptoms (odds ratio, OR=2.32, 95% CI 1.04–5.14) and was inversely associated with a decrease in severity of symptoms during follow up (OR=2.33, 95% CI 1.2–5.88) [7]. For those using corticosteroids, the cumulative rate of a second course of corticosteroids was 34%, 73%, and 77% at 1, 5, and 10 years, respectively, from diagnosis. In the second 5 years of disease, only 42% used corticosteroids. Also evident from their data was that by 5 and 10 years of disease, 25% of patients had never used corticosteroids. Furthermore, as disease duration extends, disease activity seems to lessen with less need for corticosteroids. In this study, corticosteroids did not impact on the need for surgery so their use only partly predicted the nature of disease course.

In a review of population-based studies from North America (mostly based on data from Olmsted County, Minnesota), it was suggested that less than half of subjects require corticosteroids at any one point and during any year, and approximately 10% are treated with corticosteroids [19]. These data from Scandinavia and North America are considerably different than data from pediatric studies, where the vast majority of children are treated with corticosteroids early in their disease course (see below).

There are also studies that report an increased risk of complications from corticosteroid use. In a study of 432 subjects, systemic corticosteroid therapy was associated with an increased risk for developing intra-abdominal or pelvic abscesses (OR 9.03, 2.4–33.98), with a greater risk evident at doses of prednisolone of ≥ 20 mg/day and also with continuous use for over 3 months as opposed to shorter duration use [20]. Retrospective studies have also identified systemic corticosteroids as a risk factor for developing postoperative sepsis complications [21, 22]. The interpretation of retrospective analyses of these sorts is complicated by the inability to dissect whether it is the corticosteroids per se or the fact that corticosteroids are used in subjects with greater disease burden and perhaps at greater risk for complications.

Thiopurines

Studies examining disease progression, disease activity, or the “natural history” prior to the 1990s included the evolution of disease prior to widespread use of immunosuppressives. Even in the 1990s uptake of thiopurine use was slow. In the IBSEN study (incident cases 1990–1994, [7]), azathioprine was used by 6% within the first year, 21% by 5 years, and 33% by 10 years. A French study is often cited as evidence that use of azathioprine over time has not reduced the need for surgery [23]. In this study while the probability of using azathioprine increased dramatically from 0 in

1978–1982 stepwise to 56% in a 1998–2002 cohort, the cumulative risk for surgery did not change. However of surgical patients in this study only 9% were using azathioprine prior to surgery so perhaps those who came to surgery were less aggressively treated or that the azathioprine was simply initiated too late in the course of disease to prevent progression to require surgery.

Anti-TNF Therapy

Antitumor necrosis factor (TNF) therapy has been the first medical therapy shown in a placebo controlled fashion to lead to fistula improvement and even closure [24]. Other clinical trial data reported that hospitalizations and surgeries do occur less commonly in infliximab- or adalimumab-treated patients than in placebo/episodic-treated patients [25–28]. Clinical trials are somewhat artificial situations where some patients are excluded for specific clinical characteristics; other interventions may be delayed in the hope that the enrollees may complete the full study and to date the available reports present data at a maximum of 1 year postinitiation of therapy. Two other observational studies have shown a reduction in hospitalization and surgery up to 1 year postinfliximab in fistulizing but not for luminal disease [29, 30]. Conversely, in a national US study using the Nationwide Inpatient Sample during 1998–2005 (after infliximab was introduced and increasingly gained in use) hospitalization for Crohn's disease increased and surgeries did not decrease [31]. Hence, there was no signal from this study that the advent of infliximab use was associated with reductions in these parameters; however, these data would need to be placed in context of trends of Crohn's disease prevalence rates during that period. In a population-based study from Manitoba, it was reported that the likelihood of surgery for up to 3 years postdosing was greater in persons prescribed infliximab compared to a cohort of subjects newly prescribed azathioprine or to a cohort of subjects using none of infliximab, immunosuppressives, or corticosteroids [32]. In this study it took 2 years for physician visits to reduce to those seen 2 years preinfliximab dosing and up to 2 years to reach hospitalization rates evident in the infliximab cohort 2 years prior to initiating the drug (or to that evident in the cohort of the newly prescribed azathioprine users). Hence, where population-based data are available it is not evident that infliximab will reduce health care utilization and including important milestones like surgery or hospitalization. Whether it will reduce adverse disease outcomes such as fistulas remains to be seen. For persons who respond it can improve quality of life.

It has been suggested that earlier initiation in disease course (before irreversible damage occurs) is more likely to promote better long-term outcomes [16]. This was suggested in a study using certolizumab where subjects dosed before 1 year of disease had better response rates than those dosed after 4 years of disease [33]. It was also suggested by the first “step up versus top down” study reported [34]. In this study of persons with early onset Crohn's disease, one group receiving an induction regimen of infliximab plus azathioprine and then episodic infliximab were compared to a group who received corticosteroids followed by repeat corticosteroids plus azathioprine if disease activity flared and then ultimately rescue infliximab. In a subset studied with endoscopy, complete mucosal healing was evident in 75% of the “top down” group versus 21% of the “step up” group at 2 years. It remains to be seen whether mucosal healing will in fact lead to less aggressive and less complicated disease over time. It is not known whether mucosal healing also is associated with transmural healing. In a nested sample of this study cohort who underwent endoscopy at the end of 2 years ($n=49$), the presence of mucosal healing at 2 years significantly predicted a greater likelihood of steroid-free remission in those followed out to 3–4 years ($OR=4.35$, 1.10 – 17.22) [35]. Of interest though when mucosal healing was diagnosed at 2 years only 52% of those in clinical remission ($n=31$) had mucosal healing (reconfirming older information of the potential to feel clinically well while having active mucosal disease). Perhaps more perplexing was that of 11 patients without a clinical remission 73% had mucosal healing. Hence do these patients have active mucosal disease out of the colonoscope's reach or do they have functional symptoms?

Surgery

Even though surgery is not curative it has an important place in the management of Crohn's disease. Firstly, we have gained etiologic insights from surgical procedures. Studies from the UK were the first to show that a diverting ileostomy as treatment for colonic Crohn's disease was effective [36, 37]. Further, when ileal contents were infused into the diverted colon the disease could be activated; the first suggestions that the gut microbiome may have a role in disease activation. Thirty years later a Belgian study showed that infusing intestinal contents into the diverted ileum causes recurrent disease in the ileum suggesting that the colon contents were potentially more harmful than the ileal contents [38]. Secondly, surgery can be associated with long-lasting remission [9]. However, surgery is associated with recurrence which has been reported at 30%, 50%, and 60% at 5, 10, and 15 years, respectively, from a Stockholm County cohort from 1955 to 1974 [39]. When updated to 1989 surgical rates to 5 and 10 years had not appreciably changed [40].

In an early Danish cohort, the cumulative probability of surgery was 55% at 10 years [41]. In an update to this it was reported that 35% had surgery during the year of diagnosis, with a rate of 5% per year after the second year from diagnosis. The cumulative probability of surgery after 10 years was 61% and that of more than one surgery was 39% [6]. In an assessment of incident cases diagnosed between 2003 and 2005 in Denmark, only 12% underwent surgery within the first year of diagnosis [42]. So this series of Danish studies may suggest that more recently surgery had been pursued less often within 1 year from diagnosis. Is this because of more aggressive medical therapy or a change in disease behavior?

In the IBSEN population-based cohort of patients enrolled 1990–1994 in Norway, the cumulative probability of surgery at 1, 5, and 10 years was 14%, 27%, and 38%, respectively. Terminal ileal location, stricturing disease, penetrating behavior, and age younger than 40 years at diagnosis were all significant independent risk factors for surgery [7]. Sex, the need for systemic steroids or smoking at diagnosis did not impact on the need for surgery. Of those subjects who had surgery by 10 years, 9% had at least 2 surgeries.

In the EC-IBD where subjects were enrolled in 1991–1993, 40% of patients underwent surgery within 10 years of diagnosis [43]. In a large cohort ($n=2,008$) from France followed from 1978 to 2002, 15% underwent surgery within the first 3 months, however, after the first 3 months the annual operative rate was 3.3–7.5% with little change over 26 years [23].

Among Olmsted County residents diagnosed with Crohn's disease in 1940–2001, 58% underwent surgery at some time to a median follow up of 13 years, and of these, 50% had at least 2 resections [44]. The cumulative risk for intestinal surgery was 24%, 49%, and 64% at 1, 10, and 30 years, respectively.

Other US data from 1998 to 2005 from the Northern California Kaiser Permanente (when anti-TNF therapy was emerging as a key therapeutic option) suggested that surgery rates did not change over that 7-year period [14]. In a population-based inception cohort study from Manitoba of 201 newly diagnosed patients with Crohn's disease, 45% had surgery by 15 years of follow up, of which two thirds or 29% had their surgery within 5 years.

Hence overall it seems that in the prebiologic era from population-based studies that approximately 40–50% of subjects will have undergone intestinal surgery within 10 years from diagnosis.

In a large case series of 507 subjects with isolated colorectal Crohn's disease diagnosed between 1955 and 1989, the surgery rate at first presentation was 18%. The probability of major surgery in this group was 33% by 5 years and 48% by 10 years and this did not change with time. The probability of surgical resection was increased with the presence of fistulas (relative risk, $RR=1.7$, 95% CI 1.3–2.2) and decreased with left-sided disease ($RR=0.6$, 95% CI 0.4–0.8) [45]. Those with proximal colonic disease had the highest surgery rates. The nature of the surgery

can impact on recurrence rates. For patients undergoing proctocolectomy the rate of ileal operations at 5 years was 19% whereas at 10 years it was 24% [46]. In patients who underwent subtotal colectomy with ileorectal anastomosis, an ultimate proctectomy with ileostomy creation was necessary in 24% by a median of 12.5 years [47]. In a different population it was reported that persons undergoing colonic or anorectal resection had a rate of reoperation at 15 years of 50% [48]. The implication is that reoperation is lower in persons who have their entire colons removed but of course this involves accepting a permanent stoma. However if followed out long enough, the rates of reoperation among proctocolectomy patients may approach that of those treated with segmental resection [49]. Hence, further long-term studies on recurrence rates postoperatively in large sample sizes are required to fully understand if the type of surgical resection can predict recurrence rates. Overall though, these data do not suggest lower surgical rates in those with isolated colorectal disease than that reported from all-comers with Crohn's disease including those with small bowel disease.

Disease Localization

In the Norwegian IBSEN cohort of 200 subjects, at diagnosis, the distribution of disease location was isolated ileitis (27%), isolated colitis (45%), ileocolitis (26%), and upper gastrointestinal disease (4%). The disease distribution reported from the EC-IBD was only minimally different in terms of less isolated ileitis and more ileocolitis. Of 358 patients the at-diagnosis location distribution was ileitis (15%), colitis (42%), ileocolitis (37%), and upper gastrointestinal disease (6%) [8]. The IBSEN study reported on changes in location over 5 years and found the distribution was isolated ileitis (23%), isolated colitis (37%), ileocolitis (38%), and upper gastrointestinal disease (6%). This amounted to a change in location in 14% [50]. This is not dissimilar from what Louis et al reported in a referral population where location changed in only 16% by 10 years and 20% changed overall [51]. At diagnosis 53% had ileal disease (the highest report from anywhere of isolated ileal disease), 27% had colonic disease, 19% had ileocolonic, and 4% had upper gastrointestinal disease [51]. At most recent assessment (median of 9 years of disease duration) 38% had ileal disease, 22% had colonic disease, 33% had ileocolonic disease, and 6% had upper gastrointestinal tract disease. Of all location changes 83% was to evolve to ileocolonic disease with a median duration to change of 5.5 years. From the Northern California Kaiser Permanente in the years 1998–2005 of the 2,892 prevalent Crohn's disease patients, 17% had isolated small bowel involvement, 41% had small and large bowel disease, and 32% had colonic involvement only, and 5% had upper gastrointestinal tract disease (10% were unknown) [14].

Ileal location was associated with an increased risk for surgery in a large French cohort (HR=2.78, 95% CI 2.19–3.15) [23]. Absence of rectal involvement was associated with a decreased risk for surgery in this cohort (HR=0.34, 95% CI 0.27–0.43).

In a Danish population-based cohort assessing incident cases diagnosed between 2003 and 2005, 32% had ileal disease, 39% colon only, 22% ileocolon, and 7% had upper gastrointestinal disease. Comparing adults to children younger than 18 years there was with more pure colonic disease and pure ileal disease in adults whereas children had more ileocolonic disease [42].

In an analysis of 507 subjects with isolated colonic disease over 35 years from Sweden, colonic distribution was segmental in 40%, left sided in 26%, and total in 31%. Small bowel disease developed in 24% over time [45]. Patients diagnosed between ages 0 and 29 years were more likely to have total colonic disease than older patients (37.4% vs. 23%, $P<0.05$) and an increased tendency to left-sided disease among those diagnosed over age 60 years. Of patients presenting with rectal sparing, 27% had subsequent rectal inflammation.

Perianal

Perianal fistulas initially had fallen within the domain of disease behavior and were lumped together with other fistula types as being penetrating disease [52]. The Montreal Classification revised that to give perianal fistulas special status as a specific disease location independent of the luminal location of disease and considered penetrating disease only that which occurred in the lumen [53]. In a Swedish cohort followed from 1990 to 2001, 14% had anorectal fistulas by the time of diagnosis but it was less clear how many developed perianal disease over time [54]. In an Olmsted County population-based cohort by 5 years of disease, only 15% had perianal fistulas but by 20 years of disease to 1993, 26% had perianal fistulas [55]. Half of those perianal fistulas were evident at diagnosis and the remaining presented at a median follow up of 5.5 years. In a population-based cohort from Manitoba, Canada, the prevalence of perianal disease was only 14% at most [56]. In the Northern California Kaiser Permanente from 1998 to 2005, among 2,892 Crohn's disease patients only 6% had perianal disease [14]. An updated cohort from Stockholm (1990–2001) reported an incidence of perianal disease of only 13.7% [54]. In a Danish population-based cohort assessing incident cases diagnosed between 2003 and 2005, 2.4% had internal fistulas and 6.3% had external fistulas at diagnosis (it was not specified how many of these were perianal but clearly <6% were) [42]. Overall it seems that recent population-based studies report rates of approximately 15%; however, long-term follow up may ultimately show these rates to increase toward 30%.

Disease Behavior

Overall duration of disease and location of disease are predictive of disease behavior pattern. In the Norwegian IBSEN cohort at diagnosis, 28% had stricturing disease and 12% had penetrating disease, leaving 61% with inflammatory disease [50]. At 5 year follow up, 33% had stricturing disease, 21% had penetrating disease, and hence 47% had inflammatory disease. So 14% had a change in disease behavior. At 10 years, of those being followed, 53% developed either stricturing or penetrating disease (31% had stricturing disease and 22% had penetrating disease) [7]. Complicated disease (both stricturing and penetrating disease) occurred in 86% of those with isolated ileal disease and only 30% of those with colonic disease and 60% of those with ileocolonic disease. Age at diagnosis less than 40 years was significantly associated with penetrating disease at 10 years but not stricturing disease. Of those who developed penetrating disease about half had luminal fistulas and half had perianal fistulas. In the EC-IBD at diagnosis, 16% had stricturing disease and 8% had penetrating disease and 2% had both. Hence 74% had inflammatory disease, a much higher proportion than the Norwegian cohort [8].

In a referral population, it was reported that 90% of patients present with inflammatory disease but the majority will evolve to either stricturing or penetrating disease by 10 years from diagnosis. Of inflammatory phenotypes, 30% became stricturing or penetrating by 5 years (equal percentage becoming stricturing as penetrating by 5 years) and overall 69% and 88% had stricturing or penetrating disease by 10 and 25 years, respectively. Of those with stricturing disease, the majority remained stricturing over time [51]. In this study, 46% changed behavior over 10 years. Of patients with inflammatory disease at diagnosis, a near equal number evolved to stricturing (27%) as to penetrating disease (29%). At most recent assessment (median of 9 years of disease duration), 28% had inflammatory disease, 28% had stricturing disease, and 43% had penetrating disease (although this study included perianal disease as penetrating). Penetrating disease was more common from a colonic only or ileocolonic location than from an ileal only location [51]. Age at diagnosis did not influence disease behavior or location [51]. In another French referral cohort of 1,199 Crohn's disease patients followed for a mean of 8.6 ± 7.9 years, 60% developed stricturing or penetrating

disease [57]. Of these, 13% had stricturing disease, 27% had perianal penetrating disease, and 20% had luminal penetrating disease. The 20-year actuarial rates of having inflammatory disease, stricturing disease, and penetrating disease were 12%, 18%, and 70%, respectively. Stricturing disease was associated with ileal (HR 2.5, 95% CI 1.9–3.3) or jejunal disease (HR 3.2, 95% CI 2.2–4.7) and penetrating disease with colonic disease on univariate but not multivariate analysis. At 20 years of disease, ileal location was associated with a complication in 94%. Age less than 40 at diagnosis predicted penetrating disease. Having perianal disease was a strong predictor of penetrating disease (OR 5.02, 95% CI 3.40–7.42) [56].

In the Olmsted County cohort diagnosed between 1970 and 1993, 35% developed at least 1 fistula which translated to a risk of any fistula of 33% after 10 years and 50% by 20 years [55]. In contrast to referral-based studies, only 34% developed recurrent fistulas. Fistulas were more likely to be seen in those with ileocolonic disease than in patients with disease at other sites.

So while there is an apparent discrepancy in comparing population-based cohort samples with referral center ones, there is a flaw in this type of analysis regardless of the population being studied. There is a lack of uniformity in assessing the behavior of disease at diagnosis in most centers. Some patients have early surgery where previously undiagnosed fistulas are identified and hence early on are labeled as having penetrating disease whereas their diagnostic imaging studies may not have identified this. The lack of uniformity in methods used to establish the extent and behavior of disease limits the reliability of phenotype data established at time of diagnosis. The variation of disease behavior from even one center is best reflected in a large French cohort of 2008 patients evaluated within 3 months of diagnosis between 1978 and 2002 [23]. In each 5-year period, the prevalence of stricturing disease ranged from 5 to 26% with a high of 26% in 1978–1982 and a low of 5% in 1998–2002, likely accounted for because some with stricturing either had surgery for stricture segment removal or evolved to penetrating disease. The prevalence of penetrating disease ranged from 29 to 54%. A recent review stated that at diagnosis up to one third had evidence of stricturing or penetrating disease and by 20 years half of all patients had these complications. This seems to be a reasonable summary [44].

Smoking

Smoking deserves its own discussion since it is the one factor that has been shown in several different studies to adversely impact on the outcome of persons with Crohn's disease. Smokers have more aggressive disease and an increased risk for surgery and postoperative recurrence [58, 59]. A large cohort of French patients was followed over 12–18 months. During this period, 46% of smokers flared compared to 30% of nonsmokers and 23% of former smokers [58]. This group reported on a study where smoking cessation counseling was undertaken in 447 smokers with Crohn's disease. While only 12% quit smoking for at least 1 year, of those who quit there was a 65% reduction in risk of relapse compared to those who did not quit [60].

Pediatrics

In a Danish population-based study of children diagnosed between 1962 and 1987, 22% had isolated small bowel disease, 30% isolated colon disease, and 39% had ileocolon disease; however, this was in an era prior to the use of colonoscopy in the majority of children at diagnosis. Of the 23 children with childhood onset Crohn's disease, the mean yearly operation rate was 13% with a cumulative probability of surgery at 20 years of disease of 47% [61].

In 116 children followed for a mean of 3.4 years from 6 tertiary referral centers in the UK, 12% had abdominal surgery after a mean of 1.8 years. Extraintestinal diseases were present in only 6% of which only 2% had arthritis (9% had arthralgias) [62].

In a French population-based cohort of 404 patients diagnosed between 1988 and 2002 and followed for a median of 84 months [63], at diagnosis 63% had disease in the ileum and colon and 36% had upper gastrointestinal disease. On follow up, disease extension to at least one other segment occurred in 31% (including an additional 12% who had upper gastrointestinal disease). This is such a high prevalence of upper gastrointestinal tract disease that it suggests that perhaps they were considering any histologic inflammation as being pathological. At diagnosis 9% had perianal disease and at follow up this increased to 27%. Complicated behavior occurred in 29% at diagnosis (including 4% with penetrating disease) and 59% at follow up (including 15% with penetrating disease). The cumulative incidence of surgery was 20% at 3 years and 34% at 5 years. Stricturing behavior at diagnosis was associated with an increased risk for surgery (HR=2.54, 95% CI 1.58–4.01). Treatment with corticosteroids occurred in 85% and proved to increase the risk for surgery (HR=2.98, 95% CI 1.64–5.41). However, the 15% who were not treated with corticosteroids likely had much milder disease and hence had possibly a lower likelihood to head to surgery anyway. Azathioprine decreased the risk for surgery (HR=0.51 (95% CI 0.33–0.78). In the nested cohort from 1988 to 1994, 32% received immunomodulators while this increased to 68% in the cohort from 1995 to 2002. Infliximab was used in 24% of cases and did not impact on whether surgery ultimately occurred or not. The 5 year rates of surgery were 26% in users and 35% in nonusers. The lack of significant difference may reflect a Type II error. Intestinal resection occurred in 44%. The cumulative probability of surgery was 34% by 5 years (95% CI 30–40%). Extraintestinal diseases occurred in 23% at diagnosis and in 48% at follow up. While it was not clear how many of these subjects had arthralgias as opposed to frank arthritis a remarkable 30% at follow up were said to have skin disease. These data form an instructive dataset on pediatric Crohn's disease. However, the rates of upper gastrointestinal tract disease are so high, of extension in location so common, and the use of infliximab so high that it is questionable if this simply reflects something very different about pediatric from adult Crohn's disease or whether there is something different about this specific French population.

In a single American institution study of children with Crohn's disease from 1968 to 1994, the probability of surgery was 28.8% within 3 years from diagnosis and 47.2% by 5 years [64]. Ileal and cecal disease were more likely to undergo surgery than disease localized in the colon or upper gastrointestinal tract. Symptomatic recurrence rate was 44% and occurred at a median of 1.8 years, postresection.

In a study of 989 Crohn's disease patients enrolled in 2000–2003 from 6 different American pediatric IBD centers, patients were followed for a mean of 3.6 ± 3.1 years. The cumulative annual risk for surgery was 5.7% at 1 year, 17% at 5 years, and 28.4% at 10 years [65]. Being female (HR=1.49, 95% CI 1.04–2.14), having an initial diagnosis of UC (HR 3.63, 95% CI 1.91–6.9), having poor growth at presentation (HR=2.16, 95% CI 1.24–3.77), having an abscess (HR=1.9, 95% CI 1.18–3.09), and having a stricture (HR=3.41, 95% CI 2.35–4.94) were all predictive of having surgery. Younger age at diagnosis (3–5 years, HR=0.26 95% CI 0.09–0.74, 6–12 years HR=0.62, 95% CI 0.42–0.89) was predictive of decreased risk for surgery. While this study suggested a protective effect of 5-ASA and infliximab on surgery, these data would have to be reconciled with the general consensus that 5-ASA has limited efficacy in Crohn's disease and that subjects requiring surgery may have been too ill to get infliximab.

In an incident cohort of pediatric patients from 2000 to 2001 from Wisconsin of 129 patients diagnosed with Crohn's disease, isolated ileal disease was present in 25%, isolated colonic disease in 32%, ileocolonic disease in 29%, and significant upper gastrointestinal disease in 14%. Perianal fistulas were present in 8% at diagnosis. Only 1 patient had primary sclerosing cholangitis while 2% had arthritis, 1.5% had inflammatory skin disease. Ninety percent had inflammatory disease, 8% had penetrating disease, and 2% had structuring disease [66].

Corticosteroids are used as the main agent of choice to treat moderately to severely ill pediatric patients [67]. Up to 90% of children with Crohn's disease have rapid improvement with corticosteroids [68]. However, corticosteroids should be limited in children with profound growth retardation or delayed puberty [67]. The rate of steroid dependence and resistance can be reduced from 50% to 20% with addition of thiopurines [67].

It has been suggested that earlier more aggressive therapy in children leads to better outcomes. A study comparing corticosteroids plus 6-mercaptopurine versus corticosteroids plus placebo led to higher rates of sustained remission and more steroid sparing in children with newly diagnosed Crohn's disease [69]. Subsequently, in an observational study it was shown that 80% of children diagnosed with Crohn's disease with moderate to severely active disease were started on immunosuppressive therapy within 1 year and these patients had more steroid sparing and possibly fewer hospitalizations than the group started on immunosuppressives beyond 1 year from diagnosis [70]. A better response to infliximab was seen in a small pediatric study where children were dosed within 1 year of diagnosis compared to beyond 1 year from diagnosis [71]. Similar findings were evident in an earlier pediatric study assessing infliximab use [72].

Putting It Together: Risks for Aggressive Disease

Most could agree that the development of luminal fistulas or abscesses (penetrating disease) or requiring repeated hospitalizations, requiring surgery or having debilitating extraintestinal diseases could be considered aggressive disease. A French group defined aggressive disease as requiring more than 2 courses of corticosteroids, hospitalization, disabling perianal disease or extraintestinal diseases, need for immunosuppressive therapy or surgery within 5 years from diagnosis as aggressive disease. The predictors at diagnosis included age younger than 40 years at diagnosis, need for corticosteroid treatment at diagnosis and perianal disease [73]. In the Norwegian IBSEN cohort being less than 40 years at diagnosis, having stricturing or penetrating disease and terminal ileum disease were associated with higher risk for surgery [7]. It was also shown that age less than 40 years at diagnosis and need for corticosteroids during the first presentation in IBSEN and age less than 40 years at diagnosis in the EC-IBD were associated with higher relapse rates [7, 8]. While it is useful to know that persons less than 40 years at diagnosis have more aggressive disease than older persons at presentation, since the peak age of incidence is the third decade and the majority of Crohn's patients have presented by age 40, more refined predictors than age less than 40 years would be required to help identify those requiring earlier, more aggressive therapy. No studies have reported that sex impacts on disease outcome except for a Swedish study that suggested that females have a higher likelihood of postoperative recurrence [40].

Perianal disease is often debilitating and for sure impacts on quality of life. In a cohort of Crohn's disease patients with disease localized to the colon diagnosed in Stockholm between 1955 and 1989, perianal fistulas were present in 37% at diagnosis, a much higher rate than reported for other cohorts that included subjects with diseases at all sites [45]. Patients with perianal fistulas in this study were at an increased risk for chronic continuous disease and also for surgery. In Manitoba subjects with isolated colitis were more than three times as likely to develop perianal fistulas as those with ileitis [56]. In general, it is considered that colonic disease, and particularly subjects with active rectal disease have the highest risk for developing perianal disease as opposed to isolated ileal or ileocolonic disease [74]. Having perianal fistulas increased the risk for postoperative recurrence in Sweden [40] as did penetrating disease (including both luminal and perianal fistulas) compared to stricturing disease in a US referral center population [75].

Having small bowel disease seems to pose other disease outcome risks compared to colonic disease. Patients with small bowel only and ileocolonic disease have an increased risk of

hospitalization compared to patients with isolated colonic disease [13, 44], an increased risk of evolution from inflammatory to stricturing or penetrating disease [44], an increased risk for surgery [8], and an increased risk of postoperative recurrence [40].

The new hot area for consideration of predicting outcomes is the development of mucosal healing. In the IBSEN cohort, 141 patients with Crohn's disease underwent endoscopic evaluation at 0.5 and 2 years postdiagnosis and 50 underwent endoscopy at 5 years [76]. Mucosal healing at 0.5 years did not predict relapses, complications, or surgery. Mucosal healing at 1 year, however, was associated with less endoscopic disease at 5 years and less need for subsequent corticosteroids. Corticosteroid use at diagnosis was associated with a significant reduction by 70% of mucosal healing at 1 year. However, conclusions from this retrospective study are confounded by the issue as to whether corticosteroids per se reduce mucosal healing or that corticosteroids are used for more severe disease. Further, only 20% of this cohort had isolated ileal involvement and assessment of predictability of mucosal healing for this subgroup was not undertaken [76].

Mucosal healing or by corollary mucosal recurrence is also a hot area in terms of postoperative recurrence. The clinical recurrence of disease after surgery is also a hallmark of aggressive disease since surgery impacts greatly on quality of life and costs in the short term, early recurrence can be frustrating. At 1 year postoperatively endoscopic recurrence rates have ranged as wide as 28–73% [77, 78]. Can drugs prevent postoperative recurrence? Mesalamine may prevent recurrence in approximately 10% at 1 year and thiopurines may be more effective than mesalamine by approximately 10%. In a meta-analysis of thiopurine trials for postoperative disease it was reported that there was a 15% benefit at reducing mild endoscopic disease but no benefit at reducing severe endoscopic disease [79]. One year of ornidazole has been shown to reduce endoscopic disease at 1 year from 79% (placebo group) to 54% (ornidazole group) (OR 0.31; 95% CI 0.10–0.94) and clinical recurrence at 1 year from 38% (placebo) to 8% (ornidazole group) (OR 0.14 95% CI, 0.04–0.55) [80]. This seems to provide the most robust benefit but long-term tolerance of this drug is an important drawback of this approach. A summary of the key messages for long-term outcomes for Crohn's disease can be found in Table 26.1.

Table 26.1 Summary statements for disease course in Crohn's disease

1. Follow-up studies are available to about 2004 from population-based studies. Through the 1970s to the 1990s it could be estimated that 15% of patients remained in long-lasting remission, while 50% had mild disease, or rare or occasional relapse. At least two thirds of patients had easily managed disease from an era of no biological drugs and little use of immunosuppressives. It was only 10% or so that had chronically active or difficult to manage disease. The remaining 25% had fluctuating disease with substantive relapses interspersed with periods of remission. It is this one third of patients that may be most impacted by more aggressive therapy
2. In general, at least from North America, there seems to be a recent trend toward reduced hospitalization rates. In the prebiologic era, from population-based studies, approximately 40–50% of subjects will have undergone intestinal surgery within 10 years from diagnosis and the risk of postoperative recurrence may be about 50% by 10 years
3. Up to half of adults with Crohn's disease use corticosteroids early on but as many as 25% never use them. A number of studies have reported increased complications among corticosteroid users but these reports are confounded by bias as to whether it is truly the corticosteroids causing the complications or the fact that the corticosteroids were prescribed in persons with the most aggressive disease and hence at greatest risk for complications. As thiopurines and anti-TNF therapies are increasingly used, trends in hospitalizations and surgeries will need to be revisited to determine what impact these agents have on these negative markers of disease evolution
4. In Crohn's disease, the location of disease is mostly stable over time with 15–20% having a change in disease location when followed over time. While ileitis has been a hallmark of Crohn's disease, in population-based studies the colon is involved more often at a rate of up to 75%. Perianal disease is present in about 15% of subjects and when followed over time up to 30% may develop perianal penetrating disease. Ileal disease location is more likely to be associated with complicated disease such as development of strictures or fistulas. At diagnosis up to one third have evidence of stricturing or penetrating disease, but by 20 years half of all patients had these complications. Since there is a lack of uniformity in examining for disease behavior at all sites at time of diagnosis, the true incidence of evolution over time to complicated behavior is unknown

(continued)

Table 26.1 (continued)

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5. The main differences in pediatric onset disease versus adult onset disease are a higher prevalence of upper gastrointestinal disease in children and a greater use of corticosteroids to induce remission. While it has been shown that disease onset prior to age 40 is more aggressive than after age 40 years it is not clear that pediatric onset disease is necessarily more aggressive or has a different phenotype than adults presenting prior to age 40 years
 6. Unequivocally, smoking seems to worsen the outcome of Crohn's disease
 7. Mucosal healing seems to be a desired effect of therapy and its presence may reduce some negative outcomes. However, to what extent it occurs in small bowel disease is unknown and whether it can ultimately be associated with long-term reduction in complications (strictures or fistulas), surgery, and other comorbidities remains to be proven
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UC

One aspect of considering disease evolution in UC is to assess the extent of colonic involvement including the progression to a greater extent over time and even regression and how disease extent may impact on disease outcomes. In an era when colonoscopy was not the diagnostic modality, 1,116 patients with UC were followed from 1960 to 1983 at the Cleveland Clinic with a mean follow up of 12.7 ± 5.3 years [81]. Forty-six percent had proctosigmoiditis, 17% left-sided colitis and 37% pancolitis. In 54% the disease progressed including 34% of those diagnosed initially with proctitis. Disease extension was increased in persons who presented with fulminant colitis (OR=14.8, 95% CI 3.5–63.1), with left-sided disease at diagnosis versus proctitis (OR=2.5, 95% CI 1.6–3.9), and those with arthralgias or arthritis (OR=3.7, 95% CI 1.6–8.6). In this study, 38% underwent colectomy although 58% of those whose disease progressed underwent surgery [81]. The rates of colectomy at 5, 10, and 20 years, respectively, were 24%, 34%, and 44%. The 10- and 20-year colectomy rates for those with pancolitis were 55% and 68%, respectively. At final follow up two thirds of all patients were asymptomatic. Only 37% remain on any medication, mostly sulfasalazine. This study may give more of a natural history glimpse of UC with less aggressive therapy available, but some of these outcomes may be worse than that what may occur in a more recent era with more aggressive therapy available.

In a population-based Danish study, 1,161 patients with UC were followed from 1962 to 1987 [82]. Proctosigmoiditis was evident in 44% at diagnosis, substantial colitis (disease beyond the splenic flexure) was evident in 36%, and total colitis in 18%. Disease progression in those originally diagnosed with proctosigmoiditis was 27% after 5 years and 53% after 25 years (disease extent was defined by sigmoidoscopy plus barium enema and not biopsies). Disease progression among those with substantial colitis to total colitis was only 9%. Disease regression was also evident in approximately 75% of those who presented with either substantial or total colitis. The authors concluded that disease extent was not static and changed over time in at least half of patients. This study is flawed by relying on barium enema for disease extent. In a Danish population-based cohort assessing incident cases diagnosed between 2003 and 2005, 31% had proctitis, 42% left-sided disease, and 27% had extensive disease [42]. These data show that with more modern era diagnostics disease extent will be shown to be more extensive.

In a study from 13 Italian centers of 273 patients diagnosed with ulcerative proctitis endoscopically, proximal extension occurred in 27% at a mean of 68 months. Only 4% had evolution to total colitis [83]. The cumulative rates of proximal extension to the sigmoid colon and beyond the splenic flexure by 10 years were 30% and 10%, respectively, and by 20 years were 56% and 40%, respectively. Again in the absence of histological analysis it is unclear what the true rate of proximal extension is but 27% would be a minimum rate. Of those patients who initially had endoscopic ulcerative proctitis, 14% had what was thought to be refractory disease and refractory disease was the only factor on multivariate analysis that predicted disease proximal extension or proximal extension beyond the splenic flexure.

In the IBSEN study of 423 UC patients enrolled 1990–1994 in Southeastern Norway who completed 10-year follow up, the initial extent of disease included proctitis (32%), left-sided colitis (35%), and extensive colitis (33%) [84, 85]. However, this study relied on endoscopy but not biopsy to determine disease extent which at least was some advance over older studies that just relied on sigmoidoscopy plus barium enema. Nonetheless, our current approach to staging disease extent with histology would likely reduce the percentage of those presenting with proctitis. Rates of disease progression are also likely underestimated as only one fifth of patients with proctitis or left-sided colitis at diagnosis progressed to extensive colitis by 10 years. In this study, the relapse rate was 83% (and nearly half were relapse free for the second 5 years). One half reported achieving remission or having only mild disease after the first presentation while 37% reported fluctuating disease and 6% reported chronic continuous symptoms. After the first disease presentation, 22% did not have another relapse. The majority of patients (57%) had no intestinal symptoms at 5 years and only 7% had symptoms that interfered with activities of daily living. Of those who reached 5 years of disease without having surgery, 41% were not using any kind of medical therapy at 5 years and only 50% were using a 5-ASA. Of interest 19% had never used sulfasalazine or 5-ASA at any time during their follow up. While it is not stated in the series of reports from this group as to how many subjects were treated at or near diagnosis with corticosteroids, the cumulative rate of subjects getting a second course of steroids was 13% at 1 year and 48% by 10 years.

The cumulative colectomy rate by 1 year was 3.5% and by 10 years was 10%. Extensive colitis at diagnosis was associated with colectomy (HR=3.57, 95% CI 1.6–7.96) (the rate in this subgroup was 19%) and age at diagnosis of ≥ 50 years was associated with decreased risk of surgery (HR=0.28, 95% CI 0.12–0.65). The latter may suggest that the disease is milder as one presents at an older age. This study also reported on the disconnect between endoscopic evidence of disease and symptoms. Symptoms at 5 years were more frequent in those with macroscopic disease (60%), but the presence of symptoms was not predicted by extent of disease. Furthermore, 40% of subjects with endoscopic evidence of disease at 5 years had no intestinal symptoms. While mucosal healing has become a study endpoint for clinical trials in UC it is unclear if treating an asymptomatic patient until mucosal healing is achieved will change prognosis and it would require treating a large number of persons who otherwise had no symptoms.

The 10-year rate of surgery of 10% was much lower than that seen in older studies from Sweden (28%) [86] or Denmark (24%) [87]. Age could have influenced treatment and approach to earlier surgery in those jurisdictions. In the Swedish study of 1,586 patients followed from 1955 to 1984, the 5-year colectomy rate was 20% and the 25-year rate was 45% [86]. Patients with total colitis had colectomy rates of 32%, 42%, and 65%, respectively, at 5, 10, and 25 years of disease while those with proctitis had colectomy rates of 14%, 21%, and 34% at 5, 10, and 25 years, respectively. In almost all patients initially diagnosed with proctitis who came to colectomy, the histology of the resected specimen revealed pancolitis. Higher rates of colectomy amongst those with pancolitis were also evident in older literature from the UK and Denmark [88–90]. Colectomy rates also lessened after the first 2 years [90]. Colectomies occurred in 10% within the first year and 4% within the second year from diagnosis with 1% per year thereafter. Hence 66% of colectomies were undertaken within 2 years from diagnosis and it remains unclear if this would still be the case in a modern therapy era. The duration of disease before colectomy decreased during the study period and the number of acute colectomies increased during the study period. This may have been a reflection of an era with little option for medical therapy (particularly for steroid-resistant disease) and improved surgical approaches and perioperative supportive therapy making it a more attractive option.

In the EC-IBD, the 10-year cumulative clinical relapse rate after diagnosis was 67% [91]. Age at diagnosis less than 30 years was associated with higher relapse rates but disease extent at diagnosis was not. This cohort study also reported a decline in surgery rates [91, 92]. In the EC-IBD, the 10-year cumulative colectomy rate was only 8.7% with an increased rate in extensive colitis versus proctitis at diagnosis (HR=4.1, 95% CI 2.0–8.4).

In a Manitoba population-based cohort of 187 newly diagnosed persons with UC in 1987 followed for 15 years, 31% were admitted at least once to hospital for an IBD-specific diagnosis and of those admitted 51% got readmitted at some time for a second time [12]. Twenty-three percent of patients had their admissions within the first 5 years. Only 15% experienced a UC-related surgery within 15 years corroborating the lower rates reported from Norway and two thirds or 10% occurred within the first 5 years from diagnosis.

Surgery rates declined in Northern California Kaiser Permanente 1998–2005 by 50%. In this cohort, there were 656 patients hospitalized with severe UC in 1996–2004 [93]. Of these 20% underwent colectomy during their initial hospitalization. By 1 year after initial hospitalization, 29% of those who had not undergone colectomy at first hospitalization were re-hospitalized for UC (most of which occurred within the first 3 months postdischarge) and an additional 10% required colectomy. By 5 years after initial hospitalization, 39% of those who had not undergone colectomy after initial presentation were re-hospitalized for UC and an additional 15% required colectomy. Almost uniformly this hospitalized cohort of severe UC patients were treated with steroids and at 1 year and 5 years postinitial discharge, 34% and 26% received a further course of steroids. It could not be discerned from this report what disease duration was so it is unclear how that might impact on ultimate course (i.e., do those with severe colitis at initial presentation have different outcomes versus those who present this way at some other time in their disease course). A recently published review of short-term colectomy rates in severe hospitalized UC suggested that the short-term colectomy has remained stable at 27% [94].

Data were reported from Olmsted County where among 185 patients diagnosed with UC 34% received corticosteroids (of whom 54% achieved complete remission, 30% achieved partial remission, and 16% had no response) and 1 year after treatment, 49% were off corticosteroids with a prolonged response, 22% were corticosteroid dependent, and 29% had undergone surgery [18]. Note that this rate of surgery of 29% is only among the one third of patients who received corticosteroids.

In summary, surgery rates at 10 years from diagnosis are approximately 10% which is much lower than reported colectomy rates in studies completed prior to 1990. Short-term colectomy rates in severe hospitalized UC have remained stable at 27% for several years. The rates of long-term remission require further modern day analyses.

Pediatrics

In a Danish population-based study of children diagnosed between 1962 and 1987, 29% had total colitis, 25% had proctitis which was a much higher rate of total colitis than an adult onset cohort from this region during that era (total colitis=16%). Of 20 patients with proctitis at diagnosis in 65% the proctitis spread proximally. Overall, the cumulative probability of progression of disease was 18% by 4 years, whereas the probability of regression of disease was 61%. This is similar to data from an adult cohort from this region and era. However, regression was determined in many cases radiologically and not histologically. Of the 80 children with childhood onset UC, the cumulative colectomy rate was 6.4% at 1 year, 26% at 10 years, and 29% at 20 years. The cumulative probability of colectomy for proctitis cases at 5 years was 15% whereas it was 38% for those with pancolitis [61].

In a population-based incident cohort from France of children diagnosed between 1988 and 2002, 113 patients with UC were followed for at least 2 years (median=77 months) [95]. At diagnosis 28% had proctitis, 35% left-sided colitis, and 37% colitis beyond the splenic flexure. After a median of 6.5 years, 60% had extensive colitis. Disease extension occurred in 54% of those with initial diagnosis of proctitis and in 49% of those with initial diagnosis of left-sided colitis. The main predictor of disease extension was a family history of IBD ($R=11.8$, 95% CI 1.3–111.3). During the

Table 26.2 Summary statements for disease course in UC

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1. In the Norwegian IBSEN study of those who reached 5 years of disease without having surgery, 41% were not using any kind of medical therapy at 5 years and only 50% were using a 5-ASA. Of interest, 19% had never used sulfasalazine or 5-ASA at any time during their follow up
 2. Surgery rates at 10 years from diagnosis are approximately 10% from recent studies which is much lower than reported colectomy rates in studies completed prior to 1990
 3. Short-term colectomy rates in severe hospitalized UC have remained stable at 27% for several years
 4. Pancolitis is a risk factor over lesser disease extent for requiring surgery
 5. Children seem to have higher rates of extensive colitis at diagnosis than adults. There also seems to be higher rates of colectomy in children than for adults (i.e., at least 20% at 10 years) and perhaps this reflects a higher rate of extensive disease. However, both disease extent documentation with colonoscopy and biopsy and colectomy rates in an era of more aggressive medical therapy requires verification with more recent studies
 6. While mucosal healing has become a study endpoint for clinical trials in UC it is unclear if treating an asymptomatic patient until mucosal healing is achieved will change prognosis and it would require treating a large number of persons who otherwise had no symptoms
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follow-up period, 35% were hospitalized and 24% had colectomy. The cumulative rate of colectomy was 8% at 1 year, 15% at 3 years, and 20% at 5 years. The highest colectomy rate was in persons with extensive colitis. The presence of extraintestinal diseases (7% at diagnosis and 22% cumulatively) increased the risk for colectomy (HR=3.5, 95% 1.2–10.5). For those who experienced disease extension, the risk of colectomy was also increased (HR=13.3, 95% CI 1.7–101.7). Sixty-eight percent received steroid therapy and at 1 year 26% were steroid dependent and 10% were steroid resistant. The 5-year cumulative probability of prescription for immunosuppressives increased from 0.11 (1988–1994 cohort) to 0.34 (1995–2002 cohort). In an incident cohort of pediatric patients from 2000 to 2001 from Wisconsin of 60 patients diagnosed with UC, left-sided colitis was present in 10% with disease beyond the splenic flexure in 90%. Only one child had an extraintestinal disease (arthritis) [66].

In summary, children seem to have higher rates of extensive colitis at diagnosis than adults. There also seems to be higher rates of colectomy in children than for adults (i.e., at least 20% at 10 years) and perhaps this reflects a higher rate of extensive disease. However, both disease extent documentation with colonoscopy and biopsy, and colectomy rates in an era of more aggressive medical therapy require verification with more recent studies.

Mucosal Healing in UC

Mucosal healing has become an important target of clinical trials although it is unknown as yet if it definitively predicts an improved prognosis. In the IBSEN cohort of 354 UC patients, 50% had mucosal healing confirmed at a mean of 14.7 months (SD 3.5) [84]. Having a high school education (OR=2.08, 95% CI, 1.27–3.43) and extensive disease (2.56, 95% CI, 1.38–4.75) at diagnosis were predictive of mucosal healing at 1 year. It is unclear how to reconcile either of those factors with enhancing mucosal healing unless higher education in some way contributed to enhanced treatment adherence and more extensive disease at diagnosis contributed to more aggressive treatment. Mucosal healing at 1 year was a significant predictor of reduced colectomy rate (OR=0.22, 95% CI 0.06–0.79). This is a very positive outcome; however, mucosal healing did not significantly predict any reduction in relapses, inflammatory activity at the 5-year visit, and use of corticosteroids at any subsequent time. It should also be noted that in this study treatment at diagnosis with steroids or 5-ASA did not predict mucosal healing. A summary of the key messages for long-term outcomes for UC can be found in Table 26.2.

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Chapter 27

Conventional Medical Management of Crohn's Disease: Sulfasalazine

Miquel A. Gassull and Eduard Cabré

Keywords Sulfasalazine • Crohn's disease • Mesalazine • Therapy • Postoperative recurrence

Introduction

Sulfasalazine (salazosulfapyridine, SASP) is a prodrug composed by a molecule of 5-aminosalicylic acid (5-ASA) and sulfapyridine, linked by an azo bond. It was aimed towards the treatment of rheumatic diseases such as rheumatoid arthritis and ankylosing spondylitis. When taken orally, anaerobic intestinal bacteria split the azo bond releasing the two components 5-ASA and sulfapyridine, the former being the active anti-inflammatory component [1]. Because the lower G-I tract – terminal ileum and colon – is the area of the gut with the highest commensal bacteria concentration (10^{10} – 10^{14} cfu/mm³), it was assumed that the 5-ASA moiety would exert its anti-inflammatory activity in the inflamed and ulcerated areas of the intestine where the active compound would be released [2, 3].

Mechanisms of Therapeutic Action

The mechanism of action of SASP and its metabolites is not well understood, especially their systemic effect on rheumatic diseases, since 5-ASA is poorly absorbed into the bloodstream [3]. The fact that the active molecule is particularly effective when administered topically into the rectum, as enema or suppository, supports this concept. It has been reported that SASP and its metabolites decrease eicosanoid synthesis [4–6], cytokine expression [7–9], and NF-κB activation [10, 11].

M.A. Gassull, MD, PhD (✉)

Health Science Research Institute, Germans Trias i Pujol Foundation,
Carretera de Can Ruti, camí de les escoles s/n, Badalona, Catalonia 08916, Spain
e-mail: magassull@igtp.cat

E. Cabré, MD, PhD

IBD/G-I Unit, Department of Gastroenterology, Hospital Universitari Germans Trias i Pujol,
Badalona, Catalonia, Spain

The antioxidant effects of SASP are well established which is probably due to its scavenging effects against reactive oxygen and nitrogen species (ROS and RNS), as well as metal chelating properties, and its inhibitory effects over neutrophil oxidative burst [12, 13]. A recent article compared the potential scavenging activity mediated by SASP and its metabolites 5-ASA and sulfapyridine on ROS and RNS, using validated *in vitro* screening systems [14]. SASP and its metabolite 5-ASA, but not sulfapyridine, showed ROS- and RNS-scavenging effects which may be a contributing mechanism of its anti-inflammatory effects through the prevention of the oxidative/nitrative/nitrosative damages caused by these species [14].

Clinical Evidence

As mentioned, the fact that the effect of anaerobic bacteria is necessary to “activate” SASP explains why it has been used in ulcerative colitis where it has been shown to be effective in both inducing the remission of active disease and preventing relapse in inactive patients. However, the role of SASP in the treatment of mild or moderate Crohn’s disease is controversial till now [15].

SASP was used in the treatment of active Crohn’s disease on the belief that it would benefit in a treatment that has been effective in active ulcerative colitis, given the similarity of mucosal lesions between both diseases. On the other hand, in the early sixties there were no other drugs available for such conditions, except for corticosteroids. Since Crohn’s disease may affect independently the colon, the terminal ileum, or both together, and both areas contain a high anaerobic bacteria concentration, it is logical to assume that the prodrug could be split by them in both locations and locally release 5-ASA. Some observational studies were published in the sixties and early seventies [16–18] to assess the effectiveness of SASP for both inducing and maintaining remission in Crohn’s disease. Also, in that decade, controlled trials were also performed with different designs to ascertain its effect as adjunctive therapy in active disease or as maintenance treatment, most of them with a small and heterogeneous group of patients [19, 20].

The first solid evaluation of the effect of SASP on Crohn’s disease comes from two large studies: the American National Cooperative Crohn’s Disease Study (NCCDS) [21] and the European Cooperative Crohn’s Disease Study (ECCDS) [22].

The NCCDS compared the efficacy of SASP, prednisone, and azathioprine with placebo, and showed that prednisone (0.25–0.75 mg/kg adjusted to disease activity) was superior to SASP (1 g/15 kg), and that SASP, but not azathioprine (2.5 mg/kg), was superior to placebo, in inducing 16-week clinical remission of Crohn’s disease [21]. Subgroup analyses suggested that patients who had been treated with steroids previously failed to respond to SASP, while those who had not taken steroids at entry responded to SASP significantly better than placebo [21]. Patients with involvement limited to the colon responded to SASP better than to placebo, whereas those who had disease located only in small bowel were less likely to benefit from this therapy [21]. None of the treatments – including SASP – proved to be better than placebo for preventing clinical relapse in inactive disease [21].

The ECCDS randomized patients to treatment with 6-methylprednisolone (48 mg/day weekly, tapering to 8 mg/day) alone, 6-methylprednisolone in combination with SASP (3 g), SASP alone, or placebo. Effects on active disease were assessed at 6 weeks, whereas inactive patients were on therapy for 2 years or until relapse. 6-Methylprednisolone proved to be the most effective therapy for inducing remission [22]; in subgroup analyses it was significantly more effective than SASP for patients who had been previously treated with steroids, for those who had disease only in the small bowel, and for those with disease in the small bowel and colon [22]. The combination of steroids plus SASP was most effective in previously untreated patients and when the disease was located in the colon [22]. SASP alone was the least effective active treatment regimen. No therapeutic regimen was better than placebo for inactive disease [22].

Other controlled trials in patients with active Crohn's disease have confirmed some of the conclusions of these subgroup analyses by demonstrating that SASP is not an effective adjunctive therapy to prednisone [20], whereas prednisolone is an effective adjunctive therapy to SASP [23], probably because corticosteroids are more effective therapies than SASP. In another small, comparative study of SASP (4–6 g/day) with placebo, van Hees et al. showed that 62% of patients treated with SASP had a favorable response after 26 weeks compared with 8% of placebo-treated patients [24].

A recent meta-analysis has pooled the available data on the effect of different salicylates in mild-to-moderate active Crohn's disease [25]. SASP was more likely to induce remission (RR: 1.38; 95% CI: 1.02–1.87; $n=263$) compared to placebo, with benefit confined mainly to patients with colitis, whereas it was less effective than corticosteroids (RR: 0.66; 95% CI: 0.53–0.81; $n=260$) [25]. Interestingly, however, the performance of SASP was better than that of mesalazine, either at low or high dose. Low-dose mesalazine (1–2 g/day) was not superior to placebo (RR: 1.46, 95% CI: 0.89–2.40; $n=302$) and was less effective than corticosteroids. High-dose mesalazine (3–4.5 g/day) was not superior to placebo for induction of remission (RR: 2.02; 95% CI: 0.75–5.45) or response (weighted mean difference: –19.8 points; 95% CI: –46.2 to 6.7; $n=615$). The authors conclude that SASP shows modest efficacy for the treatment of active Crohn's disease [25]. However, a direct comparison between SASP and mesalazine in mild-to-moderate active Crohn's disease has never been performed.

Another setting where aminosaliclates have been assayed is the prevention of postoperative recurrence of Crohn's disease after resection and ileo-colonic anastomosis. A very recent meta-analysis has pooled the data of eleven trials, five of them using SASP [19, 26–29], with a total of 1,282 patients [30]. The RR of relapse of Crohn's disease in remission after surgery with aminosaliclates vs. placebo or no therapy was 0.86 (95% CI: 0.74–0.99) (NNT=13). As opposite to the treatment of active disease, SASP was of no benefit in preventing relapse (RR: 0.97; 95% CI: 0.72–1.31; $n=448$), whereas mesalazine was more effective than placebo or no therapy (RR: 0.80; 95% CI: 0.70–0.92; $n=834$), with a NNT=10 [30]. The authors conclude that mesalazine has only a modest effect in preventing postoperative recurrence in Crohn's disease and should be used in patients in whom immunosuppressive therapy is either not warranted or contraindicated [30].

Recommendations for Clinical Practice

On the light of the available evidence, both experts [31] and consensus-based guidelines [15] recommend the use of high-dose (3–6 g/day) SASP in Crohn's disease only for patients with mild disease confined to the colon. Other authors, however, do not give any chance to SASP and its metabolites in the management of active Crohn's disease [32]. Anyway, SASP should be used in the short term, and active disease beyond 16 weeks of therapy should be considered a therapeutic failure. In addition, SASP is ineffective as maintenance therapy after both medically and surgically induced remission (in this later setting, mesalazine may have a minor role).

In addition, SASP may have a role in the management of Crohn's disease patients with associated arthropathy. However, recent review of the available evidence indicates that its usefulness is confined to some patients with peripheral arthropathy, or in those with early ankylosing spondylitis (i.e., those with higher levels of ESR Erythrocyte Sedimentation Rate or active disease) [33]. Advanced disease does not benefit from this drug since no effect on physical function, pain, spinal mobility, or enthesitis has been observed [33].

Side effects of SASP include headache, epigastric pain, nausea, vomiting, skin rash, fever, hepatitis, autoimmune hemolysis, aplastic anemia, leucopenia, agranulocytosis, pancreatitis, pharmacological systemic lupus erythematosus, sulfonamide-induced toxic epidermal necrolysis,

Stevens–Johnson syndrome, pulmonary dysfunction, and male infertility [34, 35]. Most of the side effects of SASP can be attributed to the systemic absorption of sulfapyridine and the adverse effects occur more frequently in patients who are genetically predisposed to “slow” acetylation of sulfapyridine to *N*-acetylsulfapyridine in the liver [34]. Some of the side effects (headache, nausea, vomiting, and epigastric pain) are dose related and can be minimized by gradual dose escalation [35]. It is well known that SASP reduces folate absorption [36, 37]. Thus, folate supplements should be administered in patients on SASP therapy. Nephrotoxicity, mainly as interstitial nephritis, has been reported in patients on aminosalicylate therapy. However, a systematic review of studies with regular monitoring of serum creatinine and creatinine clearance showed that its frequency is low, with a mean annual rate of only 0.26% (95% CI: 0.13–0.5%) per patient-year [38]. The lack of relationship of this complication with dose suggests that it depends on idiosyncratic mechanisms [38]. Anyway, in spite of its low frequency, periodic monitoring of serum creatinine is advised [39].

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Chapter 28

Conventional Medical Management of Crohn's Disease: Corticosteroids

A. Hillary Steinhart

Keywords Crohn's disease • Steroids • Prednisone • Prednisolone • 6-Methylprednisolone • Budesonide • Side effects

Introduction

Steroids are a class of hormones that are normally produced in a variety of organs in the human body. They have a number of different physiologic properties that are determined by the nature of the molecular substitutions at several points around the backbone ring structure that is common to all steroids. The steroid hormone cortisone is produced by the adrenal cortex and, in addition to having catabolic activity, it also has anti-inflammatory activity. It is this property that provides the potential for therapeutic use of steroids in Crohn's disease and a variety of other inflammatory and autoimmune disorders. Cortisone is rarely used clinically to treat Crohn's disease, but a number of synthetically altered forms of steroids are frequently utilized therapeutic agents. These pharmacologic agents typically have modifications that enhance their potency by increasing their affinity for binding of the steroid receptor, that alter their excretion or elimination, or that change their relative glucocorticoid and mineralocorticoid effect. The most commonly used systemically administered and active steroids are prednisone, prednisolone, methylprednisolone, and hydrocortisone. Budesonide, a highly potent and topically active steroid, is also used in the form of controlled release preparations for treating Crohn's disease confined to the terminal ileum and the ascending colon.

Induction Therapy

Conventional Steroids

Steroids are very effective at reducing inflammation and alleviating symptoms in patients with active intestinal Crohn's disease. They have been in use for the treatment of Crohn's disease for well over half a century and generally have the advantage of being effective in treating active inflammation in

A.H. Steinhart, MD, MSc, FRCP(C) (✉)
Department of Medicine, University of Toronto, IBD Centre, Mount Sinai Hospital,
Room 445, 600 University Avenue, Toronto, ON, Canada M5G 1X5
e-mail: hsteinhart@mtsinai.on.ca

a number of different locations throughout the gastrointestinal tract as well as treating some of the extraintestinal manifestations of Crohn's disease. Despite the widespread use of steroids, there have been relatively few randomized controlled trials that have both demonstrated their effectiveness and provided clear guidance with respect to optimal dosing, duration of therapy, and tapering regimens. The effectiveness of steroids in treating active Crohn's disease was confirmed by the first part of the National Cooperative Crohn's Disease Study (NCCDS) in the USA [1] and by the European Cooperative Crohn's Disease Study (ECCDS) [2].

In the NCCDS, Part 1, Phase 1 the effectiveness of prednisone, sulfasalazine, and azathioprine were compared to placebo in treating patients with symptoms of active Crohn's disease over a 17-week study period [1]. In the steroid-treated arm, the dose of prednisone was based upon the baseline disease activity with 0.5 mg/kg used for those patients with mild to moderate disease activity (Crohn's disease activity index score between 150 and 300) and 0.75 mg/kg for those with more severe disease activity (Crohn's disease activity index score greater than 300). The maximum daily dose of prednisone was 60 mg. A complex analysis of outcome rankings demonstrated superiority to placebo for both prednisone and sulfasalazine. Using that analytical method, it appeared that prednisone was particularly effective in patients with small intestinal involvement but not terribly effective in patients with only colonic involvement. When the more conventional assessment of proportion of patients in remission by the end of the 17-week study period was used, only prednisone was found to be superior to placebo with approximately 62% of patients in remission (Crohn's disease activity index below 150) compared with approximately 30% in the placebo arm using life table analysis. Using simple proportional analysis, 47% of prednisone-treated patients and 26% of placebo-treated patients were in remission at week 17.

In the induction phase of the ECCDS, patients with active Crohn's disease were randomized to receive 6-methylprednisolone, sulfasalazine, combination of 6-methylprednisolone and sulfasalazine or placebo for 6 weeks [2]. The 6-methylprednisolone was initiated at 48 mg per day and tapered to a dose of 12 mg per day over the 6-week study period. Patients initially randomized to the steroid arm, who were in remission at the end of the acute phase (CDAI < 150), were then continued on maintenance dose of 6-methylprednisolone 8 mg per day. Two increases in steroid dose back to 48 mg per day followed by repeated attempts at tapering were permitted in the 6-methylprednisolone-treated patients who were not in remission by the end of 6 weeks. Treatment with 6-methylprednisolone was found to be effective in inducing remission and was superior to sulfasalazine when Crohn's disease was located in the small intestine or both small and large intestine. In patients with only colonic disease location, the combination of 6-methylprednisolone and sulfasalazine was the most effective treatment. Overall, 83% of patients in the steroid-treated arm were in remission at the end of 18 weeks when compared with 37.9% of patients in the placebo arm.

Pooled analysis of the steroid induction studies demonstrated a twofold increased likelihood of remission induction with steroids when compared with placebo and a number needed to treat of 3.33 [3].

Many potential side effects of prednisone were recognized and described within the context of Part 1, Phase I of the NCCDS with moon face reported in 47% of patients, striae in 6%, ecchymoses in 17%, acne in 30%, infection in 27%, muscle weakness in 9%, hirsutism in 7%, and polyuria in 15% [1]. However, the use of prednisone in that trial was not consistent with what would currently be considered to be standard practice in that no tapering of the dose was permitted over the 17-week period. This may have contributed to the very high rate of steroid-associated side effects given the high cumulative dose of prednisone. Combining the adverse event results of the NCCDS and ECCDS demonstrated an almost fivefold increased risk of adverse events in steroid-treated patients when compared with placebo [3]. Although most of these did not result in patient withdrawal from the studies, the use of steroids was associated with a trend toward greater withdrawal rates in steroid-treated patients.

The optimal form of systemically acting steroid therapy has not been determined and the choice of specific steroid preparation typically depends upon local experience. Steroids can also be administered

intravenously (e.g., hydrocortisone and 6-methylprednisolone) to hospitalized patients with more severe disease or more acute presentations but the superiority of intravenous administration has not been proven. Its main advantage is that it can be given to patients who are not able to tolerate oral intake.

The optimal dose of steroids for the treatment of active Crohn's disease has not been established. In the NCCDS, where up to 0.75 mg/kg per day, up to a maximum of 60 mg of prednisone, was used, remission was achieved in 62% of patients over 17 weeks of treatment [1]. However, in an unblinded single arm study from France, prednisolone was used at a dose of 1 mg/kg for between 3 and 7 weeks with the induction of remission observed in 92% [4]. This raises the possibility that the steroid dose-response relationship has not been completely elucidated and that doses greater than the usual 40 to 60 mg per day of prednisone, or its equivalent, may provide additional benefit.

In the acutely ill patient, it is important to sufficiently exclude the presence of an abscess or systemic sepsis before initiating any form of systemic steroid. In the ECCDS, of the five deaths that occurred during or shortly after the study period, three occurred in patients treated with steroids who had a palpable abdominal mass prior to the initiation of therapy [2]. No routine imaging was performed prior to study entry and, as a result, it is quite likely that these masses could have represented Crohn's-related abscess that were not properly managed with drainage and antibiotics prior to the initiation of prednisolone.

Topical rectally administered steroids, in the form of liquid or foam enemas, can also be used as adjuvant therapy in controlling local symptoms in patients with distal colonic or rectal Crohn's disease. However, there is no evidence that this is an effective strategy and their use is based upon an extension of the data from ulcerative colitis.

Budesonide

For patients with ileal or ileocolic Crohn's disease, the use of the controlled ileal release preparation of the topically active steroid budesonide is an effective treatment for controlling symptoms of active disease. When administered at a dose of 9 mg per day budesonide was shown to be more effective than placebo [5] and mesalamine [6]. Another placebo-controlled trial demonstrated a trend towards higher remission rates on budesonide 9 mg once daily (46.2%) and 4.5 mg twice daily (51.9%) when compared with placebo (31.7%) but this was not statistically significant [7]. Doses greater than 9 mg per day do not appear to have any further therapeutic gain and may be associated with a greater incidence of steroid-associated side effects and adrenal suppression [5].

Another trial compared 16 weeks of budesonide 9 mg daily to mesalamine 2 g twice daily for control of active ileal or ileocolic Crohn's disease and demonstrated clear superiority for budesonide with 62% of budesonide-treated patients and 36% of mesalamine-treated patients in remission at the end of the study period [6]. Severe and serious adverse events were observed less often in budesonide-treated patients. Ten percent of budesonide-treated patients had abnormal ACTH-stimulated cortisol testing at the end of 16 weeks of treatment.

Budesonide that is absorbed from the intestinal lumen into the portal circulation undergoes high first pass metabolism through the liver leading to the production of inactive metabolites. It is this high first pass metabolism that likely results in the favorable short-term safety profile with steroid-associated side effects seen no more frequently on a 9 mg daily dose when compared with patients receiving placebo. However, the 9 mg daily dose of budesonide resulted in a lowering of basal plasma cortisol levels in two trials [5, 6] and in another trial a smaller proportion of patients receiving budesonide had normal adrenal function after 8 weeks (53% vs. 83%) [7]. Both placebo-controlled trials demonstrated a reduction in ACTH-stimulated plasma cortisol levels in patients treated with

budesonide [5, 7]. Given the demonstrated subclinical degree of adrenal suppression the use of adrenal replacement therapy for patients undergoing surgery or other severe physiologic stresses should be considered in patients currently or recently on budesonide at a dose of 9 mg per day.

When compared directly with the use of conventional systemic steroids in patients with ileal and ileocolic Crohn's disease, it appears that budesonide-controlled ileal release formulation may produce somewhat less reduction in disease activity [8] although another study showed roughly equivalent efficacy [9]. In both studies, the induction of remission was slightly less frequent with budesonide than with prednisolone, although the differences were not statistically significant [8, 9]. In the study of Rutgeerts and colleagues, fewer steroid-associated side effects and less suppression of morning cortisol levels were observed on budesonide when compared with conventional steroids [8]. In the study of Campieri, steroid-associated side effects were observed at similar frequencies in patients treated with budesonide and those treated with conventional steroids, although moon face was observed much more frequently on prednisolone [9]. Adrenal suppression, as measured by short ACTH stimulation test, was more frequent in patients treated with conventional systemic steroids [9].

A third small study of the controlled ileal release preparation of budesonide was carried out in pediatric patients with active Crohn's disease [10]. In that study, the starting dose of prednisolone, the comparator conventional steroid, was adjusted between 20 and 40 mg per day according to the patient's body weight. All patients in the budesonide arm received 9 mg per day for 8 weeks. The proportion of patients who achieved clinical remission by the end of 8 weeks was higher in the prednisolone-treated arm (71% vs. 55%) but this was not statistically significant [10]. Possible steroid-associated side effects were noted in 77% of the prednisolone-treated patients compared with 50% of the budesonide-treated patients. In particular, moon face and acne were much less frequent in the budesonide-treated patients. Similar to the adult studies, mean morning cortisol levels were higher in the budesonide-treated patients. Sixty-two percent of patients receiving budesonide had abnormal ACTH-stimulated cortisol test results at the end of 8 weeks, indicating some degree of adrenal suppression, but this prevalence was less than the 89% seen in prednisolone-treated patients.

Budesonide has also been formulated using a pH-dependent release mechanism and has been compared directly with mesalamine [11] and with conventional systemic steroids in patients with active Crohn's disease [12, 13]. The pH-dependent release form of budesonide was found to be non-inferior to mesalamine 4.5 g per day in 309 patients with mild to moderately active Crohn's disease with 69.5% of patients treated with budesonide 9 mg once daily or 3 mg tid achieving remission after 8 weeks of therapy compared with 62.1% of patients treated with mesalamine 4.5 g per day [11]. However, in this study, patients with only colonic involvement, including the left side of colon and rectum, were included. In another study, the pH-dependent release form of budesonide, given as 3 mg three times daily, resulted in remission almost as frequently as prednisone 40 mg per day followed by a tapering schedule after 2 weeks (51% vs. 52.5%) [13]. In this study, patients with only colonic involvement, including distal disease, were included. In patients with only colonic involvement, prednisone was much more effective than budesonide. Another smaller trial of pH-dependent release form of budesonide 9 mg once daily found a non-significant trend toward higher response rates on the conventional steroid, 6-methylprednisolone given as 48 mg daily for 1 week followed by a tapering schedule over 8 weeks (72.7% vs. 55.9%) [12].

One trial also examined the proportion of patients who achieved remission while not experiencing any side effects and found that 30% of budesonide-treated patients met this goal, whereas only 14% of prednisone-treated patients did so [13].

Pooled analysis of the studies that have compared budesonide to conventional steroids for the induction of remission demonstrates a likelihood of remission on budesonide that is approximately 85% of that observed on conventional steroids [14].

Maintenance Therapy

Conventional Steroids

It is generally accepted that steroids are neither safe nor effective for maintenance of remission in Crohn's disease. Several controlled trials have shown no improvement in remission rates in patients treated with maintenance low-dose steroids when compared with placebo [1, 2, 15]. Although these trials may have not demonstrated a statistically significant reduction in relapse rates on steroids, it is possible that this could have been due to inadequate sample size within the individual studies. The largest study to examine this question was the NCCDS Part II in which patients with preexisting quiescent disease were randomly assigned to receive placebo, prednisone 0.25 mg/kg per day, sulfasalazine or azathioprine for up to 2 years [1]. However, this study included patients with both remission following medical therapy ($n=226$) and remission following surgical resection within the previous year ($n=48$). Of the total 274 patients participating in the trial, 101 were randomized to receive placebo, 61 to prednisone, 58 to sulfasalazine, and 54 to azathioprine. The heterogeneous patient population, with inclusion of patients with both medical and surgical-induced remission may have diminished the trial's ability to find an overall treatment effect. However, combining the results of all three conventional steroid maintenance trials in a meta-analysis did not demonstrate any significant reduction in relapse rates in patients with quiescent Crohn's disease treated with steroids [16]. In addition to the fact that they do not appear to reduce relapse rates, chronic use of steroid therapy is associated with significant toxicity and potential adverse events. In the NCCDS Part II in which patients were treated for up to 2 years, 26% of patients experienced side effects that were rated as being moderate or severe. Moon face was observed in 25%, hirsutism in 8%, acne in 19%, polyuria in 15%, and muscle weakness in 3% [1]. Although not measured in that trial, the deleterious effects of steroids on bone density are well known [17].

Although the major trials clearly showed no overall benefit of maintenance conventional steroids, there did appear to be a subset of patients within the ECCDS whose active disease was brought under control with a 6-week course of steroids who seemed to do better when they remained on low dose of 6-methylprednisolone [2]. This seems to be consistent with the clinical experience of many physicians who find that there is a small proportion of patients who seem to be dependent on steroid therapy and, as such, are not able to reduce the dose below a certain threshold without causing recurrence of symptoms. In some cases, these symptoms are due to the adrenal suppression produced by chronic steroid use, but in some cases it is clearly a recurrence of the Crohn's disease-related symptoms. Nevertheless, the effect of low-dose steroids on the course of Crohn's disease has not been shown to be beneficial and, given the potential long-term side effects of steroids, even at low doses, other steroid sparing strategies and maintenance therapies are currently favored. These include the use of immunomodulators such as azathioprine, 6-mercaptopurine, or methotrexate and the use of anti-TNF α agents such as infliximab, adalimumab, or certolizumab.

Budesonide

The side-effect profile of the topically active steroid, budesonide, is more favorable than that of conventional systemically administered and available steroids, but budesonide, at a maintenance dose of 6 mg per day, has been shown only to delay the recurrence of Crohn's disease symptoms in patients who achieved remission on an 8-week acute course of budesonide [18]. Despite the fact that the occurrence of steroid-associated side effects necessitating discontinuation of therapy was uncommon over 1 year of maintenance therapy, patients were no more likely to be in remission on

budesonide by the end of 1 year of therapy than if they had received no active therapy. Other studies have confirmed the lack of efficacy of budesonide at doses of 3 or 6 mg per day in maintaining remission that has been achieved by an induction course of budesonide or conventional steroids [19–22]. Meta-analysis of the budesonide maintenance trials did not provide any evidence for the effectiveness of budesonide in maintaining remission [23]. Furthermore, even though the side-effect profile of budesonide is better than that of conventional steroids with possibly less loss of bone mineral density [24], patients on budesonide were approximately 50% more likely than patients on placebo to experience an adverse event deemed to be related to treatment [23]. In addition, an abnormal ACTH stimulation test was almost three times more likely in patients on budesonide than those on placebo [23].

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Chapter 29

Conventional Medical Management of Crohn's Disease: Azathioprine and 6-Mercaptopurine

Gil Kaplan and Lloyd R. Sutherland

Keywords Crohn's disease • Azathioprine pharmacology • Azathioprine or 6-mercaptopurine • Mechanism of action • Induction of remission • Maintenance of remission • Prevention of recurrence (medical) • Prevention of post-surgical recurrence • Recommended dose • Safety monitoring

Introduction

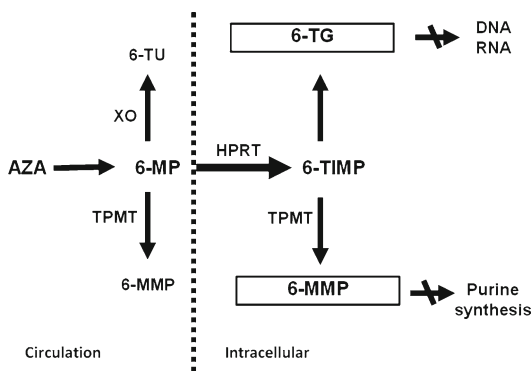
For years, the only treatment for Crohn's disease was 5-aminosalicylates and corticosteroids followed by surgery if these medications failed. In the last two to three decades, the introduction of the immunosuppressive agents, Azathioprine (AZA) and 6-mercaptopurine (6-MP) became one of the mainstay therapeutic options in the treatment of Crohn's disease. In this chapter, we review the pharmacology, evidence for indication of use, adverse events, monitoring, and role in pregnancy of AZA and 6-MP in the treatment of Crohn's disease. The use of these medications in pregnancy is reviewed in Chap. 52.

Pharmacology and Metabolism

AZA and 6-MP are purine antimetabolites that suppress the immune system by impairing nucleic acid synthesis. AZA is taken once daily with a biologic half-life of 24 h. Figure 29.1 summarizes the metabolism of AZA and 6-MP [1]. In the serum AZA is metabolized to 6-MP. Hypoxanthine phosphoribosyl transferase catalyzes 6-MP to 6-methyl-thioinosine 5'-monophosphate, which is converted to 6-thioguanine (6-TG) that interferes with nucleic acid synthesis leading to immunosuppression. In the presence of the enzyme thiopurine methyltransferase (TPMT), 6-methyl-thioinosine 5'-monophosphate is transformed to 6-methylmercaptopurine (6-MMP).

G. Kaplan, MD, MPH • L.R. Sutherland, MDCM, FRCPC, FACP (✉)
Division of Gastroenterology, Department of Medicine, University of Calgary,
Room 3D08, TRW Building, 3280 Hospital Dr NW, Calgary, AB, Canada T2N 4Z6
e-mail: lsutherland@ucalgary.ca

Fig. 29.1 Metabolism of azathioprine and 6-mercaptopurine. (Based on data from [1])



Indication of Use

Since the first randomized controlled trial of AZA for Crohn's disease was conducted in 1970s [2], several studies have demonstrated a therapeutic role for AZA and 6-MP in the treatment of Crohn's disease. Currently, the literature supports the role of AZA or 6-MP in Crohn's disease for the indications described in Table 29.1.

Induction and Maintenance of Remission in Crohn's Disease

Crohn's disease patients with moderate–severe active Crohn's disease are often treated with prednisone to induce remission of disease activity. Natural history studies have demonstrated that approximately one-fifth of Crohn's patients prescribed corticosteroids were steroid refractory (i.e., no response within 2 weeks of initiating high-dose corticosteroids) and one-third were steroid dependent (i.e., unable to discontinue prednisone or early recurrence following discontinuation) [3, 4]. Thus, several therapeutic agents have undergone clinical trials to determine efficacy for the treatment of steroid refractory or dependent Crohn's disease.

A meta-analysis supports the therapeutic role of AZA and 6-MP in induction of remission of steroid-dependent and refractory Crohn's disease. The overall odds ratio of response for AZA and 6-MP was 3.09 (95% CI: 2.45, 3.91) in active Crohn's disease. AZA and 6-MP demonstrated a steroid sparing effect in Crohn's patients who were corticosteroid dependent or refractory ($OR=3.69$; 95% CI: 2.12, 6.42) [5]. The summary of randomized, placebo-controlled trials also highlighted the importance of appropriate dose and duration of use in maximizing efficacy of AZA and 6-MP. The optimal dose of AZA was 2.0 to 2.5 mg/kg, whereas 1.0 to 1.5 mg/kg in 6-MP in both active and inactive Crohn's disease. Efficacy was greatest among active Crohn's disease patients who used AZA or 6-MP for at least 17 weeks [5]. A recent *Cochrane Database Systematic Review* also confirmed the role of AZA and 6-MP in the treatment of Crohn's disease. The number needed to treat (NNT) of Crohn's disease patients with AZA or 6-MP to observe efficacy in active Crohn's disease was five and the NNT to achieve a steroid sparing effect was three [6].

AZA and 6-MP have also been shown to be effective in maintaining remission of Crohn's disease. A meta-analysis demonstrated that studies using AZA or 6-MP in Crohn's disease patients with quiescent disease were over twofold likely to maintain remission as compared to placebo ($OR=2.27$; 95% CI: 1.76, 2.93) [5]. Furthermore, withdrawal studies have also supported the role of AZA and 6-MP in sustaining remission. Among Crohn's patients who respond and tolerate AZA or 6-MP, discontinuation of the drug leads to higher rate of disease recurrence. In one study the rate of recurrence

Table 29.1 Indications for azathioprine and 6-mercaptopurine in Crohn's disease

-
- Induction of remission in steroid refractory or steroid-dependent disease
 - Maintenance of remission in moderate–severe Crohn's disease
 - Fistulizing Crohn's disease
 - Prevention of post-operative recurrence of Crohn's disease
 - Concomitant therapy with infliximab
-

of Crohn's disease following discontinuation of AZA or 6-MP in patients in long-term remission were 14 and 53% at 1 and 3 years, respectively [7]. Several studies have shown that withdrawal of AZA or 6-MP after 2 years of remission was associated with greater relapse rates [8, 9]. As well, data support the continued use of AZA after 3 years of sustained remission [10]. Crohn's disease patients in remission on AZA or 6-MP for at least 3.5 years were randomized to continue AZA versus discontinue (i.e., placebo). The 18-month cumulative relapse rate among patients who continued AZA (8%) was lower than those who discontinued the drug (21%) [10].

Abundance of evidence supports the role of AZA and 6-MP in treating active Crohn's disease patients, particularly those who are corticosteroid refractory or dependent. Crohn's disease patients who respond and tolerate AZA or 6-MP should continue the medication for at least 3 years of sustained remission.

Fistulizing Disease

A perianal fistula is a complex complication of Crohn's disease that often requires a combination of medical and surgical approaches to manage. Randomized controlled studies of the efficacy of AZA or 6-MP in treating fistulizing Crohn's disease are limited; most studies are small in sample size and fistula healing was not the primary indication of the study. Consequently, the role of AZA or 6-MP in treating perianal fistulae was established following pooling of randomized controlled studies in a meta-analysis [5]. Fistula response was evaluated in five studies and was defined as complete healing or decreased discharge. Compared to placebo, Crohn's disease patients treated with AZA or 6-MP were over fourfold ($OR=4.44$; 95% CI: 1.50–13.20) more likely to experience a fistula response [5].

Prevention of Post-operative Recurrence

Approximately 75% of Crohn's disease patients will require at least one intestinal resection to manage their disease [11]. Furthermore, the 1-year endoscopic recurrence of Crohn's disease following resection with ileo-colonic anastomosis is greater than 70% [12], which results in a subset of Crohn's disease patients requiring multiple resections [13]. Consequently, many studies have evaluated the efficacy of medications in preventing post-operative Crohn's disease including several randomized controlled studies of AZA or 6-MP. A meta-analysis pooled four randomized controlled studies of AZA or 6-MP and these drugs significantly reduced post-operative recurrence at year 1 and 2 by 8 and 13%, respectively [14]. However, AZA and 6-MP were less likely to prevent very severe post-operative recurrence [14]. In subgroup analysis of the individual studies, Crohn's disease patients were more likely to respond to immunomodulators when they had a history of perforating phenotype [15] or multiple prior resections [16].

Concomitant Therapy with Biologics

Monoclonal anti-bodies to tumor necrosis factor (TNF) are widely used in the treatment of Crohn’s disease. While a comprehensive discussion of biologic therapy for Crohn’s disease can be found in Chap. 31–35 (Biologics and Treatment of Crohn’s Disease), the role of concomitant AZA or 6-MP with biologics use will be highlighted in this section. Early trials of infliximab in Crohn’s disease demonstrated reduced efficacy of infliximab among Crohn’s patients receiving episodic rather than scheduled therapy [17]. Higher levels of antibodies were detected among patients who were prescribed infliximab in an episodic regimen. Concomitant use of AZA or 6-MP with infliximab was shown to reduce antibody production and improve efficacy among patients receiving episodic infliximab [17]; however, the beneficial effects of concomitant therapy were not observed in Crohn’s patients receiving scheduled infliximab [18]. The role of concomitant therapy became more controversial when studies demonstrated increased risk of infections [19] and with the recognition of rare hepatosplenic T-cell lymphomas [20] that were associated with combination therapy. Consequently, combination therapy of anti-TNF agents with AZA or 6-MP has become a widely debated and inconsistently practiced [21].

Randomized controlled studies were conducted to address the role of concomitant therapy. The SONIC study randomized Crohn’s disease patients who were naïve to biologics and immunomodulators to AZA alone, infliximab alone, or a combination of infliximab and AZA. Because patients enrolled into the study were naïve to biologics and immunomodulators, the cohort of patients represented an early diagnosis population (median disease duration of 2 years). At 26 weeks (primary endpoint) Crohn’s patients randomized to infliximab and AZA had a significantly higher rate of steroid-free clinical remission (57%) as compared to infliximab alone (45%) and AZA alone (30%). At 50 weeks, 46% of combination users were maintained in steroid-free remission as compared to 35% of infliximab alone and 24% of AZA alone. This was the first randomized controlled study to support the use of combination therapy [22]. In a second study 80 Crohn’s disease patients received infliximab and an immunosuppressant, and then were randomized to discontinue or continue the immunosuppressant after 6 months of therapy. Withdrawal of immunosuppressant did not lead to increased rates of altering infliximab dose or discontinuing infliximab. However, immunogenicity (i.e., antibody production) was higher among patients who discontinued immunosuppressants and the study may not have been powered ($n=80$) to detect smaller differences in efficacy.

Side Effects

While AZA and 6-MP are well tolerated by most Crohn’s disease patients, important adverse effects need to be considered by Crohn’s patients and prescribing physician. Overall, in pooled analysis 8.9% of Crohn’s patients will discontinue AZA or 6-MP secondary to an adverse event [5]. The most prevalent adverse events are listed in Table 29.2. A systematic review reported that approximately

Table 29.2 Adverse events associated with AZA and 6-MP

Adverse event	Frequency (%)
Liver injury [23]	3.4
Allergic or hypersensitivity reactions [5]	2.0
Leukopenia [5]	1.7
Nausea [5]	1.3
Severe infection [5]	0.3

3.4% of users of AZA and 6-MP experienced liver injury [23]. The most common type of liver injury were hypersensitivity reaction-induced hepatitis and idiosyncratic cholestasis. Rarely Crohn's patients developed endothelial cell injury leading to increased portal pressures (e.g., veno-occlusive disease) [23]. In pooled analysis, allergic, or hypersensitivity reactions occurred in 2.0% and often manifested with fever, rash, and/or arthralgia. Leucopenia is an important side effect that is most commonly associated with genetic variants of the TPMT enzymes impairs drug metabolism. In a meta-analysis, 1.7% of patients discontinued AZA or 6-MP due to leucopenia with 0.3% developing a serious infection [5]. Pancreatitis is a serious idiosyncratic reaction that occurs in 1.7% of drug users and often recurs if the drug is re-introduced [5]. Gastrointestinal symptoms, including nausea, may be complained by patients though, nausea leading to discontinuation of AZA or 6-MP occurred in only 1.3%.

A serious concern for physicians and Crohn's disease patients is the potential risk for lymphoproliferative disorders. A meta-analysis of six observational studies reported that the risk of lymphoma among Crohn's disease patients taking AZA and 6-MP were at an over fourfold increased risk of lymphoma (relative risk = 4.18; 95% CI: 2.07–7.51). The absolute risk of developing lymphoma was low and age-specific. The authors calculated that among 20 to 29-year-olds 4,357 Crohn's disease patients need to be treated with AZA or 6-MP for one patient to develop lymphoma in contrast, among 70 to 79-year-olds the number needed to harm was 355 [24].

Drug Dose and Monitoring

AZA and 6-MP are dosed based on weight: 2.0–2.5 mg/kg and 1.0–1.5 mg/kg, respectively [1]. AZA and 6-MP are started at low dose to ensure that Crohn's patient tolerates the medications (e.g., rule out hypersensitivity reaction) and to monitor the patient for leucopenia [1]. The majority of leucopenia is based on decreased enzyme activity of TPMT. Approximately 11% of the population carries heterozygote variants of the TPMT gene, and 1:300 are homozygous [25]. Two approaches are commonly used in monitoring drug dosing of AZA or 6-MP. First, TPMT genotype and enzyme activity can be commercially tested to facilitate drug dosing. Patients with reduced enzyme activity or known genetic heterozygosity of TPMT should have their drug dose reduced (e.g., by 50%) and then drug dose is tailored to the WBC count [26]. However, homozygotes should avoid AZA and 6-MP [26]. Close monitoring of the WBC and neutrophil count should be performed with each dose increase of AZA or 6-MP [26]. In alternate approach, physicians who do not test for TPMT genotype or enzyme activity may adjust AZA and 6-MP based on CBC and neutrophil counts. Commonly, patients are started on low dose of the drugs and then CBC are monitored for dropping WBC/neutrophils on a weekly basis until therapeutic dose is achieved and stable blood counts are established [26]. Irrespective of approach, once the dose of drug is established, then most patients are monitored on a regular basis. Important blood work to monitor includes CBC and liver enzymes. Patients are also warned of other side effects (e.g., pancreatitis), which are then followed clinically.

Physicians may consider measuring metabolites of AZA and 6-MP in Crohn's disease patients who have not achieved an appropriate clinical response after several months [26]. Two most commonly measured metabolites are 6-TG and 6-MMP. 6-TG levels can be measured to determine if a patient's dose is sub-therapeutic and to confirm compliance with medication [27]. Patients with sub-therapeutic 6-TG levels can be considered for dose modifications. However, a subset of patients taking AZA or 6-MP will shunt their metabolism away from 6-TG and towards 6-MMP. This subgroup may have increased risk of toxicity and lower rates of efficacy [27]. However, routine monitoring of drug levels is not widely advocated [26].

Summary

AZA and 6-MP are commonly used medications in the treatment of Crohn's disease. The drugs are efficacious in a number of Crohn's disease indications including steroid-dependent or refractory Crohn's disease, fistulizing Crohn's disease, post-operative prevention, and concomitant therapy with biologics. However, important adverse events are associated with AZA and 6-MP including leucopenia, liver injury, allergic reactions, nausea, pancreatitis, and lymphoma. Consequently routine clinical and laboratory monitoring is recommended for all patients on AZA and 6-MP.

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Chapter 30

Conventional Medical Management of Crohn's Disease: Methotrexate

Eoin R. Storan and Laurence Egan

Keywords Methotrexate • Crohn's disease • Treatment

Introduction

Methotrexate (MTX) was first developed for use as a chemotherapeutic agent in cancers such as leukemia, lymphoma, and choriocarcinoma. Subsequently, it became well established in treating two chronic inflammatory diseases, rheumatoid arthritis [1, 2], and psoriasis [3, 4].

In 1989, Kozarek et al. reported the first use of MTX for the treatment of refractory inflammatory bowel disease (IBD) [5]. This study involved 21 patients with IBD (7 with ulcerative colitis, 14 with Crohn's disease) and showed encouraging results with 16 of the 21 patients showing objective improvement. Since then there have been several randomized controlled trials showing MTX to be efficacious in the treatment of refractory Crohn's disease.

Pharmacology of Methotrexate

MTX is a folate analogue originally synthesized in the 1940s which acts as an anti-metabolite drug but also has anti-inflammatory properties, depending on the dose used. The principal cellular action of MTX is the reversible competitive inhibition of dihydrofolate reductase (DHFR) [6]. Parent MTX, polyglutamated MTX metabolites (MTXPG), and 7-hydroxymethotrexate (7-OH-MTX) are all folate analogues with inhibitory activity against many of the enzymes involved in the metabolic pathway of folate [7] (Fig. 30.1). These folate-dependent enzymes are vital for the de novo synthesis of purines, pyrimidines and the transmethylation of DNA, RNA, phospholipids, and proteins [7]. Anti-proliferative effects dominate with high dose MTX therapy [6, 8] while low dose MTX has an immunosuppressive role that may be independent of the inhibition of cell proliferation via DHFR blockade [7, 9, 10]. In cell culture systems, MTX has been shown to lead to the accumulation of

E.R. Storan, MB, BCh, BAO • L. Egan, MB, BCh, BAO (✉)
Department of Pharmacology, Clinical Science Institute, University Hospital Galway,
Newcastle Road, Galway, Ireland
e-mail: laurence.egan@nuigalway.ie

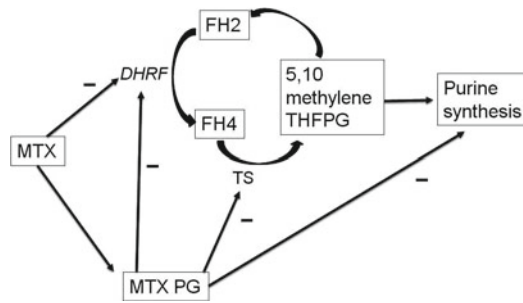


Fig. 30.1 *Methotrexate (MTX) metabolism.* MTX and its polyglutamate metabolites (MTXPG) potentially inhibit dihydrofolate reductase (DHFR), impairing the conversion of dihydrofolate (FH2) to tetrahydrofolate (FH4). MTXPG also inhibit folate-dependent enzymes distal to DHFR such as thymidylate synthase (TS). 5, 10, methylene-tetrahydrofolate polyglutamate (5, 10 methylene THFPG)

adenosine, a lymphotoxic and immunosuppressive autacoid [11]. However, a putative mechanism of action of MTX relating to adenosine has not been supported in IBD, where no rise in plasma or rectal levels of adenosine was found following administration of MTX [12].

At the cellular level, MTX has been observed to induce apoptosis and clonal deletion of activated T-lymphocytes in a mechanism that is independent of the APO-1/Fas (CD95) receptor/ligand system [13]. MTX also acts as a strong differentiation factor for immature monocytes which in vitro is associated with natural cytokine inhibitor release and a simultaneous down-regulation of interleukin (IL)-1 β effects [14].

MTX decreases the production of the pro-inflammatory cytokines IL1, IL6 [15–17], IL2, interferon- γ [18] and tumor necrosis factor (TNF)- α [14] and increases *IL4* and *IL10* gene expression, both of which have anti-inflammatory effects [18]. MTX was shown to inhibit IL-1 activity by blocking the binding of IL-1 to its receptor [19]. There is evidence in patients with rheumatoid arthritis that MTX indirectly inhibits COX-2 synthesis [20] and neutrophil chemotaxis [21]. It is likely that the anti-inflammatory effects of MTX in IBD are underpinned to an extent by combinations of these different actions.

Methotrexate Use in Crohn's Disease

The treatment of steroid-dependent IBD is problematic. In a population-based study on the natural history of corticosteroid therapy for IBD [22], 58% of patients with Crohn's disease treated with corticosteroids were in complete remission after 30 days of therapy and 1 year outcomes revealed that 28% of patients were steroid dependent. Corticosteroids are not indicated in the maintenance of IBD because of their side effect profile with 32% of patients on high dose and 26% on prophylactic dose requiring withdrawal or dose reduction of therapy secondary to side effects [23].

MTX has been studied for both induction and maintenance of remission in patients with chronic active Crohn's disease. There have been five randomized controlled trials reported on the use of MTX for induction of remission in refractory Crohn's disease.

The largest trial, by Feagan et al. in 1995, reported substantial benefit [24]. This trial used intramuscular administration of MTX 25 mg once weekly in patients with chronic active, steroid-dependent Crohn's disease. After 16 weeks, 39.4% of patients were in clinical remission compared to 19.1% with placebo. MTX also induced a substantial benefit in quality of life in these patients, as well as lower requirements for corticosteroid administration.

A study of 54 patients with chronic active, Crohn's disease by Ardizzone et al. compared using intravenous MTX 25 mg once weekly for 3 months, followed by 3 months of oral therapy at the same dose, to therapy with oral azathioprine [25]. The study showed no statistically significant difference in induction of remission between MTX and azathioprine [25]. Adverse reactions resulting in withdrawal of therapy were the same in both treatment groups (11%) but more adverse effects that did not require withdrawal of therapy occurred in the MTX group (44% vs. 7%).

Several trials have evaluated the oral administration of MTX at lower doses. Oren et al. compared oral MTX 12.5 mg once weekly to oral 6-mercaptopurine (6-MP) in chronic steroid-dependent Crohn's disease [26]. There was no statistically significant difference between the drugs for induction or maintenance of remission.

Mate-Jimenez 2000 evaluated the efficacy and tolerance of oral MTX 15 mg once weekly to 6-MP and 5-aminosalicylic acid (5-ASA) [27]. This study similarly failed to show a benefit with oral MTX compared to 6-MP in inducing or maintaining remission in chronic active Crohn's disease. It did show a statistically significant benefit over therapy with 5-ASA. A study by Arora et al. which also investigated using 15 mg oral MTX once weekly in steroid-dependent Crohn's disease similarly failed to show a benefit [28]. This suggests that lower doses of 12.5–15 mg MTX per week and oral route of administration may be less effective than higher, parenterally administered doses. However, no firm conclusions can be drawn from these studies as the numbers involved were small and failure to respond may have been due to inadequate dose and oral route being utilized [26–28].

A randomized trial by Egan et al. investigated the optimum dose of MTX for induction of remission in patients with steroid-dependent IBD [29]. Of these patients, 80% were refractory to corticosteroid therapy and 70% had previously failed adequate therapy with 6-MP or azathioprine. Overall, 17% of IBD patients investigated entered remission and there was no benefit to 25 mg over 15 mg of weekly subcutaneous MTX. The likely explanation for the low level of response seen in this group of IBD patients is the refractory nature of their disease. Interestingly, 11 of the patients who did not respond to treatment with 15 mg weekly after 16 weeks subsequently had their dose escalated to 25 mg weekly MTX and 36% of these patients had an improvement in clinical status although there was no increase in the rate of remission. Toxicity was not different between the two groups [29].

A large study by Feagan et al. 2000 investigated maintaining remission using intramuscular MTX 15 mg once weekly in patients with chronic active Crohn's disease who had already achieved remission after 14–24 weeks treatment with intramuscular MTX 25 mg once weekly [30]. This study showed that relapse occurred in 35% after 40 weeks of therapy with MTX compared to 61% with placebo. The number needed to treat to prevent one relapse was four. The mean time to relapse was >40 weeks compared to 2 weeks with placebo [30]. This study followed on to treat 22 of the 36 patients who relapsed (14 in the MTX group and 22 in the placebo group) with 25 mg intramuscular MTX for 40 weeks. Of this group, 55% were in remission at 40 weeks compared to 14% of the remainder of the group not treated with MTX [30].

For an overview of MTX clinical trials in Crohn's disease, see Table 30.1.

Administration of Methotrexate

Subcutaneous administration of MTX has been shown to have similar pharmacokinetics compared to the intramuscular route [34, 35]. Bioavailability approaches 100% with parenteral administration in contrast to oral route which has shown 50–90% bioavailability in other chronic inflammatory conditions [36–38]. The subcutaneous route has been shown to be well tolerated by patients and to have few local complications at the injection site [34, 39, 40].

Table 30.1 Evidence base for MTX in Crohn's disease

Study	Methods	Patient selection	Category	Intervention	Primary endpoint	Outcome
Feagan 1995 [24]	Randomized, double-blind placebo-controlled, multicenter study	Chronic active CD despite minimum 3 months therapy with prednisone. <i>n</i> =141	Induction of remission CD	IM MTX at 25 mg per week versus placebo for 16 weeks	Clinical remission at 16 weeks defined as off corticosteroids and CDAI < 150 [31]	39.4% remission with MTX versus 19.1% with placebo
Feagan 2000 [30]	Randomized, double-blind placebo-controlled, multicenter study	Chronic active CD previously achieving remission (CDAI ≤150) with MTX 25 mg per week IM for 16–24 weeks. <i>n</i> =76	Maintenance of remission CD	IM MTX 15 mg per week versus placebo for 40 weeks Relapsed patients subsequently treated with MTX 25 mg IM per week for 40 weeks	Occurrence of a relapse at 40 weeks (increase of 100 in CDAI above baseline or initiation of prednisone or an anti-metabolite)	Relapse in 35% MTX group, 61% placebo group Relapsed patients treated with 25 mg MTX per week, 55% achieved remission versus 14% in untreated group
Mate Jimenez 2000 [27]	Randomized, controlled unblinded single center, 3-arm study	Induction arm: steroid-dependent CD and UC Maintenance arm: achieved remission at 30 weeks on MTX from induction arm of study <i>n</i> =72 (38 CD)	Induction and maintenance of remission CD and UC	Oral MTX 15 mg per week 6-MP 1.5 mg/kg/day 5-ASA 3 g/day Induction: 30 weeks Maintenance: if achieved, MTX 10 mg once per week given for 76 weeks	Clinical remission at 30 weeks, CDAI <150 Maintenance of remission at 76 weeks	Differences not statistically significant
Egan 1999 [29]	Randomized, single-blind study	Steroid-dependent IBD resistant to azathioprine and 6-MP <i>n</i> =32 (22 CD)	Induction of remission CD and UC	Compared 25 mg and 15 mg once weekly subcutaneous MTX for 16 weeks. Non-responders in 15 mg group escalated dose to 25 mg for 16 weeks	Compare clinical remission (IBDQ <170 [32]) at 16 weeks between 15 mg and 25 mg once weekly dose of MTX	No significant difference between different doses of MTX 17% from each group achieved remission Dose escalation did not improve remission or increase toxicity
Arora 1999 [28]	Randomized, double-blind placebo-controlled trial	Steroid-dependent CD <i>n</i> =33	Induction and maintenance of remission CD	Oral MTX 15 mg once weekly for 1 year Non-responders had MTX titrated up to 22.5 mg/weekly	Remission (CDAI <150) and reduction in corticosteroid use	Non-significant trend towards fewer flares and increased side-effects in MTX group
Oren 1997 [26]	Randomized, double-blind placebo-controlled, multicenter trial	Chronic active, steroid-dependent CD (Harvey Bradshaw Index ≥7 [33]) <i>n</i> =84	Induction and maintenance of remission CD	3 arms: oral MTX 12.5 mg weekly Oral 6-MP 50 mg daily placebo	Induction of remission and not receiving corticosteroids	No statistically significant difference between the groups

CD: Crohn's disease, UC ulcerative colitis, 6-MP 6-mercaptopurine, 5-ASA 5-aminosalicylic acid, IM intramuscular, MTX methotrexate, IBD inflammatory bowel disease, CDAI Crohn's disease activity index, IBDQ inflammatory bowel disease questionnaire

Adverse Effects

Adverse effects experienced with MTX can be categorized as being due to bone marrow suppression [41], idiosyncratic reactions such as rash or pneumonitis [42], or secondary to MTX-induced fibrosis for example, pulmonary fibrosis or hepatic fibrosis.

There is evidence that the anti-inflammatory effect of MTX may be offset at higher doses by MTX-induced enterotoxicity [43, 44]. MTX causes morphological and functional abnormalities of the small intestinal mucosa [45, 46]. Two studies reporting on jejunal biopsies taken from children with acute lymphoblastic leukemia treated with MTX revealed striking structural abnormalities of the Paneth cells with marked vacuolar dilatation of the cytoplasm [44] and striking distension of the lateral basal intercellular spaces, cell vacuolation, and patchy necrosis [43]. This effect may have been due to a direct toxic effect of MTX therapy or interference with crypt cell generation [43]. There are also several case reports of MTX itself (or aminopterin, a related anti-folate compound) causing severe colitis [47–51].

Up to 18% of patients in IBD trials discontinue the drug due to MTX related toxicity [24, 52]. In the largest study by Feagan et al. side effects were observed in similar frequency at 45% in the MTX treated group versus 42% in placebo group; however, 17% withdrew from treatment because of adverse events (including nausea and asymptomatic elevation in serum aminotransferase) compared to 2% in the placebo group [24]. The patients who withdrew secondary to adverse effects improved after withdrawal of MTX.

The Oren 1997 study found that there was no statistically significant difference in withdrawals and adverse events between patients treated with MTX and 6-MP [26].

Importantly, liver fibrosis and cirrhosis can develop in some patients that are treated chronically with weekly MTX. MTX polyglutamates accumulate in hepatocytes and hepatotoxicity with MTX is thought to be due to the direct toxic and steatogenic effects of MTX on the liver [53, 54].

Patients with psoriasis treated with MTX appear to be at the highest risk of developing hepatic toxicity. In that disease, liver biopsies are typically performed at cumulative MTX doses of 1.5 g [55, 56]. In contrast, patients with rheumatoid arthritis seem to be at a lower risk with the risk of cirrhosis after 5 years of continuous therapy estimated at only 1/1,000 [54, 57].

Studies have suggested that patients with IBD appear to be at a lower risk of developing MTX-induced hepatic toxicity than patients with psoriasis [58, 59]. Te et al. showed that cumulative doses of up to 5,410 mg MTX given for up to 281 weeks in patients with IBD were associated with minimal hepatotoxicity, and that abnormal liver function tests did not identify patients with fibrosis [58]. They suggested that surveillance liver biopsies based on cumulative MTX doses are not warranted in patients with IBD [58]. However, it is the practice of other authors to perform biopsies at cumulative doses of 1.5 g MTX [29]. Whether or not IBD patients on chronic MTX should undergo liver biopsies remains controversial.

Monitoring for Toxicity

Ninety percent of MTX is renally excreted within 24 h of administration [39]. The remainder exists primarily as the pharmacologically active intracellular polyglutamate derivatives in many cell types, including erythrocytes. However, there is no evidence that therapeutic monitoring of erythrocyte or plasma MTX or 7-hydroxymethotrexate levels can be used to guide therapy [29].

Current guidelines advise routine monitoring of patients with complete blood counts and liver function tests to assess for bone marrow suppression and hepatic fibrosis. Persistently abnormal liver biochemistry tests above two times the upper limit of normal warrant discontinuation of the drug or liver biopsy [60]. For suggested guidelines regarding monitoring of patients receiving long-term MTX therapy, see Table 30.2.

Table 30.2 Guidelines for monitoring of patients receiving long-term MTX therapy

Baseline investigations
CBC, creatinine concentration, serum AST, ALT ^a , alkaline phosphatase, albumin, bilirubin levels, Hepatitis B and C and HIV serologies, pregnancy test, chest X-ray
Consider liver biopsy if
Heavy alcohol consumption
Elevated serum liver enzyme activity
Type 2 diabetes mellitus
Toxicity monitoring during treatment
CBC and creatinine concentration weekly initially, with interval gradually increased to 2–3 months after therapy is stabilized
Serum ALT, AST, and albumin levels every 4–6 weeks
Liver Biopsy if:
More than half of regularly checked AST values are more than twice the upper limit of normal
Progressive increase in serum liver enzyme activity
Indications to discontinue use of MTX
Clinically evident liver disease
Fibrosis or cirrhosis on liver histology at biopsy

^a CBC complete blood count, ALT alanine transaminase, AST aspartate transaminase

(Based on data from guidelines developed for rheumatoid arthritis and psoriasis [61, 62])

Recommendations for Clinical Practice

MTX has been proven to be effective for the induction of remission in patients with chronic active, steroid-dependent Crohn's disease. It should be started at a dose of 15–25 mg once weekly by subcutaneous injection for 12–16 weeks. An initial response after this period is unlikely. If there is failure to enter remission after this period on 15 mg once weekly, dose escalation to 25 mg once weekly for another 12–16 weeks may be considered if MTX is being well tolerated.

Baseline investigations such as complete blood count, serum creatinine concentration, and liver biochemistry tests should be performed as listed in Table 30.2.

Patients at risk for steatohepatitis, such as those with a prior history of high alcohol intake, increased weight, type 2 diabetes mellitus, or who have any chronic liver disease are at increased risk of developing hepatic fibrosis [53, 54]. We caution against starting MTX in such patients.

Contraindications to MTX therapy include known liver and renal disease, active infection, and alcohol excess and obesity. Also, MTX is contraindicated in patients who are pregnant or lactating, and in women of childbearing age unless effective contraception is employed. Concomitant use of certain medications such as non-steroidal anti-inflammatory drugs [63, 64] and trimethoprim-sulfamethoxazole is contraindicated and patients should avoid alcohol entirely.

Maintenance of remission with MTX in Crohn's disease patients is indicated for those who enter remission with this drug. Subcutaneous MTX therapy is continued at a dose of 15 mg once weekly. Regular monitoring of complete blood count as well as serum creatinine and liver function tests is indicated to detect cytopenias and patients developing hepatotoxicity.

Mild degrees of leukopenia or thrombocytopenia can be reversed by temporarily stopping MTX therapy and recommencing at a lower dose. Intravenous leucovorin is indicated in more serious or life threatening pancytopenia [42]. The administration of concomitant oral folic acid with MTX in patients with rheumatoid arthritis or psoriasis has been found to reduce haematological complications and gastrointestinal side effects such as stomatitis and gastrointestinal upset [65] without an apparent reduction in clinical efficacy [66]. However, this strategy has not been tested in patients with IBD.

In summary, MTX has been proven to be effective for the induction of remission in patients with chronic active, steroid-dependent Crohn's disease and for the maintenance of remission in patients who enter remission with this drug.

MTX and Ulcerative Colitis

There is a paucity of evidence for using MTX in the treatment of ulcerative colitis (UC). There is only one randomized controlled trial reporting on the use of MTX for the induction and maintenance of remission in UC. This trial by Oren et al. 1996 looked at 67 patients with chronic active, steroid-dependent UC who were randomized to oral MTX 12.5 mg once weekly or placebo for 9 months [67]. There was no statistically significant difference between the two groups with 47% of the MTX group versus 49% of the placebo group entering remission.

Kozarek et al. reported a retrospective study of patients with steroid-dependent UC was treated with parenteral MTX 25 mg once weekly [68]. Seventy percent of patients with UC showed an initial response but only 40% continued to have a response at 59 weeks.

The failure of MTX to show benefit in the treatment of UC may be due to inadequate doses administered or the oral route being utilized in these trials. Alternatively, it may be that MTX is not an effective drug in the treatment of UC, as indicated by the poor rates of response seen with intramuscular 25 mg weekly MTX in the study by Kozarek [68].

Two randomized controlled trials are currently in preparation and in progress, namely the MERIT-UC trial, and the METEOR trial which is investigating intramuscular MTX at 25 mg per week (www.Clinical-Trials.gov; NCT00498589). These trials will more accurately guide therapy in the future. There is currently insufficient evidence to support the use of MTX for the treatment of UC.

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Chapter 31

Biologic Treatment of Crohn's Disease: Adalimumab

Remo Panaccione

Keywords Crohn's disease • Anti-TNF • Biologicals • Adalimumab • Inflammatory bowel disease • Treatment

Introduction

Crohn's disease (CD) is a chronic relapsing inflammatory disorder characterized by transmural inflammation, which can affect the gastrointestinal tract from mouth to anus. Common symptoms include abdominal pain, intestinal cramping, diarrhea, weight loss, and fatigue. The development of fibrostenosis of the intestinal lumen, fistulization, and the presence of significant extraintestinal manifestations may complicate the disease [1]. Many of the complications lead to the need for hospitalization and surgery. The ideal therapy for CD should be safe and effective. This therapy would effectively induce and maintain clinical remission without need for corticosteroids, halt the development of complications, and reduce hospitalizations and surgeries. This would lead to positive effects on quality of life (QoL) while enjoying a favorable safety profile [2]. Furthermore, demonstrating the ability to heal the mucosa is increasingly being appreciated as an important therapeutic endpoint with hopes that this may lead to a disease-modifying effect [3].

Advances in the understanding of the pathogenesis of IBD has led to the development of several agents specifically aimed at key elements of the inflammatory cascade. The development of antagonists to tumor necrosis factor- α (TNF- α) have played a major role in the advancement of therapy in CD. Infliximab (REMICADE[®], Centocor Ortho Biotec Inc., Malvern, PA, United States) [4] and adalimumab (ADA, HUMIRA[®], Abbott Laboratories, Abbott Park, IL, United States) [5] have been shown in pivotal clinical trials to induce and maintain clinical remission in patients with moderate to severe CD [6–11], and certolizumab pegol (CIMZIA[®], UCB Inc., Smyrna, GA, United States) [12] has been shown to reduce signs and symptoms of CD and maintain clinical response [13, 14]. In this chapter, the efficacy data from the major randomized controlled trials, open label studies, and safety supporting the use of ADA in the treatment of CD will be reviewed and its use in clinical practice put into context.

R. Panaccione, MD, FRCPC (✉)
Department of Medicine, Foothills Medical Centre, 3280 Hospital Drive NW,
Rm 6D3Z, TRW Building, Calgary, AB, Canada T2N 4N1
e-mail: rpanacci@ucalgary.ca

Background

ADA (Humira™, Abbott, USA) is a recombinant fully human IgG₁ monoclonal antibody that binds to TNF- α which can be self-administered subcutaneously (such). It consists of human-derived heavy and light chain variable regions and human IgG₁:k constant regions. TNF- α is a 51-kd pro-inflammatory cytokine formed by three soluble 17-kd monomer proteins. It is secreted by monocytes, macrophages, and T-cells [15–17]. The binding of TNF- α to its receptors leads to a number of intracellular events culminating in the activation of nuclear factor κ B and the production of pro-inflammatory cytokines such as interleukin (IL)-1 and IL-6, activation of neutrophils, and promotion of leukocyte migration, all of which are key elements of the inflammatory cascade in CD [18–20].

Pharmacodynamics

ADA binds with high affinity to TNF- α , thus blocking its interactions with its receptors. In binding both soluble and transmembrane TNF- α , ADA fixes complement, causes antibody-dependent cell-mediated cytotoxicity, and induces T-cell apoptosis which is thought to be a key mechanism by which both infliximab and ADA exert their effect [21–23].

Pharmacokinetics

In a randomized, double-blind, placebo-controlled trial of ADA for the induction of remission for CD, clinical assessment of ADA safety and efficacy as studied for the induction of CD (CLASSIC-I), mean serum concentrations (microgram per milliliter) at week 4 were 2.79 ± 1.48 ($n=66$), 5.65 ± 3.06 ($n=65$), and 12.61 ± 5.25 ($n=67$) for the 40/20 mg, 80/40 mg, and 160/80 mg groups, respectively [9]. Concomitant use of 6-mercaptopurine or azathioprine was not associated with differences in mean serum concentrations [9]. In a randomized, double-blind, placebo-controlled trial of ADA for the induction of remission in patients who have had loss of response or intolerance to infliximab, gauging adalimumab efficacy in infliximab non-responders (GAIN), the median ADA concentration was 12.2 mg/ml following administration of 160 mg at week 0 and 80 mg at week 2 [11]. The bioavailability of ADA is 64% with a $T_{1/2}$ of approximately 2 weeks in healthy volunteers.

Immunogenicity

The immunogenicity of infliximab is a well-described phenomenon owing, in part, to its chimeric nature. In a study of ADA for the treatment of rheumatoid arthritis, the development of anti-adalimumab antibodies (AAA) occurred in 5% of patients overall (1% for patients on concomitant methotrexate and 12% for patients on ADA monotherapy) [24]. No data was collected, in this study, on the influence of antibodies on ADA levels and clinical response. A later study in rheumatoid arthritis, however, demonstrated that 17% of patients developed AAA [25]. Moreover, the presence of AAA was associated with lack of clinical response and lower serum ADA levels. Patients with AAA had a median serum ADA concentration of 1.2 mg/l (range: 0.0–5.6) vs. 11.0 mg/l (range: 2.0–33.0) for those with no antibodies. The presence of AAA and measurement

of ADA levels in this study were assayed using a technique developed by the authors and is not performed in the context of an industry-sponsored clinical trial so the assays may not be directly comparable. In the CLASSIC-II trial, a maintenance trial of ADA for CD, 2.6% of patients (7/269) for which data was available developed AAA. All AAAs occurred in patients on monotherapy as none of the 84 patients on concomitant anti-metabolites developed antibodies [26].

Clinical Efficacy of Adalimumab in Crohn's Disease

Randomized Controlled Trials for the Induction of Remission and Response

In CLASSIC I [9], 299 patients with moderate to severe CD defined by a CD activity index (CDAI) score of 220–450 were randomized to one of three induction regimens of subcutaneous (such) ADA (ADA 160 mg week 0, 80 mg week 2; ADA 80 mg week 0, 40 mg week 2; ADA 40 mg week 0, 20 mg week 2) or placebo such at weeks 0 and 2. Primary endpoint was the proportion of patients who achieved clinical remission as defined by CDAI of less than 150 points at week 4 in the two highest dosing regimens compared to placebo. Secondary endpoints included responses as defined by both 70 and 100 point drops in the CDAI. Statistical differences were observed in the primary endpoint of remission between to 160/80 mg ADA and combined (160/80 mg ADA and 80/40 mg ADA) groups compared to placebo. At the highest dosing group (160/80 mg ADA), 36% of patients achieved remission compared to 12% in the placebo group ($p=0.001$). Fifty percent of patients in the 160/80 mg group had a response defined by a 100-point decrease in CDAI and 59% had a response defined by a 70-point drop in the CDAI both superior and statistically significant to placebo.

In GAIN [11], 325 patients with moderate to severe CD who had previously been exposed to infliximab and lost response or became intolerant to infliximab were randomized to receive either ADA such 160 mg at week 0, followed by 80 mg at week 2 or placebo. The primary endpoint was remission as defined by the CDAI at week 4. Twenty-one percent of ADA treated at patients entered into remission, 52% had a decrease of 70 points in the CDAI, and 38% had a decrease of 100 points in the CDAI at week 4 (7, 34, 25% for placebo, respectively, $p<0.05$). Based on the results of these studies, ADA was granted expedited review by regulatory agencies in the United States, Canada, and the European Union.

Randomized Controlled Trials for the Maintenance of Remission and Response

Two hundred seventy-five patients from CLASSIC I enrolled in CLASSIC II and received open-label ADA 40 mg at weeks 0 and 2 (Weeks 4 and 6 of CLASSIC I) [26]. Patients who were in remission at both week 0 (end CLASSIC I/beginning CLASSIC II) and week 4 were re-randomized to 40 mg every other week (e.o.w.), weekly, or placebo through 56 weeks. In the re-randomized cohort, 79% who received ADA 40 mg e.o.w. and 83% who received 40 mg weekly maintained remission through week 56, vs. 44% for placebo ($p<0.05$ for both ADA groups vs. placebo).

In Crohn's trial of the fully human antibody adalimumab for remission maintenance (CHARM), 854 patients with moderate to severe CD received open label ADA SC at doses of 80 mg at week 0 and 40 mg at week 2 [10]. Patients who had been exposed to IFX in the past and either lost response or had become intolerant to infliximab were eligible for this trial. Patients, who responded, as defined by a drop in CDAI of 70 points, were then randomized to one of three treatment arms; ADA 40 mg e.o.w., ADA 40 mg weekly, or placebo. Approximately 60% of patients responded to the initial 80/40 mg induction dose at week 4 and were randomized. At week 26, 40% of the ADA 40 mg e.o.w.,

47% of the ADA 40 mg weekly, and 17% of the placebo group were in remission ($p=0.001$ for both groups compared to placebo, no difference between active groups). The benefit was maintained out to week 56 with 36% ADA 40 mg e.o.w., 41% ADA 40 mg weekly, and 12% placebo group remaining in remission ($p=0.001$). A difference was not appreciated in the proportion of patients who were able to maintain remission or response according to their previous infliximab exposure.

Within the CHARM study population 117 patients had active perianal fistulizing disease. Although the study was not specifically designed or powered to evaluate patients with fistula, one-third of patients treated with ADA had complete healing of fistula.

Effect of Adalimumab on Quality of Life and Hospitalization

QoL in patients with CD is often assessed using the inflammatory bowel disease questionnaire (IBDQ); a validated measure of QoL in patients with IBD assessing systemic features, bowel system, emotional, and social function. Scores can range between 32 and 224 with a score of >170 on the IBDQ correlating with clinical remission as defined by a CDAI <150 [27]. In patients continued on ADA 40 mg SC e.o.w., or weekly in CLASSIC-II, IBDQ >170 were maintained, whereas placebo-treated patients had IBDQ scores that rapidly declined demonstrating that sustained response to ADA was associated with improved QOL [28]. In patients in the GAIN study, scores in the ADA-treated patients were significantly higher in all four domains of the IBDQ than in placebo-treated patients again demonstrating improved QoL associated with treatment with ADA [29].

An important measure of success with respect to managing patients with CD is an ability to reduce the rate of hospitalization for management of the disease. In a study evaluating patients enrolled in the CHARM study, a secondary analysis evaluated rates of hospitalization in patients treated with placebo vs. those treated with ADA [30]. At 56 weeks, the actuarial hospitalization rate in placebo and ADA-treated patients was 13.9 and 5.9%, respectively ($p<0.01$), with treatment with ADA being the only independent factor associated with reduced risk [30].

Effect of Adalimumab on Mucosal Healing

In the 52-week *EXTend* the safety and Efficacy of adalimumab by eNDoscopic healing (EXTEND) trial, mucosal healing (defined as absence of mucosal ulceration observed by endoscopy) at weeks 12 and 52 was 27 and 24% ($p=0.056$), respectively, for the ADA maintenance group compared with 13 and 0% ($p<0.001$), respectively, for patients who received ADA 160/80 mg for induction only followed by placebo [31]. A *post-hoc* analysis of mucosal healing rates by baseline disease duration showed a greater treatment effect (ADA vs. placebo) at week 12 for patients with a disease duration <5 years vs. ≥ 5 years ($p=0.029$) [32].

Safety and Tolerability

Safety of Anti-TNF Agents as a Class

With a few exceptions, the safety data with respect to anti-TNF agents falls into two broad categories: class-specific and agent-specific effects. For the most part, safety concerns are a class effect. The safety issues related to the use of anti-TNF agents includes infusion/injection reactions, infections, autoimmunity, malignancies (both hematological and solid organ) and other events such as

demyelinating disease, congestive heart failure (CHF), hematologic, and hepatic abnormalities including reactivation of hepatitis B.

Infusion reactions in infliximab-treated patients in clinical trials occurred in 2–16% of cases [6–8]. Pre-medication with corticosteroids and anti-histamine may prevent subsequent reactions [33]. Injection site reactions can occur with the subcutaneously administered anti-TNF agents at a rate of up to 6% as reported in clinical trials [9–11, 24]. Delayed hypersensitivity reactions are well described in the setting of the administration of infliximab and can occur at any time point and are characterized by arthralgia, myalgias, fever, urticaria, and rash and generally occur several days after an infusion.

Infectious complications that occur with the use of anti-TNF agents can be thought of as minor infections and serious infections. Most minor infections comprise of non-specific upper respiratory tract infections or urinary tract infections and occur in approximately one-third of patients and are comparable across all three agents in the class [34]. Generally, minor infections may delay a scheduled treatment, but do not lead to discontinuation of therapy. Serious infections have been reported with the use of anti-TNF agents and include pneumonia, cellulitis, sepsis, and abscess formation. In clinical trials of infliximab, serious infections occurred in 4–5% of patients. Opportunistic infections, such as pneumocystis carinii pneumonia, listeriosis, histoplasmosis, and mucormycosis have been reported but are rare [35–38]. However, it is important to recognize that patients using anti-TNF agents are often taking concomitant therapy with other potentially immune-suppressive medications including corticosteroids. Data from the TREAT registry (CD Therapy, Evaluation and Assessment Tool), a large voluntary registry of patients with CD treated with a variety of therapies, revealed that serious infections were linked more to the use of corticosteroids and narcotics than to the use of infliximab or immunosuppressive therapy when evaluated by multivariate logistic regression analysis [39]. The use of anti-TNF agents is known to be associated with reactivation of latent tuberculosis and appropriate screening in advance of administration is mandatory [40].

The development of antinuclear antibodies (ANAs) is known to be associated with the use of anti-TNF agents [41]. In a cohort of 125 patients exposed to infliximab, the incidence of ANA at 24 months was 57% [41]. Drug-induced lupus with positive ANA and Anti-dsDNA has been described, but is rare [42].

The potential for the development of a malignancy in association with anti-TNF therapy has always been a theoretical concern owing to their immunosuppressive nature. The majority of reported malignancies temporally associated with anti-TNF therapy have been Non-Hodgkin's lymphomas. In the TREAT registry, 0.42 patients per 100 patient years in the infliximab-treated group developed malignancies compared to 0.51 patients per 100 patient years in those not treated with infliximab. With respect to lymphomas specifically, the incidence per 100 patient years was 0.062 in the infliximab-treated group compared to 0.057 in those not treated with infliximab [39]. The difference was not statistically significant between groups with either comparison. A recent meta-analysis of randomized trials examined the risk of serious infection and malignancy in patients with rheumatoid arthritis treated with infliximab and ADA [43]. This study concluded that the odds ratio for malignancy with anti-TNF therapy was 3.3 (95% confidence interval, 1.2–9.1) compared to placebo. However, the study has been criticized with respect to how trials were selected for inclusion, the statistical methods employed, the lack of control for potential confounders, and the inclusion of non-melanotic skin cancers into malignancy estimates. How this controversial data translates into the IBD population is unknown. More recently, there has been concern related to the reporting of a number of cases of a rare form of $\delta\gamma$ T cell lymphoma (hepatosplenic T cell lymphoma) in CD patients treated with anti-TNF agents [44]. These cases all occurred in younger patients between the ages of 12–31 with the majority being on or having recently received thiopurine therapy (6-MP/AZA). Given the rarity of the event, the true relevance of this as it pertains to the use of anti-TNF therapy in CD is unknown but remains an issue worthy of ongoing vigilance.

Patients with pre-existing New York heart CHF Class III or IV, or those with a history of multiple sclerosis or optic neuritis should not receive anti-TNF therapy as worsening of CHF and demyelinating syndromes have been described [45–48].

Safety of Adalimumab in Open-Label and Controlled Studies

A recent study pooled all data from controlled trials of ADA in CD and assessed the rates of adverse events (AE) [49]. Data was available for 1,459 patients with a total of 1,506 patient years of ADA exposure. For those patients in induction studies, there was no difference in infectious AE at any dose of ADA vs. placebo. For patients in maintenance studies, there was a significantly greater number of AE leading to discontinuation of therapy in the placebo-treated patients than in ADA-exposed patients. In the total cohort of exposed patients, there were two cases of demyelinating disease, three cases of tuberculosis, one case of CHF, three cases of lupus-like reaction, and two deaths. The two deaths were due to pulmonary embolism in an elderly patient and acute myeloid leukemia in a patient on AZA.

With respect to autoimmunity, increases in titers of ANAs and Anti-dsDNA antibodies have been reported to occur in 5.3 and 12.9% of ADA-treated RA patients [50].

The Use of Adalimumab in Clinical Practice

For those patients who require biologic therapy, ADA is a very good option in both patients who are bio-naïve and those patients who have lost response to or become intolerant to infliximab. The efficacy of ADA with respect to both induction and maintenance of CD is comparable to that of infliximab. This is supported by data from the pivotal randomized controlled trials CLASSIC I, CHARM, and GAIN. Moreover, the safety concerns of the two agents are generally equivalent with the exception of agent-specific issues such as infusion vs. injection site reactions. For these reasons either agent is an acceptable choice as a first-line biologic agent for patients with luminal disease.

The induction dosing in the North American labels and Europe are slightly different. The approved induction dosing in North America is 160/80 mg at weeks 0 and 2 as opposed to Europe where the 80/40 mg dosing regimen is cited as the preferred induction except for patients with severe disease where the 160/80 mg dosing is suggested. Of interest, recent studies demonstrate that in large cohorts that the 160/80 mg dosing is likely superior both in short and long term. A single-center analysis of ADA use for CD in 168 infliximab-exposed patients showed that the 160/80 mg induction dose was associated with more frequent CRP normalization ($p=0.04$), more frequent ($p=0.004$) and longer sustained clinical benefit ($p=0.04$), and less frequent primary non-response ($p<0.0001$) compared with the 80/40 mg regimen [51]. In addition, two real-world observational analyses found that patients who received the 160/80 mg induction regimen were approximately half as likely to receive dosage intensification (i.e., weekly dosing) compared with other induction regimens ($p<0.0001$) [52, 53]. Based on the clinical trial data as well as these recent reports, the author always uses the 160/80 mg induction regimen.

In addition, it appears that a subgroup of patients may require longer than the “4 week induction period” to achieve full efficacy. Although many patients respond to ADA by week 4, some may require a longer duration of therapy to achieve initial clinical response and remission. For non-responders in CHARM and GAIN, defined as failure to achieve a ≥ 70 -point decrease in CDAI at week 4, more than 50% achieved a response by week 8 and at least 60% achieved it by week 12 [54]. Rates of clinical remission for the CHARM initial non-responders were 26% by week 8 and 28% by week 12; the corresponding rates for the GAIN non-responders were 19 and 25%, respectively [54]. Clinicians should consider continuing ADA 40 mg e.o.w. therapy for up to an additional 8 weeks after induction, before deeming the patient a treatment failure. Options for potential non-responders include increasing the dosage to 40 mg weekly or switching to an alternative agent if symptoms persist after dosage adjustment.

The durability of response beyond 1 year may be a differentiating feature between the anti-TNF agents where it appears that ADA may have an advantage. ADA clinical trial data have demonstrated sustained clinical remission, improvements in QoL, reductions in hospitalization, steroid-sparing effects, and fistula healing during long-term treatment for CD for up to 3 years [55–57].

Patient preference may play a large role in which anti-TNF agent is preferred by the patients [58]. In a young patient population such as CD, the e.o.w., subcutaneous administration of ADA may represent a significant potential advantage over the intravenous administration of infliximab. It allows for more flexibility regarding travel, work, and education [58].

ADA is the only biologic available for patients who have become intolerant to, or lost response to infliximab. This represents a significant proportion of patients after 1 year of therapy. Data from the GAIN study indicates that ADA has efficacy in this population. However, the expected response rate is approximately 8–15% lower than in infliximab-naïve patients.

One possible advantage of infliximab is in those patients with fistulizing CD. The data for ADA demonstrates efficacy but as a secondary endpoint in a smaller number of patients. Infliximab represents the only biologic agent for which there is randomized controlled trial data with fistula response as a primary endpoint. It remains unclear as to which agent, if any, is superior in these patients.

An obvious temptation of both patients and physicians will be to consider switching from intravenously administered infliximab to subcutaneously administered ADA in those patients who have demonstrated and maintained a response to the former agent. It is worth noting that there is no data to support this approach and, given the relative lack of other available biologic therapies at the present time, a recommended approach would be to optimize currently successful therapy before switching to an alternate agent. This is an area that warrants study in a controlled fashion.

In summary, ADA represents an important step in the evolution of anti-TNF therapy and is a welcome addition to our therapeutic armamentarium for CD. Its convenient subcutaneous administration coupled with its proven efficacy and safety will likely position it as the dominant biologic agent for luminal CD, especially if it delivers on other evolving areas of biologic therapy such as mucosal healing. Although the impact of anti-TNF therapy in the treatment of CD has been substantial, one-third of patients do not respond to these agents. This underscores the need for better defining not only which patients will best respond to these agents, but also which patients would benefit from earlier use of a biologic agent as well as the need to continue to develop novel biologic agents with alternate mechanisms of action.

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Chapter 32

Biologic Treatment of Crohn's Disease: Certolizumab

Wojciech Blonski and Gary R. Lichtenstein

Keywords Crohn's disease • Anti-TNF therapy • Certolizumab • Infliximab

Introduction

Anti-TNF therapy has become a mainstay of therapy for patients with Crohn's disease (CD) unresponsive to conventional medical therapy. There now exist three anti-TNF agents approved in the USA for the treatment of CD. Infliximab is administered intravenously and adalimumab and certolizumab pegol are administered subcutaneously. Certolizumab pegol previously known as CDP870 is a polyethylene glycolated Fab' fragment of humanized anti-TNF monoclonal antibody. This monoclonal antibody is known to bind TNF- α without inducing T-cell or monocyte apoptosis. The pegylation process may lead to reduced immunogenicity and subsequently permits higher serum levels with associated longer duration of efficacy [1]. Overall certolizumab was approved in April 2008 by the US food and drug administration (FDA) for reducing signs and symptoms of CD and for maintaining clinical response in adult patients with moderate to severe activity of the disease with insufficient response to conventional therapy [2].

Pharmacokinetics

Certolizumab pegol is administered subcutaneously every 2 weeks for the first three injections followed by once every 4-week administration [2]. Certolizumab pegol has been found to have a linear pharmacokinetic profile in humans with the volume of distribution of 45 mL/kg, clearance rate of 0.72 mL/h/kg, elimination half-life of 311 h, and at least 100% bioavailability after either intravenous or subcutaneous administration in healthy male volunteers [3].

W. Blonski, MD, PhD • G.R. Lichtenstein, MD (✉)
Center for Inflammatory Bowel Diseases, Division of Gastroenterology,
Department of Medicine, Hospital of the University of Pennsylvania,
3400 Spruce Street, 3rd Floor Ravdin Building, Philadelphia, PA 19104, USA
email: grl@uphs.upenn.edu

Clinical Trials

Certolizumab pegol was evaluated in two dose–response Phase II ($n=384$) [4, 5] and two Phase III (PRECISE 1 and PRECISE 2) [6, 7] ($n=1,330$) randomized placebo-controlled trials in adult patients with CD [5–7] (Table 32.1).

The first Phase II trial that enrolled 92 patients with moderate to severe CD assessed efficacy of certolizumab over 12 weeks period after single 30-min intravenous infusion of either certolizumab (CDP870) (1.25, 5, 10, or 20 mg/kg) or placebo [4]. Clinical response was defined as decrease in CDAI score at least by 100 points whereas clinical remission was defined as CDAI score lower or equal to 150 points [4]. Treatment with active drug demonstrated similar efficacy to placebo in achieving primary endpoint at week 4 (clinical response) (60% for 5 mg/kg, 58.8% for 10 mg/kg, 47.8% for 20 mg/kg vs. 56% for placebo) [4]. Likewise, no difference between any dose of CDP870 and placebo was observed with respect to secondary endpoints (clinical response, remission, CDAI score ≥ 70 points at weeks, C-reactive protein (CRP) levels at weeks 2, 8, 12) [4]. However, the superiority of active drug over placebo was found for 10 mg/kg dose of CDP870 in inducing remission at week 2 (47.1% vs. 16%, $p=0.041$) [4].

The second Phase II 12-week trial evaluated efficacy of certolizumab pegol (100, 200, or 400 mg) or placebo given subcutaneously at week 0, 4, and 8 among 292 patients with moderate to severe CD [5]. Treatment with certolizumab pegol was not superior over placebo at primary endpoint (clinical response at week 12) with the response rates among active drug arm of 36.5% (100 mg), 36.1% (200 mg), and 44.4% (400 mg) and in the placebo arm of 35.6% [5]. On the other hand, certolizumab pegol was significantly more efficacious than placebo in achieving clinical response at week 2 (100, 200, and 400 mg), 4 (200 and 400 mg), 8 (100 and 400 mg), and 10 (400 mg) with the highest rates for 400 mg dose [5]. Likewise, certolizumab pegol showed superiority over placebo in inducing clinical remission at week 4 (100, 200, and 400 mg) and week 8 (100 and 400 mg) but not week 12 [5]. Of note, post hoc analysis revealed that treatment with the highest dose of certolizumab (400 mg) was superior to placebo in achieving primary (53.1% vs. 17.9%, $p=0.005$) and secondary endpoints in patients with high baseline levels of CRP (≥ 10 mg/L) whereas no significance of certolizumab over placebo was observed among patients with low baseline CRP [5]. Therefore, it was suggested that efficacy of certolizumab over placebo might not have been reached due to the high placebo response rate in the large cohort of patients with low baseline level of CRP [5].

The latter findings were taken into consideration in a subsequent Phase III 26-week PRECISE 1 trial [6]. PRECISE 1 trial evaluated the efficacy of certolizumab pegol in 662 patients with moderate to severe CD who were stratified based on low (<10 mg/L) or high (≥ 10 mg/L) baseline CRP levels and then randomized to subcutaneous injections of either certolizumab 400 mg or placebo administered every 2 weeks through week 4 and then every 4 weeks through week 26 [6]. Certolizumab pegol was found to be significantly more efficacious in achieving primary endpoint (clinical response defined as at least 100 point decrease in CDAI score at week 6 and both weeks 6 and 26 in a cohort of 302 patients with high baseline CRP levels) with rates of 37 versus 26% (week 6, $p=0.04$) and 22 versus 12% (both week 6 and 26, $p=0.05$) [6]. Among all evaluated patients regardless of baseline CRP level treatment with certolizumab was superior to placebo in achieving clinical response at week 6 (35% vs. 27%, $p=0.02$) and both weeks 6 and 26 (23% vs. 16%, $p=0.02$) [6]. On the other hand, no significant difference was found between active arm and placebo arm with respect to achieving remission at any time point among all studied patients (week 6: 22% vs. 17%, $p=0.29$; both week 6 and 26: 13% vs. 8%, $p=0.24$) or patients with high baseline CRP level (week 6: 22% vs. 17%, $p=0.17$; both week 6 and 26: 14% vs. 10%) [6]. The researchers did not find any association between concomitant immunosuppressants or corticosteroids use or previous infliximab therapy or smoking status and response rates at the aforementioned time points [6].

Table 32.1 Randomized placebo-controlled trials of certolizumab in induction and maintenance of remission in patients with moderate to severe Crohn’s disease

Study	Design	Primary endpoint definition	Results		No. of patients with AEs		No. of patients withdrawn due AEs	
			CP	placebo	p-value	CP	placebo	p-value
Sandborn et al. PRECISE 1 [6]	• Double blind placebo controlled	Clinical response (decrease in CDAI score ≥ 100 points) in patients with baseline CRP ≥ 10 mg/dL	37% (54/145)	26% (40/154)	0.04	81% (269/331)	79% (260/329)	0.47
	• Patients with CD for ≥ 3 months, CDAI 220–450 points		/week 6	/week 6				
	• Certolizumab (400 mg) or placebo s.c. at week 0, 2, 4 and then every 4 week through week 26		22% (31/144)	12% (19/154)		11% (36/331)	12% (39/329)	
Sandborn et al. PRECISE 2 [7]	• Double blind placebo controlled	Clinical response (decrease in CDAI score ≥ 100 points) in patients with baseline CRP ≥ 10 mg/dL at week 26	62% (69/112)	34% (34/101)	<0.001	65% (140/216)	67% (143/212)	not reported
	• Patients with CD for ≥ 3 months, CDAI 220–450 points							
	• Induction Rx with certolizumab 400 mg s.c. week 0, 2, 4							
	• Responders (decrease CDAI ≥ 100 points) at week 6 randomized to certolizumab (400 mg) or placebo s.c. at week 8, 12, 16, 20 and 24 followed through week 26					8% (18/216)	13% (28/212)	Not reported

CP certolizumab pegol, AEs adverse events

The presence of antibodies to certolizumab was observed in 8% of patients who received certolizumab with a 4% rate in patients treated with certolizumab and concomitant immunosuppressive agents and at a rate of 10% in patients treated with certolizumab without concomitant immunosuppressive agents [6].

The subsequent PRECISE 2 trial observed the beneficial effect of certolizumab in maintaining response and achieving clinical remission in patients with moderate to severe CD who responded to induction therapy with certolizumab [7]. There were 428 patients enrolled who responded at week 6 to initial open-label induction therapy with three single doses of 400 mg certolizumab given subcutaneously every 2 weeks [7]. These patients were stratified according to baseline CRP levels and randomized to receive subcutaneously either certolizumab 400 mg or placebo administered every 4 weeks through week 24 with follow-up through week 26 [7]. Therapy with certolizumab was superior to placebo in maintaining response to treatment through week 26 in both patients with high levels of CRP ($n=215$, 62% vs. 34%, $p<0.001$) and in the intention to treat population ($n=425$, 63% vs. 36%, $p<0.001$) [7]. Moreover, certolizumab was also significantly more efficacious than placebo (48% vs. 29%, $p<0.001$) in achieving clinical remission at week 26 [7]. Patients receiving continuous certolizumab treatment were less likely to develop antibodies to certolizumab than those who received only induction treatment followed by placebo maintenance (8% vs. 18%) [7]. The development of antibodies to certolizumab was further reduced by concomitant use of immunosuppressive agents (2% vs. 12% for certolizumab maintenance arm and 8% vs. 24% for placebo maintenance arm) [7].

Recent additional analysis of PRECISE 2 data demonstrated that short duration of CD (less than 1 year) was associated with higher response and remission rates to certolizumab at week 26 [8]. Although patients with short (<1 year) and long (≥ 5 years) duration of CD who received certolizumab maintenance therapy had significantly higher response (89.5% vs. 37.1%, $p<0.01$ and 57.3% vs. 32.7%, $p<0.001$, respectively) and remission (68.4% vs. 37.1%, $p<0.05$ and 44.3% vs. 23.5%, $p<0.001$) rates than placebo recipients at week 26 the benefit was more visible among patients with short duration of the disease [8]. In addition, absence of prior intestinal resection, lack of prior infliximab use, duration of CD less than 2 years, and use of corticosteroids use at baseline were found to be independent predictors of maintenance of response to certolizumab at week 26 [8]. These interesting observations need to be further evaluated in large prospective placebo-controlled trials. Data from PRECISE 2 trial have also shown that patients with CD may benefit from treatment with certolizumab irrespectively of prior use of other anti-TNF agent, infliximab [9]. Although certolizumab was significantly more effective than placebo as maintenance therapy at week 26 in patients with previous exposure to infliximab (response: 44.2% vs. 25.5%, $p=0.018$; remission: 32.7% vs. 13.7%, $p=0.008$) and in infliximab naïve patients (response: 68.7% vs. 39.6%, $p<0.0001$; remission: 52.8% vs. 33.3%, $p<0.001$), the effect was higher in the latter subgroup of patients [9].

Promising results of PRECISE 2 trial [7] yielded conduction of two open label extension trials, namely PRECISE 3 and PRECISE 4 trials [10, 11]. PRECISE 3 trial enrolled patients who completed 26 weeks of PRECISE 2 trial and assessed the efficacy of subcutaneous administration of certolizumab at the dose 400 mg given every 4 weeks for 54 weeks in patients who received either maintenance therapy with certolizumab (PRECISE 2 continuous arm, $n=141$) or placebo (PRECISE 2 drug interruption arm, $n=100$) following successful induction of remission with certolizumab [10]. Although rates of sustained response and remission measured by Harvey-Bradshaw Index were similar between continuous and drug interruption arms 26 (sustained response: 74.4% vs. 79.7%, respectively; sustained remission: 72.8% vs. 73.5%, respectively) and 54 (sustained response: 66.1% vs. 63.3%, respectively; sustained remission: 62.1% vs. 63.2%, respectively) weeks after onset of PRECISE 3 trial, drug interruption arm was characterized by higher incidence of adverse events related to certolizumab [10]. Therefore, it has been suggested that continuous administration of certolizumab is advocated due to its effectiveness and more favorable safety profile [10]. On the other hand, PRECISE 4 trial assessed efficacy of certolizumab among patients who

experienced relapse in PRECISE 2 trial and withdrew from it before week 26 [11]. All enrolled patients ($n=124$) received in PRECISE 4 trial three doses of certolizumab 2 weeks apart with subsequent administration every 4 weeks up to week 52 and were divided into continuous ($n=49$) and drug interruption ($n=75$) arm based on the fact whether they relapse during PRECISE 2 trial while receiving maintenance treatment with certolizumab or placebo [11]. Certolizumab was equally efficacious in both treatment arms with 63.3 and 65.3% response rates at week 4 and with 54.8 and 59.2% further maintaining this response at week 52, respectively [11].

There is recent evidence which suggests that certolizumab is also an effective agent in patients with moderate to severe CD who have lost their response or became intolerant to another widely used anti-TNF agent, infliximab. Data from an open label induction Phase IIIb WELCOME trial of 6-week duration have shown that 62% (334/539) and 39.3% (212/539) of such patients achieved clinical response and remission, respectively, at week 6 after three doses of certolizumab (400 mg) administered 2 weeks apart [12]. A double-blind maintenance part of WELCOME trial comparing efficacy of every 2 week ($n=161$) and every 4 week ($n=168$) administration of certolizumab during further 20-week interval has demonstrated that sustained response (36.6% vs. 39.9%, respectively $p=0.55$) and remission (30.4% vs. 29.2%, respectively, $p=0.81$) rates were comparable in both dosing regimens at the end of the trial (week 26) [12].

Conclusion

Current evidence strongly suggests that certolizumab pegol is an effective and safe therapy for patients presenting with moderate to severe CD either without previous exposure to infliximab or with secondary loss of response or intolerance to infliximab. Certolizumab has certainly enriched an anti-TNF spectrum available in the treatment of CD showing not only promising clinical efficacy but also improving health-related quality of life for patients [13].

Abbreviations

Anti-TNF	Antitumor necrosis factor
CD	Crohn's disease
PRECISE	The pegylated antibody fragment evaluation in Crohn's disease: safety and efficacy
WELCOME	26-Week open-label induction, double-blind maintenance, placebo-controlled trial evaluating the clinical benefit and tolerability of certolizumab pegol induction and maintenance in patients suffering from CD with prior loss of response or intolerance to infliximab.

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Chapter 33

Biologic Therapy of Crohn's Disease: Infliximab

Frank Hoentjen, Atsushi Sakuraba, and Stephen Hanauer

Keywords Tumor necrosis factor • Azathioprine • Crohn's disease • Fistula • Immunosuppression • Infections • Infliximab

Introduction

Infliximab is a chimeric monoclonal antibody targeting tumor necrosis factor α (TNF- α). It is the first biological agent developed and marketed around the world to treat Crohn's disease and has also been highly efficacious for the treatment of other immune-mediated inflammatory disorders including rheumatoid arthritis, spondyloarthropathies, psoriasis, and ulcerative colitis. Infliximab has been highly efficacious for the treatment of refractory Crohn's disease and has had demonstrated effects on symptoms, signs, mucosal healing, and fistulization such that successful treatment has reduced the number of surgeries and hospitalizations. By virtue of the impact on TNF- α the safety profile of infliximab includes an increased risk of infections, in particular infections by intracellular pathogens, as well as an increased production of autoantibodies. There is also an association with neoplasia, particularly associated with combination therapy with other immunosuppressives.

This chapter will outline the proposed mechanisms of action, clinical efficacy, and side effects of infliximab therapy in Crohn's disease.

Mechanisms of Action of Infliximab (See Table 33.1)

Crohn's disease had traditionally been accepted as a T-helper 1 (Th1)-mediated form of inflammation [1, 2]; however, recent investigations have led to the recognition of both Th-1 and Th-17 contributions to the immune-mediated inflammatory disorder [3]. One of the proximal events leading to the

F. Hoentjen, MD, PhD

Section of Gastroenterology, Hepatology and Nutrition, Pritzker School of Medicine,
University of Chicago, 5841 S. Maryland Ave., MC 4076, Chicago, IL 60637, USA

Department of Gastroenterology, Free University Medical Center, Amsterdam, The Netherlands

A. Sakuraba, MD • S. Hanauer, MD (✉)

Section of Gastroenterology, Hepatology and Nutrition, Pritzker School of Medicine,
University of Chicago, 5841 S. Maryland Ave., MC 4076, Chicago, IL 60637, USA
e-mail: shanauer@uchicago.edu

Table 33.1 Summary of mechanism of action of infliximab in Crohn's disease

Proposed mechanism	Reference
Blocking soluble TNF- α	[12, 13]
Blocking membrane TNF- α	[14–16]
Induction of apoptosis of peripheral blood and lamina propria lymphocytes	[17–22]
Induction of apoptosis of monocytes	[24, 25]
Suppression of proinflammatory cytokine production	[26–31], [33], [42]
Induction of anti-inflammatory cytokine production	[34, 35]
Induction of regulatory T cells	[35–38]
Blockade of CD40-CD40L pathway	[39]
Suppression of apoptosis and inflammatory cytokine production by epithelial cells	[41, 42]
Regulatory effect on myofibroblast	[43, 44]
Regulatory effect on macrophages and dendritic cells	[34], [45–48]

chronic inflammation is an increase in interleukin (IL)-12 and IL-23 produced by the antigen-presenting cells, such as macrophages and dendritic cells [4]. The downstream effects from these cytokines are increases in the production of the Th-1 cytokine, interferon- γ , and the Th-17 cytokine, IL-17 by CD4+T cells [3]. During this process, TNF- α , an inflammatory cytokine, is also produced by a broad range of immune cells, including macrophages/monocytes and T cells. Indeed, evidence suggests that the mucosa of Crohn's disease is infiltrated with Th-1 and Th-17 cells, as well as with TNF- α producing cells, suggesting their important role in the pathogenesis of Crohn's disease [5–7].

TNF- α is a member of the TNF and TNF-receptor superfamilies. TNF- α is first produced as a 26-kDa transmembrane form with an intracellular tail, which is then cleaved by the TNF- α converting enzyme (TACE) to be secreted as a 17-kDa soluble form. This form aggregates to form trimers that interact with its receptors. Monocytes/macrophages are believed to be the largest source of TNF- α , although T lymphocytes and other kinds of cells are also able to produce significant amounts of TNF- α . In response to a variety of inflammatory stimuli, including enteric bacteria or bacterial products, TNF- α -producing cells can be activated, at both the transcriptional and translational level, to produce TNF- α . In the intestinal mucosal inflammation of Crohn's disease, TNF- α is relevant to the disruption of the epithelial barrier, coagulation, and fibrinolysis, as well as formation of inflammatory granulomas. TNF- α can also activate macrophages, stimulate B lymphocytes, transmit signals between immune cells and other cells, and promotes the migration of circulating neutrophils to inflamed sites by upregulating the expression of adhesion molecules in endothelial cells. Indeed, TNF- α is elevated in stool samples of Crohn's disease and is a marker of intestinal inflammation [8].

The biological effects of TNF- α are mediated by specific binding to either a p55 type I (TNFR1) or a p75 type II (TNFR2) receptor. Both of these receptors are expressed on the surface of many cell types. Binding of TNF- α to these receptors initiates the activation of intracellular signaling processes that result in diverse cellular responses, including differentiation, activation, production of proinflammatory cytokines, and apoptosis. An imbalanced cytokine milieu is the hallmark of Crohn's disease, and therapies targeted toward the Th1 and Th-17 pathways are currently either in the clinic or under development.

Biologic agents are large-sized proteins that have been manufactured to target a specific protein, antibody, or receptor. They may be a cytokine, antibody, or fusion protein and carry the advantage of being more specific and targeted than conventional, systemic therapies. Specifically anti-TNF- α agents bind to both circulating and membrane-bound TNF- α , thus preventing its proinflammatory actions during the disease progression (see below). Infliximab was the first anti-TNF- α agent investigated in Crohn's disease. It is a chimeric IgG1 monoclonal antibody comprised of 75% human and 25% murine sequences that carries a high specificity for and affinity to TNF- α [9]. It does not interact

with TNF- β (lymphotoxin- α), a related cytokine that shares the same receptor site [10]. The 25% murine sequence that specifically recognized human TNF- α is at the variable Fab' region of the antibody. The Fc portion of the antibody is comprised of a human IgG1 constant region. The human region of the molecule decreases immunogenicity and preserves functional immune capacity [11].

There are several mechanisms that have been proposed to explain the actions of infliximab. It is a TNF- α neutralizer, binding both monomeric and trimeric forms of the soluble TNF- α [12]. It was assumed that infliximab works by blocking the action of TNF- α by preventing it from binding to its receptor on the cell surface [13]. Besides binding and neutralizing soluble TNF- α , infliximab also binds to and blocks membrane TNF- α [14–16], which, through reverse signaling, induces sufficient secretion of proinflammatory cytokines to cause colitis.

Etanercept, a recombinant TNF receptor/immunoglobulin G fusion protein that mainly neutralizes soluble TNF- α , is highly effective in rheumatoid arthritis but not in Crohn's disease. Thus, TNF- α -neutralization is not solely responsible for the powerful action of infliximab in Crohn's disease. Van den Brande et al. have sought the additional mechanism that underlies between infliximab and etanercept [17]. They showed that, while both agents effectively neutralized soluble TNF- α , only infliximab induced apoptosis of activated peripheral blood lymphocytes and lamina propria T cells via activation of caspase 3. Other studies have shown that complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity also play a role in infliximab-induced apoptosis [18, 19]. Programmed cell death/apoptosis of TNF- α -expressing activated T cells [20–22] in the peripheral blood as well as in the mucosa by infliximab explains its efficacy in Crohn's disease and other granulomatous disorders. However, this difference may also explain for the increased incidence of tuberculosis and histoplasmosis with the use of infliximab compared to etanercept [19, 23]. Infliximab also induces apoptosis of monocytes [24], and the number of peripheral blood monocytes decreases after treatment [25].

Infliximab treatment has been shown to alter the cytokine balance in the serum and lamina propria of Crohn's disease. Decreases in the production of proinflammatory cytokines IL-1, IL-6, IL-18, GM-CSF, and IFN- γ by peripheral blood and lamina propria lymphocytes have been observed [26–31]. Soluble TNF receptor types 1 and 2 that were elevated in the serum of patients with active Crohn's disease declined with infliximab treatment [32]; however, the precise physiological significance of circulating soluble receptors remains undetermined. Till et al. showed that infliximab reduced signal transduction and activation of transcription 3 phosphorylation in cultured T cells taken from patients with Crohn's disease through neutralization of soluble TNF [33]; which may lead to decreased cytokine production. Ebert also demonstrated that infliximab increased the peripheral blood monocyte and lamina propria lymphocyte production of the anti-inflammatory cytokine IL-10 [34], further providing evidence that infliximab reconstitutes the unbalance between pro- and anti-inflammatory cytokines. Treatment with infliximab also resulted in a significant increase in the numbers of circulating regulatory T cells [35–38] that was accompanied by significant increases of serum TGF- β 1 and IL-10 [35].

Danese et al. showed that infliximab significantly reduced expression of CD40 and vascular cell adhesion molecule-1 levels on intestinal endothelial cells [39]. CD40, also a member of the TNF receptor family, is found on the surface of B cells, epithelial and endothelial cells, and CD40–CD40L ligation results in the upregulation of cell surface markers and inflammatory signals. Blockade of CD40–CD40L by infliximab resulted in decreased levels of lymphocyte recruitment and proinflammatory cytokine production.

Epithelial cells possess barrier function and are also part of the innate immune response in Crohn's disease [40], and can also be a target of infliximab. Zeissig et al. showed that patients with Crohn's disease had a higher index of apoptotic epithelial cell foci than healthy controls that returned to normal levels after infliximab treatment [41], suggesting a protective role of infliximab on epithelial cells. Bouchaud et al. showed that infliximab decreased the serum levels of the proinflammatory cytokine IL-15, through trans-membrane TNF reverse signaling that activated TACE,

leading to the release of soluble IL-15R α by epithelial cells that subsequently neutralized circulating IL-15 [42].

Treatment of infliximab has been recognized to induce greater degrees of mucosal healing compared to traditional medications. Ex vivo model of gut T-cell mediated injury indicated that infliximab inhibited production of matrix metalloproteinase that facilitates tissue damage [43]. Infliximab also enhanced the production of tissue inhibitor of metalloproteinases, and induced the migration of myofibroblasts, which may reduce metalloproteinase activity and facilitate the process of wound healing [44].

Recent studies have focused on monocytes/macrophages and dendritic cells, the major antigen-presenting cells in immune-mediated disorders. As mentioned earlier, infliximab induces apoptosis of monocytes and reduces their capacity to produce inflammatory cytokines. Vos et al. showed that treatment with infliximab induced CD206 positive regulatory macrophages that have immunosuppressive properties and an important role in wound healing [45]. Studies in rheumatoid arthritis patients have shown that infliximab reduced the expression of TNFR2 and the activating Fc γ receptor I on monocytes [34, 46]. A decrease in the percentages of circulating CD11c⁺ and CD123⁺ dendritic cells has also been observed and expression of the activation marker CD83 was also reduced on dendritic cells in rheumatoid arthritis patients treated with infliximab. Furthermore, after 6 months of treatment, clinical improvement was associated with a less active phenotype of dendritic cells [47]. This study supports the role of anti-TNF- α in preventing the maturation and activation of dendritic cells. Infliximab reduced TLR-2 and TLR-4 expression on monocytes of spondylarthritis and rheumatoid arthritis patients leading to lower levels than in controls, and to impaired lipopolysaccharide-induced TNF- α production [48].

Clinical Efficacy of Infliximab Therapy in Crohn's Disease

Induction of Remission

Infliximab (initially termed cA2) was first demonstrated to have efficacy in rheumatoid arthritis in 1994 [49]. Subsequently, the first reported open-label experience with infliximab in 1995 described nine patients with steroid-refractory Crohn's disease who were treated with a single infusion of infliximab 10 mg/kg. The investigators reported remissions in eight patients after 4 weeks [50]. Two years later, the efficacy of infliximab remission was confirmed in a larger randomized placebo-controlled trial that enrolled 108 patients with moderate-to-severe Crohn's disease to receive a single infusion of placebo or 5, 10, or 20 mg/kg of infliximab. The primary endpoint was a reduction in Crohn's Disease Activity Index (CDAI) of 70 points or more after 4 weeks. Response rates were 17, 81, 50, and 64%, respectively. Thirty-three percent of patients who received infliximab achieved remission (CDAI <150) compared to 4% in the placebo group. At week 12, the end of the follow-up period, an increasing trend for C-reactive protein (CRP) and CDAI scores was observed in the responders; indicating a potential relapse of inflammatory activity. Initial nonresponders to the first dose of infliximab received a second infusion of infliximab, leading to response and remission rates of 34% and 17%, respectively.

Maintenance of Remission

Rutgeerts et al. [51] began to study the potential maintenance benefits of infliximab by re-treating 73 patients who had responded to the initial infusion of infliximab (or placebo) from the "Targan"

study [52] who randomized to receive four subsequent infusions of infliximab, 10 mg/kg, or placebo every 8 weeks. The clinical benefits were “maintained” throughout the period of retreatment in nearly all of the infliximab-treated patients, but not with placebo infusions. The clinical benefits were associated with maintenance of infliximab serum concentrations and the treatments were well tolerated [51].

The efficacy of infliximab for maintenance of remission in Crohn's disease was more formally investigated in A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen (ACCENT-I) [53]. Initially, 573 patients with active Crohn's disease were enrolled and received one, open-label, 5 mg/kg, infusion of infliximab. Three hundred and thirty-five patients responded within 2 weeks (CDAI reduced by 70 points) and were randomized to receive subsequent every 8-week infusions of placebo or infliximab, 5 or 10 mg/kg. The primary endpoint in this study was remission (CDAI < 150) at week 30. A secondary endpoint was the time to loss of response up to week 54 in initial responders. At week 30, remission rates were 21, 39, and 45%, respectively, for patients who were receiving placebo, infliximab 5 or 10 mg/kg. This trend continued to week 54 as the time to loss of response was longer for patients on infliximab (19 weeks on placebo, 38 weeks at 5 mg/kg, >54 weeks at 10 mg/kg). In addition, more patients were able to discontinue steroids while receiving infliximab vs. placebo: 29% vs. 9% [53].

Subsequently, in a single-center cohort of 614 Crohn's disease patients, 89% initially responded to infliximab and were followed with a median follow-up time of 4.6 years [54]. Sixty-three percent of the infliximab responders maintained benefit until the end of the follow-up period. Infliximab was discontinued in 37% of the patients; the most frequently reported reason was loss of response (22%).

Fistulizing Crohn's Disease

As several patients in the “Targan” study were noted to have improvement in fistula drainage, infliximab was evaluated for the treatment of draining in a randomized placebo-controlled trial that enrolled 94 Crohn's disease patients with draining abdominal or perianal fistula [55]. Patients received three doses of infliximab 5 or 10 mg/kg, or placebo at 0, 2, and 6 weeks. The primary endpoint was a reduction of 50% or more in the number of draining fistulas at two or more consecutive study visits within the 14-week trial. The primary endpoint was reached by 68%, 56%, and 26% of patients, respectively. The beneficial effect of infliximab was mostly noticed within 2 weeks of the first infusion and lasted for approximately 3 months.

The ACCENT II study then assessed the maintenance benefits of infliximab on recurrence of fistula drainage [56]. Three hundred and six patients with one or more draining Crohn's disease fistula initially received infliximab 5 mg/kg at weeks 0, 2, and 6. Those who responded (50% reduction in draining fistula at weeks 10 and 14) were randomized to continue with every 8-week infusions of infliximab 5 mg/kg or placebo. The 195 responders and 87 nonresponders were then randomized to receive placebo or infliximab, 5 mg/kg every 8 weeks up to week 54. The time to loss of response (primary endpoint) was significantly prolonged for patients receiving maintenance infliximab (median >40 weeks) compared to responders who were randomized to placebo maintenance (14 weeks, $p < 0.001$). After 1 year (week 54), 36% of patients receiving maintenance infliximab had a complete absence of draining fistula compared with 19% of patients who received placebo maintenance infusions.

Although patients in the ACCENT II study may have “failed” prior treatment with antibiotics a small follow-up study has suggested that the addition of ciprofloxacin to infliximab for treatment of fistulizing disease leads to higher response rates. After 12 weeks of combined treatment, 73% of patients had a >50% reduction in draining fistula compared to 39% in the group that received infliximab monotherapy [57].

Prevention of Postoperative Recurrence

Endoscopic recurrence rates for Crohn's disease after surgical resections are as high as 85% [58]. Inflammation typically returns at, or proximal to, the anastomotic site. In a small randomized, placebo-controlled "pilot" study, 24 patients who had undergone an ileal or ileocolonic resection were randomized to postoperative therapy with infliximab or placebo [59]. Patients who had been receiving preoperative thiopurine therapy were allowed to continue postoperatively. The primary endpoint was the proportion of patients at 1 year that experienced recurrence of disease. At 1 year, 9% (infliximab) vs. 85% (placebo) were diagnosed with endoscopic recurrence of disease, despite the higher rate of smokers and the lower rate of immunosuppressives in the infliximab group. Further studies are needed in larger trials to confirm these data and to determine the optimal duration of treatment.

Controversies Regarding Infliximab Therapy for Crohn's Disease

Mucosal Healing

Mucosal healing, the absence of gross or deep ulcerations, has been associated with improved long-term outcomes in Crohn's disease [60]. Several trials have demonstrated the potential for infliximab to induce mucosal healing in Crohn's disease. Rutgeerts et al. assessed mucosal healing in a subgroup of patients enrolled into ACCENT I who agreed to pre- and posttreatment ileo-colonoscopy. Complete mucosal healing at week 10 was observed in 31% of week 2 responders who had received three doses of infliximab compared with 0% of week two responders who subsequently received placebo maintenance. Further, at week 54, 50% of patients receiving maintenance infliximab infusions had complete mucosal healing compared with 7% of patients who received "episodic" infliximab infusions. While there was a poor correlation between the endoscopic findings and clinical outcomes, complete mucosal healing was associated with fewer hospitalizations and surgeries during the 1-year period of observation [61].

The Belgian group then demonstrated that, in their single-center cohort of 183 patients with active Crohn's disease that responded to infliximab therapy and underwent a colonoscopy before and during infliximab therapy, 68% had partial or full mucosal healing observed at follow-up colonoscopy [62]. In their experience, patients with mucosal healing had more clinical benefit from infliximab (65% vs. 40%), required less abdominal surgery (14% vs. 38%) and hospitalizations (42% vs. 59%) compared to patients on infliximab without mucosal healing.

Most recently, the SONIC trial also demonstrated the ability of infliximab induction and maintenance therapy to induce mucosal healing after 30 weeks with infliximab monotherapy (30%) or combination therapy with azathioprine (44%) than patients who received azathioprine monotherapy (13%) [63].

Scheduled vs. Episodic Treatment

The ACCENT trial also afforded the ability to assess scheduled, every 8-week maintenance therapy with infliximab compared to patients who lost response and were treated on an "episodic," essentially as needed basis. Those patients receiving regularly scheduled infusions had more mucosal healing and fewer hospitalizations and surgery than patients who received episodic infliximab treatment [61].

Similarly, in the Belgian cohort of 614 Crohn's disease patients that responded to infliximab, scheduled treatment led to higher steroid withdrawal rates, lower hospitalization rates (26% vs. 47%), and less need for surgery when compared to patients on episodic treatment [54].

Concomitant Immunomodulation

From the onset of clinical investigations with infliximab in Crohn's disease studies have primarily enrolled patients who were *refractory* to conventional agents, including thiopurine and methotrexate immunosuppressives. Similarly, the development program for infliximab in rheumatoid arthritis evaluated infliximab in combination with methotrexate vs. methotrexate alone, and infliximab monotherapy vs. infliximab and methotrexate combination therapy [64, 65].

In the initial trials, described earlier, with infliximab in Crohn's disease the patients who were receiving azathioprine (or methotrexate) prior to enrolling in the clinical studies were allowed to maintain therapy with the immunosuppressive. Although there were no differences in clinical outcomes for patients who were receiving combination therapy after failing "immunosuppressive monotherapy" in the ACCENT study [53] it became clear that patients who were receiving concomitant immunosuppressives had lower rates of immunogenicity (i.e., development of antibodies to infliximab) [51, 66–68]. Immunogenicity to infliximab has been associated with infusion reactions (acute and delayed) and reduced duration of response to infusions [66, 67]. Nevertheless, the lack of impact of immunogenicity on clinical outcomes at 1 year [69] along with the increased risk of serious infections in patients treated with combination therapy [70] and the potential for lymphomas (see below) raised the issue of the benefit/risk of combination therapy [71].

Prospective trials evaluating the potential benefits and risks of combination therapy were initiated, first, in Europe. Lemann and colleagues [72] randomized 112 steroid-refractory patients to receive an induction schedule of infliximab (three infusions of 5 mg/kg) or placebo infusions. The patients were stratified according to whether they had been receiving azathioprine or 6-mercaptopurine (6-MP) for more than 6 months (55 patients) or were naïve to prior treatment with these medications (57 patients). The latter group was started on azathioprine or 6-MP at the start of the study. The primary endpoint was steroid-free remission at week 24 at which time the success rate was higher in the infliximab group (57%) vs. placebo (29%). Patients with, and without, previous immunomodulator exposure showed higher response rates to infliximab compared to placebo; however, after a follow-up period of 4 years, 73% of the previously azathioprine-naïve patients had relapsed, indicating the potential need for continuation of infliximab therapy in this group of patients [73].

Around the same time as the aforementioned study was underway, investigators in Belgium tried to determine whether long-term combination therapy was necessary by randomizing a group of patients who were in a stable remission on combination infliximab and azathioprine to withdrawal of azathioprine (infliximab monotherapy) vs. continuation of combination therapy [74]. The Infliximab Maintenance Immunosuppressives Discontinuation trial (IMID) enrolled 80 Crohn's disease patients that had originally failed immunomodulators and were subsequently started on infliximab. After 6 months, patients were randomized to either continue or discontinue their thiopurine therapy. The primary endpoint of this study was the proportion of patients that either stopped infliximab due to loss of response or required a dosing interval dose adjustment. After the 18-month follow-up period, similar rates for stopping or adjusting infliximab therapy were observed in both groups (60% continued, 55% discontinued), indicating the lack of significant benefit of combination therapy for maintenance purposes after at least 6 months of successful combination therapy. However, in the group that discontinued azathioprine, the mean CRP levels were higher and infliximab trough levels were lower than the group who maintained combination therapy, suggesting that a longer follow-up period might have been necessary to confirm advantages to long-term combination therapy.

While the prior studies confirmed the effectiveness of maintenance therapy with thiopurines, they did not directly address the prospective benefits or risks of initiating combination therapy in patients who were immunosuppressive naïve. This question of “comparative effectiveness” was addressed by the “study of biologic and immunomodulator naïve patients in Crohn’s disease,” “SONIC” study [63]. The SONIC trial enrolled 509 patients with moderate-to-severe Crohn’s disease who were naïve to immunosuppressive and biological therapy and, who may also have been failing low-dose steroids. These patients were randomized to receive infliximab, azathioprine, or combination (infliximab and azathioprine) therapy. The primary endpoint was steroid-free remission at week 26. At the end of the trial, 57% of patients on combination therapy with infliximab and azathioprine were in steroid-free remission. Monotherapy with azathioprine or infliximab led to steroid-free remission rates of 30% and 44%, respectively. Results from week 50 showed a similar trend. Further analyses revealed that only patients with an increased CRP or endoscopic inflammation at baseline showed benefit from the combination therapy. Of equal importance, there were no increased signals of toxicity for patients randomized to combination therapy and, indeed, overall side effects were less than patients receiving monotherapy (as many side effects related to Crohn’s disease were reduced with more effective therapy). Based on this study, patients with active Crohn’s disease, naïve to immunosuppressive therapy, can benefit from combination therapy to achieve steroid-free remission.

Top-Down vs. Step-Up Therapy with Infliximab

To date, the clinical trials leading to the worldwide marketing of infliximab for Crohn’s disease have enrolled *refractory* patients who have had active disease despite therapy with conventional agents. Meanwhile, trials in early rheumatoid arthritis that have demonstrated the ability to *modify* disease behavior by preventing the development or progression of joint erosions or joint space narrowing [75] have led to consideration of earlier interventions in Crohn’s disease. Investigators from the Benelux countries designed a clinical trial to try to address whether a “step-up” approach using corticosteroids vs. early introduction of infliximab induction followed by azathioprine maintenance therapy would lead to improved clinical outcomes and reduced steroid exposure in steroid-naïve patients with moderately active disease. One hundred thirty-three steroid-naïve Crohn’s disease patients were randomized to receive three infusions of infliximab and azathioprine, and received additional infusions of infliximab or finally steroids, if necessary. This was compared to a conventional sequential strategy with two courses of steroids, followed by azathioprine and then infliximab if needed [76]. The primary endpoint for this trial was steroid-free remission at 6 and 12 months. At 52 weeks, 62% of patients receiving early combination therapy were in steroid-free remission, compared to 42% in the step-up approach. Mucosal healing rates were compared in a separate sub-study, showing that complete mucosal healing was achieved in 73% of patient on early combination therapy compared to 30% of patients on step-up therapy. Further follow-up after 4 years observed that 71% of the patients with complete mucosal healing at year 2 were still in steroid-free remission, compared to 27% of patients with endoscopic disease activity [77].

The above-mentioned trial is important in identifying the potential for infliximab therapy to spare corticosteroids and induce mucosal healing. Unfortunately, from the standpoint of comparative effectiveness it does not address optimal management with the individual treatment options. Future studies might compare steroid induction with a thiopurine from the onset to infliximab induction and maintenance, to infliximab induction followed by azathioprine maintenance. Similar studies are also needed to determine whether patients with a poor prognosis according to clinical, serologic, and/or genetic criteria will benefit from earlier intervention with anti-TNF therapy.

Loss of Response to Infliximab

Approximately 10–30% of Crohn's disease patients do not respond to induction therapy with infliximab. It is critical to ascertain that patients embarking on infliximab (or any therapeutic change) have active inflammation as patients enrolled into clinical trials without evidence of active mucosal disease or elevations in CRP had no difference in response with infliximab compared to placebo [61, 63]. For the most part, determination of response or loss of response can be determined after the first two infusions [66] and these primary nonresponders generally do not benefit from additional infliximab infusions or switching to a different anti-TNF agent [78].

Similarly, patients that lose response after obtaining initial benefit from infliximab, so-called secondary nonresponders, require further assessment to determine the cause for recurrence of symptoms. It is critical to comprehend the concept of a maintenance agent that prevents relapse and that the pharmacokinetics of infliximab are quite variable amongst individuals (see above). Hence, we query all of our patients on infliximab maintenance therapy whether they feel any different the week(s) before their infusion compared to the week(s) after. It was recognized from the ACCENT I experience [53, 61] that a proportion of patients were able to regain (lost) responses by increasing doses from 5 to 10 mg/kg. Similar dose adjustments are frequently required in the setting of rheumatoid arthritis [79, 80] and, in clinical practice, many Crohn's patients require dose adjustments to maintain response [81]. Again, reconfirmation of active inflammation is essential before considering patients as secondary nonresponders. Once a patient has a documented loss of response in the presence of active inflammation, assessments of immunogenicity and serum levels of infliximab can be useful in determining appropriate measures to regain response [78, 82].

Patient with low trough levels of infliximab usually respond to a dose increase to 10 mg/kg or a shortening of the infusion interval [78]. In the ACCENT I trial, 88% of the patients that lost response to infliximab after initial benefit regained response with a dose increase to 10 mg/kg [83]. The Leuven single-center experience suggests that it may be possible to reverse the dose increase or the interval shortening [54]. The reduction of the infliximab infusion interval in 108 Crohn's disease patients was successfully reversed in 31 patients. Reversal of a dose increase was possible in 103 of 144 patients that either underwent a dose increase or re-induction schedule with infliximab. Sometimes this was accomplished by the introduction of a thiopurine or methotrexate.

A common contributor to the reduced pharmacokinetics of infliximab is development of immunogenicity, that is, antibodies to infliximab (previously termed HACA; human antichimeric antibodies) [82, 84–86]. While antibodies to infliximab may be neutralizing or nonneutralizing and do not impact on the short-term responses to infliximab [87] in patients who do lose response, the measurement of infliximab blood levels may provide rationale for either dose escalation or switching to an alternative anti-TNF agent or mechanistic target [78, 88].

Infliximab Trough Levels and Immunogenicity

Infliximab, similar to all biologics is a nonnatural foreign protein and, further, a partial mouse-human chimeric monoclonal antibody [66, 87]. The formation antibodies to infliximab has been associated with infusion reactions and shortened duration of response [67] but, as mentioned earlier, was not associated with long-term outcomes in the ACCENT I study [69]. Taken together, despite the interpretation from ACCENT I, the development of antibodies to infliximab is not a “positive” outcome [87] and several strategies have been identified that can reduce the formation or presence of antibodies to infliximab [68, 84] including induction and regularly scheduled maintenance infusions (compared with episodic infusions), pretreatment with corticosteroids, and the use of

concomitant immunosuppressives [82]. In the ACCENT I trial, scheduled infliximab treatment led to 9% antibody formation vs. 28% in patients that were treated episodically [83]. Concomitant immunosuppression also reduces antibody formation to infliximab. In a cohort of 125 Crohn's disease patients treated with episodic infliximab, 75% of patients without immunosuppressive therapy developed antibodies compared to 43% of patients on immunosuppressives [67]. The same trend was observed in the ACCENT I trial [89].

The latter study underlined the importance of prevention of antibody formation to infliximab by showing the inverse correlation between antibody formation and long-term clinical efficacy of infliximab. Although there were no statistical differences in the proportion of responders who were antibody to infliximab-positive vs. -negative, the duration of response to infliximab was reduced from 71 to 35 days if antibodies to infliximab were present, and this was associated with an increased risk of infusion reactions and consequently lower infliximab trough levels. Concomitant immunosuppression reduced antibody formation and was associated with increased infliximab trough levels with episodic therapy [67] and, in patients enrolled in the SONIC trial, those on combination therapy, compared to infliximab monotherapy, had lower rates of antibodies to infliximab (1% vs. 14%) and higher trough levels (3.5 vs. 1.6 mg/ml) [63] that were associated with higher rates of steroid-free remission rates at week 26 when compared to patients with lower or undetectable trough levels (74% vs. 59%). Ultimately, the trough levels may be more predictive of long-term responses than presence, or absence, of antibodies to infliximab [87].

Discontinuation of Infliximab Therapy

It is unclear how long Crohn's disease patients should continue infliximab therapy. In a cohort of 547 Crohn's disease patients that responded to infliximab, only 20% were able to successfully stop infliximab therapy while in remission and the long-term outcome of these patients has not, yet, been described [62]. In contrast, a study that recruited 115 patients in steroid-free remission for more than 1 year while on azathioprine and infliximab showed a relapse rate of 57% at 1-year after stopping infliximab [90]. While patients who achieve a complete response (steroid tapering and mucosal healing) have the greatest likelihood of a prolonged response after discontinuing infliximab the data are still insufficient to determine individual risk stratification whom to cease therapy with infliximab and/or an immunosuppressive to minimize the long-term risk of disease recurrence.

Safety of Infliximab Therapy in Crohn's Disease

The assessment of complications from infliximab therapy in Crohn's disease is complicated by risks associated with the duration of follow-up, underlying disease, disease-related complications, age, and concomitant medications [91–93]. Nevertheless, TNF is important for maintenance of immunity, particularly against intracellular organisms [94, 95].

Infections

In any series of patients, infections are likely to be the most commonly reported adverse event. Fortunately, most are not serious. It has been debated whether infliximab therapy is associated with an increased risk of infectious complications. In the ACCENT I trial in which all patients had been exposed to infliximab, by week 54, 32% of all patients required antimicrobial treatment for infections.

Although patients in the placebo-maintenance group had more infections that required antimicrobial treatment, this difference was not statistically significant [53] and the risk of infection was not related to the number of infliximab infusions. Serious infections occurred in 3–4% in both the placebo as well as the infliximab-maintenance treatment groups.

In a series of 500 Crohn's disease patients treated with infliximab at the Mayo Clinics [96], fifteen had serious infections including eight patients with pneumonia, two cases were fatal. A subsequent report described the safety of infliximab in 734 IBD patients treated over a mean follow-up time of 54 months compared to a group of 666 "control" IBD patients not treated with infliximab [97]. Fifty-nine serious infections were observed in the infliximab group vs. 77 in the control group. Concomitant therapy with azathioprine or methotrexate did not influence the risk for infection, but a significant increased risk occurred in periods with concomitant steroid use. Similarly, the TREAT registry, including over 3,000 Crohn's disease patients treated with infliximab compared to patients who had not received infliximab confirmed that the use of steroids and narcotic medication, but not infliximab itself, were independent risk factors for infections during infliximab therapy [98].

The risk of opportunistic infections during infliximab therapy is increased but the absolute numbers are relatively low and vary between 0.3 and 0.9% [99]. In a case-control study, 100 patients with IBD and opportunistic infections during infliximab therapy were compared to 200 matched controls without opportunistic infections [70]. The risk of opportunistic infections was significantly greater when infliximab was combined with concomitant immunosuppressive therapy. The odds ratio for an opportunistic infection during infliximab therapy was 4.4, compared to 14.5 when combined with steroids and thiopurine therapy [70]. It should also be noted that in this series from the Mayo Clinic the risk of opportunistic infections also increased with age; the odds ratio was 3.0 for patients above the age of 50 receiving infliximab compared to younger patients. The impact of age on the risk of infections for patients receiving infliximab was confirmed in a retrospective study that also demonstrated that patients older than 65 receiving infliximab or adalimumab were at greater risk of serious infections compared to younger patients (11% vs. 2.6%) [100].

Somewhat surprising, but also reassuring were outcomes in the SONIC trial where the rate of serious infections was 5.6% for patients receiving azathioprine, 4.9% with infliximab, and 3.9% for patients on the combination therapy [63]. The lower rate of infectious complications in the combination group is likely due to the reduced rate of infections attributed to disease activity, for example, abscesses. Thus, corticosteroid therapy and poor control of disease activity should be considered factors that increase the risk of serious infections in patients with Crohn's disease, whereas, infliximab therapy, by virtue of controlling the disease and reducing steroid exposure may, in fact, reduce the risk of serious infections. However, despite these observations patients still require monitoring for infections and, in particular, older patients and those on steroids or other immunosuppressives.

In addition, opportunistic infections with intracellular pathogens, including tuberculosis, histoplasmosis, cryptococcosis, coccidiomycosis, listeriosis, etc., have been reported with infliximab therapy. In particular, tuberculosis has been an important preventable infection for patients embarking on infliximab therapy. Subsequent to the introduction of infliximab in 1998, 70 cases of tuberculosis were reported from clinical trials by 2001 [94]. Subsequently, with active screening of patients with skin tests (PPD) and chest X-rays the rate of tuberculosis was markedly diminished and of the initial cases, 48 patients developed tuberculosis within the first three infusions. From 2001 to 2006, 130 patients on infliximab therapy were diagnosed with active tuberculosis in the USA [101]; the most important risk factor for activation of tuberculosis was concomitant immunosuppressive therapy. In the latter series, 34 patients had a negative PPD before initiation of infliximab therapy which has led to the recommendation that every patient should be screened by a careful history of prior tuberculosis contact, as well as a PPD and chest X-ray prior to starting infliximab therapy. Patients who have received a BCG vaccine may be screened with an interferon-based assay to rule out active tuberculosis [93]. Patients with latent tuberculosis should be started on isoniazid before starting infliximab, and active tuberculosis should be fully treated before initiating anti-TNF therapy.

It should also be considered that patients receiving infliximab may also have worsened outcomes from opportunistic infections; again, potentially related to age, complications of Crohn's disease (including malnutrition), and concomitant medications [93]. Reactivation of hepatitis B is another potential infectious/immunologic complications requiring evaluation for hepatitis B prior to initiating therapy.

Vaccinations

Prior to initiating infliximab therapy an evaluation is required to assess for risk factors for infections. As mentioned, patients should be assessed for latent (or active) tuberculosis and hepatitis B. Concurrently, and preferably, prior to initiating any immunosuppressive therapy an update on the vaccination status is strongly advised [102, 103]. Pneumococcal and influenza vaccines should be updated routinely. Young women should be vaccinated against HPV and students for meningococcus.

In contrast, live vaccines, including mumps, measles, rubella, yellow fever, typhoid, polio, and tuberculosis, are contraindicated [103]. Household contacts of Crohn's disease patients on infliximab should undergo their routine vaccinations as well.

Infusion Reactions

Infusion reactions to infliximab can be categorized as either acute or delayed [104, 105]. Acute reactions typically occur during the infusion but may present within 4 h after completing administration. These reactions are most typically characterized by dizziness, headache, diaphoresis, and nausea but more severe reactions can present with fever, dyspnea and/or stridor, palpitations, or hypotension. The exact nature of these reactions is unclear but, in the absence of urticaria and bronchospasms, these reactions mostly do not fulfill the criteria of a true anaphylactic reaction. Most infusion reactions are mild and can be managed by slowing the infusion rate and the use of acetaminophen, antihistamines, and iv fluids [105]. More severe reactions require discontinuation of the infusion and intravenous hydrocortisone. As described in a retrospective series of 682 IBD patients receiving infliximab, most patients can successfully be retreated with premedication with hydrocortisone, acetaminophen, and/or diphenhydramine [97]. While 115 patients experienced acute infusion reactions after a median of two infusions, only 19 patients had to discontinue their therapy due to these reactions. However, it should be noted that the presence of acute infusion reactions does not bode well from a prognostic standpoint as, in another study, Crohn's patients with infusion reactions were less likely to be in remission at 1 and 5 years and were more likely to require surgery [106]. Antibodies to infliximab increase the risk of acute infusion reactions but are not necessary for the reactions to occur. In the ACCENT I trial, 16% of the infusions resulted in infusion reactions in patients positive for antibodies, compared to 8% and 3% for a negative or indeterminate antibody status [53].

Delayed-type infusion reaction can occur from 1 to 14 days after infusion. Symptoms include joint pains, myalgia, and skin rashes and are more typical of serum sickness-like reactions and, while the pathogenesis is unknown, the formation of immune complexes has been suggested [105]. Most mild reactions will resolve spontaneously although treatment options include acetaminophen and a short course of steroids. In 50 of 682 IBD patients receiving infliximab, a delayed-type infusion reaction occurred after a median of three infusions, and in 46/50 required discontinuation of infliximab therapy either due to the infusion reactions or loss of response [97].

Autoimmunity

Tumor necrosis factor inhibits autoantibody formation; hence, inhibition of TNF would be expected to increase the likelihood of developing autoantibodies. Furthermore, it is likely that apoptosis may increase immune exposure to antinuclear antibodies. Hence, not unexpectedly, infliximab is associated with the formation of autoantibodies, including antinuclear antibodies (ANAs) [107, 108]. In 125 Crohn's disease patients on infliximab, 57% tested positive for ANAs after 2 years. Forty-three ANA-positive patients were further subtyped, 33% had double-stranded DNA, 40% had single-stranded DNA, and 21% had antihistone antibodies. Only two patients, both positive for antibodies to histone and double-stranded DNA, developed drug-induced lupus [108]. The development of autoantibodies does not affect the efficacy of infliximab therapy but, on occasion lupus-like reactions may lead to transient discontinuation of therapy during which time ANA titers will decline [109].

Malignancies

Similar to the risk of infectious complications, neoplastic complications such as lymphomas may be related to the underlying disease, concomitant medications, or exposure to infliximab [110, 111]. Furthermore, the relatively short duration of observation (usually less than 1 year) in clinical trials may falsely lower the apparent risk.

The TREAT registry with >3,000 IBD patients on infliximab indicated a lymphoma risk of 0.06 per 100 patient-years compared to 0.05 per 100 patient-years for IBD patients not receiving infliximab [98]. Of the first 500 Crohn's disease patients treated with infliximab at the Mayo clinic followed for a median of 17 months, one patient developed a Hodgkin's lymphoma and one patient developed a non-Hodgkin's lymphoma [96]. However, 75% of the patients in this cohort were also taking thiopurines, which by themselves increase the rate of lymphomas [112]. In a retrospective series of 734 IBD patients on infliximab, 15 malignancies and eight nonmelanoma skin cancers were observed [97], including one non-Hodgkin's and one Hodgkin's lymphoma. The control group, consisting of 667 IBD patients who never received infliximab, experienced 37 malignancies and five nonmelanoma skin cancers, including three non-Hodgkin's lymphomas. Although the above-mentioned studies failed to show an increased risk for lymphomas during infliximab therapy, an increased risk was reported in a large meta-analysis [113] that included 26 studies with 8,905 Crohn's disease patients on anti-TNF therapy. Thirteen lymphomas were detected, yielding a rate of 6.1 per 10,000 patient-years, significantly more than the expected 1.9 per 10,000 patient-years. This increased risk translated to one lymphoma per 685 treated patients. However, almost all lymphoma patients were or had been previously treated with thiopurines and the authors continue to conclude that "the absolute rate of these events remains low and should be weighed against the substantial benefits associated with treatment."

While most lymphomas are treatable and curable, the hepatosplenic T-cell lymphoma variant is a rare type of non-Hodgkin's lymphoma with an aggressive course and mostly fatal outcome. Until recently, only 36 patients' cases have been reported in the IBD population: most patients were less than 35 years old, males, on thiopurine therapy (100%), and on infliximab (56%) [114]. The pathogenesis of this type of lymphoma is currently unclear, although chromosomal abnormalities have been suggested as a risk factor related to thiopurine therapy [114]. Although the absolute incidence of this malignancy is very low, the aggressive course warrants careful consideration before initiating dual immunosuppressive therapy in young males.

Infliximab has been identified as a risk factor for nonmelanoma skin cancer in rheumatoid arthritis patients [115], but multiple randomized controlled trials and cohort studies did not show this trend in Crohn's disease patients [96]. Furthermore, infliximab does not seem to be a risk factor for solid tumors although there is one intriguing case report of a patient with chronic obstructive pulmonary disease who developed a nonsmall cell lung cancer that resolved spontaneously after discontinuation of infliximab therapy [116].

Pregnancy

Infliximab is classified by the FDA as a class B drug for use during pregnancy, but the data on safety of infliximab during pregnancy is limited. Ninety-six pregnant women on infliximab, either for Crohn's disease or rheumatoid arthritis, have been reported from a pregnancy registry [117]. The numbers of live births, miscarriages, and therapeutic terminations were comparable in Crohn's disease patients on or off infliximab. However, it should be noted that most women stopped infliximab after conception. The TREAT registry reported 33 pregnancies during infliximab therapy [98]. The numbers for miscarriages and neonatal complications were similar between women on infliximab compared to the control group without infliximab therapy.

Large-sized immunoglobulins do not pass the placenta in the first trimester of pregnancy. However, transfer is possible in the second and third trimester. Infliximab is an IgG1 antibody and does cross the placenta, presumably in the third trimester. Transfer was indirectly demonstrated in five mothers on infliximab therapy during the last trimester of pregnancy. All newborns had detectable blood levels of infliximab after delivery and the decreasing levels during follow-up suggested placental transfer rather than transfer by breast milk. To our current knowledge, the use of infliximab to maintain maternal health in appropriate patients outweighs any risk to the fetus [118, 119]. A strategy of discontinuation of infliximab in the last trimester of pregnancy has been proposed in order to prevent placental transfer of infliximab; however, to date, no reports have described the success (or complications) of this approach. Long-term follow-up is needed to determine potential long-term side effects of infliximab in newborns and, similar to patients receiving infliximab, infants exposed to infliximab should not receive live vaccines.

Other Safety Issues

Skin eruptions are frequently reported side effects of infliximab therapy. In a retrospective series of 734 patients on infliximab, 150 patients reported dermatologic side effects [97]. In the latter group, 39 patients were diagnosed with psoriasiform dermatitis. Only two patients had to discontinue infliximab therapy due to these side effects although more recent reports describe this uncommon, but troublesome complication that can lead to discontinuation of therapy [120].

Anti-TNF and infliximab therapy are also contraindicated for patients with evidence of demyelinating disorders, including multiple sclerosis, Guillan-Barré syndrome, and optic neuritis [121–123].

Similarly, infliximab therapy is contraindicated in patients with severe, AHA Class III-IV, heart failure due to potential worsening [124].

Abnormal liver enzyme tests during infliximab therapy have been reported with descriptions of both cholestatic and more hepatitis-like patterns [125, 126]. While most liver enzyme abnormalities will be secondary to concomitant medications or conditions, it is recommended to stop infliximab if liver enzymes exceed three times the upper limit of normal; usually LFT normalize after cessation of therapy and infliximab should not be used in patients with chronic hepatitis B infections [127].

Summary and Conclusion

Infliximab, the first biologic therapy approved for the treatment of Crohn's disease, has markedly changed the prognosis for patients with refractory disease and is beginning to be used earlier in the course to prevent steroid-related complications. For the majority of patients, infliximab can be used safely to induce and maintain remissions, reduce fistula drainage and heal fistula, reduce steroid requirements, and to induce mucosal healing. Effective therapy with infliximab also has been shown to reduce hospitalizations and surgeries. The dosing needs to be individualized to maintain sufficient blood levels throughout the infusion intervals. Immunogenicity is also an important factor related to loss of response and infusion reactions that can be minimized by induction and regularly scheduled maintenance dosing, pretreatment with steroids, and/or concomitant immunosuppressives.

Important issues in daily practice that can limit infliximab's efficacy and safety include: an increased relative risk of opportunistic infections (particularly in older age patients and with concomitant steroid and/or immunosuppressive therapies) and a small risk of lymphoma that is more often associated with thiopurines (including fatal hepatosplenic T cell lymphomas). Higher trough levels and reduction of antibody formation are associated with higher steroid-free remission rates and, in immunosuppressive naïve patients combination therapy with azathioprine has been more effective than monotherapy. For every individual patient, the risks and benefits of infliximab and combination therapy should be weighed to optimize therapy and maintain remission with minimal side effects. Future research is needed to optimize strategies for initiation, continuation, and discontinuation of infliximab therapy.

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Chapter 34

Biologic Treatment of Crohn's Disease: Briakinumab and Ustekinumab

Peter Mannon

Keywords Interleukin-12 • Interleukin-23 • Briakinumab • Ustekinumab

Background

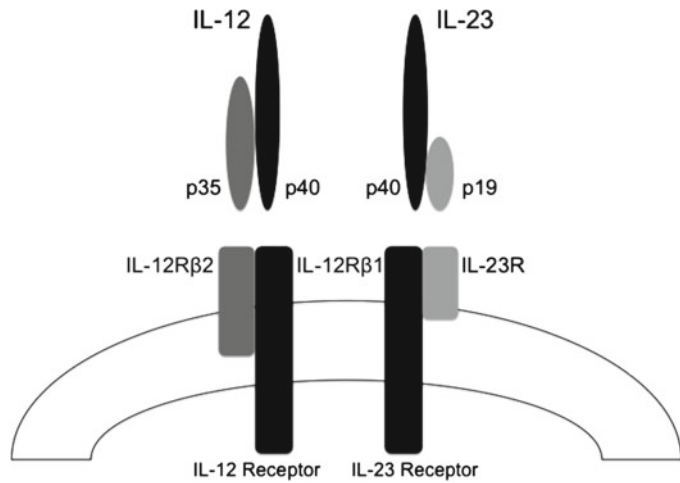
The introduction of IL-12/IL-23 anti-p40 antibodies as a novel therapy for Crohn's disease is a case study in basic immunologic observations derived from animal models of human disease thereby propelling innovative, targeted treatment into the clinic. In this case, IL-12 was initially suggested as the driving force behind the dysregulated immune response in Crohn's disease (IL-23 was discovered sometime later). Based on work from Mosmann and Coffman [1], reactive inflammatory T cell responses were separated into two major types, so-called Th1 T cells that produce interferon-gamma (IFN γ) and Th2 cells that produce IL-4, IL-5, and IL-13. An excess of Th1 cytokine production by lamina propria monocytes and T cells was seen in animal models of Crohn's disease as well as in Crohn's patients [2, 3]. Th1 cells are directly induced by interleukin-12. IL-12 is secreted by antigen-presenting cells activated by particular microbial components; naïve T cells encountering a cognate ligand presented by a monocyte in the presence of IL-12 develop into Th1 cells and secrete IFN γ as part of the Th1 inflammatory response after encountering the same ligand thereafter. Interleukin-12 is a heterodimeric protein composed of two covalently bound subunits, a p35 and p40 protein named for their relative molecular sizes (Fig. 34.1). Interleukin-12 binds to a specific cell surface receptor on T cells, the IL-12 receptor, another heterodimeric protein composed of the IL-12 receptor beta 1 (IL12R β 1) and 2 (IL12R β 2) subunits.

In the late 1990s, data from several studies using animal models of colitis confirmed an important role for IL-12 effects on Th1 T cells in the induction and maintenance of intestinal inflammation [4–6]. In a murine model of Crohn's disease, trinitrobenzene sulfonic acid (TNBS) colitis, administration of anti-IL-12 at Day 0 (before inflammation began) or Day 21 (when colitis is established) prevented or reversed colitis [4]. Another model of Crohn's disease, cell transfer colitis, was also treated by blockade of IL-12 when administered early or late in the induction of colitis [7]. Other studies showed that anti-IL-12 induced apoptosis in large numbers of lamina propria CD4 T cells,

P. Mannon, MD, MPH (✉)

Division of Gastroenterology and Hepatology, University of Alabama at Birmingham,
1825 University Blvd., SHEL613, Birmingham, AL 35294, USA
e-mail: pmannon@uab.edu

Fig. 34.1 The IL-12 and IL-23 cytokines share the p40 subunit but bind to specific receptors. IL-12 (p40 and p35 heterodimer) binds to the IL-12 receptor (IL-12R β 1 and IL-12R β 2 heterodimer) and IL-23 (p40 and p19 heterodimer) binds to the IL-23 receptor (IL-12R β 1 and IL-23R heterodimer)



suggesting a mechanism of action for anti-IL-12 therapeutic effects. So the fact that patients with Crohn's disease have increased lamina propria IL-12 production coupled with the success of targeting IL-12 activity in animal models of disease (especially in treating established colitis) provided compelling evidence for trials of such an agent in Crohn's disease.

The discovery of IL-23 along with its control of IL-17, another cytokine newly appreciated for its role in autoimmune inflammatory disease, forced a revision of the view that IL-12 and Th1 cells were solely responsible for Crohn's disease (and therein pointed to other potential targets for novel therapies). In fact, these discoveries helped to explain some inconsistencies in the Th1–Th2 dichotomy of T cell inflammation (such as why some Th1 animal models of autoimmune disease get worse with blockade of interferon γ [8, 9]), and spurred interest in investigating the roles of IL-23 and IL-17 in IBD. IL-23 is related to IL-12 in that IL-23 is largely produced by cells of the innate immune system and, like IL-12, is a heterodimeric protein composed of a p19 subunit covalently bound to a p40 subunit, the subunit common to the IL-12 molecule (Fig. 34.1). While IL-12 induces Th1 T cells, IL-23 is more important for the maintenance and function of IL-17-secreting cells (Th17 cells) [10, 11]; Th17 T cells are induced by IL-6 and TGF β , whereupon differentiation into Th17 cells is accompanied by expression of cell surface IL-23 receptor making them responsive to IL-23 stimulation. However, given the shared p40 subunit, an anti-p40 antibody would target both IL-12 and IL-23. Therefore it is easy to postulate that IL-12- and IL-23-driven inflammation could both be interrupted by administration of an anti-p40 antibody.

In fact, a role for IL-23 and IL-17 in human IBD, particularly Crohn's disease, was suggested by certain animal models. In the IL-10-deficient mouse model of spontaneous colitis, specifically eliminating IL-23 or IL-17 effects could successfully treat the colitis, whereas eliminating IL-12 did not prevent inflammation [12]. In the cell transfer model of colitis, where colitogenic naïve CD4CD45RB^{high} T cells from wild-type mice are infused into T cell-deficient mice (recombination activating gene (RAG) knockout or severe combined immunodeficiency (SCID) mice), this results in gut inflammatory activity that displays a more pronounced dependency on IL-23. In this transfer model, T cell-deficient recipient mice that also lacked IL-23 (RAG/p19 double knockout) were protected against developing severe colitis (which did not happen with IL-12-deficient RAG/p35 double knockout recipients) [13]. Similarly, mice that received CD4CD45RB^{high} T cells that also lacked the IL-23 receptor and therefore were defective in developing robust Th17 cells [14] were also protected from developing colitis. However, despite the important role of the IL-23/IL-17 axis in the transfer model of colitis, blockade of IFN γ activity (either by treating mice with an anti-IFN γ antibody [15] or transferring T cells defective in IFN γ production [16]) also prevents development of gut inflammation.

Furthermore, there are data that show that an intact IL-23/IL-17 cytokine axis may limit inflammation in animal models since IL-17-deficient T cells used in the transfer model of colitis have earlier onset weight loss and higher expression of inflammatory cytokines in the gut while IL-23-deficient (p19 knockout) mice have more severe TNBS-induced colitis [17, 18].

In addition to the preclinical data, the IL-23/IL-17 axis is associated with human Crohn's disease since, like IL-12 and IFN γ , production of both IL-23 and IL-17 are significantly elevated [19, 20]. Moreover a low-prevalence polymorphism in the coding region of the IL-23 receptor gene confers protection from Crohn's disease, and although the mechanism of this effect is not clear, it has been proposed that the polymorphism results in the production of a soluble receptor splice variant that acts to antagonize IL-23. In summary, while animal models have provided guidance to inflammatory pathway aberrancies in Crohn's disease, namely the IL-12/IFN γ and IL-23/IL-17 cascades, it is currently unclear whether there is a clear benefit to targeting one pathway over the other, but it is clear that an anti-p40 strategy has the potential to block important initiating and supporting cytokines that drive these pathways.

Clinical Trials with Briakinumab in Crohn's Disease

Briakinumab (ABT-874/J695) is a fully human IgG $_1$ λ antibody raised against the p40 subunit of IL-12/IL-23. This agent has been used in two completed Phase II trials in human Crohn's disease that tested efficacy and measured safety. The first trial enrolled 79 patients with active Crohn's disease and treated them with subcutaneous injections of placebo, 1 mg/kg, and 3 mg/kg [21]. Two cohorts received study drug as seven injections, the first treatment group was administered a single injection followed by 6 weekly injections and the other group was administered 7 consecutive weekly injections. Patients were followed for 18 weeks after the final injection. The primary objective of this study was observation of safety endpoints. Overall, the patients tolerated the anti-p40 well where 84% of patients completed all seven injections and 87% completed at least six injections. While there were a number of adverse events that occurred in more than 10% of both briakinumab and placebo patients (nausea, vomiting, abdominal pain, arthralgia, urinary tract infection, bronchitis, cough, headache, fever, and fatigue), these were not significantly different between placebo and briakinumab, and there were no serious infections. However, 77–88% of patients receiving 1 or 3 mg/kg and 25% of placebo recipients reported local injection site reactions, the majority (88%) of which were considered mild. Though nine severe adverse events occurred (seven briakinumab group, two placebo) none were adjudged related to the study drug.

For this study the secondary endpoints included rates of response (drop in the baseline Crohn's disease activity index (CDAI) ≥ 100 points) and remission (CDAI ≤ 150 points) at the end of treatment and at the end of follow-up (18 weeks after the final injection). The first treatment group showed response rates of 38, 63, and 56% and remission rates at the end of treatment of 38, 31, and 44% for the placebo, 1, and 3 mg/kg groups respectively, without any significant differences among them. The first treatment group showed response rates of 25, 19, and 50% and remission rates at the end of follow-up of 13, 19, and 50% for placebo, 1, and 3 mg/kg respectively, without any significant differences among them. In the second dose cohort, at the end of treatment the response rates were 25, 27, and 75% and the remission rates were 0, 8, and 38% for placebo, 1, and 3 mg/kg, respectively, with significant differences seen between placebo and 3 mg/kg response rates ($p=0.03$). Furthermore, at the end of the 18-week follow-up period the response rates were 25, 19, and 69% and remission rates were 0, 13, and 38% for placebo, 1, and 3 mg/kg, respectively, with differences seen between placebo and 3 mg/kg response rates that tended toward but did not reach significance ($p=0.08$). These data showed that clinical responses and remissions could be rapidly achieved (2–4 weeks) and could be durable after therapy was stopped.

Anti-drug antibodies (ADAs) were identified in three patients from the 1 mg/kg dose (two in the first treatment group, and one who had them detected before receiving drug). These ADAs were associated with low serum levels of briakinumab and quick clearance but due to certain technical difficulties with this assay of measuring ADAs in the presence of anti-p40 drug, the actual rate of ADA formation may have been underestimated, as they were not detected in three other subjects who also had unusually low briakinumab levels.

Changes in lamina propria mononuclear cell cytokine secretion and improvement in mucosal histology at the end of treatment were measured in a subset of nine patients. Of the eight patients who received briakinumab, seven reported a clinical response and all of these patients showed significantly decreased production of IL-12, IFN γ , and TNF α at the end of treatment, the sole primary non-responder in this group had low pre-treatment levels of IL-12, an increase in IFN γ production at the end of treatment and no change in TNF α . Similarly, primary responders to briakinumab had significantly decreased production of IL-23 and IL-17 at the end of treatment [20]. So these data show that anti-p40 was well tolerated by Crohn's patients. Additionally, there was efficacy at a higher dose and using a dosing strategy of uninterrupted administration.

With this data in hand, a second briakinumab Phase IIb, randomized, double-blind, parallel group, placebo-controlled dose ranging study was conducted. Using a 12-week induction phase and a 12-week maintenance phase, the objective was to test the efficacy and safety of inducing and maintaining remission in Crohn's disease [22]. Patients with active Crohn's disease (CDAI 220–450) received intravenous infusions of briakinumab (400 and 700 mg) or placebo every 4 weeks for 12 weeks; at the end of treatment, responders (drop in CDAI of ≥ 70 points) were eligible for a maintenance extension phase at the same dose (only patients receiving 700 mg IV every 4 weeks in the induction phase were re-randomized to placebo, 200, or 700 mg IV every 4 weeks) and treated for 12 weeks when clinical assessments were made at week 24. Patients who were not in clinical response at week 12 were eligible for enrollment in open-label treatment using 700 mg IV every 4 weeks (for up to 2 years from week 0) in addition to patients who were not in clinical remission at week 24 (CDAI < 150), and patients who relapsed during the maintenance treatment or follow-up. The primary endpoint was rate of remission at week 6; secondary endpoints included remission rates at week 12, week 24 (among those responding at week 12), and response rates (drop in CDAI of ≥ 100 points) at weeks 6, 12, and 24. There were 48 patients receiving placebo, 45 receiving 400 mg, and 139 receiving 700 mg at the start of the study. The primary endpoint of remission at week 6 was not met, with no differences among rates for placebo (9%), 400 mg (13%), or 700 mg (17%); however, at week 12 there were significant differences among remission rates for briakinumab comparing placebo (11%) to 400 mg (29%, $p=0.03$) (but not vs. 700 mg (22%), $p=0.087$). At neither week 6 nor 12 were there any significant differences among clinical response rates for placebo, 400, or 700 mg: 17, 36, and 37%, and 20, 31, and 40%, respectively. At the end of the 12-week maintenance treatment, there were no significant differences between placebo and the 400 or 700 mg doses either for response (36, 62, and 71%, respectively) or remission (29, 48, and 57%). When looking at the subset of patients with elevated C reactive protein (CRP) serum levels (CRP ≥ 1 mg/dL), a group repeatedly seen to have lower placebo response rates across many trials, the 6-week remission rates showed a trend toward improved results without reaching significance: 6-week remission rates for placebo, 400, and 700 mg doses in the high CRP group were 5, 21, and 18% compared to 11, 5, and 17%, respectively. Stratifying patients according to anti-TNF α experience (naïve, responder, and non-responder) did not show significant differences in remission rates at week 6 either, although by this point the diminishing numbers in certain categories precluded adequate powering of the analysis. In terms of adverse events, there were no significant differences between rates in placebo or study drug groups; the infectious adverse events in both groups during induction and maintenance dosing were rather high (25–34%), but the rate of serious infectious adverse events and opportunistic infections, specifically oroesophageal candidiasis, were lower at less than 5%. Injection site reactions were infrequent (0–6.5%) consistent with the intravenous administration and eight serious infusion reactions occurred in the open-label extension phase.

In summary, the first of these two studies gave an indication that targeting the IL-12/IL-23 pathways could induce clinical improvement in Crohn's symptoms in patients with long-standing disease. Furthermore, this outcome was supported by significant decreases in the effector cytokines (IL-12, IFN γ , IL-23, and IL-17) thought to be specifically driving the gut inflammation in Crohn's disease. In contrast, the follow-up study using larger doses and long-interval intravenous infusion failed to show significant differences in early clinical benefit; however, there were trends showing benefit at 12 weeks and beyond, even reaching rates of clinical response seen in the first trial. Briakinumab has subsequently been shown to be highly effective in psoriasis [23] but have more limited efficacy in multiple sclerosis [24].

Ustekinumab

Ustekinumab is a fully human monoclonal IgG κ antibody that can bind to IL-12, IL-23, and the p40 monomer [25]. Binding of ustekinumab to IL-12 or IL-23 interrupts coupling of the cytokines to the cell surface IL12 receptor (via the IL-12R β 1 subunit) and prevents intracellular signaling and T cell activation. While this agent is currently approved for use in psoriasis, it has also been investigated for multiple sclerosis, psoriatic arthritis, and Crohn's disease [26]. The only published trial of ustekinumab in Crohn's disease randomized 104 patients to a double-blinded, cross-over, placebo-controlled Phase IIa induction study [27]. Patients were randomized to one of four dose regimens: (1) 4 weekly SC doses of placebo, then after a 4-week washout, 4 weekly doses of 90 mg ustekinumab SC ($n=25$), (2) 4 weekly SC doses of 90 mg ustekinumab SC, then after a 4-week washout, 4 weekly SC doses of placebo ($n=25$), (3) IV placebo followed by 4.5 mg/kg IV ustekinumab 8 weeks later ($n=27$), or (4) 4.5 mg/kg IV ustekinumab followed by IV placebo 8 weeks later ($n=26$). The primary endpoint was the rate of clinical response at week 8 (overall decrease in CDAI by ≥ 70 representing a $\geq 25\%$ drop from the pre-treatment score). Secondary endpoints included rates of clinical response at weeks 4 and 6, and remission rates (CDAI ≤ 150 points) and "100-point response" rates at weeks 4, 6, and 8 (there was also an endpoint assessment at week 16 for those randomized to the third arm, so that response to the single dose of ustekinumab at week 8 could be measured).

The week 8 clinical response primary endpoint was not met in this study, but patients receiving the single intravenous dose of ustekinumab had a significantly higher response rate at week 6 compared to placebo (54 vs. 22%, $p=0.024$). Using the data combined from both routes of administration, rates of response were significantly higher at weeks 4 and 6 (53 vs. 30%, $p\leq 0.02$), and a 100-point response was seen at week 6 (49 vs. 25%, $p=0.01$). There were no differences in rates of remission. Interestingly, when analyzing the subset of patients ($n=49$) who had previously been exposed to infliximab (regardless of response to infliximab), the clinical response rates for ustekinumab were significantly higher at every time point compared to placebo, 55–59% versus 15–26%, respectively. In an open-label extension study of ustekinumab in patients who reported primary or secondary non-response to infliximab, the clinical response to either a single intravenous 4.5 mg/kg dose ($n=14$) or 4 weekly SC injections of 90 mg ($n=13$) was similar at week 8 (54 vs. 43%), but there were higher response rates for the IV administration at all the earlier time points perhaps reflecting a quicker onset of action related to the route of administration. Additional analyses based on changes in CRP after treatment, show that ustekinumab administration is associated with decreased CRP serum levels, higher baseline CRP is associated with higher ustekinumab response rates, and prior infliximab experience may amplify these effects [28].

Safety results showed that while there were no significant differences in adverse event rates through week 8 between the blinded placebo and treatment groups, the total adverse event rates were rather high (68–85%). However, of those adverse events reported more than 10% of the time, the placebo patients reported more nausea, worsening of Crohn's symptoms, fatigue, and pharyngolaryngeal pain.

Placebo patients also reported higher rates of infection but no one had a serious or opportunistic infection or malignancy. Serious adverse events were mostly related to complications or worsening of Crohn's disease. During the follow-up phase, disseminated histoplasmosis was seen in a ustekinumab recipient who was taking azathioprine and prednisone concomitantly, and viral gastroenteritis occurred in another ustekinumab subject. A 54-year-old male subject with an elevated prostate-specific antigen serum level at baseline was diagnosed with prostate cancer 2 months after intravenous ustekinumab. A female patient was diagnosed with basal cell carcinomas 6 months after her final ustekinumab injection.

These ustekinumab trial data show that this agent can induce a clinical response in patients with active, established Crohn's disease, particularly in patients who have a history of infliximab treatment. Similarly, treatment with ustekinumab induces a biologic response, decreasing CRP, but we do not know if this effect is actually associated with clinical response versus non-response, independent of baseline CRP values or infliximab exposure history [28].

Conclusions

The discovery of IL-12 and IL-23 and the key roles they play in the inflammation in animal models of human disease have positioned them among the top targets for developing novel drugs for Crohn's disease. However, there is a notable gap between the success of blocking IL-12/IL-23 p40 in animal models of Crohn's disease and the reports of successful treatment of human disease. Obviously, an advantage of the animal model is that a more uniform inflammation is induced by the use of timed colitogenic exposures in genetically identical subjects to test the effects of a specific therapy. In human disease and therapeutic trials, subjects have various phenotypes of disease, with different durations of disease and have genetically disparate backgrounds. These factors can make blocking IL-12/IL-23 p40 of differential importance to early versus late disease, to recurrent versus persistent disease, or to one individual versus another independent of duration or course of disease. Unfortunately there are no biomarkers yet known that would predict which patient would derive the better outcome from IL-12/IL-23 p40 targeting.

Many other influences on the therapeutic effect of targeting IL-12/IL-23 p40 in Crohn's disease also have not been worked out. These include the differential pharmacologic effects of early, constant, prolonged high concentrations of IL-12/IL-23 p40 immunoneutralization on cytokine cell surface receptor and intracellular signaling as compared to peak and trough dosing. The results of the first briakinumab study in Crohn's disease would suggest this is important. In addition, IL-12/IL-23 p40 is secreted in large amounts as a monomer and homodimer that can be recognized by antibodies, and ustekinumab recognizes the p40 monomer [25]. Binding to these molecules could act as a sink for the drug and leave the unbound IL-12 and IL-23 heterodimers still active; alternatively since p40 homodimers can act as an antagonist to IL-12, the differential binding of a therapeutic antibody to the p40 homodimer could alter what might have been a beneficial balance of homo- and heterodimers even in an established inflammation [29]. Finally, it is not at all clear whether there is a hierarchy of cytokine targeting, for example, IL-12/INF γ versus IL-23/IL-17, at various stages of Crohn's disease or among individuals, this is important in that INF γ , IL-17, and the regulatory cytokine IL-10 seem to exist in a balance, one that might be unpredictably disturbed with the use of anti-p40 antibody.

The data from animal models and human trials using anti-IL-12/IL-23 p40 antibodies in Crohn's disease suggest that there is a future for this targeted approach in establishing new therapies. Furthermore, because there are a large number of legitimate questions about how best to administer these drugs and monitor its activity, these trial results are only a first attempt at estimating efficacy. Additional knowledge about immunotypes and associated biomarkers that identify

patients at highest probability for response as well as strategies that can position patients for improved response will ultimately fulfill the promise of targeting IL-12/IL-23 p40 in Crohn's disease.

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Chapter 35

Biologic Therapy of Crohn's Disease: Natalizumab, Vedolizumab, CCX282-B

Alvin Chung and Peter R. Gibson

Keywords Crohn's disease • Adhesion • Antiadhesion • Trafficking • Migration • $\alpha 4$ integrin • Chemokine receptor • Natalizumab • Vedolizumab • CCX282-B • Alicaforfen • MAdCAM-1 • ICAM-1 • Progressive multifocal leukoencephalopathy

Introduction

As our understanding of the pathogenesis of inflammatory bowel disease (IBD) increases, new therapies for these conditions are moving away from classical nonspecific immunosuppressive and immunomodulatory agents to more targeted therapies. Soluble mediators believed to be critical in the inflammatory cascades have been major targets in recent years with the therapeutic and commercial success of inhibitors of tumor necrosis factor- α fostering a large pharmaceutical investment in modulating other soluble targets, but with only minor success to date. One step in the process of inflammation in IBD involves leukocyte recruitment from the blood stream to the gastrointestinal mucosa. This chapter reviews the therapeutic agents targeting this step.

Theoretical Concepts

The presence of leukocytes at the site of inflammation is critical to the inflammatory process. Leukocytes, including lymphocytes, monocytes, eosinophils, and neutrophils, reach the tissue involved by migrating from the circulation across endothelium. If the migration of leukocytes could be inhibited, this would decrease the density of leukocytes within the tissue, reduce the secretion of proinflammatory soluble mediators, and diminish cellular immune events, which ultimately attenuate tissue inflammation. The processes by which circulating leukocytes migrate across the endothelium are now well described. Free leukocytes must make the initial contact with the endothelium of high endothelial venules and then undergo a process of tethering, rolling, adhering firmly, spreading

A. Chung, MBBS, FRACP • P.R. Gibson, MD, FRACP (✉)
Department of Gastroenterology and Hepatology, Eastern Health Clinical School,
Eastern Health and Monash University, Box Hill Hospital, 5 Arnold Street, Box Hill, VIC 3128, Australia
e-mail: peter.gibson@monash.edu

then migrating through the endothelium [1]. This process must be controlled on two levels: the first is that leukocytes (principally lymphocytes) that are primed to function in a specific location are able to home to that tissue, and second, that leukocyte migration increases where needed (e.g., in the presence of tissue injury or infection) [2]. Molecules controlling the attraction of leukocytes to the endothelial surface and subsequently adhering to it and the mechanisms of tissue specificity are being increasingly understood. This has enabled pharmacological agents to be designed to inhibit such processes, and there is now animal and human evidence that the application of these agents can modulate chronic inflammatory states.

The Target Molecules

Adhesion Molecules

Integrins are a superfamily of cell-surface glycoproteins that mediate adhesion, migration, and activation of leucocytes to the endothelial wall allowing migration from the blood stream to the site of inflammation [3]. Integrins consist of noncovalently linked α and β chains. The dimers have specific ligands expressed on the endothelial surface (Fig 35.1). There are three factors that dictate the degree of adhesion in any vascular bed [4, 5].

- *The expression of the integrin:* Different vascular beds express different ligands in a basal, unstimulated state. This may dictate to which tissue endothelium the leukocyte can adhere.

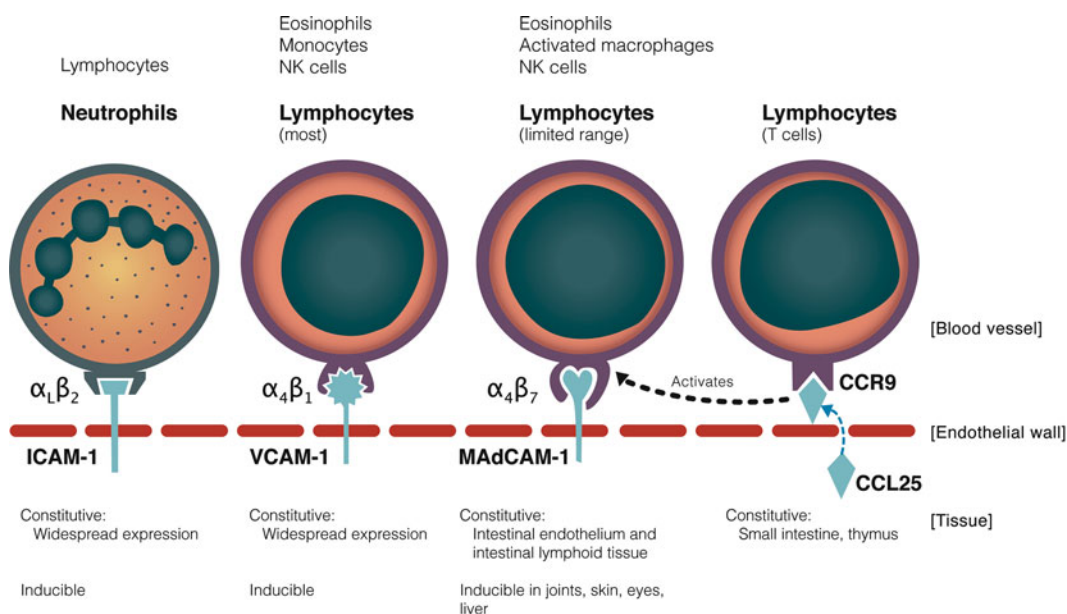


Fig. 35.1 The major adhesion molecules and their respective ligands that have been targeted for treatment in Crohn's disease. The cell types expressing the receptors and the tissue localization and effect of inflammation on expression of the ligand by the endothelium are also shown. CCL25 is a chemokine that is secreted in the tissue and is immobilized on the endothelial surface to bind to its receptor (CCR9) on the lymphocyte. This binding activates $\alpha_4 \beta_7$ to increase its adhesiveness

- *The affinity of the integrin for its receptor:* This increases during the process of adhesion via interaction with other receptors and factors, especially chemokines and their ligands (see below).
- *The receptor expression/density on the endothelium:* This may vary across tissues. Receptors can also be induced by cytokines in injured or inflamed tissues, even if they are not normally expressed in that tissue.

$\beta 2$ integrins

The $\beta 2$ integrins are expressed widely on lymphocytes, neutrophils, monocytes, and eosinophils [6]. The heterodimer $\alpha_L\beta_2$, also known as lymphocyte function-associated antigen-1 (LFA-1) is the predominant β_2 integrin on neutrophils and lymphocytes. One of the ligands for LFA-1 is intercellular adhesion molecule-1 (ICAM-1), which is constitutively expressed on endothelium and can be induced in association with inflammation or injury. This appears to be especially the case in ulcerative colitis where neutrophil migration may be more important.

$\alpha 4$ integrins

The $\alpha 4$ integrins are particularly important in recruitment of lymphocytes and monocytes [3]. The $\alpha 4$ chain dimerizes with the $\beta 1$ or $\beta 7$ chain, and each dimer has different ligands:

- *$\alpha 4\beta 1$ integrin:* This binds to vascular-cell adhesion molecule-1 (VCAM-1) and fibronectin. This receptor–ligand system appears to be relatively nonspecific. $\alpha 4\beta 1$ is expressed by nearly all lymphocytes, monocytes, and eosinophils [3]. Likewise, VCAM-1 is expressed widely by endothelium and is inducible by cytokines. Not surprisingly then, this interaction is responsible for the recruitment of leukocytes at sites of inflammation.
- *$\alpha 4\beta 7$ integrin:* This binds to mucosal addressin-cell adhesion molecule-1 (MAdCAM-1). $\alpha 4\beta 7$ is expressed on a more limited range of lymphocytes (e.g., it is present on IgA-expressing B cells but not on memory cells not of gut origin), activated macrophages, natural killer cells, mast cells, and eosinophils. The expression of MAdCAM-1 is predominantly on the endothelium of high endothelial venules in intestinal tissue. Evidence is now strong that $\alpha 4\beta 1$ -MAdCAM-1 interaction is the major route for lymphocyte trafficking to the intestine [7]. MAdCAM-1 expression is also increased in the presence of inflammation and cytokine exposure. This can occur in extraintestinal sites such as joints, eyes, skin, and liver.

In both experimental and human IBD, expression of the ligands is increased as might be anticipated. Indeed, enhanced $\alpha 4$ -integrin-dependent leukocyte binding by human intestinal microvascular endothelial cells from inflamed tissue has been demonstrated [2]. The importance of $\alpha 4\beta 7$ integrin in chronic intestinal inflammation and its potential as a target for therapy have been demonstrated in experimental models of colitis, such as that in cotton-top tamarins, where inhibition of binding to its ligand has led to attenuation of colitis [8, 9]. However, this has not been the case in all models, indicating that other pathways of lymphocyte recruitment or stage-specific differences in recruitment pathways may be present.

It is important to note that the above discussion is likely to be an oversimplified view of the immunological role of $\alpha 4\beta 7$ -MAdCAM-1 interaction since other cells, such as follicular dendritic cells, also express MAdCAM-1 and its blockade may have other effects, such as effects on lymphocyte co-stimulation, polarization, and survival, effects that are not necessarily anti-inflammatory [10]. This is beyond the scope of this review.

Chemokine Receptors

The other important mechanism for T cell homing to the intestine is via expression of chemokine receptors by lymphocytes and the chemoattraction offered by chemokines. Binding of chemokines to its receptor can activate integrins to increase adhesion. Chemokines secreted in the tissue localize to the surface of endothelium by binding to amino glycosaminoglycans that appear to have tissue specificity [11]. The chemokines also act via classic chemoattractant properties enabling migration of cells along an ascending concentration gradient, and by activation of integrins in the tissue matrix thereby enhancing tissue retention. The chemokine system that has attracted most interest in Crohn's disease has been CCL25, also known as thymus-expressed chemokine (TECK), which is expressed specifically in small intestine and is overexpressed in inflamed intestinal tissue [12]. CCR9 is expressed by most T lymphocytes and some IgA-secreting cells and its interaction with CCL25 activates $\alpha 4\beta 7$. Proof of concept that CCR9 may have a quantitatively important role in the inflamed intestine was shown in the reduction of early chronic murine ileitis with antibody blockade of CCL25/CCR9 [13].

Candidate Therapeutic Agents

A few pharmacological agents that interfere with leukocyte adhesion to endothelium and subsequent migration into intestinal tissue have undergone evaluation in patients with IBD. Natalizumab, a monoclonal antibody that blocks binding of $\alpha 4$ integrins to their ligands, has been extensively studied and is now available as a therapeutic agent for Crohn's disease. Vedolizumab (MLN-02), a monoclonal antibody to $\alpha 4\beta 7$ integrin, and CCX282-B (Traficet-ENTM), a small molecule that selectively blocks CCR9, are currently under active evaluation. Alicaforfen (ISI 2302), an antisense phosphorothioate oligonucleotide to human ICAM-1 RNA, has shown a disappointing lack of efficacy in Crohn's disease.

Natalizumab

Natalizumab is a recombinant humanized (5% murine) IgG₄ monoclonal antibody to $\alpha 4$ integrin, which is expressed at a high level on the majority of lymphocytes and to a lesser degree on eosinophils and monocytes. The mode of action of natalizumab is inhibition of the binding of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ to their corresponding endothelial receptors, VCAM-1 and MAdCAM-1. Natalizumab has been shown to increase circulating lymphocytes numbers presumably by interrupting the $\alpha 4$ integrin-dependent migration of lymphocytes to extravascular sites [14, 15]. However, neutrophils, a major component of the inflammatory infiltrate in CD, do not normally express $\alpha 4$ integrins. While the physiological stimulus of transmigration can invoke expression of $\alpha 4\beta 1$ on human neutrophils after the cells leave the blood vessels, it is unclear whether natalizumab would have activity on neutrophils at this stage [16]. What is apparent is that T-lymphocytes are required for ongoing neutrophil recruitment via secretion of cytokines and chemokines and inhibition of this step in the pathway may explain the anti-inflammatory effect of natalizumab [8].

Randomized Controlled Trials in Crohn's Disease

To date there have been five randomized, double-blinded, placebo-controlled trials published [14, 15, 17, 18]. The details of the studies are given in Table 35.1. A Cochrane review of natalizumab for

Table 35.1 Details of fully published randomized placebo-controlled trials for natalizumab and vedolizumab in patients with Crohn's disease

Natalizumab		
Gordon et al. 2001 [14]	<i>Design</i>	Two centers. Thirty patients with active CD with a CDAI 151–450. Randomized 2:1 to 3 mg/kg natalizumab ($n=18$) or placebo ($n=12$). Primary endpoint was the change in CDAI at week 2
	<i>Efficacy</i>	At 2 weeks, mean reduction in CDAI of 45 points (from 258 to 213; $p=0.02$) natalizumab. This was not significantly different from the 11 point mean reduction for placebo ($p=0.2$). The majority of patients in the study had commenced rescue medication by 4 weeks (50% natalizumab, 67% placebo). Pharmacokinetics showed that natalizumab had a reduced half-life in patients with active CD of 4.8 days compared with 8.7 days in healthy volunteers
	<i>Safety</i>	No differences in the adverse effect profile between the two groups. Natalizumab infusions generally well tolerated
	<i>Conclusions</i>	Natalizumab well tolerated No signal of efficacy. A single dose 3 mg/kg may be suboptimal and the authors called for further study of the drug
Ghosh et al. 2003 [15]	<i>Design</i>	35 centers. 248 adults with moderate to severe CD (CDAI ≥ 220 , ≤ 450). Randomization to one of four treatments: two infusions of placebo, one infusion of 3 mg/kg of natalizumab then one infusion of placebo, two infusions of 3 mg/kg natalizumab or two infusions of 6 mg/kg of natalizumab. Infusions given 4 weeks apart. Primary endpoint was the rate of clinical remission (CDAI < 150) at week 6
	<i>Efficacy</i>	The group given two infusions of 6 mg/kg had significantly higher remission rates at 4 and 8, but not 6 weeks. Patients receiving two infusions of 3 mg/kg had significantly higher remission rates at all 4, 6, 8, and 12 weeks. Clinical response (CDAI decrease ≥ 70 from baseline) was higher in all three natalizumab groups compared with placebo. Patients treated with natalizumab, but not placebo, had a decline in CRP
	<i>Safety</i>	No differences in adverse events. Antibodies to natalizumab found in 7% of patients and two patients had mild rash and itching, both during the second infusion with only one patient testing positive for antibodies
	<i>Conclusions</i>	Natalizumab efficacious for reducing signs and symptoms of Crohn's disease in the short term
Sandborn et al. 2005 (ENACT-1) [17]	<i>Design</i>	905 patients with moderate to severe Crohn's disease (CDAI ≥ 220 , ≤ 450) were randomized at a ratio of 4:1 to either infusions of 300 mg natalizumab ($n=724$) or placebo ($n=181$) at weeks 0, 4, and 8. The primary outcomes were response defined as a reduction in CDAI from baseline ≥ 70 . One of the secondary outcome measures was the rate of remission (CDAI < 150) at week 10
	<i>Efficacy</i>	Rate of clinical response was 56% for natalizumab compared with 49% for placebo ($p=0.05$). Remission at week 10 in 37% for natalizumab, 30% for placebo ($p=0.12$). In 660 patients with elevated CRP at baseline, response in 58% for natalizumab, 45% for placebo ($p<0.05$), and remission in 40% for natalizumab and 28% for placebo ($p<0.05$)
	<i>Safety</i>	Acute infusion reactions occurred in 11% ($n=83$) given natalizumab. Patients who tested positive for antibodies against natalizumab had a higher rate of infusion reactions compared with patients who tested negative for antibodies (45% vs. 9%, $p<0.001$)
	<i>Conclusions</i>	Efficacy for natalizumab as induction therapy was only observed in patients with an elevated CRP Infusion reactions associated with antibody formation to natalizumab

(continued)

Table 35.1 (continued)

Natalizumab		
Sandborn et al. 2005 (ENACT-2) [17]	<i>Design</i>	338 patients in remission (CDAI 0–220) who achieved natalizumab or placebo-induced clinical response at both weeks 10 and 12 in ENACT-1 randomized 1:1 to receive either 300 mg natalizumab or placebo every 4 weeks from weeks 12–56. Primary outcome was sustained response through to week 36. Loss of response defined as an increase in CDAI score of 70 after week 12, absolute CDAI of >220, or the need for rescue intervention after week 12
	<i>Efficacy</i>	61% achieved a sustained response with natalizumab compared with 28% with placebo ($p<0.001$). Adjustment for significant patient characteristic differences between the two groups did not affect the outcome of the result
	<i>Safety</i>	Patients on natalizumab significantly more likely to develop influenza (5% vs. 12%, $p<0.05$). One patient developed PML and died. The patient received 17 doses of natalizumab in combination with azathioprine
	<i>Conclusions</i>	Natalizumab has efficacy in maintaining a natalizumab or placebo-induced response over 12 months Signal of increased susceptibility to viral infection
Targan et al. 2007 (ENCORE) [18]	<i>Design</i>	509 patients with moderate to severe Crohn's disease (CDAI ≥ 220 , ≤ 450) and active inflammation (elevated CRP) randomized to 300 mg natalizumab or placebo given at weeks 0, 4, and 8. Primary endpoint was response (CDAI decrease ≥ 70 from baseline) at weeks 8 through to 12. Secondary endpoints were the rate of sustained remission, and response and remission over time
	<i>Efficacy</i>	Rate of sustained response from week 8 to 12 was 48% for natalizumab and 32% for placebo ($p<0.001$). Sustained remission in 26% for natalizumab, 16% for placebo ($p<0.001$). Normal CRP at week 12 in 23% for natalizumab, 8% placebo
	<i>Safety</i>	Higher rate of infections in the natalizumab-treated patients (35%) compared with patients on placebo (30%) primarily due to nasopharyngitis. No PML or other opportunistic infection. 9.5% developed antibodies against natalizumab. Numerically higher incidence of acute infusion and hypersensitivity reactions in patients with antinatalizumab antibodies compared with those without
	<i>Conclusions</i>	Natalizumab has efficacy as an induction therapy in patients with active Crohn's disease, as evidenced by an elevated CRP Infection rate, particularly viral respiratory, is elevated
Vedolizumab		
Feagan et al. 2008 [35]	<i>Design</i>	185 patients with moderately severely active Crohn's disease (CDAI ≥ 220 , ≤ 400) randomized to two infusions of vedolizumab 2 or 0.5 mg/kg, or placebo in a 1:1:1 ratio given 4 weeks apart. Primary endpoint was a ≥ 70 point reduction in CDAI compared with baseline measured at day 57. Secondary endpoints included rate of clinical remission (CDAI ≤ 150) at day 57 and rate of enhanced response (≥ 100 point reduction in CDAI compared with baseline)
	<i>Results</i>	Response for vedolizumab at either dose (53% response for 2 mg/kg and 49% for 0.5 mg/kg) not superior to placebo (49%). Enhanced response 47% for 2 mg/kg dose versus 31% for placebo ($p=0.05$) and remission 37% vs. 21% ($p=0.04$)
	<i>Safety</i>	Vedolizumab well tolerated. Adverse events similar to placebo. One infusion reaction to vedolizumab. At the arbitrary cut-off titer ($>1:125$), human antihuman antibodies (HAHA) observed in 34% for 0.5 mg/kg of vedolizumab (34%) compared with 12% 2 mg/kg
	<i>Conclusions</i>	Signal for efficacy, but primary endpoint not reached. Suggestions of a dose-dependent effect Vedolizumab well tolerated but HAHA were observed commonly, especially with the lower dose

induction of remission in Crohn's disease was published in 2007 and updated in 2009 [19]. Pooled data suggested that natalizumab given as 300 mg or 3–4 mg/kg was efficacious for inducing clinical response and remission in patients with moderate to severe Crohn's disease with a significant benefit seen for 1, 2, or 3 infusions. The benefit appeared to be more significant in patients with objective evidence of active inflammation or chronically active disease despite conventional therapy. Natalizumab appeared overall to be well tolerated and safe.

Predictors of Response to Induction Therapy with Natalizumab

Subgroup analysis of ENACT-1 suggested that those with active CD leading to an elevated CRP are more likely to respond to natalizumab. In order to identify other potential factors that might indicate a higher or lower likelihood of response to natalizumab as an induction therapy, further post hoc analyses of data from the relevant randomized controlled clinical trials have recently been reported in preliminary form. As found for anti-TNF therapies, patients with disease duration less than 3 years seemed to be more responsive to induction therapy with natalizumab; remission rates at week 8 were 39% for natalizumab vs. 21% for placebo ($p < 0.05$), and at week 12, 52% vs. 27% ($p < 0.05$) [20]. Unlike infliximab, concomitant use of immunosuppressants did not appear to improve rates of sustained response and remission in Crohn's disease patients [21]. Patients with very high disease activity (CDAI ≥ 330) appeared to be responsive to natalizumab, the sustained response rates from weeks 8 through to 12 were 51% in the natalizumab-treated patients compared with 27% for the placebo-treated patients ($p < 0.002$) [22]. Furthermore, natalizumab appeared to be efficacious in patients in the ENCORE study who had previously failed infliximab; 172 patients considered to have failed infliximab therapy had a significantly greater sustained response and remission rates to natalizumab (38 and 17%) compared with placebo (15 and 5%) through weeks 8–12 [23].

Concurrent Infusions of Natalizumab and Infliximab

Concurrent infusions of natalizumab have been shown to be safe and well tolerated in patients with Crohn's disease who are not in remission while receiving infliximab [24]. In the only published report, 79 patients with active CD despite infliximab treatment were randomized 2:1 to receive three infusions of natalizumab or placebo every 4 weeks. There was a positive, although not significant trend towards an improved response on combination therapy than treatment with infliximab alone. There was a similar rate of adverse events in both groups.

Postregistration Safety Information

All five randomized controlled trials showed that natalizumab was well tolerated by the majority of patients with CD. Common adverse events included headache, worsening of Crohn's disease, abdominal pain, arthralgia, influenza-like symptoms, nausea, vomiting, fatigue, and infusion reactions, mostly at frequencies similar to those in placebo-treated patients. The ENACT-2 trial showed a significant increase in risk of influenza in patients receiving maintenance natalizumab over 48 weeks compared with placebo (12% vs. 5%). There have also been six cases of serious hepatic injury reported from November 2004 to June 2008. In all cases, natalizumab was discontinued with a corresponding improvement in liver biochemistry [25]. Infusion reactions, which are a common adverse event, occur more frequently in patients with antinatalizumab antibodies.

Reports of progressive multifocal leukoencephalopathy (PML) in patients who have received therapy with natalizumab have dominated concerns regarding the safety of this agent. PML, a devastating neurological disorder caused by the JC virus, occurs primarily in immunocompromised patients. The most common clinical features are limb weakness, gait disturbance, speech impairment, visual disorders, and cognitive decline [26]. In the vast majority of patients, this rapidly progressive disorder leads to significant permanent disability or death.

Natalizumab was first approved for the treatment of relapsing-remitting multiple sclerosis in December 2004 but it was suspended in February 2005 as a result of three reported cases of PML and the subsequent death of two of those patients. A study was commissioned to determine if further cases of PML occurred in all patients exposed to natalizumab. No further cases of PML were detected from 3,116 patients exposed to a mean of 17.9 monthly doses. This number reflected 91% of all patients ever exposed to this drug [27]. The USA FDA subsequently approved the use of natalizumab in CD in January 2008 with access to the medication under the tysabri outreach: unified commitment to health (TOUCH) program. As of December 2010, there have been 79 confirmed cases of PML worldwide in approximately 75,500 patients who have received natalizumab in the postmarketing setting. Sixteen (20%) have died and the survivors have apparently all suffered neurological disability despite reversal of the immunosuppression. The overall risk of PML infection is 1.00 per 1,000 patients (95% CI: 0.79–1.25 per 1,000 patients) [28]. Accumulated data show that longer treatment duration and concurrent immunosuppressant use increase the risk of PML.

It appears likely that disruption of lymphocyte recruitment and adhesion plays a major role in PML and this enhances our understanding of this rare disease. At this stage, there is no reliable method of predicting or preventing PML in patients with CD. Because the JC virus infects the majority of the population during adolescence, seropositivity to the JC virus will not be a useful screening test [29]. Some authors suggest regular screening for the JC viremia and subsequent dose reduction or cessation of the drug if the virus is detected [30]. However, most immunocompromised patients with JC viremia do not develop PML and there have even been cases of active brain disease without corresponding JC viremia. In the absence of a reliable predictor for this disease, the current goal of treatment for this condition is early recognition and reversal of immunosuppression.

Conclusions Regarding the Use of Natalizumab in Crohn's Disease

As an induction agent, natalizumab is superior to placebo, but the magnitude of the effect does not appear substantial. This is not surprising given that natalizumab targets only one pathway for leukocyte adhesion and migration, and has little effect on migration of neutrophils and other leucocytes via non- $\alpha 4\beta 7$ -dependent mechanisms up-regulated during inflammation. What is more impressive is its ability to maintain remission in patients in whom remission was induced by natalizumab. Again, this is consistent with its ability to specifically inhibit intestinal T-lymphocyte trafficking. Whether it shows such excellent maintenance properties after remission induced by other agents, such as corticosteroids, require elucidation.

The major issue regarding the use of natalizumab in Crohn's disease is the 1 in 1,000 risk of developing a disabling neurological disease with a high mortality rather than its efficacy or tolerability. This issue is similar to the risks associated with antitumor necrosis (TNF) factor drugs and immunomodulators, where severe infection and lymphoma are potential life-threatening adverse events. The question of what constitutes an acceptable risk–benefit ratio has been addressed in a study of American patient's with Crohn's disease [31]. The mean maximal acceptable annual risk for such severe adverse events, if that therapy were to lead to remission in patients with moderate to severe symptoms, ranged from 0.39 to 0.81%. This figure is within the 0.1% occurrence of PML in all patients with any exposure to natalizumab. In other words, these findings suggest that the risk

is acceptable to most patients whose quality of life is miserable due to symptoms associated with the disease. This is well in line with current clinical practice of targeting patients with poorer predicted outcomes with other biological therapies.

On the basis of current data, it is difficult to recommend natalizumab as first- or second-line biologic therapy for patients with moderate to severe disease that has responded poorly to standard immunomodulatory therapy, based on its modest efficacy as an induction agent. However, real-life clinical experience will be invaluable in determining its impact in patients with Crohn's disease where response to other medical therapy has been suboptimal.

Vedolizumab

Vedolizumab (MLN0002) is a humanized monoclonal antibody to the $\alpha 4\beta 7$ integrin. It interferes with the binding of $\alpha 4\beta 7$ integrin, present primarily on lymphocytes, with its endothelial receptor mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [32]. Studies show that T lymphocytes and B lymphocytes cells that express high levels of $\alpha 4\beta 7$ migrate selectively to the gut [33]. It has also been shown that MAdCAM-1 is preferentially expressed in the gastrointestinal tract and its associated lymphoid tissue [34].

Vedolizumab differs from the $\alpha 4$ -integrin antagonist, natalizumab, by recognizing only the $\alpha 4\beta 7$ heterodimer. Vedolizumab is, therefore, a highly selective adhesion molecule antagonist for lymphocyte adhesion and migration to the gut. The theoretical advantages of vedolizumab compared with the less specific natalizumab are greater selectivity, reduced systemic immune system effects, and a decreased risk of PML.

Only one randomized controlled trial of vedolizumab in Crohn's disease has been published. The details of this study are given in Table 35.1 [35]. From a safety point of view, too few patients have been studied to be confident that vedolizumab is safe; however, together with safety information from the phase 2 study in patients with ulcerative colitis [36], vedolizumab does appear to be well tolerated with few drug-related serious adverse events. Infection rates appear not to be increased (apart from a suggestion of increased upper respiratory tract infection) and no cases of PML have been reported. Antibody formation appears to occur commonly, but the clinical significance of this has yet to be determined. The key question of efficacy in active disease and in maintenance remains open, but sufficient optimism of clinical benefit from the phase 2 studies has prompted large multi-center phase 3 trials in both Crohn's disease and ulcerative colitis.

CCX-282

CCX-282 (Traficet-En™), an antagonist to the chemokine receptor CCR9, is currently under clinical study for treatment of Crohn's disease. As described earlier, the chemokine receptor CCR9 and its ligand CCL-25 appear to play an important role in lymphocyte migration and homing in the small intestine. The CCL-25 receptor appears to be expressed in the small bowel and not in the large bowel, which, in theory, should restrict the therapeutic effect of CCX-282 to the small intestine [12].

The results of the randomized controlled clinical trial of CCX-282 in Crohn's disease (PROTECT-1) and its extension study have yet to be fully published. PROTECT-1 is a multicenter, randomized, double blinded, placebo-controlled trial comparing CCX-282 at three different doses to placebo in 436 patients with moderately severe Crohn's disease [37]. CCX-282 at a dose of 500 mg daily was significantly superior to placebo in achieving the primary endpoint of CDAI reduction of ≥ 70 compared with baseline. The results were also significant when assessing for

reduction in CDAI ≥ 100 compared with baseline and endoscopic improvement with the Crohn's disease endoscopic index of severity (CDEIS).

In the extension study, 241 patients were re-randomized to CCX-282 at a dose of 250 mg twice daily or placebo for 36 weeks [38]. The proportion of patients in clinical remission (CDAI ≤ 150) remained stable at about 50% over 36 weeks in the CCX-282 group but fell from 50 to 31% in the placebo group. The difference in proportion of patients remaining in remission at 36 weeks was statistically significant ($p=0.01$).

CCX-282 appears to be safe. Adverse events occurred at a similar rate in the placebo and drug groups. In particular, no CCX-282-specific adverse effects were evident.

It is somewhat curious that efficacy was apparent both in patients with small and large bowel disease, which would not have been expected given the lack of CCL25 receptors in the large bowel. However, there is much excitement about a novel therapy that can be taken orally and is well tolerated. Further information, especially full publication of the results, is eagerly awaited.

Alicaforsen (ISIS 2302)

Alicaforsen is a first-generation phosphorothioate antisense oligodeoxynucleotide, which hybridizes selectively with messenger RNA encoding human intercellular adhesion molecule-2 (ICAM-1). The heterodimer formed is cleaved by the ubiquitous nuclease RNase-H causing a reduction in the expression of ICAM-1 in a variety of human leukocytes [39].

Following a pilot study [40] that suggested a positive effect of intravenous ISUS 2302 in 20 patients with moderately severe, steroid dependent CD, two multicenter, randomized, placebo-controlled trials failed to show efficacy with the very strict primary endpoint of steroid-free remission (CDAI ≤ 150) at week 14. The first, a study involving 75 patients from Germany was terminated early following an interim analysis; only 3% (2 of 60) of patients in the treatment arm achieved steroid-free remission [41].

In the second study, 299 patients from North America and Europe were randomized to placebo or ISIS2302 2 mg/kg intravenously 3 $\times$ a week for 2 weeks or 4 weeks [42]. Steroid-free remission rates were equivalent for the 2 and 4 week alicaforsen groups (20 and 21%) and the placebo group (19%). However, retrospective analysis showed a correlation between the rate of steroid free remission and plasma levels. Only a relatively small number of patients attained high plasma levels of ISIS 2302 in this trial prompting a subsequent study evaluating this drug at a higher dose. Alicaforsen (ISIS 2302) given 3 $\times$ a week for 4 weeks at a fixed dose based on the patient's weight (> 50 kg received 300 mg, 35–50 kg received 200 mg) was no better than placebo (33.9% vs. 34.5%, $p=0.89$) in achieving remission in 331 patients with Crohn's disease [43]. Results of these studies show a lack of clinical efficacy of this class of medications in the management of Crohn's disease.

Conclusions

The concept of inhibition of adhesion molecules is exciting and has the potential to selectively modulate immune events in the intestinal compartment, something that few pharmacological agents can offer. Indeed the efficacy of natalizumab in maintenance of remission is impressive. However, hurdles to overcome include the fact that there are multiple adhesion molecules involved in the migration of leukocytes into inflamed tissue and that the therapeutic agents target only one of these molecules. The other major hurdle is the real risk of serious, fatal infection that has been associated with at least one of the less selective adhesion molecule inhibitors. Nevertheless, this approach is

worthy of considerable effort as there is an unmet need for new, safer, and more effective therapies. Large multicenter randomized controlled trials will provide some of the answers, but the desire for all therapies to excel as both induction and maintenance agents, as demonstrated by trial designs for all agents so far evaluated, may severely impair progress and ongoing investment in this novel approach before it has a chance to prove its worth in the real-world management of patients with Crohn's disease.

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Chapter 36

Conventional Medical Management of Ulcerative Colitis: Sulfasalazine and 5-Aminosalicylates

Reena Khanna and John K. Marshall

Keywords Sulfasalazine • 5-ASA • Ulcerative colitis • Ulcerative colitis therapies

Chemical Structure/Pharmacokinetics

Sulfasalazine consists of 5-ASA linked to a sulfa moiety by a di-azo bond. After oral administration, a portion of the dose is absorbed in the small bowel. Of this fraction, the majority enters enterohepatic circulation and returns to the small intestine via bile, while a minute amount is excreted in the urine [2]. Most of the ingested sulfasalazine reaches the colon where bacterial diazoreductase cleaves 5-ASA from sulfapyridine. Sulfapyridine is absorbed and undergoes hepatic acetylation and hydroxylation, with subsequent urinary excretion. Most 5-ASA is eliminated in the feces, but a small fraction is absorbed and inactivated by acetylation in intestinal epithelial cells and in the liver [3]. Acetyl-5-ASA enters the systemic circulation and undergoes urinary excretion [4].

5-ASA is the major active therapeutic component of sulfasalazine [5]. Although sulfapyridine has been suggested to exert independent therapeutic effects through induction of T-cell apoptosis [6, 7], it also contributes to the poor oral tolerability and adverse effect profile of sulfasalazine [3]. Alternate 5-ASA formulations were developed to improve tolerability while optimizing delivery to specific segments of the gastrointestinal tract. Despite such chemical modifications, newer formulations of 5-ASA appear to retain similar pharmacokinetic profiles to sulfasalazine.

Balsalazide consists of a 5-ASA molecule linked to an inert carrier, 4-aminobenzoyl-L-alanine, by a di-azo bond [3]. Similarly, olsalazine joins two 5-ASA molecules with a di-azo bond. Like sulfasalazine, olsalazine and balsalazide have low bioavailability with almost complete delivery to the colon where the di-azo bond is cleaved by bacterial azoreductase. Per weight, olsalazine delivers two times the amount of 5-ASA to the colon as sulfasalazine [3].

Mesalamine refers to 5-ASA monomers that are delivered to various segments of the gastrointestinal tract using pH-mediated or time-based coatings and release mechanisms. One formulation uses ethylcellulose-coated granules to release 5-ASA throughout the small intestine and colon. Others use the acrylic resin Eudragit as a coating to release drug in the distal small bowel and colon

R. Khanna, MD, FRCPC • J.K. Marshall, MD, MSc, FRCPC, AGAF (✉)
Division of Gastroenterology, Farncombe Family Digestive Health Research Institute,
McMaster University, 1280 Main Street West, Hamilton, ON, Canada L8N 4K1
e-mail: marshallj@mcmaster.ca

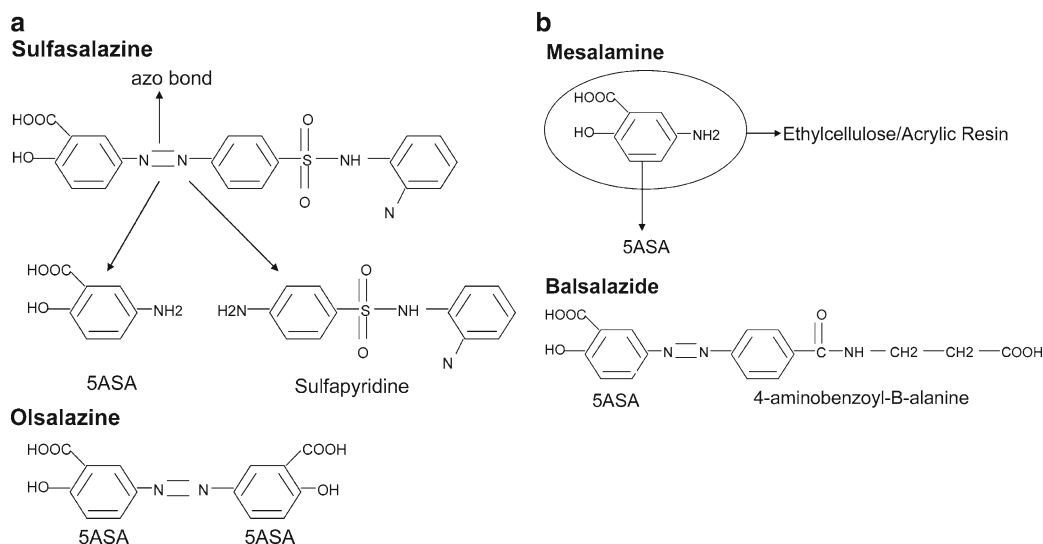


Fig. 36.1 (a, b) Chemical structures of sulfasalazine and 5-ASA derivatives

as pH rises. A recent formulation uses a multimatrix structure to encapsulate 5-ASA in an inner lipophilic matrix, a hydrophobic matrix, and an outer polymethacrylate coating. The result is a time- and pH-based release of 5-ASA in the terminal ileum and throughout the colon (Fig. 36.1) [2, 8].

Mechanism of Action

Sulfasalazine has been suggested to exert its clinical effects through anti-inflammatory, immunosuppressive, and bacteriostatic mechanisms. Downregulation of the inflammatory response by sulfasalazine is mediated by inhibition of granulocyte functions including degranulation, chemotaxis, migration [2, 9], and superoxide production [10]. It also acts on several arachidonic acid metabolism pathways resulting in a net increase in prostaglandins which possess both anti-inflammatory and immunosuppressive effects, and a decrease in pro-inflammatory lipoxigenase [11] titers. Immunologically, sulfasalazine inhibits the activity of natural killer cells and T cells [6, 9]. The sulfapyridine moiety has *in vitro* bactericidal activity in human blood [1, 7].

5-ASA has been shown to decrease leukotriene production, remove free radicals, and inhibit leukocyte chemotaxis *in vitro* and *in vivo* models. 5-ASA has also been postulated to inhibit the nuclear factor-kappa B pathway, decrease oxidative stress-induced cell injury and apoptosis, increase heat shock protein response, decrease leukotriene production, and alter prostaglandin metabolism in colonic epithelial cells [3]. Patients with ulcerative colitis who are treated with 5-ASA have lower fecal concentrations of prostaglandin E2 [12].

The primary mechanism of action of 5-ASA may be activation of gamma-form peroxisome proliferator-activated receptors (PPAR-gamma) in colonic epithelial cells. These receptors play a role in inducing transcription in response to environmental cues, and are activated *in vivo* primarily by colonic bacteria. Evidence for PPAR-gamma involvement in ulcerative colitis is derived from the observation that heterozygote knock-out mice develop colonic inflammation. Interestingly, PPAR-gamma is known to have anti-proliferative effects, while influencing differentiation and apoptosis. Cumulatively, these may protect against the development of colonic dysplasia and neoplasia in ulcerative colitis [3].

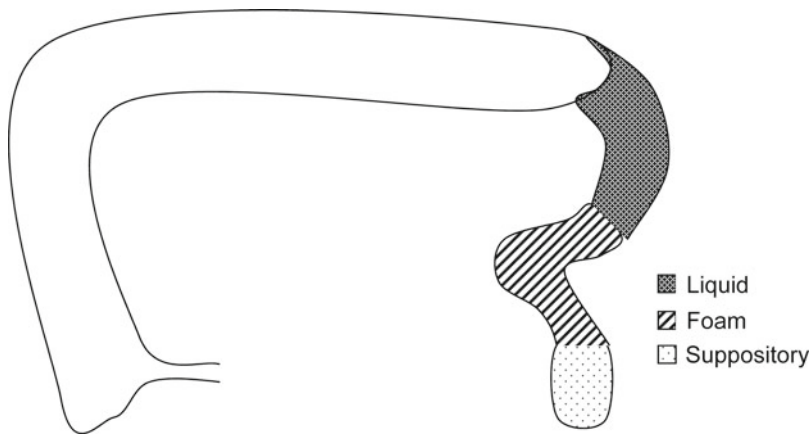


Fig. 36.2 Proximal distribution of rectal 5-ASA therapy [Modified by permission from MacMillian Publishers Ltd: *Am J Gastroenterol*. Marshall JK, Irvine EJ. Putting rectal 5-aminosalicylic acid in its place: the role in distal ulcerative colitis. 95(7):1628–36. © 2000]

Formulations

5-ASA is believed to act topically, in that direct mucosal contact is required for its therapeutic effect. Accordingly, 5-ASA is available in both oral and rectal preparations. Although, oral therapy is more commonly used in clinical practice [13], rectal therapy is effective in treating left-sided disease [14]. As rectal administration cannot deliver drug reliably beyond the splenic flexure, this defines the proximal margin for primary rectal therapy [13]. However, rectal therapy may still augment the efficacy of oral 5-ASA in patients with more extensive disease [15]. Rectal therapy also delivers medication to the site of maximal inflammation, while limiting systemic absorption and associated side effects. However, uptake of rectal therapy may be limited by poor patient acceptance [13].

The proximal distribution of 5-ASA differs among suppositories, foams, and liquid enemas [13]. Suppositories deliver drug only to the rectum, as evidenced by scintigraphic studies [16, 17]. Foam preparations reach the sigmoid colon [17, 18], while enemas deliver medication up to the splenic flexure [13, 17, 19]. The proximal spread of an enema is dependent upon its volume [13] and viscosity [20]. All formulations of 5-ASA are equally effective in inducing remission [21], but foam-based products offer more uniform colonic coating [22] and greater patient comfort [23] than liquid enema. Enema retention optimizes efficacy and is improved with greater viscosity [20] but reduced by disease severity [24] (Fig. 36.2).

Efficacy

Oral Formulations

Controlled trials report symptomatic remission in up to 61% of patients taking oral 5-ASA [25–27], and a meta-analysis reported a significant odds ratio of 0.4 (95% CI 0.3–0.53) for failure of 5-ASA to induce remission [28]. For the induction of remission with 5-ASA the number need to treat has been estimated to be 10, while the number needed to treat to maintain remission is 6 [29].

Both sulfasalazine and 5-ASA are used to induce and maintain remission of ulcerative colitis and have roughly similar efficacy for both indications [30]. However, 5-ASA may induce clinical and endoscopic improvement more quickly and is better tolerated [27]. For maintenance of remission, sulfasalazine was superior to 5-ASA in a meta-analysis, however, the individual trials have been criticized for enrolling patients known to be tolerant of this medication [31].

Rectal Formulations

Rectal 5-ASA is a first-line therapy for both induction and maintenance of remission in patients with mild to moderately active distal ulcerative colitis [13]. A meta-analysis of five trials found topical 5-ASA to be superior to placebo, with pooled odds ratio 7.36 (95% CI 4.72–11.47) for symptomatic improvement and 10.37 (95% CI 5.72–18.8) for symptomatic remission. Rectal 5-ASA also induced mucosal healing, with odds ratios for endoscopic and histologic remission of 8.23 (95% CI 4.08–16.58) and 12.47 (95% CI 3.75–41.43), respectively [14, 32]. No dose–response relationship has been defined for rectal 5-ASA, and efficacy does not appear to differ among suppository, foam and enema preparations.

Rectal 5-ASA is more effective than rectal corticosteroids, with pooled odds ratios for symptomatic, endoscopic and histologic remission of 2.42 (95% CI 1.72–3.41), 1.89 (95% CI 1.29–2.76) and 2.03 (95% CI 1.28–3.2), respectively [32, 33]. When compared to oral 5-ASA therapy, rectal treatment is at least as effective in inducing remission, and may be superior as indicated by physician and patient global improvement scores [13, 32, 34].

Rectal 5-ASA is more effective than placebo for maintaining remission of distal ulcerative colitis [34]. A meta-analysis pooling data from four placebo-controlled trials found that rectal 5-ASA had a pooled odds ratio of 5.6 (95% CI 3.0–10.5) for maintaining symptomatic remission [13] and was superior to oral 5-ASA in three trials with a pooled odds ratio of 2.3 (95% CI 3.0–10.5) [13].

Combination Therapy

The combination of oral and rectal 5-ASA is superior to either monotherapy [15, 35, 36], even among patients with extensive or pan-colitis. This may reflect either increased local concentrations of active drug or improved distribution of drug through the affected segments. Combination therapy is superior in patients at high risk for relapse, as the addition of rectal 5-ASA to oral therapy results in significantly fewer recurrences [35, 36], clinic visits, hospitalizations, and endoscopic evaluations [36]. Combination 5-ASA therapy also decreases steroid usage [36], making dual therapy an effective alternative for patients judged to be at high risk of disease exacerbation. The combination of rectal 5-ASA and topical steroids also achieves higher remission rates than either agent as monotherapy [37]. Combination strategies should be considered in patients who fail 5-ASA monotherapy as an alternative to systemic corticosteroids to avoid steroid-related adverse effects.

Dose Response

The optimal dosing of 5-ASA for both induction and maintenance of remission remains controversial. For induction of remission of mild to moderately active disease, lower daily doses (2.0–2.4 g) and higher daily doses (4.0–4.8 g) have demonstrated similar efficacy in clinical trials [38, 39].

However, the 4.8 g dosage may be more effective in some subgroups, such as patients with moderately active versus mild disease [40]. The gain in efficacy achieved by adding rectal to oral 5-ASA therapy does provide corollary evidence that increased luminal drug levels improve efficacy, although this may also reflect an improved distribution of drug throughout the colon.

Following induction, 5-ASA is efficacious as maintenance therapy [30, 41], with up to 50% of patients maintaining remission on daily 5-ASA doses of 1.5–4.8 g [41]. Although there appears to be a dose-dependent effect for sulfasalazine, 2 g daily is often prescribed due to the limited tolerability of higher doses [42]. It remains unclear whether there exists a similar dose response for oral 5-ASA formulations in maintaining remission [43, 44]. One randomized trial comparing balsalazide at 1.5 g and 3 g twice daily found remission rates of 78% with the higher dose compared to 44% with the lower dose [45]. However, no dose response was seen in a recent meta-analysis [31].

No dose response has been observed for the induction or maintenance of remission with rectal 5-ASA formulations [14].

Duration

The optimal duration of induction therapy has not been defined, and may vary among formulations of 5-ASA [25]. Most efficacy trials have evaluated 8 weeks of therapy [25–27], but response is often observed sooner [25]. Improvement in stool frequency has been reported 4 weeks after initiating sulfasalazine, with resolution of bleeding after 8 weeks [27]. Improvements in bowel frequency [25, 27], rectal bleeding [25, 26], endoscopic appearance, and physician global assessment [25] have been observed after 2 weeks of oral 5-ASA.

Similarly, the optimal duration of maintenance therapy with 5-ASA remains unknown, as no controlled trials have addressed this question. Lifelong 5-ASA therapy has been advocated both to decrease the frequency of disease flares and to reduce the risk of colorectal cancer. However, nonadherence can be problematic. Noncompliance is highest among single males, patients with left-sided colitis, and those on more complicated dosing regimens [46]. Decisions to continue long-term maintenance therapy should consider patient preferences and patient education [1].

Safety

The frequency of adverse events attributed to sulfasalazine and 5-ASA is highly variable in the literature, in part because of variations in dose, formulation, and event definition [8]. Adverse events are less common with 5-ASA than with sulfasalazine [8], likely from loss of the sulfapyridine moiety [3]. One review found no significant difference in the rate of adverse events between 5-ASA and placebo. A study that followed patients for 8 years reported drug-related adverse events in 20% of patients treated with sulfasalazine versus 6.5% of those treated with 5-ASA [47]. The most common adverse effects of sulfasalazine were dyspepsia, rash, and headache in 62, 35, and 27% of patients, respectively [47]. Of note, 40% of patients that were initially intolerant of sulfasalazine successfully underwent desensitization through a protocol of gradual dose escalation [47]. The overall rate of adverse effects with 5-ASA is 1.5% in patients never exposed to sulfasalazine, 4% in patients with prior sulfasalazine exposure, and 22% in patients with prior sulfasalazine intolerance [47]. This overlap suggests that some intolerance to sulfasalazine can be attributed to 5-ASA [48]. Common adverse effects of 5-ASA include rash, diarrhea, headache, and fever, which occur in 37%, 25%, 17% and 17% of patients, respectively [47]. Other organ-specific adverse effects are summarized in Table 36.1.

Table 36.1 Summary of the adverse events reported with sulfasalazine and 5-ASA

Renal
Asymptomatic elevation in creatinine
Asymptomatic decline in creatinine clearance
Nephrotic syndrome
Tubulointerstitial nephritis
Sulfapyridine stones
Hematologic
Leucopenia
Anemia
Heinz body anemia
Hemolysis
Immune complex hemolytic anemia
Folate deficiency anemia
Cardiac
Pericarditis
Myocarditis
Reproduction
Spermatic abnormalities
Motility
Form
Concentration
Folate deficiency
Diarrhea in the infant
Gastrointestinal
Dyspepsia
Diarrhea
Pancreatitis
Respiratory
Pulmonary eosinophilia
Neurological
Benign headaches
Intracranial hypertension
Sensorimotor neuropathy
Immune
Lupus-like syndrome
Erythroderma
Toxic epidermal necrolysis
Hypersensitivity reactions
Hepatotoxicity
Jaundice
Thrombocytopenia
Peripheral eosinophilia
Topical
Discomfort
Inconvenient drug administration

Renal

A variety of renal complications of 5-ASA therapy have been reported. However, it is important to note that renal disease may also be a primary complication of inflammatory bowel disease itself. Known renal manifestations of inflammatory bowel disease include nephrolithiasis, urinary obstruction, fistualization, glomerular disease, protein-losing nephropathy, secondary amyloidosis, and renal failure [49].

Long-term 5-ASA and sulfasalazine have been associated with a gradual increase in serum creatinine levels and a decline in creatinine clearance. These changes have been correlated with both the duration of therapy and the total daily dosage. One study reported a decline in creatinine clearance from 104.6 to 93.1 ml/min, and suggested that baseline renal dysfunction was predictive of subsequent renal decline [50]. However, the clinical significance of such small changes and their causal relationship to 5-ASA exposure has yet to be established. Still, patients on long-term 5-ASA therapy have been advised to have annual testing for renal function.

Sulfasalazine-induced nephrotic syndrome is an unusual complication of 5-ASA therapy that is believed to be mediated via podocyte toxicity and may be reversible with systemic corticosteroids [51]. Sulfapyridine stones have also been reported [52] following acetylation and renal excretion. Tubulointerstitial nephritis has been reported in patients receiving 5-ASA therapy, but its pathogenesis is unclear and it may be an extra-intestinal manifestation of inflammatory bowel disease [47, 50].

Hematologic

Hematologic toxicities have been attributed more to sulfasalazine than to 5-ASA. A reversible leucopenia has been associated with serum sulfapyridine concentrations greater than 50 mcg/mL. Sulfapyridine is acetylated to form *N*-acetylsulphapyridine by *N*-acetyltransferase 2 (NAT2). Case reports suggest that a NAT2 variant results in slow acetylation which subsequently increases serum sulfapyridine levels and predisposes to drug toxicity [53]. Sulfasalazine is known to induce Heinz body anemia in patients with hemoglobinopathies, as well as hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency. Rare idiosyncratic immune complex hemolytic anemia in patients without an underlying disease process has also been described [54]. Sulfasalazine can also induce folate deficiency (see below), although inadequate folate intake is uncommon in patients consuming unrestricted diets [55].

Cardiac

Both sulfasalazine and 5-ASA have also been associated with isolated pericarditis as a hypersensitivity reaction within 2 weeks of medication initiation or following dose escalation. Sulfasalazine has also been associated with pericarditis in the context of a systemic lupus-like reaction. There is variable experience with recurrence of sulfasalazine-induced pericarditis when patients are re-challenged with 5-ASA. Sulfasalazine and 5-ASA-induced pericarditis appear to resolve with medication discontinuation [56].

Myocarditis is a rare but life-threatening extra-intestinal manifestation of inflammatory bowel disease, but has also been reported after 5-ASA therapy. A case report describes eosinophilic cardiac infiltrates consistent with a hypersensitivity reaction, improvement of cardiac function with withdrawal of 5-ASA, and recurrence of myocarditis with 5-ASA re-challenge. 5-ASA-induced myocarditis has been successfully treated with steroids and azathioprine [57].

Reproductive

Fertility is affected in all male patients treated with sulfasalazine as determined by seminal fluid analysis. Abnormalities in sperm motility, form, and concentration occur in 92, 42, and 40%, respectively. There is no known correlation with sulfasalazine dose [58], but form and concentration have been observed to improve when patients are switched to 5-ASA [47, 58]. Motility improves to

a lesser degree. Men have been encouraged to discontinue sulfasalazine or switch to 5-ASA 3 months prior to conception as the life span of sperm is 120 days [59].

Sulfasalazine and most 5-ASA products carry an FDA class B designation for use in pregnancy, meaning that there is no evidence of fetal risk but randomized controlled data are lacking. Olasalazine, in contrast, is a class C medication. Although, there is no evidence to suggest an increase in congenital abnormalities with sulfasalazine therapy, the potential for medication-induced folate deficiency exists. This has prompted the recommendation for women on sulfasalazine to take oral folate supplements prior to conception and throughout pregnancy [59]. Unlike other sulfa-based medications, sulfasalazine does not lead to the displacement of bilirubin or kernicterus in the infant. Thus breast-feeding is considered low-risk [59].

A meta-analysis of pregnancy outcomes in women with inflammatory bowel disease exposed to 5-ASA during gestation showed nonsignificant trends toward higher rates of congenital abnormalities, stillbirths, spontaneous abortions, preterm deliveries, and low birth weight, when compared with those who had not received medications [60]. Although, 5-ASA from breast-milk can lead to diarrhea in the infant, this is not a contraindication to continuing treatment during lactation, provided that attention is given to stool changes and infant hydration [59]. Overall, the evidence supports the safety of sulfasalazine and 5-ASA during pregnancy and lactation. The risks of these medications are thought to be less than the harm associated with severe disease exacerbations during pregnancy [59].

Gastrointestinal

Gastrointestinal symptoms are the most common adverse effect associated with sulfasalazine and 5-ASA. Up to 62% of patients on sulfasalazine experience dyspepsia, while up to 25% of those on 5-ASA develop paradoxical diarrhea [47]. 5-ASA-induced diarrhea is idiosyncratic and can occur at any dosage, and even after a single administration [61–63]. A possible risk factor for this is extensive or pan-colitis [61]. Several mechanisms for this effect have been invoked, including accelerated orocecal transit [61] and increased small intestinal fluid secretion [61, 64]. The observation that an individual may develop diarrhea with one 5-ASA formulation, while remaining tolerant to another [61, 62] suggests intestinal sensitivity to an inert compound within the medication [62] as the causative mechanism in a subgroup of patients. It is challenging to distinguish disease activity from adverse effect, except that drug-induced diarrhea is generally mild and responds to medication withdrawal [47, 62, 63].

Case reports have associated acute pancreatitis with wide range of 5-ASA and sulfasalazine doses [65] (0.8–5 g daily) [66] and treatment durations [65, 67] (2 days to 2 years) [66], suggesting an idiosyncratic reaction [65, 66]. Although cases are generally mild, fatal necrotizing pancreatitis has also been described with sulfasalazine [66]. An analysis of population-based hospital discharges, however, found no attributable risk of acute pancreatitis for either sulfasalazine or 5-ASA. This study suggested that the underlying inflammatory bowel disease may be causative [68]. Disease-related pancreatitis is thought to be mediated by cholesterol and pigment stones due to ileal disease, structural abnormalities of the duodenum, and immunological derangements [66].

Respiratory

Although sulfasalazine-induced pulmonary toxicity is rare, its most commonly described manifestation is pulmonary eosinophilia attributed to the sulfapyridine moiety. This appears to resolve with

discontinuation of sulfasalazine [69] and relapses on re-challenge with sulfasalazine but not 5-ASA [70]. However a similar clinical presentation, including biopsy proven interstitial pulmonary fibrosis has been reported with 5-ASA [71].

Neurological

Benign headaches are common with sulfasalazine therapy [47], but rare reports have associated intracranial hypertension with both sulfasalazine and 5-ASA [72]. A sensorimotor neuropathy mediated by sulfasalazine and, much less frequently, 5-ASA has been reported. A case report temporally associated lower limb dysesthesia and gait abnormality with 5-ASA [73]. The mechanisms for such neurological toxicities remain unknown, but there is reported improvement with drug withdrawal [72, 73].

Immune

Sulfasalazine and 5-ASA have been associated with the development of lupus-like syndromes, including lupus nephritis [74]. Both agents have also been associated with erythroderma and toxic epidermal necrolysis. Caution should be exercised in using 5-ASA products in patients with severe reactions to sulfasalazine, even when topical therapy has previously been tolerated [75]. Other hypersensitivity reactions have manifested as hepatotoxicity, thrombocytopenia and peripheral eosinophilia, even after several years of drug exposure [76].

Rectal Therapy

The extent of systemic absorption from rectally administered 5-ASA is dependent upon the acidity of the topical preparation, and is lower in active disease states. Despite this variability, topical 5-ASA results in substantially lower systemic drug distribution than oral formulations as determined by urinary excretion and serum metabolite analysis. Thus, rectal formulations result in significantly fewer systemic adverse effects than oral therapy. In fact, the majority of adverse events attributed to topical 5-ASA is due to discomfort and inconvenience from drug administration [13].

Cancer Prevention

The risk of colorectal cancer in patients with ulcerative colitis increases with duration of disease and the extent of inflammation. Additional risk factors in this population include concomitant primary sclerosing cholangitis, folate deficiency, and a family history of colorectal cancer [77, 78]. Currently, management of this risk involves colonoscopic surveillance and colectomy for dysplasia [79, 80]. This has prompted the search for more cost-effective, less invasive methods for primary prevention [79].

In ulcerative colitis, the development of dysplasia is believed to be secondary to the increased cellular turnover associated with chronic inflammation [81, 82] and altered prostaglandin metabolism [83]. Despite this, corticosteroids have not been shown to decrease cancer risk [77]. 5-ASA has been postulated to exert primary chemoprotective effects via mechanisms other than its control of disease

activity [81]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with a decreased rate of colon cancer in the general population, in patients with familial adenomatous polyposis [78], and in animal models [78, 81]. Mesalamine, instilled as an enema in patients with sporadic colon cancer induces apoptosis in tumor cells [84], which may be mediated by inhibition of nuclear factor kappa B [81]. Additionally, NSAIDs and 5-ASA activate peroxisome proliferator-activator receptor gamma which leads to anti-inflammatory and anti-proliferative effects in colonic epithelium [81, 82]. These agents also display antioxidant properties [81]. Cumulatively, this evidence has prompted investigation of 5-ASA as a means of chemoprevention in ulcerative colitis.

Of note, an increase in colorectal cancer among patients with ulcerative colitis is not seen in Denmark, which has been attributed to widespread and longstanding use of 5-ASA maintenance therapy [78]. Observational data have associated 5-ASA therapy with a 70% reduction in colorectal carcinoma [77, 80, 85]. This protective effect is greatest when mesalamine is taken at doses greater than 1.2 g daily [77, 85]. One study suggested that the risk of colorectal carcinoma or dysplasia in these patients decreases by 16% for each 1,000 g increase in the cumulative dosage of 5-ASA and by 56% for each 1 g incremental increase in daily dose [85]. Although protective effects are seen with daily sulfasalazine doses greater than 2 g, its protective effect seems less robust [77]. This may be secondary to alterations in folate metabolism, as folate deficiency is an independent risk for colon cancer [81].

Two recent cohort studies have questioned the role of 5-ASA in primary chemoprevention. In a nested case-control analysis of patients enrolled in a rigorous surveillance program at St. Mark's Hospital in the United Kingdom, histological inflammation was the only significant factor in the development of colorectal cancer [83]. A similar study of a cohort of patients followed in the United States reached a similar conclusion [86]. Neither analysis identified 5-ASA or sulfasalazine as an independent risk factor, suggesting that the chemopreventive benefit of these agents lies in their ability to control disease activity.

Cost-Effectiveness

The cost-effectiveness of 5-ASA therapy for ulcerative colitis has been evaluated in several economic analyses. A cost-utility decision analysis concluded that a daily 5-ASA dose of 4.8 g was less costly and more effective than a daily 5-ASA dose of 2.4 g over a limited time horizon of 12 weeks in patients with moderately active disease, with fewer hospitalizations and surgeries [87]. Maintenance 5-ASA therapy has also been estimated to cost approximately \$8,810 (US) for each flare that is prevented [88], although this estimate was highly sensitive to variations in 5-ASA cost, rates of flare, rates of colectomy, and health state utility. In a Markov simulation model, 5-ASA therapy allowed cost-effective extension of colonoscopic surveillance intervals from 1 to 3 years [89].

Conclusion

Despite new developments in the management of inflammatory bowel disease, sulfasalazine and 5-ASA have maintained a prominent role in the treatment of ulcerative colitis. Over time, both oral and topical formulations have proven their efficacy as induction and maintenance therapies [13, 28, 31] and possibly in chemoprevention of colorectal cancer [77, 85]. As these agents are well-tolerated [47] and cost-effective [35], sulfasalazine and 5-ASA are advocated as first-line therapies for mild to moderate disease activity, and will likely maintain their key role in management algorithms.

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Chapter 37

Conventional Medical Management of Ulcerative Colitis: Corticosteroids

A. Hillary Steinhart

Keywords Ulcerative colitis • Steroids • Cortisone • Hydrocortisone • Prednisone • Prednisolone • Methylprednisolone • Topical therapy • Intravenous therapy

Systemic Steroids for Moderate to Severe Disease

The effectiveness of cortisone was initially proven in one of the first randomized placebo controlled trials of any therapy in any condition [1]. This led to the widespread adoption of steroids as standard therapy for patients with ulcerative colitis, particularly in those with moderate to severe disease activity [2–4]. Although the original randomized placebo controlled trial from Truelove and Witts demonstrated the effectiveness of cortisone 100 mg for up to 6 weeks in patients with symptoms of ulcerative colitis with clinical disease activity ranging from mild to severe, based on the Truelove–Witts disease activity index developed for that trial (Table 37.1), the use of systemic steroids has generally been limited to patients with moderate to severe disease activity or to those with less severe disease that is refractory to sulfasalazine or 5-aminosalicylic acid-based therapies. In the subgroup of patients with moderate and severe disease remission was obtained in 27/85 patients (32%) and improvement was obtained in an additional 26/85 patients (31%) [1]. Although cortisone was the steroid that was first used in the Truelove and Witts trial, other steroid preparations, including prednisone, prednisolone, hydrocortisone, and methylprednisolone have been used with similar remission and response rates obtained in other controlled trials as well as in uncontrolled observational and retrospective series [5–13]. The most commonly used oral preparations in clinical practice are prednisone and prednisolone. The doses of systemic steroids used in the clinical trials and cohort studies have generally ranged between the equivalents of 20–60 mg of prednisone given daily. Within that range there appears to be an increase in response rates although side effects tend to be more frequent at the higher doses [6, 9]. In general, daily doses equivalent to 40–60 mg of prednisone are used for induction therapy in the treatment of moderate to severe flares of ulcerative colitis in out-patient clinical practice. Compared to the usual once-daily administration, there does not appear to be any advantage with respect to efficacy or side effects in

A.H. Steinhart, MD, MSc, FRCP(C) (✉)

Department of Medicine, IBD Centre, Mount Sinai Hospital,

University of Toronto, 600 University Avenue, Toronto, ON, Canada M5G 1X5

email: hsteinhart@mtsinai.on.ca

Table 37.1 Ulcerative colitis severity criteria

Variable	Mild disease	Severe disease
Stool frequency	4 or less per day	6 or more per day
Blood in stools	No more than small amounts of macroscopic blood	Macroscopic blood
Fever	No fever	Mean evening temperature more than 37.5°C or a temperature of more than 37.8°C or more on at least 2 days out of 4
Pulse	No tachycardia	Pulse more than 90 per min
Anemia	Not severe	Hemoglobin 75% or less
ESR	>30 mm/h	ESR ≤ 30 mm/h

Moderately severe disease activity is intermediate between mild and severe

Based on data from [2]

administering systemic steroids in divided doses through the day, at least over the initial 2-week period of induction therapy [14].

Response rates, depending upon the precise definition, have been reported to be in the range of 50–80% with clinical remission rates of approximately 25–35%. Improvement in the mucosal appearance and, in some cases, mucosal healing with resolution of gross inflammatory changes has also been demonstrated in patients treated with steroids for acute flares of ulcerative colitis [1]. The optimal duration of induction therapy and tapering regimens have not been clearly elucidated by clinical trials but 8–12 weeks of steroid therapy, starting with the highest dose for 1–2 weeks followed by dose tapering over the subsequent 6–10 weeks is frequently used. Whether this gradual tapering simply avoids symptoms of steroid withdrawal and glucocorticoid deficiency or whether it reduces the risk of subsequent increase in mucosal inflammation and recurrence of disease symptoms is not known. However, it is clear that a proportion of patients who initially respond to steroids experience a flare of disease activity when the dose of steroid is tapered below a certain level and, as such, seem to require continuous steroid therapy in order to prevent disease worsening. A cohort study from Olmstead County in Minnesota, USA, has suggested that, of the 34% of the patients diagnosed with ulcerative colitis between 1970 and 1993 who required corticosteroid therapy, 84% had partial or complete remission [15]. Of these patients, only 49% had prolonged response and were corticosteroid free at 1 year with 22% being considered steroid dependent. Although some patients appear to develop steroid dependence, there has been little or no evidence to support the use of steroids for the maintenance of remission. A trial of prednisone in doses of up to 15 mg per day did not show any beneficial effect on remission maintenance [16]. Another trial which included 68 patients in whom ulcerative colitis was brought into remission on a 6-week course of oral cortisone 200 mg per day or intramuscular ACTH 80 units once daily, randomized those patients to receive cortisone 25 mg twice daily or placebo for up to 1 year [2]. Patients treated with cortisone were, if anything, more likely to experience disease relapse than patients treated with placebo, although the difference was not statistically significant [2]. A 3-month crossover trial of 24 patients already receiving sulfasalazine examined the use of prednisolone 40 mg, given on alternate days, in comparison to placebo and found that 11/24 patients relapsed while on placebo but not while on prednisolone, whereas only 1/24 relapsed while on prednisolone but not while on placebo [17]. Although this latter study seemed to suggest that high-dose alternate day steroid may improve short-term remission rates, the risk of significant adverse events at that dose appears to outweigh any potential benefit and, in those instances in which aminosalicylates are not effective maintenance therapy, particularly

following steroid induced remission, the use of the purine analogs azathioprine or 6-mercaptopurine [18] or an anti-TNF α antibody [19] should be considered.

Topically Active Steroids

Corticosteroids can be used with good clinical effect in suppository, foam, or liquid enemas in order to treat patients with ulcerative colitis limited to the rectum or rectum and sigmoid colon [20–29]. The topical therapies – which include hydrocortisone, prednisolone-21-phosphate, prednisolone metasulfobenzoate, beclomethasone dipropionate, budesonide, tixocortol pivalate – generally undergo high first-pass metabolism when absorbed and passed through the portal circulation and through the liver or they are not highly water soluble and do not undergo significant mucosal absorption. However, a study of prednisolone retention enemas demonstrated serum prednisolone levels similar to those achieved following oral administration of a similar dose [30]. Suppression of serum cortisol level was observed in a trial of beclomethasone dipropionate enemas, thus suggesting the presence of a systemic effect [31]. However, adverse events were not more frequent in patients treated with the steroid enema as compared to those treated with 5-aminosalicylic acid enemas.

Studies comparing 5-aminosalicylic acid containing enemas to steroid enemas have suggested that the 5-aminosalicylic acid enemas likely have an advantage over steroid enemas and, as a result, should be used as first-line topical therapy for distal ulcerative colitis or proctitis [32].

Orally administered corticosteroids with low systemic bioavailability have been studied in ulcerative colitis extending proximal to the sigmoid colon [13, 31, 33]. Fluticasone propionate, 20 mg daily for 4 weeks, was compared to prednisolone 40 mg daily followed by tapering to 10 or 20 mg daily in 205 patients with left-sided and extensive ulcerative colitis. Prednisolone was superior at 2 weeks but by the end of the 4-week study period the difference between the two treatments was not significant [33]. Fewer steroid-associated side effects and minimal adrenal suppression was noted on fluticasone. Another smaller trial of 72 patients with mild to moderately active left-sided and extensive colitis compared a specially formulated colonic release preparation of orally administered budesonide 10 mg daily to prednisolone 40 mg daily followed by tapering of both drugs over a 9-week period [13]. Endoscopic appearance, which was the primary outcome, was not different between the two treatments but there was a trend to better endoscopic appearance on the left side of the colon in patients treated with prednisolone. Serum cortisol levels, which were suppressed in the prednisolone-treated patients, were not adversely affected by budesonide therapy. A third trial of 119 patients with mild to moderate active left-sided or extensive ulcerative colitis compared the use of beclomethasone dipropionate 5 mg daily for 4 weeks to placebo [31]. All patients received 5-aminosalicylic acid 3.2 g per day. Disease activity index scores were better in the beclomethasone arm and a higher proportion of patients were in remission (58.6% vs. 34.4%). However, some systemic bioavailability was noted with serum cortisol levels being lower after 4 weeks of beclomethasone treatment. This biochemical adrenal suppression did not appear to translate into more frequent side effects or complications.

In summary, use of orally administered corticosteroids with low systemic bioavailability appears to have some promise in patients with extensive and left-sided mild to moderately active ulcerative colitis with clinical responses similar to that seen with conventional systemically available steroids and with apparently fewer steroid-associated side effects. However, in most instances biochemical adrenal suppression is observed with the orally administered topical steroids and the time to response may be slower than with the systemic steroids.

Intravenous Steroids for the Treatment of Severe Acute or Fulminant Ulcerative Colitis

Therapy with intravenous glucocorticoids has been the standard of care for the first-line management of severe acute or fulminant ulcerative colitis for many decades. Other therapies, such as cyclosporine and infliximab, have been introduced over the past 10–20 years but they are still relegated to second line or “rescue” therapy for patients who are not responding to therapy with steroids. The use of intravenous steroids was described by Truelove and Jewell in 1974 [3] and by Jarnerot in 1985 [4]. Truelove and Jewell described the administration of intravenous fluids, prednisolone 60 mg per day in divided doses, tetracycline 250 mg four times per day and, in some cases, parenteral nutrition. Patients were kept nil per os and were also administered hydrocortisone succinate sodium 100 mg in 120 mL of saline twice daily as a rectal drip. After 5 days patients who were in remission were switched from intravenous to oral prednisolone, 10–15 mg four times daily, and this was reduced to 5 mg four times daily after 1 week [3]. In the intensive intravenous regimen reported by Jarnerot and colleagues, patients were treated with betamethasone 3 mg, administered intravenously twice daily, and prednisolone-21-disodium phosphate, 30 mg administered rectally twice daily [4]. In addition, patients were treated with doxycycline 100 mg once daily and parenteral nutrition, albumin, blood, and additional electrolytes as indicated. Patients were permitted to take only small amounts of water by mouth. Remission rates were observed to be lower with increasing disease severity and extent such that 55.7% of patients with severe attacks achieved a clinical remission compared with 86.9% with moderate disease [4]. This impression was confirmed by a meta-analysis which found that in studies which included only patients with severe disease, the colectomy rate (i.e. the failure rate for intravenous steroids) was 34% as compared to 26% in the studies that included patients with moderately active disease [34].

In general, intravenous steroids are administered as hydrocortisone 200–400 mg daily in divided doses or methylprednisolone 40–100 mg daily in divided doses. Most studies have reported on doses of, or close to, 60 mg of methylprednisolone daily or 400 mg of hydrocortisone daily. An analysis of colectomy rates according to the dose of steroid used in individual studies found no relationship between the dose used and the colectomy rates observed [34]. When intravenous steroids are administered, the total daily dose is usually given in divided doses ranging from twice to four times per day.

Several trials have compared the use of intravenous hydrocortisone to intravenous or intramuscular ACTH for the treatment of moderate to severe ulcerative colitis [7, 8, 35]. These studies utilized hydrocortisone 300–400 mg per day and showed no overall benefit in terms of clinical improvement or remission. However, one trial demonstrated a higher rate of remission on ACTH in patients who had never received prior steroids [35]. This trial used a higher daily dose of ACTH (120 units per day) than another trial that did not demonstrate this effect of ACTH in steroid-naïve patients [7]. In the latter study, patient numbers were relatively small and not sufficient to rule out a possible treatment benefit [7]. Treatment with ACTH did not appear to provide any advantage with respect to side effects or complications [35]. In general, hydrocortisone or methylprednisolone are favored over the use of ACTH for the treatment of patients with severely active ulcerative colitis given their ease of administration, lower cost, and availability.

For patients treated with intravenous steroid therapy for moderate to severe flares of ulcerative colitis, frequent and careful monitoring for treatment response and possible complications is critical. When a patient is found to have a complication such as perforation or massive bleeding, surgical colectomy should be carried out without delay. When treatment is not effective, the patient should be considered for rescue therapy, in the form of cyclosporine or infliximab, or for surgical colectomy. It is important that the decision to move on to rescue therapy or surgery be made relatively early in the course of intravenous steroids in order to minimize the risk of opportunistic infections

and surgical complications. There are a number of predictive tools or factors that can be employed anywhere between day 1 and day 3 of intravenous steroid therapy in order to help determine the likelihood of an individual patient responding to steroid therapy so as to assist in clinical decision making [36–43]. The factors that have been found to be predictive of lack of response in these studies include number of bowel motions, endoscopic appearance with large or deep ulcers, serum albumin and serum C-reactive protein.

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Chapter 38

Conventional Medical Management of Ulcerative Colitis: Azathioprine

Barrett G. Levesque and Edward V. Loftus

Keywords Azathioprine • 6-Mercaptopurine • Ulcerative colitis • Proctocolitis • Immunosuppressive agents • Drug toxicity

Introduction

Induction therapy with cortisone for ulcerative colitis was first described by Dearing and Brown of Mayo Clinic in 1950 [1], and the final results of a randomized clinical trial by Truelove and Witts of Oxford in 1955 [2]. By 1965, Lennard-Jones and colleagues had definitively shown that prednisone was not effective in maintaining remission in ulcerative colitis [3]. Alternative immunosuppressive agents were sought which would maintain a clinical remission and spare the side-effects of long-term corticosteroids. Azathioprine (AZA) was originally demonstrated as a therapy for ulcerative colitis in 1966 by Bowen and colleagues at the University of Chicago [4]. In an open-label case series, ten hospitalized patients were treated with relatively high doses of AZA (4–6 mg/kg/day) which was then continued as an outpatient. Most patients improved; however, the results were confounded by other medications and lacked a control group [4]. In the subsequent four decades, controlled trials of AZA have revealed mixed results in induction and maintenance for ulcerative colitis [5–8]. The role of AZA in the treatment of ulcerative colitis has continued to be debated due to its evidence base of predominantly heterogeneous small clinical trials and the advent of biologic therapy. The following chapter on AZA in the treatment of ulcerative colitis emphasizes practical implications of pharmacology and metabolism, efficacy estimates from clinical trials, safety, and practical dosing and toxicity monitoring methods for clinical practice.

Pharmacology and Metabolism

AZA is a prodrug which undergoes approximately 88% conversion to 6-mercaptopurine (6MP) by nonenzymatic nucleophilic attack in red blood cells and other tissues [9, 10] (Fig. 38.1). AZA is 55% 6MP by molecular weight, and a conversion factor of 2.07 is used to convert a dose of 6MP to

B.G. Levesque, MD, MS • E.V. Loftus, MD (✉)
Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First Street, S.W., Rochester, MN, USA
e-mail: loftus.edward@mayo.edu

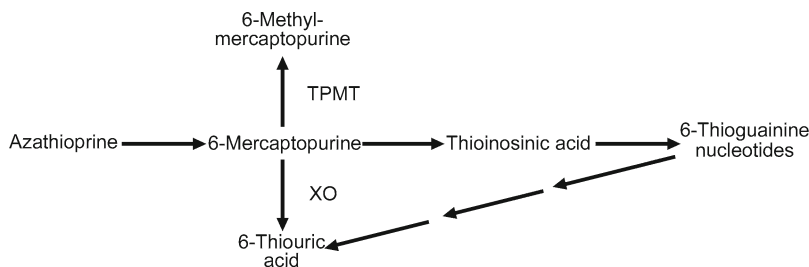


Fig. 38.1 Metabolism of Azathioprine. *TPMT*, thiopurine methyltransferase; *HPRT*, hypoxanthine phosphoribosyl transferase; *XO*, xanthine oxidase. (Reproduced from Chan GL, Erdmann GR, Gruber SA, et al. Azathioprine metabolism: Pharmacokinetics of 6MP, 6-thiouric acid and 6-thioguanine nucleotides in renal transplant patients. *J Clin Pharmacol* 1990;30:358–63. Copyright ©1960 by SAGE Publications. Reprinted by Permission of SAGE Publications)

AZA dose [11]. Xanthine oxidase, thiopurine methyltransferase (TPMT), and hypoxanthine phosphoribosyl transferase are the three enzyme systems that break down 6MP to 6-thiouric acid, 6 methylmercaptapurine (6MMP), and precursors of the active 6-thioguanine nucleotides (6-TGN) [12], respectively. The mechanism of action of AZA and 6MP has not been fully elucidated. 6-TGN are incorporated into nucleic acid and subsequently inhibit synthesis of protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) [12]. The conversion of 6MP to 6MMP by TPMT is subject to different rates due to genetic variation in TPMT activity. Approximately 0.3% of the population is homozygous for the mutant inactive form of TPMT, 11% are heterozygous with intermediate activity, and 89% have normal activity [13]. The half-life of 6-TGN in red blood cells is 3–13 days, and may take 4 days to 3 years to reach a steady state [14]. While intravenous loading doses of AZA have been shown to be feasible and initial reports seemed promising [15, 16], a randomized placebo-controlled trial of intravenous loading in Crohn's disease showed no difference in time to clinical response [17]. Interestingly, steady-state levels of 6-TGN occurred by week 2 in both groups. In general, a therapeutic response may take 2–4 months for most patients [17, 18].

Several aspects of AZA metabolism have clinical implications. An inverse association between TPMT enzyme activity and 6-TGN concentration has been suggested [15], which supports the clinical experience that patients with intermediate TPMT activity levels are more likely to respond to lower doses of AZA therapy than patients with normal TPMT activity level. Due to the risk of life-threatening myelosuppression in patients who completely lack TPMT activity, it is prudent to check a TPMT genotype or phenotype (i.e. activity level) prior to beginning AZA or 6MP. For patients with normal genotype or activity level, it is reasonable to start 2–2.5 mg/kg body weight daily of AZA or 1.0–1.25 mg/kg body weight daily of 6MP. For patients with heterozygous genotype or intermediate activity level, the recommended starting dose is 1–1.25 mg/kg body weight daily for AZA and 0.5 mg/kg body weight daily for 6MP. Beginning patients on low doses (e.g. 50 mg daily) of AZA to reduce toxicity, and then slowly increasing this dose over several weeks, is not consistent with the pharmacology, in that dose-dependent toxicity (such as bone marrow suppression) will be delayed, but not prevented, and idiosyncratic reactions (e.g. drug fever, pancreatitis, arthralgia, rash) will not be prevented [11].

Assays for 6-TGN and 6-MMP metabolites are commercially available. 6-TGN levels greater than 235–250 pmol/ 8×10^8 erythrocytes and 6-MMP levels greater than 5,700 pmol/ 8×10^8 erythrocytes have been correlated with therapeutic response and hepatotoxicity, respectively [19, 20]. Routine measurement of TPMT activity level or genotype often helps determine the correct starting dose of AZA or 6MP and likely limits the utility of universal measurement of 6-TGN metabolites. Non-responders may have high or low 6-TGN levels [19] and there is scant safety data above 2.5 mg/kg/day of thiopurine in inflammatory bowel disease (IBD). A meta-analysis of 12

studies examining the use of 6-TGN metabolite levels in IBD showed that median 6-TGN levels were $66 \text{ pmol}/8 \times 10(8)$ red blood cells higher among responders than non-responders, but there was significant heterogeneity [21]. Patients with levels above a threshold of $230\text{--}260 \text{ pmol}/8 \times 10(8)$ erythrocytes had a remission rate of 62%, while those with levels below the threshold had a remission rate of 36%. Patients with and without hepatotoxicity may have high 6-MMP levels; however, elevation in alanine aminotransferase (ALT) in patients without other liver disease on AZA often warrants decreasing the dose of AZA and following the ALT level. The 6-TGN and 6-MMP levels may have the most clinical utility in assessing patients with no or incomplete response, when patient noncompliance is suspected, and in the 10% of patients who are TPMT heterozygotes.

Allopurinol competes with xanthine oxidase, increases levels of 6TGN, and shunts metabolism away from 6MMP production, which is a metabolite associated with hepatotoxicity [22]. The addition of allopurinol may correct an unfavorable ratio of 6-TGN to 6MMP by reducing 6MMP concentrations while raising 6-TGN concentrations [23]. The addition of allopurinol to AZA, and subsequent substantial dose reduction of AZA, has been suggested in patients with hepatotoxicity [22, 24], arthralgias, or nausea [25]. This is controversial and in some experiences has been associated with high rates of opportunistic infections [26] despite small prospective studies showing safety with long-term use [24, 27]. Selected use of 6-thioguanine as a therapy has been shown to be associated with early hepatic nodular hyperplasia disease of the liver [28] and not used in our clinical practice.

Efficacy Estimates for Induction and Maintenance Therapy

The efficacy of AZA has been shown to depend on the state of ulcerative colitis activity (i.e. whether the disease is active or in remission), and the relatively small clinical trials have examined the separate issues of its use as an adjunct or alternative to steroids and/or 5-ASA therapy in these disease states. Two systematic reviews of the efficacy of AZA in ulcerative colitis have been published recently, and both concluded that the use of AZA was of modest benefit in ulcerative colitis, with pooled odds ratios ranging from 1.4 to 2.6 depending on the particular outcome studied [29, 30]. However, one could argue that the individual studies upon which these pooled analyses have been based have been heterogeneous with respect to disease activity, outcome measures, blinding of patients and investigators, controls (placebo vs. 5-ASA), and lengths of follow-up, precluding our ability to synthesize an average result across all of the trials and make an overall conclusion in either direction [31].

Overall, AZA has had mixed results in trials examining its efficacy as an induction agent in active ulcerative colitis. Controlled trials by Jewell and colleagues and Caprilli et al. in the 1970s were among the first to show that AZA was not superior to placebo as an adjunct to corticosteroids or an alternative to sulfasalazine in the treatment of active ulcerative colitis over 1–3 months [5, 6]. In 2000, Sood and colleagues examined AZA as an adjunct to starting sulfasalazine and steroids in severe relapsing ulcerative colitis. There was not a significant difference in achieving remission among the two groups over a year [32]. In 2006, Ardizzone and colleagues showed AZA to be more effective than 5-aminosalicylic acid (3,200 mg daily) in attaining steroid-free remission in steroid-dependent ulcerative colitis (53% vs. 21%, $p=0.006$); however, the patients were not blinded to the type of therapy [33]. Nonetheless, remission was defined both on clinical and endoscopic disease activity, and AZA was superior to placebo in both per-protocol and intention-to-treat analyses [33].

Controlled trials of AZA for the maintenance of remission in steroid-dependent ulcerative colitis have been more promising, but have still shown mixed results. Jewell and colleagues showed no significant difference in remission rates between AZA (1.5–2.5 mg/kg/day) and placebo (40% and 23%, respectively ($p=0.18$)), among 80 patients over 11 months, where relapse was defined as recurrent blood in the stool and endoscopic evidence of inflammation [5]. A controlled

withdrawal trial by Hawthorne et al. in 1992 again showed no significant difference between AZA and placebo as an adjunctive therapy for chronically active ulcerative colitis over 12 months [34]. However, the relapse rates among patients in remission after a year of AZA or placebo were 36% and 59%, respectively, and this small trial of 79 patients may have been under-powered [34]. In 2002, Sood and colleagues showed that the addition of AZA to steroids and sulfasalazine in 35 newly diagnosed patients with severe CUC was superior to the addition of placebo in maintaining remission over a year [35]. Rates of relapse were 24% and 56% with adjunct AZA and placebo, respectively [35]. In a small open-label study ($n=25$), Sood et al. did not show a difference in remission rates with severe ulcerative colitis [36]. The discontinuation of AZA in patients in steroid-free remission has been associated with high rates of relapse in a long-term retrospective analysis [37]. The proportion of relapsing patients at 1 year, 2 years, and 5 years was one-third, one-half, and two-thirds, respectively [37].

Safety and Monitoring

Thiopurine therapies in IBD have the potential for significant adverse reactions. Thorough patient education prior to initiating therapy is advised for clinicians initiating or continuing these medications. There are several classes of adverse reactions: dose-dependent, dose-independent, infection risk, and malignancy risk.

Myelosuppression is a dose-dependent adverse reaction that may occur both early and late in AZA therapy [38, 39]. By testing for TPMT activity level or genotype, the 0.3% of patients without any TPMT activity should be prevented from receiving AZA or 6MP, which for them could cause rapid development of life-threatening cytopenia. For patients with normal and intermediate TPMT activity, frequent monitoring of complete blood counts (CBC) and liver transaminase and alkaline phosphatase is recommended. A genetic analysis of patients with myelosuppression on AZA therapy showed that only 27% carried mutant alleles [40]. Mesalamine-containing medications may potentiate myelosuppression [41]; however, dose reductions or changes in monitoring are not typically needed. A recent large retrospective cohort study showed that severe neutropenia and thrombocytopenia occur most often in the first 8 weeks of therapy [39]; however, myelosuppression has been shown to occur even a decade after starting therapy [38]. An example of monitoring would be to check CBC at baseline, then weekly for a month, bi-weekly for 2 months, and then monthly. ALT and alkaline phosphatase may be checked at baseline, 4 weeks, and then every 3 months if normal. Increasing mean corpuscular volume is correlated with increasing 6-TGN concentration and may be an inexpensive alternative to metabolite monitoring [42]. Leukopenia warrants stopping the medication for 2 weeks and restarting at a lower dose once leukopenia has resolved, whereas borderline leukopenia may be an indication of more frequent monitoring. Even mild elevations in hepatic enzymes are concerning, and if other etiologies are excluded, warrant discontinuing the medication due to risks in the long term of progressive liver disease such as fibrosis and nodular hyperplasia. Approximately 25% of patients may have resolution of mild elevations of transaminases after changing from AZA to 6MP [43].

Dose-independent adverse reactions to AZA include drug fever, pancreatitis, hair loss, arthralgias, nausea, and rash [11]. Drug fever typically presents within 2 weeks of starting therapy, and requires immediate cessation of the thiopurine. The risk of pancreatitis in AZA therapy is approximately 3%, and also typically occurs within the first 2 weeks of therapy and requires cessation of therapy as well [44, 45]. AZA-associated pancreatitis is most often mild, but can be severe [45]. After either high drug fever or pancreatitis, it is not recommended to change from AZA to 6MP or 6MP to AZA. However, 6MP may be tolerated in 60% of patients who develop other intolerances such as flu-like illness, nausea, vomiting, and rash while on AZA [43].

Infection is of significant concern in patients with ulcerative colitis taking AZA or 6MP. Patients taking AZA for ulcerative colitis are at an increased risk of opportunistic infections [46]. Increased risk of tuberculosis is not limited to biologic therapy – a large retrospective cohort study of patients in the U.K. General Practice Research Database treated prior to the era of biologic therapy showed a greater than twofold unadjusted relative risk of active tuberculosis in patients with IBD compared to the general population, although this may have been confounded by cigarette smoking and corticosteroid use [47]. The potential risks of AZA or 6MP must be balanced against the risks of either long-term corticosteroid use, which has been associated with infection and increased mortality risk [48], or severe disease activity with its risk of toxic mega-colon and septicemia. Although often overlooked in clinical practice, vaccination for influenza virus and *Streptococcus pneumoniae* are recommended for IBD patients considering or taking AZA or 6MP [49]. Live vaccines in immunosuppressed patients are generally contraindicated; however, varicella zoster vaccination may be considered in patients taking less than 3 mg/kg body weight daily of AZA [50]. Reactivation of latent hepatitis B virus is a risk for patients treated with AZA, and hepatitis B surface antigen should be checked prior to initiating therapy for patients with any risk factors for the disease [50].

Thiopurine therapy in IBD carries an increased risk of lymphoma. A meta-analysis by Kandiel et al. in 2005 of five single-center studies and one population-based study, totaling 3,891 patients, showed a relative risk of 4.18 for lymphoma with patients treated with thiopurines for IBD compared to the general population [51]. Of note, the “number-needed-to harm” varied from 4,357 in 20–29-year-olds to 355 in 70–79-year-olds [51]. A meta-analysis of patients taking anti-tumor necrosis factor (TNF) therapy and AZA showed 13 cases of non-Hodgkin’s lymphoma among 8,905 patients with 21,178 years of follow-up (6.1 cases per 10,000 patient-years, median follow-up of 48–201 weeks) where most patients had previous immunomodulator exposure [52]. The recent results from the CESAME study group in France followed 19,486 patients with a median follow-up of 35 months (IQR, 29–40 months) [53]. The adjusted hazard ratio for lymphoma in patients treated with thiopurines for IBD compared to those without was 5.28 (95% CI, 2.01–13.9). Incidence rates of lymphoma in patients continuing thiopurine varied from 0.37 cases per 1,000 person-years in patients less than 50 years old, 2.58 per 1,000 in those 50–65 years, and 5.4 per 1,000 in patients greater than 65 years old [53]. It remains unknown if there is a constant or cumulative risk of lymphoma with AZA therapy; however, in this study there were the same number of lymphomas reported in the first and third year of the study, and in the post-transplant setting the risk of post-transplant lymphoma has been shown to be constant given a constant dose of immunosuppression [53]. Most lymphomas associated with immunosuppression are Epstein-Barr virus (EBV)-positive, as was shown in a referral-based study from Mayo Clinic [54]. Of note, there were two cases of fatal post-mononucleosis lymphoproliferative disorder in EBV-negative patients in the CESAME cohort [53], and further research is merited into whether AZA should be avoided in these patients. There have been scattered cases of fatal hepatosplenic T-cell lymphoma in almost exclusively young male patients taking the combination of AZA/6MP and anti-TNF therapy for IBD [55]. However, the absolute risk in men on antimetabolite therapy has been estimated to be 1 in 23,724 [56]. There is an increased risk of non-melanoma skin cancers in patients on AZA therapy [57, 58], with an absolute risk increase of 14% (adjusted OR 4.27; 95% CI 2.08–5.29) among patients taking thiopurines for greater than 1 year [57]. Sunscreen and regular skin exams are recommended for patients on AZA therapy.

The uncertainty of risks of AZA for ulcerative colitis during pregnancy is a challenge for clinicians and patients. Women with ulcerative colitis who plan to become pregnant seek medical care to help them reach their goals of healthy pregnancies and healthy children. Active ulcerative colitis during conception and pregnancy has been associated with an increased risk of adverse perinatal outcomes [59, 60]. A recent study group described the pregnancy outcomes of a cohort of 204 women with IBD followed prospectively in France who were treated with and without thiopurines [61]. Approximately 40% of the patients were treated with thiopurines as part of their medical

regimen and compared to patients who were not receiving them to assess primarily for differences in live births, prematurity, birth weight, and congenital abnormalities. All groups had rates of live births in the range of the general population. Furthermore, the overall rates of congenital malformations in patients receiving medication (3.5%) were not significantly greater than the general population in France. The study was limited by its power to detect only a relatively large (fivefold) relative increase in malformations in the treatment groups. Although there were higher rates of prematurity and low birth weight among all of the patients that are concerning, the severity and impact of these differences on neonatal outcomes is not clear. For example, although many studies have reported preterm deliveries in IBD, most occur after 35 weeks gestation [62, 63]. Larger prospective studies are needed to detect small incremental increase in risks of AZA during pregnancy, such as the ongoing the Pregnancy in Inflammatory Bowel Disease and Neonatal Outcomes (PIANO) registry in the United States [64]. Currently, it is recommended that risks and benefits of AZA be discussed in order to enable patients to make an informed decision regarding its use during pregnancy; however, there is not sufficient evidence to recommend discontinuing AZA for patients in whom it is indicated.

Summary and Future Directions

Thiopurines are effective medications for many patients with ulcerative colitis. Genetic variation in the population leading to variable metabolism of AZA or 6MP warrants initial and ongoing laboratory monitoring throughout the course of treatment for dose-dependent toxicities. Dose-independent toxicity often occurs in the first few weeks of therapy, and some reactions may be avoided by switching from AZA to 6MP. Infections are a primary risk of therapy which merit vaccination prior to therapy, checking for latent disease, and close monitoring for signs of infection. There is an elevated relative risk of lymphoma during therapy; however, the absolute risk is relatively low. Ongoing clinical trials may show if the combination of AZA and biologic therapy is more effective in ulcerative colitis than either therapy alone, as has been shown in the case of Crohn's disease [65].

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Chapter 39

Conventional Medical Management of Ulcerative Colitis: Cyclosporine

Gert Van Assche

Keywords Ulcerative colitis • Acute severe • Medical therapy • Cyclosporine

Introduction

The improved outcome for patients with acute severe ulcerative colitis, a potentially lethal condition in the era before the use of corticosteroids, is arguably one of the major achievements in the care of patients with IBD. Still, severe colitis not responding to steroids poses an important challenge to the surgeon and physician alike. Hospitalization should be considered in all patients who have six or more stools with visible blood a day, associated with fever, dehydration, tachycardia, malaise, and/or increased CRP [1–3]. These patients need a proactive management with intensive medical treatment or colectomy. A coordinated approach by an expert surgical and medical team is essential to ensure optimal patient outcomes.

The Medical Management of Severe Ulcerative Colitis

Patients with severe UC are started on IV corticosteroids. Pioneering studies by Truelove et al. have shown that by applying this strategy 64% of patients will enter into clinical remission and only 23% require rescue total colectomy [1, 4]. Doses higher than 60 mg or 1 mg/kg prednisolone equivalent are not recommended. Supportive therapy for this condition includes relative bowel rest and parenteral nutrition if needed. The value of antibiotics as a prophylactic or adjuvant therapy in the setting of fulminant colitis has not been established. However, patients with high fever, signs of peritonitis, or high CRP levels should receive broad spectrum antibiotics (e.g., ciprofloxacin + metronidazole). Even so, initiation of antibiotics should never postpone the decision for proctocolectomy.

When patients fail 3–5 days of IV corticosteroids at adequate doses and continue to report frequent bloody diarrhea with fever or high CRP levels, they should be considered for surgical colectomy or rescue medical treatment. Recent retrospective data indicate that the presence of several

G. Van Assche, MD, PhD (✉)

Division of Gastroenterology, Leuven University Hospitals, Herestraat 49, B-3000, Leuven, Belgium

e-mail: Gert.vanassche@uzleuven.be

additional criteria on top of the ≥ 6 bloody stools a day, increased the likelihood for colectomy [5]. Upon admission, infectious causes of colitis should be excluded. *Clostridium difficile* infection complicating ulcerative colitis is increasingly recognized as a cause of severe disease and it infers an additional risk of colectomy and mortality [6]. Also other enteric pathogens should be excluded. Unprepped sigmoidoscopy with minimal inflation performed by an experienced endoscopist provides additional information on disease severity and allows sampling of mucosal biopsies. Biopsies are particularly useful to exclude CMV colitis in patients with prolonged exposure to steroids and/or azathioprine, even if the precise role of active CMV replication in acute severe colitis is still debated.

Place in Therapy for Cyclosporine in Patients with Acute Severe Colitis

Cyclosporine is an immunosuppressive macrolide that inhibits the production of interleukin-2 by activated T-lymphocytes through a calcineurin-dependent pathway. In addition, it downregulates the synthesis of other inflammatory cytokines [7]. Intravenous cyclosporine has been shown to be an effective rescue therapy for severe attacks of UC in two controlled trials and in several uncontrolled cohorts [8–12]. In the first randomized trial by Lichtiger et al., 9 of 11 IV 4 mg/kg cyclosporine-treated patients avoided colectomy versus none of the 9 placebo-treated patients [8]. The trial was designed to recruit more patients, but was stopped prematurely due to the huge difference in outcome between the placebo and the cyclosporine group. Moreover, a double-blind controlled study from our group has demonstrated that cyclosporine as a single therapy is at least as effective as corticosteroids in the treatment of severe UC flares [9]. In a case series, Actis et al. reported as early as 1993, that low-dose cyclosporine may be equally effective as higher doses [13]. Data from a controlled trial in 73 patients proved that 2 mg/kg/d IV cyclosporine initial treatment is as effective for severe attacks of UC, although not all of these patients were failing intravenous corticosteroids [14]. Pooling of results from controlled and non-controlled trials shows that 76–85% of patients will respond to IV cyclosporine and avoid colectomy short term [8–14]. Before the initiation of IV cyclosporine, hypomagnesaemia and hypercholesterolemia should be corrected to decrease the risk of neurologic toxicity. After an initial dose of 2 mg/kg, daily cyclosporine doses should be adjusted to achieve therapeutic blood levels from day 2 onwards. Therapeutic ranges for cyclosporine blood levels may vary based on the assay used. The median time to response for IV cyclosporine is 4–5 days and in patients responding, initiation of oral cyclosporine therapy at 5–8 mg/kg divided into two doses should be considered along with gradual steroid tapering and initiation of azathioprine or 6-mercaptopurine [15]. While patients are on a triple immunosuppressive regimen prophylaxis against *Pneumocystis carinii* pneumonia should be given and alertness for opportunistic infections in general should be high [15]. Cyclosporine use in UC has been associated with mortality and most of the fatalities were due to opportunistic infections [16, 17]. Other complications of cyclosporine therapy include nephrotoxicity, tremor and convulsions, hypertension, gingival hyperplasia, and hypertrichosis. Rare cases of anaphylaxis are contributed to the castor oil solvent in Sandimmun®, the commercially available formulation of cyclosporine, and occurrence of anaphylaxis allows treatment with oral cyclosporine [18].

The calcineurin inhibitor tacrolimus (both IV and oral) and oral cyclosporine can also be considered to treat severe attacks of ulcerative colitis but only retrospective uncontrolled data are available [19, 20].

Infliximab 5 mg/kg IV is an alternative medical rescue therapy for patients with severe ulcerative colitis not responding to IV corticosteroids and its use is supported by a randomized clinical trial [21]. In this trial, significantly more patients treated with placebo (14/21) required colectomy by

Table 39.1 Medical rescue therapies for acute severe colitis

	Cyclosporine-A	Infliximab
Dosing	IV: 2–4 mg/kg d, 5 mg/kg orally Blood level monitoring	IV 5 mg/kg week 0–2–6 and Q 8 weeks maintenance
Efficacy	Evidence from RCT [7, 8]	Evidence from RCT [17]
Onset of action	Rapid (4 days)	Rapid
Complications	Serious infections Anaphylactic reaction (IV) Seizures Nephrotoxicity	Serious infections Anaphylactic reaction
Long-term options	Bridge to purine analog	Induction and maintenance

3 months as compared to those treated with a single dose of infliximab 5 mg/kg IV (7/24) [21]. Infliximab has a long half-life and dose monitoring is not required. Anaphylactic infusion reactions to infliximab can occur but are exceedingly rare at the first dose. The choice between cyclosporine, infliximab and colectomy is a medical–surgical decision mostly based on clinical signs, radiological findings, and blood analysis (CRP, serum albumin). Complications such as toxic megacolon or uncontrolled bleeding necessitate emergency colectomy. Megacolon, as defined by the dilation of the (right) colon, is not an absolute indication for surgery, and can be managed by treating the underlying colitis. However, the combination of this complication with clinical deterioration warrants surgical intervention. Predictors of early colectomy in patients treated with cyclosporine have been retrospectively assessed in a cohort treated in Paris, France [22]. A combination of clinical (mild fever and tachycardia), biological (CRP >45 mg/L), and endoscopic criteria allowed the classification of the patients into two different groups (80% vs. 20% colectomy at 6 months), indicating that a higher burden of endoscopic lesions and of systemic inflammation predicted a higher need of colectomy in patients treated with cyclosporine. In one controlled trial, only active smoking was inversely associated with the need for colectomy (OR 0.06, 95% CI 0.008–0.407) [14].

Following initial response to IV cyclosporine about 50% of patients avoid colectomy at 3 years [23–25]. Higher colectomy rates have been reported with follow-up extending to 7 years [23]. The patient population already failing adequate courses of azathioprine or 6-MP is most prone to colectomy following initial response to cyclosporine [23] and therefore cyclosporine should be typically used as a bridging strategy to the effect of azathioprine. Infliximab has been introduced more recently in the management of severe UC and colectomy data from long-term cohorts are not available. Data from the Scandinavian controlled trial, however, indicate that even after a single dose infliximab protects against colectomy at least 2 years after the initial event [26]. Even if medical rescue therapy with cyclosporine or infliximab shifts the surgical procedure from an emergency to an elective colectomy months or years down the road in a substantial proportion of patients, this should not be considered as an argument against an attempt for medical therapy since urgent rescue colectomy is associated with increased morbidity and mortality [27].

Until recently no comparative trial data are available to guide the choice between infliximab and cyclosporine in patients with acute severe colitis. However, preliminary results from a randomized open label comparative trial between infliximab and cyclosporine suggest that both drugs are equally effective to induce remission and prevent colectomy until 3 months after the start of therapy [28].

Both cyclosporine and infliximab are immunosuppressive agents associated with an increased risk of serious infections including lethal opportunistic infections [16, 17, 29]. Inevitably, previous steroid exposure adds to the burden of toxicity. The concomitant use of steroids is a proven risk factor for postoperative complications in patients who eventually come to colectomy [30]. Cyclosporine as such is not associated with an increased perioperative complication risk [31] (see Table 39.1).

Successive Use of Infliximab and Cyclosporine in Patients with Acute Severe Colitis

Even if timely colectomy should always be considered in patients with severe colitis not responding to medical rescue, consecutive use of cyclosporine and infliximab has been reported. Results from a cohort of patients treated at the Mount Sinai Hospital in New York suggested that patients receiving infliximab followed by cyclosporine or vice versa have a substantial risk of serious adverse events including mortality [32], but the data from collaborative groups in ref. [33], France, and Spain [34] suggest that successive use of both agents is a therapeutic option. Of the 86 patients in the French cohort, 65 used cyclosporine first and 62% of all patients who received both agents consecutively, avoided colectomy short term, although remission rates were below 30% and 3-year colectomy rates 63%. Also, in this study, serious infections were observed in 14% of patients and one patient died. In the smaller Spanish cohort ($n=16$) of patients treated with infliximab after failure of cyclosporine reported by Manosa et al., the short-term colectomy rate was 30% with a low incidence of side effects [34]. Clinicians should carefully consider if the number of colectomies avoided by the consecutive use of cyclosporine and infliximab outweighs the cumulative immunosuppressive risk. Even if the serum half-life of cyclosporine is less than 24 h, the impact on the risk of opportunistic infections may last much longer.

Conclusion

Patients with severe attacks of UC should be hospitalized and closely monitored by a surgical-medical team. After failing 3–5 days of intravenous corticosteroids, patients should be considered for intravenous cyclosporine (2 mg/kg d), infliximab (5 mg/kg IV), or colectomy. Patients should always be informed that colectomy may be the best alternative to treat their condition and that long-term outcomes are good. Cyclosporine is most useful as a bridge to the effect of azathioprine or 6-MP and should be particularly considered in this setting. Serious opportunistic infections are the major concern with medical rescue therapies such as cyclosporine and infliximab. Long-term colectomy free survival rates after initial response to cyclosporine are far from optimal and long-term data with infliximab are scarce. However, in patients with a dramatic response to any of the two immune therapies, a delayed elective colectomy later in the disease course is probably safer and allows patients to achieve important milestones in life.

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Chapter 40

Conventional Medical Management of Ulcerative Colitis: Tacrolimus

Daniel C. Baumgart

Keywords Tacrolimus • FK506 • Ulcerative colitis • Crohn's disease • Proctitis • Extraintestinal manifestations • Topical • T-cells • Calcineurin • Pyoderma gangrenosum • Erythema nodosum

Introduction

Tacrolimus (FK506) isolated from *Streptomyces tsukubaensis* was first described in 1987 as a novel agent [1–3]. It structurally resembles macrolides such as rapamycin and was later found to be a potent immunosuppressant [4].

While structurally entirely different from cyclosporine the drug turned out to be a potent calcineurin inhibitor. (Fig. 40.1) It inhibits the complex of calcineurin with its respective cytoplasmic receptors cyclophilin and FK-binding protein 12 (FKBP-12), both of which thereby regulate its calmodulin-dependent-phosphatase activity [5, 6]. A possible target is inflammatory key transcription factors such as nuclear factor of activated T cells (NFAT) and nuclear factor kappa B (NF- κ B) [7, 8]. Thus it may inhibit the transcription of the interleukin 2 (IL-2) gene required for T cell activation. It is well known that T cell dysfunction plays a pivotal role in the pathogenesis of inflammatory bowel disease [9]. More recently it was discovered that tacrolimus also inhibits iNOS expression and NO production in colonic epithelial cells and macrophages by an NF- κ B-independent manner [10, 11]. Tacrolimus also targets the innate immune system by suppression of T-cell-mediated recruitment of neutrophils [12].

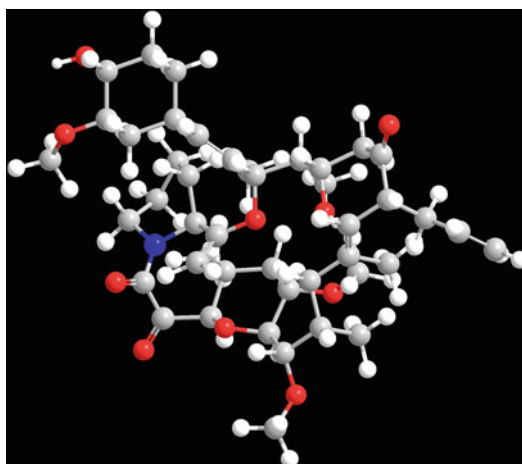
Tacrolimus is currently only approved for the prophylaxis of organ rejection in patients receiving allogeneic liver or kidney transplants. However, while not approved, it has been also widely used in small bowel transplantation [13, 14].

Early studies in animal models of inflammatory bowel disease, such as the peptidoglycan/polysaccharide (PG/PS) induced colitis in rats, in which it also attenuated extraintestinal manifestations of the disease, the dextran sulphate model of ulcerative colitis and the trinitrobenzene (TNB) sulphonic acid model of ulcerative colitis encouraged its evaluation for human inflammatory bowel disease [15–18].

D.C. Baumgart, MD, PhD (✉)

Division of Gastroenterology and Hepatology, Department of Medicine, Charité Medical Center – Virchow Hospital, Medical School of the Humboldt-University of Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: daniel.baumgart@charite.de

Fig. 40.1 Tacrolimus structure



Encouraged by positive effects of tacrolimus in sclerosing cholangitis (PSC) liver transplant recipients on their associated ulcerative colitis [19, 20] anecdotal reports and case series of its use in autoimmune enteropathy [21] and inflammatory bowel disease followed [22–26].

Tacrolimus Use in Ulcerative Colitis

Since then more experience has been gained with tacrolimus in ulcerative colitis in a number of mostly uncontrolled studies (reviewed in [27]) and one randomized controlled trial [28]. Tacrolimus can be considered as an alternative to cyclosporine in patients with severe colitis or intravenous-steroid resistant ulcerative colitis of any extent [29]. Effective doses range from 0.01 to 0.02 mg/kg i.v. or 0.1 to 0.2 mg/kg p.o. The one randomized controlled available trial suggests that it is more effective when given at a dose that achieves a trough concentration of 10–15 mg/ml. Recently, a biomarker was identified that may help predict a patient's short-term remission in steroid-refractory ulcerative colitis [30]. A number of retrospective series indicate that lower doses may be effective as well and limit its toxicity [27] (Table 40.1).

Several other studies investigated novel microsphere and nanoparticle based formulations of tacrolimus to improve drug delivery and decrease its toxicity. In the treatment of IBD, oral drug delivery using pH-dependent polymers is one of the most successful therapeutic strategies. Eudragit P-4135F, a pH-sensitive polymer for colonic delivery was used to prepare tacrolimus microparticles using an oil–oil emulsification or an oil–water emulsification technique combined with a solvent extraction or evaporation step. The oil–water technique was found to be superior to the oil–oil approach and among them solvent evaporation seemed to be more advisable, due to the higher encapsulation rate [31]. Another novel delivery system – nanoparticles (NP) – are known to accumulate at the site of inflammation in inflammatory bowel disease. Tacrolimus loaded poly (lactic-co-glycolic acid) NP entrapped into pH-sensitive microspheres (NPMS) were designed to achieve greater selectivity to their site of action when administered orally. The results from animal studies in rats suggest that the NPMS system can provide selective delivery of NP in the colon and develop a significant mitigating effect, while the control group treatments appeared to be insufficient [32, 33]. In another study drug loaded NP were prepared from either biodegradable poly (lactide-co-glycolide) (PLGA) or pH-sensitive Eudragit P-4135F by using a simple oil–water emulsification method. Tests on the therapeutic effect were conducted using dextran sulfate model colitis in mice receiving tacrolimus formulations. Tacrolimus solution groups (oral/subcutaneous) exhibited increased levels of adverse

Table 40.1 Tacrolimus studies in ulcerative colitis

First author	<i>n</i>	Year	Dose (mg/kg/day)	Concomitant medications	Response (%)	Remission (%)	Comment	References
Bousvaros	14	2000	0.1	Azathioprine or 6 mercaptopurine added 14 days later in responders	69	50		[60]
Fellermann	38	2002	0.1 to 0.2	Azathioprine, 6MP, prednisolone	47	34	Mixed cohort of 33 UC and 5 indeterminate colitis cases	[61]
Baumgart	23	2003	0.1	Azathioprine, prednisolone	96	74		[62]
Hogenauer	9	2003	0.15	Azathioprine or 6 mercaptopurine added in responders	NR	67		[63]
Baumgart	40	2006	0.1	Azathioprine, prednisolone	NR	67	Long-term outcome study	[64]
Ogata	43	2006	0.025		68	20	RCT with one high and one low trough group	[28]
Ng	6	2007	0.1	NR	67	50		[65]
Ziring	18	2007		Prednisolone	89	NR		[66]
Benson	32	2008	NR	Steroids	69	9		[67]
Lawrance	8	2008	0.3 mg/ml	NR	NR	75	Topical treatment, proctitis only	[68]
Yamamoto	27	2008	NR	NR	78	70		[69]
van Dieren	17	2009	2–4 mg enema or 2 mg supp	NR	76	NR	Topical treatment, left sided disease only	[70]
Watson	46	2011	0.2	NR	93	NR	Pediatric patients only	[71]
Yamamoto	24	2011	NR	NR	NR	71	Maintenance study	[72]

effects, whereas both NP groups demonstrated their potential to reduce nephrotoxicity. Both strategies showed similar mitigating effects while nephrotoxic adverse effects were slightly less expressed with pH-sensitive NP [34].

Tacrolimus Use in Crohn's Disease

Tacrolimus was studied exclusively in uncontrolled studies for inflammatory luminal and two controlled studies for fistulizing Crohn's disease. It was used at dose ranges similar to ulcerative colitis (see above). Few of these uncontrolled studies reported short- and long-term therapeutic advantage for steroid-refractory or steroid-dependent patients. While case reports suggested efficacy in complex enterovesical and duodenocolic fistulas in Crohn's disease [35] these encouraging observations could not be confirmed in randomized controlled trials (Table 40.2).

Overall, tacrolimus is of limited value in Crohn's disease. Based on the data available it cannot be recommended for routine clinical use in inflammatory luminal Crohn's disease and definitely not for the management of fistulas [36].

Tacrolimus Use for Pyoderma Gangrenosum and Erythema Nodosum

Tacrolimus has also been proposed for the management of some extraintestinal manifestations of inflammatory bowel disease. Topical treatment with creams and ointments as well as systemic administration has been reported effective in pyoderma gangrenosum [28, 37–43] and erythema nodosum [44]. However, excessive absorption from skin lesions can induce additional problems [45].

A novel potential mechanism for the efficacy in the management of inflammatory dermatoses was recently discovered. An experimental study demonstrated a tacrolimus dose-dependent down regulation, activation, translocation, and secretion of TNF- α from UVB irradiated keratinocytes. The degradation of I κ B induced by UVB was also inhibited by tacrolimus [46].

Special Precautions and Side Effects to Consider When Using Tacrolimus

Major limitations for long-term tacrolimus use include development of potentially life threatening infections, that is, *Pneumocystis jirovecii* pneumonia [47], hypertension, hyperglycemia, paraesthesia, neuropathy, and renal impairment [48]. Moreover, rare conditions such as eosinophilic gastroenterocolitis were reported [49, 50].

While most studies published report efficacy with lower trough levels, individual patients may require higher doses to achieve effective doses. Two studies render potential explanations for the different dose requirements among patients: tacrolimus is a substrate for the apically directed efflux transporter P-glycoprotein (P-gp) and also for cytochrome P450 3A (CYP3A). Elevated intestinal P-gp could result in decreased tacrolimus absorption, thereby leading to decreased blood concentration and clinical efficacy [51, 52].

A greater concern is the publication of a series of studies suggesting that tacrolimus could be attributed to the de novo development of ulcerative colitis in liver transplant recipients [53, 54] or Crohn's disease in solid organ transplant recipients [55–58]. The underlying hypothesis that calcineurin induced reduction of T_{reg} or TGF- β expression predisposes non-transplanted tissue to inflammation, is challenged by another study [59]. This phenomenon requires additional mechanistic research and prospective validation in other cohorts.

Table 40.2 Tacrolimus studies in Crohn's disease

First author	Year	n	Dose (mg/kg/day)	Concomitant medications	Response (%)	Remission (%)	Comment	References
Lowry	1999	11	0.15 to 0.31	Azathioprine or 6 mercaptopurine	64	NR		[73]
Casson	2000	8	0.5 mg/g	NR	87	NR	Topical use in pediatric oral or perineal Crohn's disease	[74]
Ierardi	2001	13	0.1 to 0.2	5-ASA	85	NR		[75]
Baumgart	2003	6	0.1	Azathioprine, prednisolone	67	17		[62]
de Oca	2003	19	0.1	NR	NR	66	Perianal Crohn's disease only	[76]
Sandborn	2003	48	0.2	NR	90	NR	RCT, fistulizing Crohn's disease	[77]
Gonzales-Lama	2005	10	0.1	NR	40	NR	Fistulizing Crohn's disease	[78]
Baumgart	2006	11	0.1	Azathioprine, prednisolone	91	54	Long term outcome study	[64]
Hart	2007	19	1 mg/g	Azathioprine, 6-MP or infliximab	75	NA	RCT, effective only in perianal, but not fistulizing Crohn's disease	[79]
Ng	2007	12	0.1	NR	83	42		[65]
Benson	2008	15	NR	Steroids	53	7		[67]
Tamaki	2008	14	NR	NR	14	64		[80]

NR not reported, RCT randomized controlled trial, CD Crohn's disease, UC ulcerative colitis

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Chapter 41

Biologic Therapy of Ulcerative Colitis: Infliximab, Adalimumab, Golimumab

Walter Reinisch

Keywords Ulcerative colitis • Biologics • Anti-TNF- α • Infliximab • Adalimumab

Introduction

Ulcerative colitis (UC) is a chronic inflammatory condition that progresses over time towards extensive colonic involvement and/or severe activity which renders colectomy inevitable in a substantial proportion of patients. There is a pressing demand for novel therapies, but barely any novelties have hit the market. This is best punctuated by the fact that it took almost 20 years from the introduction of mesalamine to the therapeutic armamentarium against UC before infliximab became available for use in clinical routine in 2006. Infliximab is a chimeric monoclonal anti-tumor necrosis factor α (anti-TNF- α) antibody which in EU is labeled for treatment in patients with moderately to severely active UC who have had an inadequate response to conventional therapy including corticosteroids and 6-MP or AZA, or who are intolerant to or have medical contraindications for such therapies.

Meanwhile, other monoclonal anti-TNF- α antibodies have been subjected to clinical studies in patients with UC, but none has been granted market authorization since then. Adalimumab is a fully human recombinant monoclonal antibody against TNF- α which binds specifically to the soluble and transmembrane forms of TNF- α . Adalimumab is approved to treat rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, psoriasis, and Crohn's disease in the USA and Europe. In contrast to infliximab, which is administered intravenously as a three dose infusion at weeks 0, 2, and 6 for induction and as 8-weekly infusion for maintenance, adalimumab is injected subcutaneously on a biweekly to weekly basis. Golimumab is another fully human monoclonal antibody targeting TNF- α . Golimumab is approved in North America and Europe as a once monthly subcutaneous treatment for adults with moderately to severely active rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis.

An erroneous immunological concept and the long-standing prevailing notion that UC is the less severe and debilitating among inflammatory bowel disease cured by colectomy has impeded biopharma's interest to invest in the development of anti-TNF- α compounds in this disease. UC was believed to primarily reflect a T-helper cell type-2-like mediated disease driven by interleukin-5 and

W. Reinisch, MD (✉)

Department of Internal Medicine III, Medical University of Vienna, Univ.-Klinik für Innere Medizin III,
Abteilung Gastroenterologie und Hepatologie, Währinger Gürtel 18–20, A-1090 Vienna, Austria
e-mail: walter.reinisch@meduniwien.ac.at

interleukin-13 [1, 2] for which a strategy targeting TNF- α , considered at that time as classical Th-1 cell cytokine could even deteriorate the condition [3, 4]. Thus, despite the long known fact of increased levels of TNF- α in stool samples, colonic mucosa, and serum of patients with UC, the initiation of large adequately powered placebo-controlled trials in UC immediately after the efficacy of infliximab in Crohn’s disease became evident was hampered.

Infliximab

Early Noncontrolled Experience With Infliximab

Early noncontrolled pilot studies particularly in steroid-refractory adult and pediatric patients with severe disease suggested promising effectiveness of infliximab in UC with reported response rates between 50 and 100% (Table 41.1, 5–13). In one of the largest early series embracing the experience at four institutions on a total of 27 in- or outpatients with active UC, 12 patients (44%) achieved remission and 6 patients (22%) had partial response. Five subjects subsequently underwent total colectomy. Steroid dependency was the single risk factor predictive of the response to infliximab [14].

Randomized Controlled Trials with Infliximab

Until now, in five clinical trials in UC the efficacy of infliximab was compared vs. placebo [15–18], whereas in two studies a comparison to steroids was followed [19, 20].

The first double-blind, placebo-controlled study addressed patients with severely active UC refractory to a 5 days intravenous treatment with steroids [15]. Due to slow enrolment the study was prematurely terminated after inclusion of 11 out of 60 conceived patients. Four out of eight patients (50%) who received a single infusion of infliximab at 5, 10, or 20 mg/kg body weight were considered treatment successes at 2 weeks, compared with none of the three patients who received placebo. In general, infliximab was well tolerated; however, the number of patients treated in the study was too small to allow robust conclusion.

Table 41.1 Early noncontrolled trials with infliximab in ulcerative colitis

Study	Year	<i>n</i>	Indication	IFX dose (mg/kg W0)	Outcome
Chey WY	2001	8	Severe, refractory UC	5	100% response
Kaser A	2001	6	Severe refractory UC	5	100% response short term, 4/6 long-term response
Kohn A	2002	13	Severe, refractory UC	5	77% response
Actis	2002	8	Severe refractory UC	5	50% response, 50% colectomy
Gornet JM	2003	19	Severe refractory UC	5	75% response and 43% remission at d7 but relapse 73% at 6 M
Mamula P	2004	9	Severe refractory pediatric UC	5	7/9(78%) response
Kohn A	2004	13	Severe refractory UC	5	10/13(77%) response
Eidelwein	2005	12	Severe refractory pediatric UC	5	8/12 response (concom 6-MP better)

A randomized placebo-controlled trial of infliximab (5 mg/kg) given at weeks 0 and 2 and performed in 43 patients resistant to at least 30 mg of prednisolone daily for a week did not support the use of infliximab in moderately active UC [16]. After 6 weeks, a statistically significant difference between the infliximab and placebo groups could be achieved neither in the proportion of patients with clinical remission defined as an UC symptom score (UCSS) of ≤ 2 (39% (9/23) vs. 30% (6/20); $p=0.76$) nor in the proportion of patients with mucosal healing as defined by a Baron score of 0 (13% (3/23) vs. 5% (1/19); $p=0.74$). The sample size of the study was small and based on a response rate of 81% with infliximab, which renders a type 1 error very likely.

Two large randomized, double-blind, placebo-controlled trials, each in 364 patients with moderately to severely active disease (ACT-1 and ACT-2) could finally furnish evidence for the efficacy of infliximab for induction and maintenance therapy in adults with UC [17]. In ACT-1 patients refractory to, dependent upon or intolerant of steroids and/or failing or not tolerating azathioprine/6-mercaptopurine were allowed to enter. In ACT-2, additionally patients could be enrolled, who had failed a previous treatment with oral 5-ASA compounds only. In both studies, response was defined as a decrease in the Mayo score of at least three points and at least 30%, with an accompanying decrease in the subscore for rectal bleeding of at least one point or an absolute rectal bleeding subscore of 0 or 1. Patients who received infliximab (5 or 10 mg/kg of body weight) intravenously at weeks 0, 2, and 6 and then every 8 weeks through week 46 (in ACT 1) or week 22 (in ACT 2) were more likely to have a clinical response at week 8 (ACT-1: IFX 5 mg/kg 69%, IFX 10 mg/kg 61%, placebo 37%; $p<0.001$ for both comparisons with placebo; ACT-2: IFX 5 mg/kg 64%, IFX 10 mg/kg 69%, placebo 29%; $p<0.001$ for both comparisons with placebo), at week 30 (for both studies) and week 54 (for ACT-1: IFX 5 mg/kg 45%, IFX 10 mg/kg 44%, placebo 20%; $p<0.001$ for both comparisons) than were those receiving placebo. Significantly more patients achieved clinical remission under treatment with infliximab as compared to placebo at weeks 8 and 54. An increased rate of mucosal healing, defined as an endoscopic Mayo subscore of 0 or 1, was observed under infliximab at week 8 and the difference persisted at week 54 (ACT-1: IFX 5 mg/kg 45.5%, IFX 10 mg/kg 46.7%, placebo 18.2%; $p<0.001$ for both comparisons). Mucosal healing at week 8 was associated with a fourfold increased likelihood to be in clinical remission at week 54. Under treatment with infliximab, steroid-free remission was more frequently observed (ACT-1: both IFX 21.7%) as compared to placebo (9%) at week 54. Furthermore, infliximab therapy improved health-related quality of life in patients with UC [18].

On eighty-seven percent (630 out of 728) of patients enrolled into the ACT trials, data on colectomy was available after a median follow-up of 6.2 months. The cumulative incidence of colectomy through 54 weeks was 10% for infliximab and 17% for placebo ($p=0.02$), yielding an absolute risk reduction of 7%. Furthermore, fewer UC-related hospitalizations and surgeries/procedures per 100 patient-years of treatment occurred with infliximab therapy as compared to placebo: 40 vs. 20 ($p=0.003$) and 34 vs. 21 ($p=0.03$), respectively [19].

In search of alternative rescue therapies for cyclosporine A in severe attacks of UC, Järnerot et al. performed a randomized double-blind trial of a single infusion of infliximab or placebo in patients failing intravenous steroid therapy [20]. Among the 45 patients included (24 infliximab and 21 placebo), seven in the infliximab group and 14 in the placebo group had to undergo colectomy ($p=0.017$; odds ratio, 4.9; 95% confidence interval, 1.4–17) within 3 months after randomization. Thus, also in patients with a fulminant course of disease infliximab shows potential in reducing the risk of colectomy.

Standard-Treatment Controlled Trials with Infliximab

In two small studies, efficacy of infliximab was compared to prednisolone/methylprednisolone in an open-label fashion in patients with a steroid-dependent/-refractory course of UC. From both trials it was concluded that infliximab seems to be as effective as steroids [21, 22].

Cochrane Meta-analysis on Infliximab for Induction of Remission in Ulcerative Colitis

A Cochrane meta-analysis including seven randomized controlled trials concluded that in patients with moderate to severe UC whose disease is refractory to conventional treatment using corticosteroids and/or immunosuppressive agents, infliximab is effective in inducing clinical remission (relative risk (RR) vs. placebo 3.22, 95% CI 2.18-4.76), inducing clinical response (RR 1.99, 95% CI 1.65-2.41), and promoting mucosal healing (RR 1.88, 95% CI 1.54-2.28) at 8 weeks [23].

Open-Label Experiences

A report on the first 100 patients with UC treated in a single center supported the findings from clinical studies. Early clinical response was observed in 65% of the patients. A pANCA+/ASCA-serotype and an older age at first IFX infusion was associated with a suboptimal early clinical response [24]. From a series of 83 patients with severe UC refractory to intravenous steroids, a short-term colectomy rate of 15% after infliximab treatment was described. Early colectomy rates were higher in patients receiving one infusion (9/26), compared with those receiving two/more infusions of infliximab (3/57, $p=0.001$, $OR=9.53$) [25].

Trough serum concentrations of infliximab have more recently entered the focus of interest to improve the outcome of treatment. Detectable trough serum infliximab predicts clinical remission, endoscopic improvement, and even a lower risk for colectomy (odds ratio 9.3; 95% confidence interval, 2.9–29.9; $p<0.001$) in patients with UC [26].

Comparing the colonic mucosal gene expression between responders and nonresponders to infliximab in two cohorts of patients with refractory UC, five top differentially expressed genes, osteoprotegerin, stanniocalcin-1, prostaglandin-endoperoxide synthase 2, interleukin-13 receptor $\alpha 2$, and interleukin-11, were detected, which separated response with 95% sensitivity and 85% specificity [27].

In patients failing infliximab therapy and in need of total proctocolectomy with ileal pouch anal anastomosis (IPAA), the question as to whether infliximab impacts postoperative outcome, particularly infectious complications, is controversially discussed. However, in summary of existing data, an increased risk of short-term postoperative complications associated with infliximab appears likely [28–33].

Data on the efficacy of infliximab in pediatric UC is sparse. In a cohort of 52 children receiving infliximab and documented in a collaborative research group registry, corticosteroid-free inactive disease was achieved in 38% and 21% at 12 and 24 months, respectively. By 24 months, 61% of patients had avoided colectomy [34].

Consensus Guidelines

In the evidence-based consensus on the management of UC of the European Crohn's and colitis organization (ECCO), infliximab is considered as an option for second-line treatment in severely active patients failing response to intravenous steroids [35]. Furthermore, infliximab is recommended in patients with UC refractory to immunomodulatory treatment.

Adalimumab

Early Noncontrolled Experience with Adalimumab

Several small open-label trials and case reports suggested that adalimumab can induce remission in patients with UC even in those failing previous treatment with infliximab (Table 41.2) [36–41]. In the largest of those series, 30 patients with active UC having previously failed standard medications including infliximab were treated with adalimumab 160 mg at week 0 and 80 mg at week 2, and subsequently with 40 mg every other week. In case of nonresponse maintenance dose could be increased to adalimumab 40 mg weekly. After a mean follow-up of 48 weeks, 15 patients (50%) continued on adalimumab. Patients who achieved short-term clinical response at week 12 were less likely to undergo colectomy during follow-up [41].

Randomized Controlled Trials with Adalimumab

A randomized, placebo-controlled trial on adalimumab for the induction of clinical remission in moderately to severely active UC has been published recently [42]. Three hundred and ninety patients with Mayo score of ≥ 6 points and endoscopic subscore of ≥ 2 points despite treatment with corticosteroids and/or immunosuppressants were randomized (1:1:1) to adalimumab 160/80 or adalimumab 80/40 at weeks 0 and 2 followed by adalimumab 40 mg at weeks 4 and 6, or placebo. As primary efficacy endpoint clinical remission was defined by Mayo score ≤ 2 with no individual subscore > 1 at week 8. At week 8, 18.5% of patients in the adalimumab 160/80 group ($p=0.031$ vs. placebo) and 10.0% in the adalimumab 80/40 group ($p=0.833$ vs. placebo) were in remission, compared with 9.2% in the placebo group. The authors suggested the possibility that a substantial proportion of patients with UC may have required a higher dose of adalimumab to induce remission as remission rates in patients with a body weight less than 82 kg were more than twice for patients above 82 kg in the 160/80 mg dose group. A 52 week, randomized, double-blind trial to assess the ability of adalimumab to induce and maintain remission in UC patients, including those previously exposed to infliximab, is not yet published.

As adalimumab is currently not labeled for use in UC praxis guidelines are not available as yet.

Golimumab

Golimumab, which has been formulated for both subcutaneous and intravenous administration, is currently subjected to a large research program of multicenter, randomized, placebo-controlled, double-blind studies to evaluate its safety and efficacy for induction and maintenance of remission in UC. Results are to be expected by late 2011.

Conclusion

Anti-TNF- α agents have expanded our therapeutic armamentarium against UC. Infliximab is the prototypic representative of this class of agents and currently the only label for use in UC. For infliximab we succeeded to show its potential to induce and maintain remission in patients with

Table 41.2 Early noncontrolled trials with adalimumab in ulcerative colitis

Study	Year	<i>n</i>	Indication	ADA dose	Outcome
Peyrin-Biroulet L	2007	10	Severe UC LoR or intolerant to IFX	160/80 mg induction	Week 4: 40% response, 10% remission
Oussalah A	2008	13	Severe UC LoR or intolerant to IFX	160/80 mg + 40 eow	Month 23: 32.5% maintaining ADA
Afif W	2009	20	Moderate to severe UC IFX naïve or LoR	160/80 mg 40 mg eow/w	Week 24: 50% response, 20% remission
Barreiro-de Acosta M	2009	2	Steroid-dependent UC IFX intolerant	160/80 mg 40 mg eow	Mucosal healing at 2 years treatment
Gies N	2010	25	Moderate to severe UC IFX naïve	160/80 mg 40 mg eow/w	Week 14: 80% response Maintenance: 70% response
Taxonera C	2010	30	Moderate to severe UC LoR or intolerant to IFX	160/80 mg 40 mg eow/w	Week 12: 27% remission Week 40: 50% maintained ADA

IFX infliximab, ADA adalimumab, LoR loss-of-response, eow every other week, w weekly

moderately to severely active UC as well to reduce the risk of colectomy. Nevertheless, major challenges are remaining to optimize its application in clinical routine. Right timing of treatment and tailoring its long-term use according to individual pharmacokinetic or dynamic parameters might help to improve the outcome to therapy. The potential need for its combination with immunosuppressants has to be addressed and interpreted carefully. The future of adalimumab and golimumab for treatment of UC is still uncertain. First results on adalimumab appear promising, but are also pointing to a potential dose problem in the available controlled study. Did we underestimate the inflammatory load in UC for the longest time or were basic immunologists right when being skeptical on the use of anti-TNF agents in UC? Further results on adalimumab and the golimumab studies will help improve our understanding. However, irrespective of what they will be, anti-TNF- α agents will further rescue many patients from suffering and colectomy.

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Chapter 42

Biologic Therapy of Ulcerative Colitis: Natalizumab, Vedolizumab, Etrolizumab (rhuMAb β_7)

Julián Panés, Daniel Benítez-Ribas, and Azucena Salas

Keywords Adhesion molecules • Inflammatory bowel disease • Crohn's disease • Ulcerative colitis • Integrins • Natalizumab • Vedolizumab

Introduction

The hallmark of Crohn's disease (CD) and ulcerative colitis (UC) lesions is the infiltration of the intestine by mononuclear cells, predominantly lymphocytes. This cellular infiltration results from three main components: (1) increased leukocyte recruitment, (2) cell proliferation in the inflamed organ, and (3) reduced apoptosis [1]. Therefore, adhesion molecules that are involved in regulating these processes represent novel therapeutic targets for human IBD. Recognition of the molecular determinants of leukocyte recruitment in CD and UC, development of drugs that target molecules governing this process, and identification of the most selective targets that predominantly affect recruitment to inflamed intestine and preserve the immune surveillance in other organs are current challenges in our progression to develop effective and safe drugs covering still unmet needs in IBD therapy.

J. Panés, MD, PhD (✉)

Department of Gastroenterology, Hospital Clínic Barcelona,
CIBERehd Instituto Salud Carlos III, Barcelona, Spain

Department of Gastroenterology, Hospital Clínic de Barcelona, Villarroel 170, Barcelona E08036, Spain
e-mail: jpanes@clinic.ub.es

D. Benítez-Ribas

Department of Gastroenterology, CIBERehd Villarroel 170, Barcelona E08036, Spain
e-mail: daniel.benitez@ciberehd.org

A. Salas

Department of Gastroenterology, Hospital Clínic de Barcelona, Villarroel 170, Barcelona E08036, Spain

Institut Investigacions Biomètiques August Pi Sunyer (IDIBAPS), Barcelona, Spain
e-mail: asalas1@clinic.ub.es

Leukocyte–Endothelial Cell Interactions

Leukocyte recruitment begins with interaction with the blood vascular endothelium, primarily within specialized postcapillary venules. In lymph nodes and in mucosal lymphoid organs (Peyer’s patches and appendix), the venules involved are lined by “high” endothelium [2]. In the intestinal submucosa and lamina propria venules supporting lymphocyte extravasation are less distinctive histologically, but here too the endothelial cells are highly specialized for their role in recruiting leukocytes from the blood.

Interaction between circulating leukocytes and venular endothelium comprises a multistep process in which specialized adhesion and signaling molecules participate to mediate each of a series of sequential steps. In the first step, leukocytes margined from central venular blood flow contact the endothelium and initiate rolling along the vascular lumen. Rolling delays the transit of leukocytes, allowing “sampling” of the local microenvironment for activating factors that act primarily through serpentine receptors. In this second step, these activating factors (chemokines) trigger rapid intracellular signaling in the leukocyte, leading to functional activation of cell surface adhesion molecules through conformational changes (integrins), which then mediate firm arrest of the cell on the vessel wall. Finally, transendothelial leukocyte migration can occur if a chemotactic signal is generated in the perivascular compartment (Fig. 42.1) [3]. Each stage of leukocyte recruitment, that is, rolling, firm adhesion, and transendothelial migration, involves the participation of different families of adhesion molecules, including the selectins and their ligands, integrins, and immunoglobulins superfamily.

Integrins and Their Receptors in the Process of Leukocyte Recruitment

Integrins are heterodimeric proteins consisting of noncovalently associated α and β subunits. At present, 17 α - and 8 β -chains are known. Leukocytes can express 13 different integrins from the existing repertoire with six of these being β_1 , β_2 , or β_7 integrins serving as important modulators of

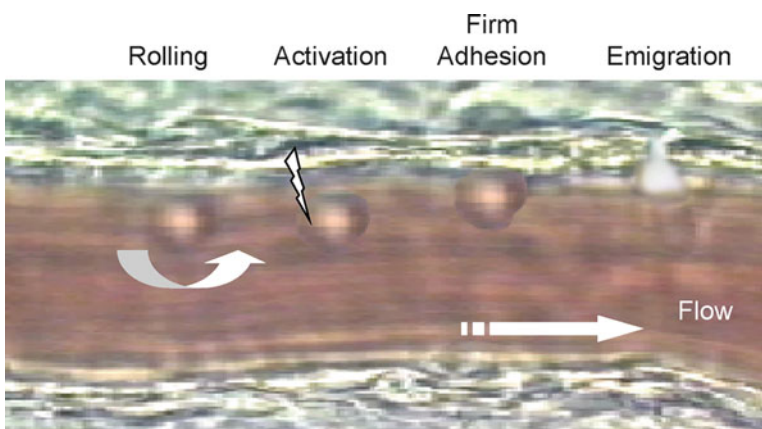


Fig. 42.1 Steps of leukocyte recruitment. Leukocyte–endothelial cell interactions. Schematic of the multistep model of leukocyte–endothelial cell adhesion. Fast moving leukocytes in the blood stream roll on activated endothelium via interactions between selectins and their ligands, or in some cases integrin (α_4)–immunoglobulin superfamily (MAdCAM-1) interactions. Selectins mediate the initial tethering and rolling interactions. Interactions between integrins and immunoglobulin superfamily members mediate firm adhesion and transmigration

Table 42.1 Integrins and their receptors

Adhesion molecule	Location	Expression		Ligand	Function
		Constitutive	Inducible		
<i>Integrin family</i>					
CD11a/CD18 (LFA-1, $\alpha_L\beta_2$)	All leukocytes	Yes	No	ICAM-1, ICAM-2	Adhesion, emigration
CD11b/CD18 (Mac-1, $\alpha_M\beta_2$)	Granulocytes, monocytes	Yes	Yes	ICAM-1	Adhesion, emigration
CD11c/CD18 $\alpha_X\beta_2$	Granulocytes, monocytes	Yes	Yes	fibrinogen, C3b	Activation, adhesion?
$\alpha_4\beta_1$ (VLA-4)	Lymphocytes, monocytes, activated granulocytes	Yes	Yes	VCAM-1, fibronectin	Adhesion
$\alpha_4\beta_7$	Lymphocytes	Yes	No	MAdCAM-1, VCAM-1, fibronectin	Rolling, adhesion
$\alpha E\beta_7$					
<i>Immunoglobulin superfamily</i>					
ICAM-1 (CD54)	Endothelium, monocytes	Yes	Yes	CD11a/CD18, CD11b/ CD18	Adhesion, emigration
ICAM-2	Endothelium	Yes	No	CD11a/CD18	Adhesion, emigration
VCAM-1 (CD106)	Endothelium	Yes	Yes	$\alpha_4\beta_1$, $\alpha_4\beta_7$	Adhesion, emigration
MAdCAM-1	Endothelium (gut)	Yes	Yes	$\alpha_4\beta_7$, L-selectin	Adhesion, emigration
PECAM-1	Endothelium, leukocytes, platelets	Yes	No	PECAM-1, $\alpha_V\beta_3$?	Adhesion, emigration
VAP-1	Endothelium	Yes	Yes	?	Adhesion

leukocyte–endothelial cell adhesion. Integrins of the β_2 subfamily involved in leukocyte–endothelial cell interactions consist of a common β subunit (CD18) which can associate with one of three immunologically distinct α subunits designated CD11a, CD11b, and CD11c (Table 42.1). The expression of β_2 integrins is restricted to leukocytes and the distribution of β_2 integrin subclasses differs among the various leukocyte populations. Peripheral blood lymphocytes express primarily CD11a/CD18 whereas neutrophils, monocytes, and natural killer cells express all three β_2 integrins. CD11a/CD18 is basally expressed on the surface of most leukocytes where it interacts with intercellular adhesion molecule (ICAM)-1 and ICAM-2 on endothelial cells to promote leukocyte adhesion [4]. Most of the CD11b/CD18 and CD11c/CD18 glycoproteins are stored in granules that can be rapidly (within minutes) mobilized to the surface of activated neutrophils and monocytes by fusion of granule membranes with the cell membrane. CD11b/CD18 interacts with ICAM-1 on endothelial cells, and is also an important receptor for complement fragment C3bi. Ligands for CD11c/CD18 include fibrinogen and iC3b; binding of the latter results in cell activation.

A second subfamily of integrins combines the β_1 (CD29) chain with variable α subunits. The $\alpha_4\beta_1$ integrin (VLA-4) is involved in the adhesion of lymphocytes, monocytes, eosinophils, and natural killer cells to cytokine-activated endothelial cells. It has also been shown that certain inflammatory conditions, such as sepsis, induce α_4 integrin expression in neutrophils, and α_4 -dependent adhesion of neutrophils to endothelial cells [5]. Ligands for VLA-4 include vascular cell adhesion molecule (VCAM)-1 and components of the extracellular matrix such as fibronectin.

An interesting function has been assigned to a member of the β_7 integrin subfamily. The $\alpha_4\beta_7$ heterodimer is highly expressed on a subset of lymphocytes that home to the gut and gut-associated lymphoid tissues. This heterodimer recognizes the mucosal endothelial ligand MAdCAM-1 and mediates lymphocyte homing to Peyer's patches [6]. In addition to binding to MAdCAM-1, the $\alpha_4\beta_7$

integrin also binds to VCAM-1 and fibronectin [7]. In contrast to the interaction of β_7 with MAdCAM-1, which mediates lymphocyte recirculation under normal conditions, the contribution of β_7 /VCAM-1 interactions to lymphocyte recruitment may occur only during inflammatory conditions.

Integrin receptors belong to the immunoglobulin superfamily of adhesion molecules, characterized by possessing multiple immunoglobulin-like domains. Five members of this family have been shown to be involved in leukocyte–endothelial cell interactions: ICAM-1 (CD56), ICAM-2 (CD102), VCAM-1 (CD106), the mucosal addressin MAdCAM-1, and platelet–endothelial cell adhesion molecule (PECAM)-1 (CD31). It is likely that additional molecules will prove to participate also in leukocyte recruitment into the intestine. In this regard, vascular adhesion protein-1 (VAP-1) may be of particular interest.

ICAM-1 recognizes CD11a/CD18 and CD11b/CD18. ICAM-1 is constitutively expressed on leukocytes, antigen-presenting cells, fibroblasts, epithelial cells, as well as endothelial cells. Endothelial cell activation with cytokines or LPS results in an increased ICAM-1 expression. In vivo studies have shown a remarkable heterogeneity in the intensity of ICAM-1 upregulation between vascular beds [8, 9]. Organs with a relatively high constitutive expression of ICAM-1 (e.g., lung) exhibit smaller increments in ICAM-1 expression after cytokine stimulation than those organs with a low constitutive expression (e.g., heart). Organs in the gastrointestinal tract exhibit robust increases in endothelial ICAM-1 expression after LPS or TNF- α stimulation, with peak expression observed at 5 h and a sustained elevation above basal values at 24 h after endothelial cell activation [8, 10].

ICAM-2 is a truncated form of ICAM-1 that has a ligand binding site for CD11a/CD18. Like ICAM-1, ICAM-2 is basally expressed on endothelial cells, but ICAM-2 expression is not increased on activated endothelial cells. The affinity of ICAM-2 for CD11a/CD18 seems to be weaker than that of ICAM-1 [3].

VCAM-1 is an important modulator of lymphocyte and monocyte trafficking. VCAM-1 is a ligand for the $\alpha_4\beta_1$ integrin (VLA-4) and also binds to $\alpha_4\beta_7$. Although VCAM-1 is absent on unstimulated HUVEC, transcription-dependent upregulation can be elicited by cytokines and LPS in these cells [8]. In murine intestine, the constitutive level of VCAM-1 expression is substantially lower than that of ICAM-1. However, profound increases in endothelial cell surface density of VCAM-1 are noted within 5–9 h of cytokine stimulation [8].

The mucosal addressin MAdCAM-1 is mainly expressed on high endothelial venules of Peyer's patches and on venules of small intestine and colon. MAdCAM-1 serves as a ligand for L-selectin and $\alpha_4\beta_7$ integrin, but not for $\alpha_4\beta_1$ (which distinguishes it from VCAM-1). MAdCAM-1 participates in lymphocyte homing to Peyer's patches and recruitment of these cells into the intestine during inflammation [3].

PECAM-1 mediates the adhesion of both leukocytes and platelets to endothelial cells as well as transendothelial leukocyte migration. This adhesion molecule is constitutively expressed on platelets, most leukocytes, and endothelial cells. The amount of PECAM-1 expressed on cultured endothelial cells does not change appreciably after stimulation with TNF- α , IL-1 or INF- γ , but in response to these stimuli PECAM-1 redistributes to the border of endothelial cells and is thought to participate in the endothelial cell–cell interactions that affect leukocyte transmigration and microvascular permeability. PECAM-1 can mediate adhesion through either homophilic and heterophilic interactions [11].

VAP-1 is known to mediate the specific binding of CD8⁺ T cells and NK cells to peripheral lymph node high endothelial venules independent of L-selectin, PSGL-1, and α_4 integrins [12], and is also able to mediate granulocyte recruitment [13]. Although VAP-1 does not function as an autonomous lymphocyte adhesive determinant, it cooperatively (with LFA-1, Mac-1, and L-selectin ligands) confers specific binding of CD8⁺ lymphocytes to lymph nodes and inflamed endothelia.

CD4⁺ cells do not bind VAP-1, but use peripheral node addressins for trafficking [12]. Together with peripheral node addressins, VAP-1 seems to be a major determinant of the flux of lymphocytes that occurs in some healthy vascular beds (e.g., lymphoid tissue) and inflamed tissue. This glycoprotein has been implicated in lymphocyte–endothelial cell interactions in inflamed joints, but is also upregulated in inflamed intestinal lamina propria [12].

Adhesion Molecules in Human IBD

The contention that vascular endothelial cells are activated in the inflamed intestine of IBD patients is supported by the observation of a profoundly increased capacity of intestinal microvascular endothelial cells isolated from IBD patients to bind leukocytes, relative to endothelial cells derived from control subjects [14]. It has also been shown that culture supernatants of colonic mucosal biopsies from patients with UC or CD induce upregulation of E-selectin and ICAM-1 in cultured human endothelial cells [15].

Immunohistochemistry studies of intestinal mucosal biopsies from patients with IBD have demonstrated an increased expression of various endothelial adhesion molecules. In keeping with findings in animal models of IBD, an increased expression of P-selectin and E-selectin in venules and capillaries has been documented in inflamed areas from biopsies and surgically resected specimens in Crohn's disease and ulcerative colitis [16–19]. Characterization of ICAM-1 expression in human IBD has produced discrepant results, with initial studies reporting an increased expression of ICAM-1 [17, 18], and later studies failing to confirm that contention [16, 19]. Contradictory results of these studies may be in part related to limitations in quantification of adhesion molecule expression by immunohistochemistry. It has also been observed that the proportion of venular endothelium within the lamina propria that expresses MAdCAM-1 is increased, compared with normal tissues, at inflammatory foci associated with ulcerative colitis and Crohn's disease [20]. VCAM-1 expression in intestinal mucosa from IBD patients has been reported to be similar to that of controls [16, 17, 19], a finding which contrasts with observations in experimental IBD showing a consistent increase in VCAM-1 expression in diverse animal models, and with studies of soluble forms of adhesion molecules, which show a marked increase in soluble VCAM-1 in association with active IBD. Probably, significant expression of endothelial VCAM-1 in active human IBD should not be completely excluded until more accurate techniques for assessment of human adhesion molecule expression are developed. Interestingly, a study performed on human intestinal microvascular endothelial cells demonstrated that ICAM-1 is constitutively expressed and VCAM-1 is not detectable in basal conditions, but a marked increase in both adhesion molecules is observed after challenge of these endothelial cells with IL-1 β , TNF- α or LPS [21], and each of these factors has been found in elevated concentrations in human IBD tissues.

As for leukocyte adhesion molecules, an increased expression of CD18 and ICAM-1 has been found on peripheral blood monocytes from Crohn's disease patients, but not in patients with ulcerative colitis [22]. Circulating mononuclear cells from patients with Crohn's disease form granuloma-like aggregates *in vitro* which mimic *in vivo* granulomas in size and organization. The formation of these aggregates, which significantly correlates with clinical activity, appears to be dependent on CD11b/CD18 and ICAM-1 [23]. Immunohistochemical studies of mucosal biopsies have shown marked increases in the number of β_2 integrins expressing cells [24], with CD11a/CD18 mainly expressed in mononuclear cells, and CD11b/CD18 in granulocytes; increased ICAM-1 has also been documented in lymphocytes, with the intensity of the adhesion molecule expression paralleling the degree of inflammation [18].

Integrins as Targets for Therapeutic Intervention

Integrin function abrogation by means of neutralizing monoclonal antibodies has proven to be a very effective strategy in limiting both acute and chronic forms of inflammation in animal models and in human IBD.

A potent effect of monoclonal antibodies directed against the α_4 integrin in attenuating the spontaneous colitis in cotton-top tamarin has been observed [25, 26]. It has also been shown that antibodies to either MAdCAM-1 or its ligand $\alpha_4\beta_7$ block lymphocyte recruitment and reduce the severity of colonic inflammatory disease in SCID mice reconstituted with CD4⁺ T cells enriched for the CD45RB^{high} subpopulation [27]. In the rat model of TNBS-induced colitis, increased leukocyte adhesion in colonic venules and clinical signs of colitis are markedly reduced by chronic administration of an anti-VCAM-1 antibody [28]. Comparison of relative value of selective adhesion molecule blockade in dextran sulfate-induced colitis showed that VCAM-1 immunoneutralization resulted in a significantly higher clinical improvement and reduction of macroscopic and microscopic lesions than blockade of either ICAM-1 or MAdCAM-1 [29].

In clinical studies three monoclonal antibodies targeting integrins have been tested, and two of them are under development. Natalizumab is a recombinant IgG4 humanized monoclonal antibody against α_4 integrin and was the first agent developed in the new selective adhesion molecule inhibitor class. A humanized anti- $\alpha_4\beta_7$ integrin antibody, vedolizumab (MLN-0002) has also progressed into clinical trials in UC and CD. The latter has an IgG1 framework, but Fc-receptor recognition and binding is deleted and this antibody inhibits specifically $\alpha_4\beta_7$ integrin binding with MAdCAM-1. Finally, the anti- β_7 integrin antibody rhuMab β_7 is being tested in a phase 1 study in UC (NCT00694980).

Natalizumab

Pharmacokinetic studies demonstrated that the mean serum half-life of natalizumab in patients with IBD varies from 3.8 to 4.8 days, which is less than that achieved in healthy volunteers [30], and this is probably related to higher uptake of the antibody by the increased number of inflammatory cells recruited at sites of intestinal inflammation. The minimum serum natalizumab concentration required to saturate at least 80% of circulating α_4 integrins was 5 $\mu\text{g/mL}$, and it was achieved in only a minority of patients at 2 weeks, suggesting that the dose used may have been suboptimal and efficacy might be improved. Hence, in phase 2 studies, two doses of natalizumab at a 4-week interval were used (either 3 mg + 3 mg/kg or 6 mg + 6 mg/kg body weight). In phase 3 studies, a uniform dose of 300 mg was used that equated to a dose of between 3 and 6 mg/kg in most patients.

In CD, the initial controlled study on 30 patients with active CD demonstrated that natalizumab 3 mg/kg was more effective than placebo in inducing remission at week 2 and the mean Crohn's disease activity index (CDAI) score was significantly more reduced in the active-treatment group. Thirty-nine percent of natalizumab-treated patients achieved remission at week 2 compared with 8% of placebo recipients. An increase in circulating lymphocyte levels after administration of natalizumab was suggestive of blocking of lymphocyte trafficking into the intestine [30]. C-reactive protein (CRP) levels were significantly lower at weeks 2 and 4 in the natalizumab-treated patients compared with controls.

In a phase 2, randomized, placebo-controlled, multicenter trial of natalizumab in CD, 248 patients with moderate to severe CD were randomized to two infusions of placebo, one infusion of natalizumab 3 mg/kg followed by placebo, two infusions of natalizumab 3 mg/kg or two infusions of natalizumab 6 mg/kg. Infusions were given 4 weeks apart [31]. At week 6, the prospectively defined primary endpoint in the efficacy analysis, the group given two infusions of 6 mg/kg of

natalizumab did not have a significantly higher rate of clinical remission (defined by a score of less than 150 on the CDAI) than the placebo group (31% vs. 27%). However, the group given two infusions of 6 mg/kg had significantly higher rates of remission at 4 and 8 weeks (29 and 39%) compared with the placebo group (14 and 27%), and the group given two infusions of natalizumab 3 mg/kg had significantly higher rates of remission at weeks 4 (29% vs. 14%), 6 (44% vs. 27%), and 8 (41% vs. 16%) compared with the placebo group. The rate of clinical response was significantly higher in all three natalizumab groups at weeks 4, 6 and 8 than in the placebo group, with the highest rate (71%) occurring at 6 weeks in the group given two infusions of 3 mg/kg. All three active treatment groups showed significant improvement in their IBD quality of life (IBDQ) score at week 6 compared with placebo [31].

In the large phase 3 trial ENACT-1, 905 patients with moderate or severe active CD were randomized 4:1 to receive infusions of either natalizumab 300 mg ($n=724$) or placebo ($n=181$) at weeks 0, 4, and 8. The trial failed to reach its primary efficacy endpoint of clinical response (decrease in CDAI ≥ 70) at week 10, but active treatment with natalizumab was statistically superior to placebo at week 12 [32]. In the ENACT-1 trial, the response and remission rates were 56 and 37% in the natalizumab-treated group compared with 37 and 30% in the placebo group at week 10 ($p=0.05$ and $p=0.12$, respectively). A high placebo response rate may have contributed to fail in reaching statistical significance and post hoc analysis of clinical response stratified by elevated CRP ($n=660$) or concurrent immunomodulator use ($n=300$) showed significant differences in clinical response rates between the natalizumab-treated and placebo groups. Interestingly, the subgroup of 358 patients who had previously received anti-TNF therapy was also more likely to have a response or remission after natalizumab treatment than after placebo.

Although the phase 2 and phase 3 natalizumab trials provided signs of efficacy of the drug for induction of response and remission at various time points, both failed to show statistically significant differences at the predefined endpoint. Because of this uncertainty regarding the efficacy of natalizumab in induction of remission in CD, a further phase 3 induction study (ENCORE; efficacy of natalizumab in Crohn's disease response and remission) was conducted. The study included 509 patients with moderately to severely active CD characterized by elevated CRP concentrations that were randomized (1:1) to receive natalizumab 300 mg or placebo intravenously at weeks 0, 4, and 8. The primary endpoint was induction of response defined as >70 -point decrease from baseline in CDAI at week 8 sustained through week 12. Results indicate that the study achieved its primary efficacy endpoint: response at week 8 sustained through week 12 occurred in 48% of natalizumab-treated patients and 32% of patients receiving placebo ($p<0.001$). Sustained remission occurred in 26% of natalizumab-treated patients and 16% of patients receiving placebo ($p<0.002$). Week 4 response rates were 51% for natalizumab and 37% for placebo ($p<0.001$). Responses remained significantly higher at subsequent assessments ($p<0.001$) in natalizumab-treated patients. Natalizumab-treated patients also had significantly higher remission rates at weeks 4, 8, and 12 ($p<0.009$) [33].

In the ENACT trial, the most promising results were seen in the maintenance phase. Three hundred and thirty-nine responders to natalizumab in the ENACT-1 trial were re-randomized to natalizumab or placebo (1:1) for up to 12 additional months (ENACT-2) [32]. Sixty-one percent of patients receiving natalizumab maintained their response for an additional 6 months compared with 28% in the placebo group ($p<0.001$), and this significant difference was maintained for an additional 12 months. The remission rate at week 36 (additional 6 months after the ENACT-1 trial) was 44% in the natalizumab-treated group compared with 26% in the placebo group ($p=0.003$). Sustained clinical remission was also significantly better in the natalizumab-treated group compared with the placebo group 12 months after the ENACT-1 trial. The rates of both sustained clinical response and remission were significantly better in the natalizumab-treated group compared with the placebo group at every time point beginning at week 20. Natalizumab was corticosteroid sparing [32]. Some details are not available, including full data regarding mucosal healing and healing

of fistulizing CD. However, the results of the ENACT-2 trial do suggest considerable promise in α_4 -integrin inhibition in the management of patients with CD.

The overall results demonstrate efficacy of natalizumab for induction of remission and response in patients with moderate and severe CD, but the magnitude of the effect of the drug is much higher for maintenance of remission. This might be related to the mechanism of action of the drug. If natalizumab exerts its beneficial effect predominantly by blocking leukocyte recruitment to sites of inflammation, once the inflammatory process is ongoing the infiltrating lymphocytes have a high resistance to apoptosis and may remain in the intestine perpetuating inflammation for considerable periods, thus requiring administration of natalizumab for a prolonged period (10–12 weeks) to have a significant effect. On the other hand, once the inflammatory cells have been eliminated, prevention of further recruitment is very effective for preventing a new relapse.

Data evaluating the efficacy of natalizumab treatment in UC are scarce. The only full publication available reports on a pilot uncontrolled study, in which ten patients with active UC, defined as a Powell-Tuck score >4 , received a single infusion of natalizumab 3 mg/kg. The median Powell-Tuck score significantly decreased from 10.0 at baseline to 7.5 at week 2 and 6.0 at week 4. Five of the ten patients achieved a clinical response defined as a Powell-Tuck score of ≤ 5 by week 2 and one more patient responded by week 4. Two patients achieved complete remission, defined as a score of 0. The median CRP at 2 weeks (6 mg/L) was lower than at baseline (16 mg/L). During the study, adverse events were infrequent and probably not related to natalizumab infusion [34]. Despite the positive efficacy demonstrated in this study, further investigation of natalizumab in UC is not likely in the future due to life-threatening safety issues with the drug (see later) and the fact that surgery is a widely accepted option for UC patients refractory to current available therapies.

Immunogenicity of Natalizumab

Immunogenicity of natalizumab is low, but associated with loss of response and infusion reactions. In the phase 2 trial in CD [31], antibodies to natalizumab were detected in 7% of patients after 12 weeks. Only two patients had minor infusion reactions: itching and skin erythema in one patient, and itching and cough in the other patient. Both occurred during the second natalizumab infusion and in one of the patients natalizumab antibodies were noted at week 12.

Acute infusion reactions occurred in 11 and 7% of the natalizumab-treated patients in the ENACT-1 and ENACT-2 trials, respectively, compared with 8% of placebo-treated patients in both the ENACT-1 and ENACT-2 trials [32]. Hypersensitivity reactions occurred in 5 and 3% of natalizumab-treated patients in the ENACT-1 and ENACT-2 trials, respectively, compared with 2 and 1% of placebo-treated patients. Antibodies to natalizumab were detected in 8% of natalizumab-exposed patients in the ENACT-1 trial and 9% of natalizumab-exposed patients in the ENACT-2 trial. Acute infusion reactions occurred in 45% of antibody-positive patients in the ENACT-1 trial compared with 9% of antibody-negative patients. Concomitant immunosuppressive therapy and corticosteroid therapy appeared to be protective against antibody formation [32]. In the ENCORE study, the development of antibodies against natalizumab occurred also at a low frequency [33]. Of the 241 patients who received at least one infusion of natalizumab and were tested for anti-natalizumab antibodies on at least one occasion, 9.5% (23 of 241) tested positive at any time during the study. In this study, acute infusion reactions occurred in 9% of patients (23 of 260) receiving natalizumab. Infusion reactions were more frequent in subjects who tested positive (22% [5 of 23]) for anti-natalizumab antibodies than in those who tested negative (8% [17 of 218] [33]). The most common infusion reactions reported in either treatment group were headache, urticaria, and erythema. Hypersensitivity-like reactions occurred in 4% of patients (10 of 260) receiving natalizumab. Also hypersensitivity-like reactions were more frequent in patients who tested positive for anti-natalizumab antibodies 17% (4 of 23) compared with 3% (6 of 218) in those who tested negative for antibodies. No delayed hypersensitivity reactions occurred in this study.

Safety of Natalizumab

During the phase 2 trial, 26 patients had serious adverse events, seven in the placebo group (11%), six in the group given one infusion of 3 mg/kg (11%), six in the group given two infusions of 3 mg/kg (9%) and six in the group given two infusions of 6 mg/kg (12%). None of these serious adverse events was considered to be causally related to natalizumab infusion.

Two patients died during or after participation in the ENACT-1 trial [32]. The first patient died from an occupational accident and the second patient died from complications of surgery for CD 28 weeks after completing the ENACT-1 trial. No patient died during the ENACT-2 trial. Basal cell carcinoma of the skin occurred in one patient in each group during the ENACT-2 trial. Influenza and influenza-like illness occurred more commonly in natalizumab-treated patients than in placebo-treated patients during the ENACT-2 trial. One case of varicella pneumonia and one of cytomegalovirus hepatitis were reported in natalizumab-exposed patients during the ENACT-2 trial.

In the ENCORE trial infections occurred in a greater proportion of patients in the natalizumab group than in the placebo group (35% and 30%, respectively); this was primarily due to the more frequent occurrence of nasopharyngitis in the natalizumab group (11%) compared with the placebo group (6%). No opportunistic infections occurred during the study. There was one case of basal cell carcinoma in a patient receiving natalizumab. No lymphomas or other malignancies occurred during the study. One patient who received three infusions of natalizumab in combination with 6-mercaptopurine in the ENCORE trial and went on to receive three additional natalizumab infusions in combination with 6-mercaptopurine as part of an open-label extension trial was diagnosed with a B-cell lymphoma more than 6 months after the last natalizumab infusion (while still taking 6-mercaptopurine).

The major setback in the clinical application of natalizumab has been reports of three serious infectious adverse events: onset of PML that occurred in two patients (one fatal) treated in clinical trials of multiple sclerosis [35, 36], and one case (fatal) on a CD patient 3 months after initiation of open-label natalizumab treatment upon completion of participation in the ENACT-2 trial [37].

PML is a rare opportunistic infection of the central nervous system caused by the JC (John Cunningham, the first patient in whom the disease was recognized) virus [38]. This virus is common and is generally innocuous in an immunocompetent host. However, in individuals with innate, acquired, or iatrogenic immunodeficiency, the JC virus can infect oligodendrocytes, leading to their lysis. Oligodendrocyte lysis leads to central nervous system demyelination, which may then result in focal neurologic deficits including hemiparesis, visual field deficits, and cognitive impairment. PML is usually irreversible and fatal. PML was first identified in 1958 in patients with lymphoma [39] but it was not until 1971 that the viral origin of this disease was recognized [38]. It remained a very rarely reported clinical entity until the 1980s and the AIDS pandemic. Indeed, deaths due to PML as a whole increased fourfold from 1979 to 1994, and most of these deaths were attributed to PML in the setting of human immunodeficiency virus (HIV) infection and AIDS [40]. Most other PML cases occur in association with hematologic malignant neoplasms or iatrogenic immunosuppression in the context of organ transplantation or autoimmune disease. It is interesting to note three recently reported cases of PML appeared in three patients with psoriasis treated with efalizumab, an antibody directed against the adhesion molecule CD11a. These three patients developed PML in the absence of any other concurrent immunosuppressive agents, and led the manufacturer to voluntarily withdraw this drug from the market [41].

The identification of the three cases of PML in patients treated with natalizumab led the Food and Drug Administration to withdraw the drug from the market in 2005. A safety evaluation in >3,500 patients with multiple sclerosis or CD, the majority treated in the context of clinical trials (91% of treated patients participated in the study) showed that there were no new cases of PML [42] and natalizumab was reintroduced to the market in 2006. Natalizumab is currently approved as monotherapy for severe relapsing–remitting MS refractory to all other treatments and for CD after

failure of anti-TNF agents. Centers where natalizumab may be used are limited, and patients treated with the drug must participate in an extensive safety monitoring program [43]. A recent report of this program revealed that, of 37,600 patients being treated with this drug in the 3 years since the reintroduction of natalizumab to the market, there have been five additional cases of PML in patients with MS [44]. Continuing safety evaluations will help to define the place of natalizumab in the therapeutic armamentarium of CD.

Vedolizumab

Since natalizumab is an anti- α_4 antibody, it blocks both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins, and therefore all VCAM-1 and MAdCAM-1 mediated leukocyte–endothelial cell interactions. It is conceivable that more selective blockade may bring about a more favorable safety profile, especially blockade of $\alpha_4\beta_7$ –MAdCAM-1 interactions given the highly predominant expression of MAdCAM-1 in the gastrointestinal tract and complete absence of expression of this molecule in brain vasculature [45]. Vedolizumab (MLN-0002) is a blocking antibody against the $\alpha_4\beta_7$ integrin, and its efficacy for treatment of CD and UC has been tested in phase 2 studies, which are followed by ongoing phase 3 trials.

The efficacy of vedolizumab for induction of remission in UC was assessed in a phase 2 multicenter, double-blind, placebo-controlled trial in which 181 patients were assigned to receive 0.5 mg/kg of vedolizumab, 2.0 mg/kg vedolizumab, or placebo intravenously on days 1 and 29 [46]. Clinical remission rates at week 6 were 33, 32, and 14% for the group receiving 0.5 mg/kg, 2.0 mg/kg vedolizumab, and the placebo group, respectively ($p=0.03$). The corresponding proportions of patients who improved by at least three points on the ulcerative colitis clinical score were 66, 53, and 33% ($p=0.002$). Twenty-eight percent of patients receiving 0.5 mg/kg and 12% of those receiving 2.0 mg/kg had endoscopically evident remission, as compared with 8% of those receiving placebo ($p=0.007$). Human anti-human antibodies developed in 44% of the patients who received vedolizumab. Overall, 24% of patients were positive for antibody at a titer of greater than 1:125; these titers were associated with incomplete saturation of the $\alpha_4\beta_7$ receptor on circulating lymphocytes and no clinical benefit of treatment. No important differences in the occurrence of adverse events were identified among the treatment groups. No deaths, cancers, or opportunistic infections were observed. A primary cytomegalovirus infection developed in one patient that recovered without treatment, and in another patient pneumonia occurred postoperatively.

The efficacy of vedolizumab in CD has been assessed in another phase 2 study with a very similar design in which 185 patients were randomized to receive vedolizumab 2.0 mg/kg, 0.5 mg/kg, or placebo on days 1 and 29 [47]. The predefined endpoint of efficacy (>70 -point decrement in the CDAI score on day 57) was not met: response rates were 53, 49, and 41% in the vedolizumab 2.0 mg/kg, 0.5 mg/kg, and placebo groups. However clinical remission rates at the same time point were 37, 30, and 21%, respectively, with significant differences between placebo and the high dose. Twelve percent and 34% of patients in the 2.0 and 0.5 mg/kg groups had clinically significant human anti-human antibody levels (titers $>1:125$). There was one infusion-related hypersensitivity reaction. Patients with high antibody titers tended to have lower remission rates compared with patients who had low or absent antibody titers, similar to the observation in the UC study.

The signals of efficacy of vedolizumab phase 2 trials are clear in UC, and less straightforward in CD, but still with higher remission rates. The development program has now progressed to phase 3 trials in both diseases.

rhuMAb β_7

rhuMAb β_7 is a humanized IgG1 monoclonal antibody targeting the beta seven integrin subunit. Therefore, while it may have similar potential therapeutic benefits to vedolizumab by blocking $\alpha_4\beta_7$ -dependent leukocyte recruitment and cell activation, it also recognizes another β_7 integrin, $\alpha_E\beta_7$ which plays other roles in gut-associated immune responses. $\alpha_E\beta_7$ is expressed by most intestinal epithelial lymphocytes (IELs) and binds to epithelial E-cadherin expressed by the epithelium. It is also expressed by other T-cell populations resident in the lamina propria (LPL) where E-cadherin is not present, suggesting that other undescribed ligand/s for this integrin may exist. Apart from IEL and LPL, a subset of dendritic cells resident in the intestine or associated lymphoid tissues express this integrin (CD103⁺CD11c⁺ cells). This subset of dendritic cells display a tolerogenic phenotype, an interaction of $\alpha_E\beta_7$ on these cells with epithelial E-cadherin may condition their function by the influence of factors released by the intestinal [48, 49]. In addition, CD25⁺CD4⁺ expressing $\alpha_E\beta_7$, at least in mice, have an increased protective phenotype [50]. In humans, a regulatory role for CD8⁺CD103^{high} T cells has also been described [51].

However whether $\alpha_E\beta_7$ on these cells has a regulatory role per se, or rather it represents a marker for a cell subset is still unclear. As seen in α_E -deficient mice, expression of $\alpha_E\beta_7$ T cells is not required on to drive their accumulation in the intestine [52], and it is not necessary for mesenteric lymph node dendritic cells to induce gut-tropic ($\alpha_4\beta_7$ and CCR9) receptors on T cells [53].

In any case, it appears that blocking of the $\alpha_E\beta_7$ integrin may potentially target both IEL, LPL, tolerogenic dendritic cells as well as Treg, with unknown consequences.

The effect of $\alpha_E\beta_7$ blocking on experimental colitis is highly dependent on the model employed. On IL-2^{-/-} colitic mice immunized with TNP-OVA, $\alpha_E\beta_7$ blocking inhibits localization of $\alpha_E\beta_7$ -expressing CD4⁺ cells in the LP preventing and ameliorating already established colitis [54]. However in the transfer model, CD4⁺CD45RB^{hi} naïve T cells from α_E ^{-/-} mice induce comparable colitis in SCID mice than wild type cells [55].

In addition, the role of CD4⁺ $\alpha_E\beta_7$ ⁺ as regulatory T cells is also dependent on the model used and these cells have been shown to protect from disease induced by CD4⁺CD45RB^{hi} naïve T-cell transfer into SCID mice [50, 55], but not in the SAMP1/YitFc ileitis [56].

In conclusion, in contrast to specifically blocking $\alpha_4\beta_7$, the potential effect of rhuMAb β_7 on IEL, Treg, and tolerogenic DC populations will have to be closely examined in patients receiving these antibodies.

Conclusions

Intervention in the initial steps of inflammation such as the means by which leukocytes adhere to venular endothelium and migrate into tissues represents an attractive novel target for the therapy of IBD. Although much has been learned of the various endothelial and leukocyte adhesion molecules involved in adhesive interactions between such cell types in animal models of IBD, comparatively little information has emerged as to the role of these molecules in human disease. Several key steps in the inflammatory cascade that result in leukocyte recruitment appear amenable to pharmacological inhibition, but the challenges posed by the potential for disruption of alternate physiological processes as well as immune suppression are significant. Nevertheless, these limitations may be overcome by research that focuses on the identification and characterization of chemical pathways that uniquely serve the process of leukocyte–endothelial cell adhesion, either at the level of receptor activation, adhesion molecule biosynthesis, and/or adhesion molecule function. The development of safe and effective drugs that target these molecular components of the inflammatory response may yield novel, improved therapies for IBD that cover unmet needs.

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Chapter 43

Probiotics, Prebiotics, and Antibiotics in Medical Management of Inflammatory Bowel Disease

Paolo Gionchetti and Fernando Rizzello

Keywords Probiotics • Prebiotics • Antibiotics • Pouchitis • Crohn's disease • Ulcerative colitis

Introduction

The rationale for using probiotics, prebiotics, and antibiotics in IBD is based on convincing evidence that implicates intestinal bacteria in the pathogenesis of the disease. The distal ileum and the colon are the areas with the highest bacterial concentrations and represent the sites of inflammation in IBD. Similarly, pouchitis, the nonspecific inflammation of the ileal reservoir after ileo-anal anastomosis, appears to be associated with bacterial overgrowth and dysbiosis. Enteric bacteria and their products have been found within the inflamed mucosa of patients with Crohn's disease (CD) [1]. The composition of the enteric flora is altered in patients with IBD. Increased numbers of aggressive bacteria, such as *Bacteroides*, adherent/invasive *Escherichia coli*, and enterococci, and decreased numbers of protective lactobacilli and bifidobacteria have been observed [2]. Manichanh et al. reported a restriction of biodiversity in the fecal microbiota of CD patients [3]. The phylum firmicutes and particularly the species *Faecalibacterium prausnitzii* are underrepresented in active CD and UC compared with healthy subjects [4], and reduction of *F. prausnitzii* is associated with higher risk of postoperative recurrence of ileal CD [5]. There is evidence of a loss of immunological tolerance to commensal bacteria in patients with IBD [6]. Patients with CD consistently respond to diversion of fecal stream, with immediate recurrence of inflammation after restoration of intestinal continuity or infusion of luminal content into the bypassed ileum [7, 8]. Furthermore, pouchitis does not occur prior to closure of the ileostomy [9].

The most compelling evidence that intestinal bacteria play a role in IBD is derived from animal models. Despite great diversity in genetic defects and immunopathology, a consistent feature of many transgenic and knockout mutant murine models of colitis is that the presence of normal enteric flora is required for full expression of inflammation [10].

All of these observations suggest that IBD may be prevented or treated by the manipulation of intestinal microflora, and increasing evidence supports a therapeutic role for probiotics, prebiotics and antibiotics in IBD [11].

P. Gionchetti, MD (✉) • F. Rizzello
Department of Internal Clinical Medicine and Gastroenterology,
University of Bologna, Policlinico S. Orsola, Via Massarenti 9, 40138 Bologna, Italy
e-mail: paolo.gionchetti@unibo.it

Probiotics

The potential benefit of probiotics in health maintenance and disease prevention has long been acknowledged. At the turn of the last century, the Russian Nobel Prize winner Elie Metchnikoff suggested that high concentrations of lactobacilli in the intestinal flora were important for health and longevity in humans [12]. Probiotics are defined as “living organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition” [13].

The bacteria most commonly associated with probiotic activity are lactobacilli, bifidobacteria, and streptococci, but other, nonpathogenic bacteria (e.g., some strains of *E. coli*) and nonbacterial organisms (e.g., the yeast *Saccharomyces boulardii*) have been used (Table 43.1). It is believed that in order to be clinically useful for probiotics it is important to be: resistant to acid and bile, metabolically active within the luminal flora, where they should survive but not persist in the long term, antagonistic against pathogenic bacteria, safe for human use, and viable following manufacturing processes [14].

Several mechanisms have been proposed to account for the action of probiotics (Table 43.2). These may include modulation of microbiota, enhancement of barrier function, and immunomodulation through direct effects of probiotic bacteria on different immune and epithelial cell types [15].

Studies in Animal Models

Encouraging results have been obtained with probiotic therapy in experimental colitis. Administration of *Lactobacillus reuteri* has been shown to significantly reduce inflammation in acetic acid- and methotrexate-induced colitis in rats [16, 17]. More recently, a mixture of species of lactobacilli was

Table 43.1 Organisms associated with probiotic activity

• Lactobacilli
• Bifidobacteria
• Streptococci
• Enterococci
• Nonpathogenic <i>Escherichia coli</i>
Nonbacterial organisms
• The yeast <i>Saccharomyces boulardii</i>

Table 43.2 Mechanisms of action of probiotics

Action	Mechanism
Inhibit pathogenic enteric bacteria	• Decrease luminal pH • Secrete bacteriocidal proteins • Colonization resistance • Block epithelial binding
Improve epithelial and mucosal barrier function	• Produce short-chain fatty acids • Enhance mucus production • Increase barrier integrity
Alter immunoregulation	• Increase IL-10 and TGF-β and decrease TNF-α • Increase immunoglobulin a production

IL-1- interleukin-10, TGF-β transforming growth factor-β, TNF tumor necrosis factor-α

shown to prevent the development of spontaneous colitis in interleukin-10 (IL-10)-deficient mice [18], and continuous feeding with *Lactobacillus plantarum* was shown to attenuate established colitis in the same knockout model [19]. A strain of *Lactobacillus*, *Lactobacillus salivarius* subsp. *salivarius* UCC18, has been reported to reduce the rate of progression from inflammation through dysplasia and colon cancer in IL-10-deficient mice [20]. Furthermore, certain strains of *Bifidobacterium infantis* and *L. salivarius* have been shown to attenuate inflammation by reducing T helper type 1 cytokine production in the IL-10 knockout model [21]. Shibolet and colleagues demonstrated that VSL#3 (VSL Pharmaceuticals, Inc., Ft. Laudersdale, FL, USA), a cocktail of probiotic bacteria, significantly attenuates inflammation by decreasing myeloperoxidase and nitric oxide synthase activity in iodoacetamide-induced colitis in rats [22]. Using the same probiotic mixture, Madsen and colleagues reported a significant improvement in inflammation, a reduction in mucosal levels of proinflammatory cytokines, and normalization of colonic barrier integrity in IL-10 knockout mice [23]. More recently, Pagnini et al. have shown that VSL#3 was able to promote gut health through stimulation of the innate immune system in a model of chronic CD-like ileitis [24].

Ulcerative Colitis

Tables 43.3 and 43.4 summarize results of clinical trials carried out with probiotics in UC. Three double-blind, controlled trials have evaluated the efficacy of the probiotic preparation *Escherichia coli* Nissle 1917 (ECN) in the prevention of relapses of ulcerative colitis (UC). In the first study, 120 patients with UC were treated for 12 weeks with either 5×10^{10} colony forming units (CFU) of ECN or 1.5 g/day mesalazine. After 12 weeks, 16% of the patients in ECN group and 11.3% in the mesalazine group relapsed. The statistical power of this study was low and duration of treatment too short, and therefore the equivalence was not demonstrated [25]. In the second study, 116 patients were treated with ECN or mesalazine at lower dose (1.2 g/day) for 1 year. Surprisingly high relapse rate occurred in both the ECN and mesalazine group (67% vs. 73%) [26]. In the third study, 327 patients were treated with either ECN or mesalazine (1.5 g/day) for 1 year. The relapse rates were, respectively, of 36 and 34% in the probiotic group and mesalazine, showing equivalence of the two treatments in an appropriate way [27].

Recently, the same preparation has been used as enemas in patients with mild-to-moderate distal UC in a double-blind study. Ninety patients were randomly assigned to receive either 40, 20 or 10 ml containing ECN or placebo for 8 weeks. In the PP analysis ECN rectal application was significantly superior to placebo and well tolerated, in contrast to ITT analysis [28].

In another small randomized controlled trial, Ishikawa et al. evaluated the efficacy of a *Bifidobacterium*-fermented milk as a dietary adjunct in maintaining remission of UC. Twenty-one patients were included in the study in the group treated with *Bifidobacterium*-fermented milk; 3 of 11 (27%) patients had a relapse of UC compared with 10 of 11 (90%) of patients in the control group [29]. Similarly, in a 4-week, open-label study, 25 patients with mild-to-moderate clinical flare-up were treated with the yeast *S. boulardii* at the dose of 250 mg three times/day for 4 weeks; 17 patients (68%) attained clinical remission [30].

Also VSL#3 has been investigated in the treatment of UC. This product contains cells of four strains of *Lactobacilli* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*), three strains of *bifidobacteria* (*B. longum*, *B. breve*, *B. infantis*), and one strain of *Streptococcus salivarius* subsp. *thermophilus*. Each packet of VSL#3 contains 450 billion viable lyophilized bacteria. A pilot study was performed using VSL#3 as a maintenance treatment in UC patients in remission who were either allergic or intolerant to sulfasalazine and mesalazine. Patients ($n=20$)

Table 43.3 Probiotics in UC: induction of remission

Study	n	Duration	Probiotic	Control	Remission (%)		p
					Probiotic	Control	
Rembacken 1999	116	4 months	Prednisone/Gentamicin + <i>E coli</i> Nissle	Prednisone/Gentamicin + 5ASA	68	75	Equal to 5ASA? Pred effect
Guslandi 2003	25	1 month	<i>S. boulardii</i>	Open label	68%		
Bibiloni 2005	34	6 weeks	VSL#3	Open label	63%		p<0.001
Sood 2009	147	12 weeks	VSL#3	Placebo	32.5	10	
Tursi 2010	144	8 weeks	VSL#3	Placebo	Improvement in UCDAI 60.5		p<0.017
Miele 2009	29	1 month	VSL#3	Placebo	92	36.4	p<0.001
Huynh et al 2009	13	8 weeks	VSL#3	Open label	56		

Table 43.4 Probiotics in UC: maintenance of remission

Study	n	Duration (months)	Probiotic	Control	Remission (%)		p
					Probiotic	Controlled	
Rembacken 1999	116	12	<i>E. coli</i> Nissle	5ASA	26	25	Relapse rates near placebo
Kruis 1997	120	3	<i>E. coli</i> Nissle	5ASA	84	89	Equivalence to 5ASA
Kruis 2004	327	12	<i>E. coli</i> Nissle	5ASA	64	66	Equivalence to 5ASA
Venturi 1999	20	12	VSL#3	Open label	75		
Ishikawa 2003	21	12	Bifidobacterium-fermented milk	Placebo	73	10	p=0.018
Miele 2009 Pediatric patients	29	12	VSL#3	Mesalamine	79.6	26.7	p=0.014

received, 1.8×10^{12} CFU VSL#3 for 12 months and were assessed clinically and endoscopically at baseline, at 6 and 12 months, or if relapse occurred.

Fecal concentrations of lactobacilli, bifidobacteria, and *S. thermophilus* were significantly increased by VSL#3. In total, 15 of the 20 patients (75%) remained in remission during the study [31]. In an open-label study, high-dose VSL#3 (3.6×10^{12} CFU) induced remission, after 6 weeks, in 63% of patients with active mild-to-moderate disease, who failed to respond to mesalazine or corticosteroids, and was associated with a positive response in a further 23% [32]. In a multicenter, double-blind, placebo-controlled trial, 147 patients with mild-to-moderate UC were randomized to receive either 3.6×10^{12} CFU VSL#3 or placebo for 12 weeks. At 6 weeks, the rate of patients with >50% reduction in UC activity index (UCDAI) (primary end-point) were, respectively, 32.5 and 10% for VSL#3 and placebo ($p=0.001$). At 12 weeks, the rate of remission was 42.9% for VSL#3 and 15.7% for placebo ($p<0.001$). The VSL# group had significantly greater decreases in UCDAI scores and individual symptoms at weeks 6 and 12 weeks compared with placebo group [33].

More recently, in a multicenter, double-blind, randomized, placebo-controlled study, a total of 144 patients with relapsing UC, while on treatment with salicylates or immunosuppressants, were treated with either VSL#3 (71 patients) at the dose of 3.6×10^{12} CFU/day or placebo (73 patients) for 8 weeks. The decrease of UCDAI scores of 50% or more and improvement in rectal bleeding were significantly higher in the VSL#3-treated group, while endoscopic improvement and remission rate did not reach statistical significance. Only few patients reported mild side-effects with placebo and VSL#3 [34].

In two small recent studies, VSL#3 has been reported to achieve remission/response in children with mild-to-moderate UC. In the first double-blind, placebo-controlled study, 29 patients with newly diagnosed UC were randomized to receive either VSL#3 (weight-based dose, range 0.45×10^{12} CFU– 1.8×10^{12} CFU) or placebo both in induction and maintenance of remission in adjunct to standard therapy. Remission was achieved in 13 patients (92.8%) treated with VSL#3 and in 4 (36.4%) treated with placebo ($p<0.001$). VSL#3 was also significantly superior in maintenance of remission [35]. In the second, open-label trial, 18 patients with mild-to-moderate active UC were treated with VSL#3 in two divided doses (the dose was based on the age of children) for 8 weeks; 10 (56%) children achieved remission after 8 weeks, and post-VSL#3 treatment demonstrated a bacterial taxonomy change in rectal biopsy. VSL#3 was well tolerated [36].

Table 43.5 Probiotics in pouchitis

Study	n	Duration	Probiotic	Control	Remission (%)		p
					Probiotic	Controlled	
Gionchetti 2002 [Maintenance: antibiotic–remission]	40	9 months	VSL#3	Placebo	85	0	<0.001
Mimura 2004 [Maintenance: antibiotic–remission]	36	12 months	VSL#3	Placebo	85	6	<0.001
Gionchetti 2003 [Post-op prevention]	40	12 months	VSL#3	Placebo	90	60	<0.05
Shen 2005 [Maintenance antibiotic-dependent]	31	8 months	VSL#3	Open label	19.4		ns
Kuisma 2003 [Acute pouchitis]	20	3 months	<i>Lactobacillus</i> GG	Placebo	0	0	ns
Gionchetti 2007 [Acute pouchitis]	29	4 weeks	VSL#3	Open label	69		<0.01

Pouchitis

Table 43.5 summarizes the results of trials carried out with probiotics in pouchitis. Total proctocolectomy with ileal-pouch anal anastomosis (IPAA) represents nowadays the surgical treatment of choice for the management of patients with familial adenomatous polyposis (FAP) and UC, and pouchitis, a nonspecific (idiopathic) inflammation of the ileal reservoir, is the most common long-term complication after pouch surgery for UC [37]. The etiology of pouchitis is still unknown, and is likely to be multifactorial; however the immediate response to antibiotic treatment suggests a pathogenic role for the microflora, and recently pouchitis was associated with a decreased ratio of anaerobic to aerobic bacteria and reduced fecal concentrations of lactobacilli and bifidobacteria [38]. Antibiotics are the mainstay of treatment, and metronidazole and ciprofloxacin are the common initial therapeutic approach and most patients have a dramatic response within few days. A double-blind study to compare the efficacy of VSL#3 with placebo in the maintenance treatment of chronic pouchitis was carried out. Patients ($n=40$) who were in clinical and endoscopic remission after 1 month of combined antibiotic treatment (2 g/day of rifaximin plus 1 g/day of ciprofloxacin) were randomized to receive either VSL#3 (1.8×10^{12} CFU) or placebo for 9 months. Patients were assessed clinically every month, and assessed endoscopically and histologically at entry and every 2 months thereafter. Stool culture was performed before and after antibiotic treatment, and monthly during maintenance treatment. Relapse was defined as an increase of at least two points in the clinical section of the pouchitis disease activity index (PDAI) and was confirmed endoscopically and histologically. All 20 patients treated with placebo relapsed during the follow-up period. In contrast, 17 of the 20 (85%) patients treated with VSL#3 were still in remission after 9 months. Interestingly, all these 17 patients relapsed within 4 months of suspension of the active treatment. Fecal concentrations of lactobacilli, bifidobacteria, and *S. thermophilus* were significantly increased within 1 month of treatment initiation and remained stable throughout the study only in the group treated with VSL#3 [39]. A subsequent double-blind, placebo-controlled study on the effectiveness of VSL#3 (at a daily dose of 1.8×10^{12} CFU) in the maintenance of antibiotic-induced remission in patients with refractory or recurrent pouchitis reported similar results [40]. After 1 year of treatment, 85% of those in the VSL#3 group were in remission versus only 6% of those in the placebo group. As regards the mechanism of action of VSL#3 in these patients, continuous administration of VSL#3 decreases matrix metalloproteinase activity, significantly increases tissue levels of IL-10, and significantly decreases tissue levels of the proinflammatory cytokines IL-1, tumor necrosis

Table 43.6 ECCO statement on probiotic therapy in pouchitis

VSL#3(18×10^{11} of eight bacterial strains for 9 or 12 months) has shown efficacy for maintaining antibiotic-induced remission [EL1b, RG B]
VSL#3(9×10^{11}) has also shown efficacy for preventing pouchitis [EL2b, RG C]

factor- α , and interferon γ [41]. In contrast, in a 3-month double-blind, placebo-controlled trial *Lactobacillus rhamnosus* strain GG (two gelatin capsules/day of $0.5\text{--}1 \times 10^{10}$ CFU/capsule) in patients with a previous history of pouchitis showed that this probiotic was not effective in preventing relapses [42].

In an open study, Shen and colleagues treated 31 patients with antibiotic-dependent pouchitis with VSL#3 at the dose of 1.8×10^{12} CFU/day after having had a clinical improvement with 2 weeks treatment with ciprofloxacin 500 mg PO BID. At 8-month follow-up, six patients were still on VSL#3, while 25 had discontinued therapy due to either recurrence of symptoms or adverse effects. This study has numerous limitations. Firstly, patients had to purchase VSL#3 which was obtained from the company's website; VSL#3 is not covered by insurance and therefore patient's adherence to therapy was a problem. Secondly, because VSL#3 was self-administered by patients medicine counts and prescription records were impossible. Further, fecal bacteriology, as in previous study was not done and this further raise the issue of adherence to therapy. Another important issue is that patients were not assessed endoscopically before starting VSL#3 treatment (differently from previous placebo-controlled studies); this would have missed residual inflammation in the pouch and may explain the high relapse rate. Finally, patients' response was based on symptoms assessment, and recurrence of symptoms while on VSL#3 does not necessarily indicate the presence of pouchitis [43].

A double-blind, placebo-controlled trial has evaluated the efficacy of VSL#3 in the prevention of pouchitis onset in patients following IPAA for UC [44]. Within 1 week after ileostomy closure, 40 patients were randomized to receive either VSL#3 (0.9×10^{12} CFU) or placebo for 12 months. Patients were assessed clinically, endoscopically, and histologically at 1, 3, 6, 9, and 12 months according to PDAI score. During the first year after ileostomy closure, patients treated with VSL#3 had a significantly lower incidence of acute pouchitis compared with those treated with placebo (10% vs. 40%; $p < 0.05$). Moreover, IBD questionnaire score was significantly improved only in the group treated with VSL#3 and among those who did not develop pouchitis, the median stool frequency was significantly lower in the VSL#3 group. More recently, an open-label study evaluated the efficacy of high dose of VSL#3 (3.6×10^{12} CFU/day) in the treatment of mild pouchitis, defined as a score between 7 and 12 in the PDAI. Sixteen of 29 patients (69%) were in remission after 4 weeks [45].

Recently, European Crohn's colitis organization (ECCO) guidelines suggested the use of VSL#3 both for maintenance of antibiotic-induced remission and for prevention of pouchitis [46] (Table 43.6).

Crohn's Disease

Tables 43.7 and 43.8 summarize the results of clinical trials carried out in CD. In a small pilot study, *E. coli* Nissle 1917 was compared with placebo in the maintenance of steroid-induced remission of colonic CD [47]. Twelve patients were treated with *E. coli* Nissle 1917 and 11 were treated with placebo. At the end of the 12-week treatment period, relapse rates were 33% in the *E. coli* group and 63% in the placebo group; unfortunately, due to the very small number of patients treated, this difference did not reach statistical significance. In a small, comparative, 6-month, open-label study, 32 patients with CD in clinical remission were randomized to receive either combination therapy with the yeast *S. boulardii* (1 g/day) plus mesalamine (2 g/day) or mesalamine (3 g/day).

Table 43.7 Probiotics in CD: maintenance of remission

Study	n	Duration (months)	Probiotic	Control	Remission (%)		p
					Probiotic	Control	
Malchow 1997	28	12	<i>E. coli</i> Nissle 1917	Placebo	70	30	ns
Guslandi 2000	32	6	<i>S. boulardi</i>	5ASA	62.5	93.75	0.04
Bousvaros 2005	75	24	<i>L. rhamnosus</i> GG + standard therapy	Placebo + standard therapy	71	83	ns
Willert 2010	38	12	VSL#3 + standard therapy	Placebo+ standard therapy	43	11	ns

Table 43.8 Probiotics in CD: prevention of postoperative recurrence

Study	n	Duration (months)	Probiotic	Control	Remission (%)		p
					Probiotic	Control	
Campieri 2000	40	12	Rifaximin × 3 months followed by VSL#3	5ASA	Endoscopic 80	60	Benefit probiotic
Prantera 2002	45	12	<i>L. rhamnosus</i> GG	Placebo	Clinical 83 Endoscopic 40	89 65	ns
Marteau 2006	98	6	<i>L. johnsonii</i> LA1	Placebo	Endoscopic 51	36	ns
Van Gossum 2006	70	3	<i>L. johnsonii</i> LA1	Placebo	Endoscopic 21	15	ns

Relapse rates were 37.5 and 6.25%, respectively, in the mesalamine monotherapy group and in the combination group [48]. In a 1-year, double-blind, placebo-controlled trial, *Lactobacillus* GG was not effective in the prevention of postoperative recurrence [49]. Similarly, in a double-blind trial *Lactobacillus* GG was shown not to be superior than placebo in prolonging remission in children with CD when given as an adjunct to standard therapy [50].

Two randomized double-blind, placebo-controlled study showed *Lactobacillus johnsonii* LA1 (4×10^9 CFU/day) was not superior to placebo to prevent endoscopic recurrence of CD [51, 52].

We performed a single-blind study to compare a sequential antibiotic–probiotic treatment with mesalazine in the prevention of postoperative recurrence of CD. Within 1 week after curative surgery, 40 patients were randomized to receive either high-dose rifaximin (a nonabsorbable wide-spectrum antibiotic) for 3 months followed by VSL#3 (1.8×10^{12} CFU/day) for 9 months, or mesalazine (4 g/day) for 12 months. Patients were assessed clinically and endoscopically at 3 and 12 months. Compared with placebo, the combined antibiotic–probiotic treatment was associated with a significantly lower incidence of severe endoscopic recurrence, both at 3 months (10% vs. 40%; $p < 0.01$) and 12 months (20% vs. 40%; $p < 0.01$) [53].

More recently, VSL#3 at the dose of 1.8×10^{12} CFU/day was shown not to be superior than placebo in maintaining remission in colonic CD, in a 12-month, randomized, double-blind trial [54].

Prebiotics

Prebiotics are dietary substances, usually nondigestible carbohydrates, which beneficially affect the host by selectively stimulating the growth and activity of protective commensal enteric bacteria (Table 43.3) [55]. Fructo-oligosaccharides (FOS), inulin, bran, psyllium, and germinated barley

foodstuff (GBF) stimulate the growth of bifidobacteria and lactobacilli, which in turn antagonize pathogenic bacteria by decreasing the luminal pH, inducing colonization resistance, and inhibiting epithelial adhesion and translocation. In addition, these substances increase bacterial fermentation, which produces SCFAs (especially butyrate) that improve epithelial barrier function [56]. These findings suggest that prebiotics are functionally equivalent to probiotic bacteria.

Studies in Animal Models

A variety of different prebiotic preparations have been tested in animal models of colitis. Lactulose has been shown to attenuate inflammation and to stimulate the growth of lactobacilli in IL-10 knockout mice [57], while administration of inulin and GBF has been shown to inhibit dextran sodium sulfate (DSS)-induced colitis in rats by increasing the luminal concentration of SCFAs, lactobacilli, and bifidobacteria [58, 59]. Experiments on FOS have produced conflicting results. Cherbut et al. reported that FOS attenuates the trinitrobenzene sulfonic acid-induced colitis in rats [60], while Moreau et al. reported no benefit of FOS in the DSS rat model of colitis [61]. Furthermore, a combination of inulin and FOS significantly decreased inflammation in HLA-B27 transgenic rats [62]. Taken together, these findings suggest that combination therapy with different prebiotics may be more effective than monotherapy, due to the fact that each agent has specific biological properties.

Human IBD Studies

A few small, controlled studies have investigated the use of prebiotics in UC, whereas there have been no studies on prebiotics in CD or pouchitis. In a small group of UC patients in remission, psyllium (also known as ispaghula or *Plantago ovata*) was shown to be superior to placebo in decreasing symptom severity, and produced a significant increase in the fecal concentration of bifidobacteria [63]. In an open-label, randomized trial, *Plantago ovata* seeds, which have previously been shown to stimulate the production of SCFAs, were tested as a maintenance treatment in UC patients in remission [64]. In this 12-month study, 105 patients were randomized to receive either *Plantago ovata* seeds alone (10 g twice daily), mesalamine alone (500 mg three times daily), or a combination of *Plantago ovata* seeds plus mesalamine at the same doses administered for monotherapy. Rates of remission were similar for the three groups, and a significant increase in the fecal concentration of butyrate was observed after *Plantago ovata* seed administration.

GBF is comprised of the glutamine- and hemicellulose-rich extracts of spent beer-brewing constituents. Use of this probiotic in patients with mild-to-moderate UC has been investigated in a small pilot study and a placebo-controlled trial [65, 66]. At a dose of 25–30 mg/day, GBF decreased clinical and endoscopic activity in these patients and significantly increased fecal concentrations of bifidobacteria. Similar results were reported by a 24-week, open-label trial [67].

Lindsay et al. [68] performed a small, open-label study in ten patients with active ileo-colonic CD using a combination of 15 g/day of oligofructose and inulin (ratio 70:30%). They found a significant reduction in disease activity, concomitant with a significant increase in mucosal bifidobacteria. Interestingly prebiotic treatment increased colonic dendritic cells expressing IL-10, toll-like receptor (TLR)-2 and TLR-4, indicating that these prebiotics affected the innate mucosal immune response. In a small placebo-controlled study, oligofructose-enriched inulin was administered as adjunctive treatment to mesalazine 3 g/day for 2 weeks in mild-to-moderate, active UC. This study showed a significant reduction of the fecal calprotectin in prebiotic-treated patients compared to placebo [69].

Antibiotics

Animal Models

In several rodent models the use of broad-spectrum antibiotics can both prevent onset and treat experimental colitis, whereas metronidazole and ciprofloxacin can only prevent experimental colitis but not reverse established disease [70–74]. Broad-spectrum antibiotics are effective in almost all models of acute and chronic colitis [74–77], however have only a transient efficacy in HLA-B27 transgenic rats [78]. Interestingly ciprofloxacin and metronidazole had selective efficacy in different colonic region in IL-10 knock-out mice, suggesting that different bacteria cause inflammation indifferent colonic segments [76]. These studies suggest that most clinical forms of IBD may respond if a proper combination of broad-spectrum antibiotics is used.

Ulcerative Colitis

Only few trials of antibacterial agents have been carried out in UC and results are controversial. Most clinicians have used antibiotics as adjuvant therapy in severe UC. Dickinson et al. have carried out a double-blind controlled trial on the use of oral vancomycin as adjuvant therapy in acute exacerbations of idiopathic colitis. No significant difference was found between the two treatment groups with only a trend toward a reduction in the need for surgery in patients treated with vancomycin [79].

Intravenous metronidazole, used as adjunctive treatment to corticosteroids, was similarly effective than placebo to induce remission in patients with severe UC [80].

In a double-blind, placebo-controlled trial in patients with acute relapse of UC, 84 patients were randomized to receive corticosteroids plus oral tobramycin or placebo. After 1 week of treatment, 74% of patient in the tobramycin treatment group versus 43% in the placebo group ($p < 0.003$) achieved a complete symptomatic remission [81]. Subsequently, the combination of tobramycin and metronidazole did not show any beneficial effect when associated with a standard steroid treatment in severely acute UC [82]. Ciprofloxacin has been tested in a randomized, placebo-controlled study; 70 patients with mild-to-moderate active UC were randomized to receive ciprofloxacin 250 mg b.i.d or placebo for 14 days. At the end of the study, 70.5% of patients in the ciprofloxacin group versus 72% in the placebo group achieved remission [83]. Similarly a short course of intravenous ciprofloxacin was not effective as adjunctive treatment to corticosteroids in severe UC in a prospective, randomized, double-blind, placebo-controlled trial [84]. Nevertheless, in a more recent randomized, placebo-controlled trial, ciprofloxacin was administered for 6 months to patients with active UC poorly responding to conventional therapy with steroids and mesalamine. At the end of the study, the treatment-failure rate was 21% in the ciprofloxacin-treated group and 44% in the placebo group ($p < 0.002$). This difference was detected using clinical criteria; while endoscopic and histological findings showed differences only at 3 months but not at 6 months [85].

The nonabsorbable, broad-spectrum antibiotic, rifaximin was tested in a small controlled study to evaluate its efficacy and systemic absorption in patients with moderate to severe active UC refractory to steroid treatment. Twenty-eight patients were randomized to receive rifaximin 400 mg b.i.d. or placebo for 10 days as an adjunct to standard steroid treatment. Although there was no significant difference in clinical efficacy between the two treatments, only rifaximin determined a significant improvement of stool frequency, rectal bleeding and sigmoidoscopic score [86].

Table 43.9 Antibiotics in luminal active Crohn's disease

Study	Patients	Weeks	Main outcome	Study design	Treatment schedules
Blichfeldt 1978	22	8	Improvement (clinical/lab score)	DB cross over study	MZ (+ SASP/CS) placebo (+ SASP/CS)
Ursing 1982	78	16	Change in CDAI and orosomucoid	DB cross over study	MZ SASP
Ambrose 1985	72	4	Improvement (clinical/lab. score)	DB RCT	MZ CO, MZ/CO, placebo
Sutherland 1991	99	16	Change in CDAI from baseline	DB RCT	MZ (10/20 mg/kg) placebo
Prantera 1996	41	12	Clinical remission (CDAI<150)	NB RCT	MZ+CIPRO steroids
Colombel 1999	40	6	Clinical remission (CDAI<150)	NB RCT	CIPRO 5ASA
Arnold 2002	47	24	Change in CDAI	NB RCT	CIPRO (+conc drugs) Placebo (+conc drugs)
Steinhart 2002	134	8	Clinical remission (CDAI<150)	DB RCT	MZ + CIPRO (+ bud. 9 mg) Placebo (+ bud. 9 mg)

Crohn's Disease

There are several studies looking at the use of antibiotics as primary therapy for luminal CD (Table 43.9). Unfortunately, the majority of these are observational, uncontrolled studies or lack sufficient power to truly detect important differences. Metronidazole has been the mostly investigated agent. In 1978, Blichfeldt et al. in a placebo-controlled, double-blind, crossover trial did not find difference between metronidazole and placebo-treated patients, but a positive trend in favor of metronidazole was observed when only the colon was involved [87]. In the National Cooperative Swedish study, metronidazole was compared to sulfasalazine as primary treatment for CD; no significant difference was found between the two groups, but, interestingly, in the crossover section of the study, metronidazole was effective in patients not responders to sulfasalazine [88]. Metronidazole was used as single therapy or associated with cotrimoxazole and compared to cotrimoxazole alone and placebo in patients with a symptomatic relapse of CD. At the end of the 4 weeks of treatment there was no difference in response among the three groups [89]. In a Canadian randomized, placebo-controlled trial, Sutherland et al. have shown that treatment with metronidazole for 16 weeks significantly decreased the Crohn's disease activity index (CDAI), but no difference was found in the rates of remission compared with placebo; benefit was dose-dependent with 20 mg/kg having a greater benefit than 10 mg/kg [90]. As in the case of the Swedish study, in the Canadian study metronidazole was effective for colonic and ileocolonic CD but not for ileitis. Metronidazole has important side-effects that includes nausea, anorexia, dysgeusia, dyspepsia, and peripheral neuropathy that limit its use in approximately 20% of patients. An antibiotic association was used in an Italian randomized controlled study in which metronidazole 250 mg four times daily plus ciprofloxacin 500 mg twice daily were compared to a standard steroid treatment for 12 weeks. No differences were reported in the rates of remission between treatments (46% with ciprofloxacin plus metronidazole vs. 63% with methylprednisolone) suggesting that this antibiotic association could be an alternative to steroid treatment in acute phases of CD [91]. Combination of metronidazole and ciprofloxacin was associated with budesonide 9 mg/day in active CD; no difference was registered compared to placebo, but surprisingly the overall response in the two groups was lower than the

previous studies on budesonide. Also in this study antibiotic treatment was more effective when the colon was involved than for isolated small bowel disease [92].

Ciprofloxacin 1 g/daily was compared to mesalamine 4 g/daily in a controlled study in mild-to-moderate active CD. After 6 weeks an equivalence in efficacy was registered (remission observed in 56 and 55% of patients, respectively, with ciprofloxacin and mesalamine), offering an alternative treatment in active CD [93]. In a small study, ciprofloxacin was shown to be effective in association with standard treatment in patients with resistant disease [94]. Other antibiotics have been tested. Shafran et al. carried out an open-label study on the efficacy and safety of rifaximin 600 mg/day for 16 weeks in the treatment of mild-to-moderate active CD. At the end of the study, 59% of patients were in remission (CDAI < 150) with a significant reduction of the mean CDAI score compared to baseline ($p < 0.0001$) [95]. In an open-label trial, Leiper et al. reported an impressive positive response (64% patients improved or were in remission after 4 weeks) of clarithromycin in a group of 25 patients with active CD, many of whom were unresponsive to other treatments [96]. As stated by ECCO, at present, antibiotics are only considered appropriate for septic complications, symptoms attributable to bacterial overgrowth, or perineal disease. Anti-mycobacterial therapy cannot be recommended on the evidence from controlled trials [97].

Antibiotics have been also tested in prevention of postoperative recurrence. Metronidazole at the dose of 20 mg/kg/day was compared with placebo in double-blind, controlled trial by Rutgeerts et al. [98]. Sixty patients were randomized to receive metronidazole or placebo for 12 weeks. At the end of the treatment, endoscopic relapse was evaluated by Rutgeerts score. Metronidazole significantly decreased the incidence of severe endoscopic relapse (grade 3 or 4) in the neoterminal ileum 6 months after surgery and the clinical recurrence rates at 1 year, with a trend toward a protective effect after 3 years. More recently, the similar antibiotic ornidazole, used continuously for 1 year, was significantly more effective than placebo in the prevention of severe endoscopic recurrence in the neoterminal ileum both at 3 and 12 months [99].

Imidazole antibiotics, as suggested by the ECCO consensus on CD management, may be a therapeutic option after ileocolic resection but are poorly tolerated [97].

Campieri et al. performed a randomized trial to evaluate the efficacy in the prevention of postoperative recurrence of rifaximin 1.8 g daily for 3 months followed by a probiotic preparation (VSL#3) 6 g daily for 9 months versus mesalamine 4 g daily for 12 months in 40 patients after curative resection for CD. After 3 months of treatment, rifaximin determined a significant lower incidence of severe endoscopic recurrence compared to mesalamine [2/20 (10%) vs. 8/20 (40%)]. This difference was maintained since the end of the study using probiotics [4/20 (20%) vs. 8/20 (40%)] [100].

A lot of studies have tried to evaluate the efficacy of antimycobacterial drugs in patients with CD, pursuing the possibility that a strain of mycobacterium might be an etiological agent in CD. Borgaonkar et al. [101] evaluated all randomized controlled trials in which antimycobacterial therapy was compared with placebo, suggesting the efficacy of antimycobacterial therapy only as a maintenance treatment in patients who obtained remission after a combined treatment with corticosteroids and antimycobacterial agents. However, the investigator emphasized the high incidence of side-effects, and that, because the small number of studies was included in the meta-analysis, the data were not conclusive and should be taken with caution.

The same antibiotics used to treat luminal CD have been reported to be beneficial in the treatment of perianal CD, but no controlled trial has been performed [102]. Metronidazole 20 mg/kg has shown rates of fistulae closure from 62 to 83% [103, 104]. The combination of metronidazole and ciprofloxacin determined an improvement in 64% of patients and fistulae closure in 21% [105]. Unfortunately, fistulae tend to recur in most patients after stopping treatment. Although the results of these uncontrolled studies are not conclusive, metronidazole, ciprofloxacin, or their combination are used by most clinicians as first-line treatment in patients with perianal disease, in combination with surgical drainage of abscesses.

Pouchitis

The awareness of the crucial importance that fecal stasis and the bacterial overgrowth may represent in the pathogenesis of acute pouchitis has led the clinicians to treat patients with antibiotics, which have become the mainstay of treatment, in the absence of controlled trials. Usually metronidazole represents the most common first therapeutic approach, and most patients with acute pouchitis respond quickly to administration of 1–1.5 g/day [106, 107]. A double-blind, randomized, placebo-controlled, crossover trial was carried out by Madden et al. to assess the efficacy of 400 mg three times a day of metronidazole per os in 13 patients (11 completed both arms of the study) with chronic, unremitting pouchitis. Patients were treated for 2 weeks, and metronidazole was significantly more effective than placebo in reducing the stool frequency (73% vs. 9%), even without improvement of endoscopic appearance and histologic grade of activity. Some patients (55%) experienced side-effects of metronidazole including nausea, vomiting, abdominal discomfort, headache, skin rash, and metallic taste [108].

More recently, Shen et al. have compared the effectiveness and side-effects of ciprofloxacin and metronidazole for treating acute pouchitis in a randomized clinical trial. Seven patients received ciprofloxacin 1 g/day and nine patients metronidazole 20 mg/kg/day for a period of 2 weeks. The results of this study have shown that both ciprofloxacin and metronidazole are efficacious as treatment of acute pouchitis: they reduced the total PDAI scores and led to a significant improvement of symptoms and endoscopic and histologic scores. However ciprofloxacin led to a greater degree of reduction in total PDAI score, to a greater improvement in symptoms and endoscopic scores; furthermore, ciprofloxacin was better tolerated than metronidazole (33% of metronidazole-treated patients reported adverse effects, none of them were ciprofloxacin-treated) [109].

Medical treatment of patients with chronic refractory pouchitis is particularly difficult and disappointing. A possible therapeutic alternative for chronic refractory pouchitis is the use of a combined antibiotic treatment. In an open trial, 18 patients with active pouchitis not responding to the standard therapy (metronidazole or ciprofloxacin) for 4 weeks were treated orally with rifaximin 2 g/day + ciprofloxacin 1 g/day for 15 days; symptoms assessment, endoscopic and histological evaluations were performed at screening and after 15 days according with PDAI. Sixteen out of 18 patients (88.8%) either improved ($n=10$) or went into remission ($n=6$); the median PDAI scores before and after therapy were 11 and 4 respectively ($p<0.002$) [110].

More recently, 44 patients with refractory pouchitis received metronidazole 800 mg-1 g/day and ciprofloxacin 1 g/day for 28 days. Thirty-six patients (82%) went into remission; the median PDAI scores before and after therapy were 12 and 3 respectively ($p<0.0001$). Patients' quality of life significantly improved with the treatment (median IBDQ increased from 96.5 to 175) [111].

Conclusions

Many clinical and experimental observations indicate that the intestinal microflora are involved in the pathogenesis of IBD.

Probiotics may provide a simple and attractive way of preventing or treating IBD, and patients find the probiotic concept appealing because it is safe, nontoxic, and natural. VSL#3, a highly concentrated cocktail of probiotics, has been shown to be effective in the prevention of pouchitis onset and relapses. Results on the use of this probiotic in UC are promising, both in terms of the prevention of relapses and the treatment of mild-to-moderate attacks. Results with probiotics in CD are poor and there is the need of well-performed studies.

It is important to select a well-characterized probiotic preparation, in view of the fact that the viability and survival of bacteria in many of the currently available preparations are unproven.

It should be noted that the beneficial effect of one probiotic preparation does not imply efficacy of other preparations containing different bacterial strains, because each individual probiotic strain has unique biological properties.

Prebiotics are an exciting potential treatment for IBD patients. They offer a safe and cost-effective approach and may be considered for long-term treatment. However, experimental evidence supporting the use of these nutraceuticals is still limited. We need to improve our knowledge on the composition of enteric flora or “the neglected organ” and on the intestinal physiology and its relationship with the luminal ecosystem.

The use of antibiotics in UC is not supported by the available studies, although large studies with broad-spectrum agents are required. Antibiotics have an essential role in treating the septic complications of CD, including intrabdominal and perianal abscesses and perianal fistulae.

There is evidence that ciprofloxacin, metronidazole or their combination are effective in Crohn’s colitis and ileocolitis, but not in isolated ileal disease, however use of antibiotics as primary therapy in CD is poorly documented, and large, controlled trials are needed for defining the optimal antibiotic regimens.

The use of antibiotics in pouchitis is largely justified although proper controlled trials have not been conducted.

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Chapter 44

Step-Up vs. Top-Down Approach in Medical Management of Inflammatory Bowel Disease

Anne Christine W. Vos and Daniel W. Hommes

Keywords Crohn's disease • Early intervention • Biological treatment

Introduction

In recent years, the medical care for patients suffering from Crohn's disease (CD) has changed considerably. Nowadays clinicians are equipped with a robust set of tools, supported by a substantial body of literature, on how to act upon diagnosing CD for the first time. There seems general consensus on the diagnostic process, using a full endoscopic workup in combination with radiologic and histopathologic assessments [1]. Once diagnosed, it is up to the team of IBD health professionals to fully inform and educate the patient and his/her relatives, and to install and monitor a vigorous medical regime.

The main treatment goals are (1) to achieve clinical remission of steroids as soon as possible, (2) to achieve endoscopic remission, (3) to avoid hospitalization and surgery, and (4) to improve the quality of life. Indeed, today we are able to monitor the success of each of the treatment goals very effectively: (1) monitoring the rate of clinical remission in daily practice can be performed on a week-to-week basis by, for example, a specialized IBD nurse, using well-defined and validated parameters of general well-being, abdominal pain, stool frequency, extra intestinal manifestations, and general symptoms like fever and weight. More and more, IBD centers are establishing *remote care* delivery systems for monitoring disease activity indices on a regular basis and thus partly allowing self-management [2]. Next to clinical parameters, a defined set of laboratory values like CRP is recommended nowadays for monitoring. In addition, most steroid tapering schedules can be completed within 8 weeks. Intervention is required much sooner than 8 weeks should patients continue to have active disease. (2) the paradigm of mucosal healing has been well accepted upon publication of a large number of studies showing the relevance of endoscopic assessments for the prediction of clinical remission [3–5]. Alternative biomarkers for mucosal healing are under development since endoscopy remains an invasive procedure, for instance, fecal calprotectin and fecal lactoferrin [6–8]. Again, these fecal tests could also be very well for home monitoring of mucosal

A.C.W. Vos, MSc (✉) • D.W. Hommes, MD, PhD
Department of Gastroenterology and Hepatology, Leiden University Medical Center,
Albinusdreef 2, Leiden 2333 ZA, The Netherlands
e-mail: d.w.hommes@lumc.nl

healing. (3) it has also been shown that induction of clinical and endoscopic remission decreases the number of hospitalizations and surgeries in CD patients [9]. (4) Similar to clinical remission, the quality of life of CD patients dramatically improves upon reaching the previously discussed treatment goals [4, 10, 11].

In summary, today's physicians are able to act immediately upon diagnosing CD with effective and safe drugs. This chapter clarifies (1) why there is this “*Window of Opportunity*” the first years after the diagnosis of CD, (2) why intensive treatment (also referred to as Top Down) is superior in selected cases, (3) which safety measures should be taken to prevent (serious) adverse events during intense therapy, (4) how to optimally monitor patients, and lastly (5) the possibilities for de-escalating therapy once deep remission is achieved.

Window of Opportunity in Crohn's Disease

When a patient is diagnosed with CD, it is an important and challenging issue how to optimally treat the patient and at which point a certain therapy should be initiated. In established CD, early disease should be distinguished from late disease, since CD is characterized by progression of inflammatory disease (early disease) to a more complicated stricturing, penetrating, and fibrotic disease (late disease) [12]. Fibrostenosis and perforations are not per se associated with early CD, usually patients with recently diagnosed CD have pure inflammatory lesions (Fig. 44.1). However, when the disease evolves, the number of complications increases and many patients will develop strictures or fistulae formation. In this stage, complications caused by tissue remodeling and fibrosis following long-standing disease are irreversible and difficult to treat with the anti-inflammatory agents. Often, surgery cannot be avoided at this stage. The progression from early to late disease is accompanied by a change in mucosal cytokine profiles. Early CD is characterized by a pronounced Th1 response, whereas in late disease Th2 cytokines are predominating [13]. The concept that mucosal T cell regulation is different in early and late disease suggests that patients with late disease respond different to therapies. Indeed, superior therapy efficacy is observed in patients with newly

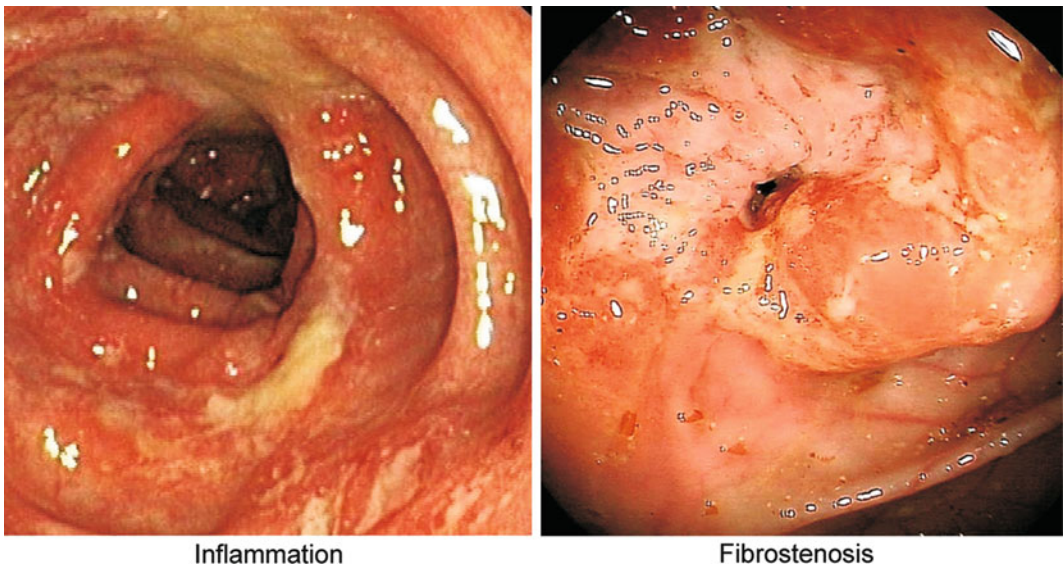
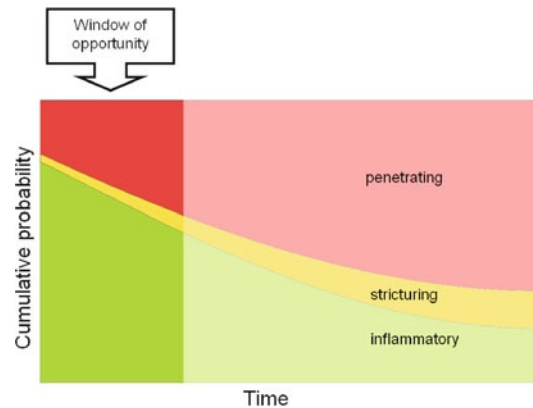


Fig. 44.1 Inflammation (*left panel*) and fibrostenosis (*right panel*)

Fig. 44.2 Window of opportunity. [Adapted from Cosnes, J., Cattan, S., Blain, A., Beaugerie, L., Carbonnel, F., Parc, R. and Gendre, J.-P. (2002), Long-term evolution of disease behavior of Crohn's disease. *Inflammatory Bowel Diseases*, 8: 244–250. with permission from Wolters [Kluwer. ©2002]



diagnosed CD compared to patients with longer disease duration [14–17]. The progression from early to complicated late disease course has also been reported in rheumatoid arthritis (RA) [18, 19]. In RA patients, therapy is aimed at preventing late disease when complications and bone destruction are irreversible. In this light, intervening in an early stage of the disease has proven to be very effective and superior to treatment in a late disease stage [19–21].

The fact that disease progresses to complicated disease suggests that there is a particular time window at which therapy is most effective and favorable. In order to prevent progression to a complicated phenotype, it is of great importance to intervene in an early stage of the disease, the so-called Window of Opportunity (Fig. 44.2). At this point, it is most likely still possible to change the course of the disease towards a less aggressive phenotype, to control symptoms, induce mucosal healing, and to induce and maintain clinical and endoscopic remission.

Early Intensive Treatment (“Top-Down”)

Selecting Patients for Early Intensive Treatment

The window of opportunity is especially important for patients who are likely to develop a disabling disease course. Risk factors that predict a disabling disease course have now been identified and confirmed in several studies (Fig. 44.3). Although “disabling disease” has various definitions in different studies, clinical factors predicting the course of disease have been independently verified. In a retrospective study involving 1,188 patients, disabling disease was defined as more than two steroid courses, hospitalization, immunomodulators, or surgery within the first 5 years of diagnosis [22]. *Young age* at diagnosis, presence of *perianal disease* at diagnosis, and initial *need for corticosteroids* were found to be predictive factors for disabling disease. In addition, when two of these factors were present at diagnosis, 84% of the patients developed disabling disease within 5 years; when three factors were present, 91% developed a disabling disease course. These factors were all independently confirmed in another study. In this study, disabling disease was defined as complex perianal disease, colonic resection, ≥ 2 small bowel resections, or a definitive stoma, also within the first 5 years of diagnosis [23]. Using one definition or the other, several factors that are present at diagnosis have been identified and confirmed that predict a disabling disease outcome, including a young age (≤ 40 y), presence of perianal disease, stricturing disease, and the initial need for steroid therapy [22–24]. Patients with these symptoms at diagnosis are at high risk for a complicated disease course.

Fig. 44.3 Predictive factors at diagnosis for selection of high-risk patients



Therefore, early intensive treatment in selected patients could prevent the development of complicated disease, and thereby prevent hospitalization and surgery.

Biomarkers predicting disease outcome in CD are currently lacking. Serological, genetic, and immunological factors are being studied and would be helpful to further adequately select patients for a certain therapy strategy. Elevated levels of CRP correlate with more severe clinical, endoscopic, and histologic disease [25]. However, CRP did not very well predict milder disease, and some patients with severe disease have normal CRP levels. Consequently, additional markers are needed. Positive anti-Saccharomyces cerevisiae antibody (ASCA) status has been associated with early surgery [26], ileal disease [27], and poor outcome [28] and might therefore be a useful marker in the selection of patients. In another study, a well-defined correlation was found between triple positive status for ASCA, anti-OmpC as well as anti-I2, and small bowel surgery [29]. Furthermore, mutations in NOD2 [30] and CARD15 [31] increase the risk for surgery in patients with pediatric-onset CD.

Large trials should further confirm the value of these markers in predicting disease course. When combining these factors, a profile can be established including phenotypic, genetic, and serologic markers to facilitate the selection of patients.

Early Intensive Strategy in Crohn's Disease

The benefit of the disease-modifying strategy has been shown in several studies. The first clues pointing towards the benefit of so-called early intervention strategies in CD result from retrospective analyses from large randomized, placebo-controlled trials. In a study evaluating the maintenance of response and remission of adalimumab, a clear difference was observed in patients with different disease durations [32]. The rate of patients with remission maintenance at week 26 was 56% in patients with a disease duration of less than 2 years, 35% in patients with a disease duration of 2–5 years, and 37% in patients with a disease duration of more than 5 years.

In a prospective, randomized controlled trial, including 130 patients with newly diagnosed CD naïve to corticosteroids, immunomodulators, or anti-TNF agents, efficacy of step-up vs. top-down therapy was evaluated [33]. The Top-Down approach involved a *treatment algorithm* starting with infliximab 5 mg/kg at weeks 0, 2, and 6 and azathioprine 2.5 mg/kg/d. Patients randomized to the Step-Up treatment algorithm were initially treated with corticosteroids (current standard around 1999), followed by azathioprine in case the corticosteroids were not able to control disease activity. Should this combination fail to avoid relapse, infliximab treatment was initiated. The co-primary endpoint in this study was clinical remission off steroids and without surgery at weeks 26 and 52.

At both time points, a significant greater proportion of patients in the early combined intervention group met this endpoint compared to the conventional therapy group. At week 26, 39 (60.0%) of 65 patients in the Top-Down group were in remission without corticosteroids and without surgical resection, compared with 23 (35.9%) of 64 Step-Up patients, with an absolute difference of 24.1% (95% CI 7.3–40.8, $p=0.0062$). Corresponding rates at week 52 were 40/65 (61.5%) and 27/64 (42.2%) (absolute difference 19.3%, 95% CI 2.4–36.3, $p=0.0278$). Furthermore, after 2 years, mucosal healing was observed in 73% of the patients in the early intervention group, whereas mucosal healing was seen in only 30% of the patients in the conventional treatment group. Importantly, 19% of the patients in the step-up group were still on steroids at this time point, whereas 0% of the patients in the early intervention group were receiving steroids. Also, this study showed that complete mucosal healing in patients with early stage CD was associated with significantly higher steroid-free remission rates 4 years after therapy began [3]. Complete mucosal healing, defined as a simple endoscopic score of 0 after 2 years of therapy, was the only factor that predicted sustained, steroid-free remission 3 and 4 years after therapy was initiated; it was observed in 17 of 24 patients (70.8%) vs. 6 of 22 patients with lesions detected by endoscopy (27.3%, Simple Endoscopic Score >0) ($P=0.036$; odds ratio=4.352; 95% confidence interval, 1.10–17.220). Fifteen of 17 patients with mucosal healing at year 2 maintained in remission without further infliximab infusions during years 3 and 4 ($P=0.032$; odds ratio=4.883; 95% confidence interval, 1.144–20.844).

Finally, in this Step-up vs. Top-down study, the number of side effects did not differ significantly between the two groups. Obviously, remission rates in the two groups did not differ significantly after 1 year since the two treatment algorithms were both designed to control disease, and allowed intensification when disease activity persisted.

Effective early intervention has also been reported in children. Twenty-nine patients receiving infliximab in either step-up (11 patients) or top-down (18 patients) regimen were evaluated 8 weeks after initiation of treatment [34]. Remission was achieved in 3/11 patients in the step-up group and in 15/18 in the top-down group. In the top-down group, significant improvement was observed in PCDAI score and perianal fistula status. Furthermore, in a retrospective study of 36 pediatric CD patients [35], relapse rate after 24 months was significantly lower in the early intervention group than in the conventional therapy group.

Finally, the SONIC trial evaluated the efficacy of infliximab monotherapy, azathioprine monotherapy, and the two drugs combined in 508 adults with moderate-to-severe CD who had not undergone previous immunosuppressive or biologic therapy [15]. Patients were randomly assigned to receive an intravenous infusion of 5 mg of infliximab per kilogram of body weight at weeks 0, 2, and 6 and then every 8 weeks plus daily oral placebo capsules; 2.5 mg of oral azathioprine per kilogram daily plus a placebo infusion on the standard schedule; or combination therapy with the two drugs. Of the 169 patients receiving combination therapy, 96 (56.8%) were in corticosteroid-free clinical remission at week 26 (the primary end point), as compared with 75 of 169 patients (44.4%) receiving infliximab alone ($P=0.02$) and 51 of 170 patients (30.0%) receiving azathioprine alone ($P<0.001$ for the comparison with combination therapy and $P=0.006$ for the comparison with infliximab). Similar numerical trends were found at week 50. At week 26, mucosal healing had occurred in 47 of 107 patients (43.9%) receiving combination therapy, as compared with 28 of 93 patients (30.1%) receiving infliximab ($P=0.06$) and 18 of 109 patients (16.5%) receiving azathioprine ($P<0.001$ for the comparison with combination therapy and $P=0.02$ for the comparison with infliximab). Thus, infliximab plus azathioprine was shown to be the superior combination for inducing remission.

In conclusion, early intensive treatment, consisting of the combination of a thiopurine with an anti-TNF, should be considered in *all* patients who have a high risk for a complicated disease course, i.e., young age at diagnosis, presentation with perianal or stricturing disease, or initial needs of steroids.

Safety Measures for Early Intensive Treatment

An important issue related to early intensive treatment is the safety of long-term use of biologics and immunomodulators. Patients are exposed to potentially toxic agents, and therefore appropriate safety measures should be undertaken before immunomodulators and biologicals are initiated (Fig. 44.4). Safety measures are aimed at prevention of infections and awareness of several rare complications.

It appears that patients treated with immunomodulators have an increased risk for influenza infections, pneumococcal infections, and *Salmonella* supp. infections. Therefore, influenza vaccination, pneumococcal vaccination, and appropriate food hygiene (avoiding raw eggs, unpasteurized milk, raw meat) are recommended. Also, reactivation of latent HBV is considered a serious risk, and therefore all IBD patients should be tested to exclude HBV [36]. Patients with active chronic infection should be treated according to standard antiviral therapy. Nucleoside/nucleotide analogues are preferred since IFN therapy might exacerbate the colitis. Seronegative patients should receive HBV vaccination; patients might need a higher dose of immunizing antigen since vaccination efficacy is affected by the number of immunomodulators.

Screening for cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Herpes simplex virus (HSV) before starting immunomodulator therapy is not recommended. In case of severe EBV infection, immunomodulator therapy should be discontinued. Latent subclinical CMV infection is also no contraindication for starting immunomodulators. However, CMV colitis should be excluded in refractory IBD cases and in case of systemic CMV, immunomodulator therapy should be discontinued [36].

Anti-TNF use is associated with an approximately 21-fold increased risk of tuberculosis (TB) without appropriate safety measures [37] (Fig. 44.5). The TB incidence has been reported to decrease with 78% when suitable safety measures were undertaken. Most cases are presented during the first 3 months of treatment and have an atypical presentation, which makes the diagnosis more complicating [38]. For that reason, international guidelines advise to assess the risk of TB before starting treatment with an anti-TNF agent, including an X-ray, tuberculin skin testing (depending on national guidelines), and careful evaluation of the TB history [36]. Latent TB may be suspected in case of a positive initial tuberculin skin test and when the patient has recently been exposed to the disease. Physicians should be aware of the possibility of false-negative skin tests, especially when patients are immunocompromised. When the patient is diagnosed with latent TB, treatment with the full therapeutic anti-TB regimen should be initiated and it is advised to delay anti-TNF treatment for at least 3 months. The full anti-TB regimen consists of isoniazid (INH) for 6–9 months. An association between isoniazid-related hepatotoxicity and methotrexate and sulphasalazine

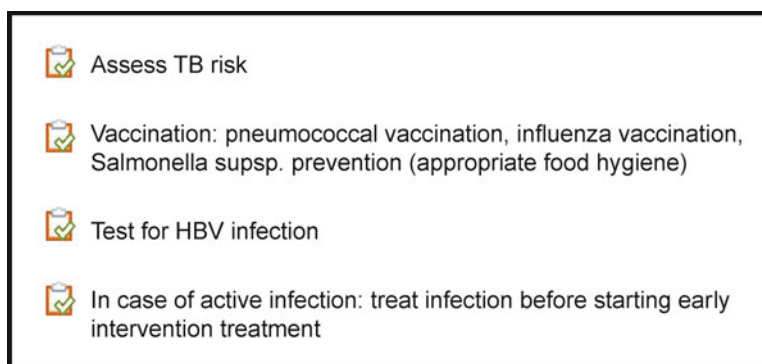
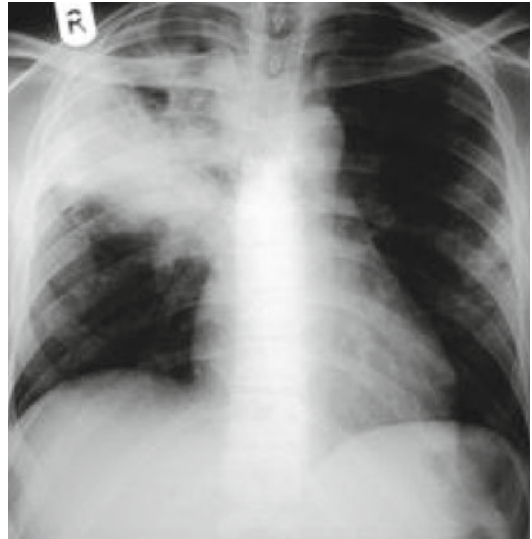


Fig. 44.4 Safety measures

Fig. 44.5 TBC

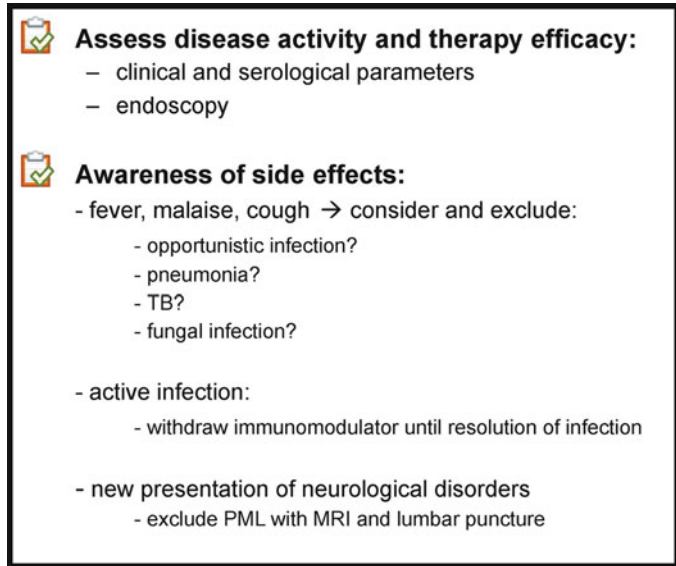
has been reported in the rheumatology setting, but this is not clear for IBD. When active TB is diagnosed, anti-TNF treatment is ideally delayed until anti-TB treatment is completed. However, solid data on the ideal timing during anti-TB treatment are lacking and in case of medical urgency, physicians may consider to start anti-TNF therapy despite positive TB diagnosis. Advice from a TB expert is in that case recommended. When TB is diagnosed during anti-TNF treatment, the anti-TNF agent should be discontinued and TB therapy should be started. Anti-TNF therapy can be resumed if needed after 2 months. In any event, supervision of a thoracic physician or infectious disease specialist is advised. 5-ASA, azathioprine, methotrexate, and steroids can be continued during anti-TB therapy. All patients should be monitored carefully for signs of cough, fever, and weight loss and treating physicians should be aware of uncommon extrapulmonary TB as well as the more common lung disease.

How to Optimally Monitor Patients

Disease Activity

Prior to installing a medical strategy, it is essential to discuss the monitoring scenario with each patient. Monitoring of both disease activity and drug safety is mandatory, irrespective of early or late stage disease. Although guidelines discuss monitoring practices, the evidence in the literature is currently lacking. Optimally, the clinical monitoring of CD activity should be done in a standardized manner using, for instance, the Crohn's Disease Activity Index (CDAI), the Harvey Bradshaw Index (HBI), or other validated indices. However, for routine clinical practice this is usually not accepted. Using simple Physician Global Assessments is very simple and easy to do, and a reasonable alternative. These clinical assessments are required on a weekly basis once remission induction is initiated, and less often once remission is achieved. In addition to monitoring clinical disease activity, clinicians should also monitor CRP each time a clinical assessment is made. Finally, the correct monitoring strategy of early intensive treatment also involves assessment of mucosal healing. It has been shown that, following surgical remission, Crohn's ulceration can be detected within 3–6 months

Fig. 44.6 Monitoring strategies for patients with early intensive therapy



following surgery preceding clinical symptoms [39]. Mucosal healing predicts long-term outcome and is associated with less surgery and hospitalization [4, 40]. In a retrospective study, it has been shown that patients with severe ulcerations at colonoscopy are more likely to have a complicated disease course [41], and in an ongoing study, a strong association was found between mucosal healing and decreased risk of relapse [42]. Therefore, assessing mucosal healing is a useful tool to predict the course of disease, to monitor disease activity, and to further optimize treatment.

Noninvasive fecal markers such as fecal lactoferrin and calprotectin are currently being evaluated for their usefulness to measure disease activity. Increased levels of calprotectin and lactoferrin have been shown to correlate with CDEIS scores [7, 43] and therefore these markers might be useful tools to assess disease activity. In addition, it has been shown that lactoferrin and calprotectin are valuable markers to predict relapse [44]. In patients with high ($>150 \mu\text{g}$) calprotectin levels or positive lactoferrin, a higher risk for relapse was observed. The value of lactoferrin and calprotectin as markers for disease activity has also been demonstrated in pediatric disease [45]. However, it has been suggested that these markers be less valuable in patients with ileal CD [46], since the disease location might interfere with accurate fecal detection. Future studies are needed to further confirm the value of these markers in daily practice, define clear cut-off values, and to finally identify a combination of markers with higher sensitivity and specificity.

Side Effects

In order to evaluate the risk benefit of the early intensive treatment, a careful monitoring of potential and sometimes avoidable adverse events is mandatory (Fig. 44.6). Patients on immunosuppressive therapy and with malnutrition are at risk for opportunistic infections [47]. For that reason, patients with fever, cough, and systemic illness should be carefully examined. In case of an active infection, it is advised to withdraw immunomodulators until the resolution of infection. In case of persistent fever and when the patient is treated with an anti-TNF agent, *mycobacterium tuberculosis* (TB) should be excluded. Patients diagnosed with pneumonia should be treated with an antibiotic covering *S. pneumoniae* [36].

Furthermore, about 60–80% of the European population has latent JC virus, which can cause progressive multifocal leukoencephalopathy (PML) upon reactivation. Since reactivation of the JC virus is associated with systemic immunosuppression, treating physicians ought to be aware of this rare situation, especially in patients presenting with new onset neurological symptoms, in which case the patient should receive an MRI scan and lumbar puncture.

Studies show divergent data on the occurrence of malignant lymphoma in IBD patients receiving immunomodulators. Whereas some studies do not show an increased risk, other studies do find a moderately elevated risk, especially in patients on thiopurine therapy [48–50]. Still, the absolute risk appears to be low and should be weighed against the beneficial effects of immunomodulator therapy. On the other hand, awareness is advised. Lethal hepatosplenic T cell lymphoma has been reported in young patients on azathioprine/infliximab combination therapy, [51–54] and therefore long-term combination therapy in younger patients is not recommended.

After all, when taking appropriate safety measures, anti-TNF and immunomodulator therapy appears to be relatively safe. Safety data from referral centers and randomized controlled trials do not show an increased risk of malignancies or infections in anti-TNF treated patients. In a large meta-analysis of 21 placebo-controlled trials including 5,356 patients, no increased risk of death, serious infection, or malignancy compared to controls was reported [55]. In line with this observation, no increased risk was found in infections, mortality or malignancy in 734 anti-TNF-treated patients compared to controls, with a median follow-up of 58 months [11]. In addition, no increased risk of malignancy was observed in patients treated with anti-TNF in a large cohort of CD patients [56]. However, long-term safety data are not available yet and therefore awareness of (serious) side effects is warranted.

Possibilities for De-escalating Therapy Once Remission Is Achieved

Once remission is achieved, it is important to know if, when, and how to de-escalate therapy. Although severe adverse events are rare, especially when appropriate safety measures are undertaken and patients are correctly monitored, they can occur and therefore establishing an individualized risk–benefit ratio is encouraged. The disadvantages of discontinuation of therapy should be taken into account, including relapse, possibly lower response to re-induction therapy, infusion reactions, and surgery. Particularly important when considering de-escalation is the earlier pattern of the disease and response to therapies. In addition, several factors may predict relapse, including smoking, previous steroid use, and elevated fecal calprotectin and CRP.

Anti-TNF and Anti-TNF/Azathioprine Combination Therapy

The question how and when to de-escalate infliximab therapy is of great interest. Trials investigating this matter are currently ongoing, and therefore there is not much data and consensus on this topic yet.

The proportion of patients with infliximab-induced remission that relapsed after discontinuing infliximab was assessed in a prospective single-center study [57]. In this study, infliximab was stopped in patients who were treated with infliximab for at least 1 year, and who were in steroid-free remission for 6 months. After the median follow-up time of 12 months, about 50% of the patients relapsed. On the other hand, 35% of the patients were still doing well up to the end of the follow-up time (nearly 7 years). Therefore, it seems that the patients that are still doing well after 7 years represent a specific group of patients. Age, gender, number of infliximab infusions, and disease location were all excluded as predictors for relapse in this study. These results were supported by data from an observational study evaluating the long-term effects of infliximab [11]. In this study,

20% of the patients who experienced a sustained clinical response to infliximab maintained remission after infliximab discontinuation.

Interim results of the STORI (infliximab discontinuation in CD patients in stable remission on combined therapy with immunosuppressors) showed similar results [58]. In this prospective study, relapse was assessed in patients on combination therapy for more than 1 year and in stable remission for ≥ 6 months after stopping infliximab therapy. About 50% of the patients relapsed within 1 year of discontinuation; patients retreated with infliximab after relapse responded well. In addition, risk factors that predict relapse were detected. A univariate analysis revealed current smoking, previous steroid treatment, lower hemoglobin, higher CDAI, ultrasensitive C reactive protein, and fecal calprotectin as predictive markers. In a multivariate analysis, CDEIS ≥ 2 , CRP > 5 mg/dl, hemoglobin ≤ 14.5 g/dl, and infliximab trough levels ≥ 2 μ g/ml were found to predict relapse. Furthermore, the risk of relapse increased as the number of risk factors increased [42].

In a study investigating the ability to stop immunosuppressives in patients treated with combination therapy, an enduring response was observed after withdrawal of immunosuppressives [59]. Importantly, they reported low infliximab trough levels before immunosuppressive withdrawal as a predictor for surgery. Risk factors for relapse after azathioprine withdrawal in patients treated with infliximab/azathioprine combination therapy were identified in another study [60]. Infliximab/azathioprine exposure duration ≤ 811 days, CRP ≥ 5 mg/L, and platelet count $\geq 298 \times 10^9$ were found to predict infliximab failure, which was defined as disease flare, hypersensitivity reactions leading to infliximab discontinuation or surgery. After 12 and 24 months, resp. 85% and 41% of the patients were infliximab-failure free.

In summary, it appears from these preliminary data that there is a distinct group of patients that does well after de-escalation therapy. It is of great interest to identify the factors that discriminate patients with sustained remission from the patients that relapse, in order to further tailor therapy and to prevent unnecessary side effects. Studies are currently ongoing, and the preliminary data need to be confirmed in large cohorts.

Thiopurines

Furthermore, there are conflicting data with regard to discontinuation of thiopurine therapy once a patient is in remission. In a large randomized, placebo-controlled double-blind trial, azathioprine discontinuation was associated with a 50% relapse rate after 54.5 months, regardless of the duration of remission [61].

In a study evaluating disease recurrence after azathioprine withdrawal, a lower rate of relapse was seen in patients who received azathioprine ≥ 4 years than in patients treated with azathioprine ≤ 4 years [62].

Concluding Remarks

Early intervention therapy in CD is beneficial in selected patients who are likely to develop a severe disease course. Young age at diagnosis, the presence of perianal disease, stricturing disease, and the initial need for corticosteroids are factors predicting a complicated disease course. When taking appropriate safety measures, this approach appears to be relatively safe. However, treating physicians should be aware of opportunistic infections and other rare complications, and patients should be monitored carefully on a regular basis. The value of serological, immunologic, and genetic markers in monitoring and predicting disease are currently under investigation and could be helpful to further optimize therapy.

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Chapter 45

Surgical Management of Crohn's Disease and Ulcerative Colitis

Robert R. Cima and John H. Pemberton

Keywords Crohn's disease • Chronic ulcerative colitis • Surgery • IPAA

Introduction

Crohn's disease (CD) and Chronic ulcerative colitis (CUC) represent the two ends of the spectrum of inflammatory bowel diseases (IBD). For both diseases, the etiology is unknown [1]. Recent research indicates that there are genetic components to both. However, genetics alone does not explain their occurrence. Many believe there is a complex interaction between genetics, environmental, dietary, and possibly infectious agents that contribute to the onset and progression of CD and CUC. While both are inflammatory conditions of the intestine, the location, extent, and histologic characteristics of the inflammation define each as distinct disease entities.

There are significant differences in the pattern of intestinal inflammation between CD and CUC that lead to different manifestations of the disease. CD is characterized by transmural inflammation that may occur anywhere along the intestine from mouth to anus. Transmural inflammation leads to a number of complications unique to CD including perforation, abscess, fistula, and stricture. Surgery is not curative in CD; therefore, operations are performed for complications of the disease or to alleviate symptoms. Unlike CD, CUC is an inflammatory disease limited to the mucosa of the colon and rectum. Since the small bowel is not involved in CUC, removal of the colon and rectum cures the intestinal manifestation of the disease. In this chapter, we discuss the indications for and some technical aspects of surgery for CD and CUC.

Surgery for Crohn's Disease

The decision to proceed to elective surgery in a CD patient needs to be a collaborative decision arising from a consensus between the surgeon, the gastroenterologist, and, most importantly, the patient. Prior to elective CD surgery, it is essential that the medical treatment for CD is optimized.

R.R. Cima, MD, MA • J.H. Pemberton, MD (✉)
Division of Colon and Rectal Surgery, Department of Surgery,
Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, USA
e-mail: pemberton.john@mayo.edu

Intestinal transmural inflammation results in a number of CD complications that may require surgical intervention. Common indications for operation include: chronic intestinal inflammation; obstruction due to an inflammatory process or chronic stricture formation; perforation; and entero-entero, entero-vesical, entero-vaginal, or entero-cutaneous fistula. Fistulizing CD in the perineum frequently results in anal ulcers, abscesses, or complex anal fistulas.

The small bowel, particularly the terminal ileum, is most commonly involved with CD. In a large series of patients with CD requiring surgery over an 11-year period, 41% had disease in the terminal ileum, 16% had perianal disease, and 16% colonic disease. Eighteen percent of patients had multiple sites of disease [2, 3]. In these series, the indications for small bowel operation were failure of medical management to control symptoms (50%), intestinal obstruction (20%), and fistulous disease (15%).

Fortunately, CD patients rarely require emergency surgery. This permits the patient to be medically optimized prior to surgery. Optimizing the patient includes ensuring appropriate CD medical therapy, improving the patient's nutritional status, and controlling any local sepsis. For patients with intra-abdominal abscesses, radiologic-guided percutaneous drainage should be performed rather than surgical drainage. This is important because it will minimize the inflammation involving non-diseased intestine, thus minimizing the amount of bowel that may require resection. Maintaining or improving a CD patient's nutritional status prior to surgery is essential. If the patient cannot be fed via an enteral route, then early institution of parenteral nutrition is appropriate. In preparation for any major CD surgery, it is advisable to have the patient marked for a possible stoma by an enterostomal therapist. Since CD patients frequently require subsequent operations for recurrent disease, the scope of surgery should be directed at the specific problem requiring intervention with every attempt made to limit the extent of bowel resection. Extensive or repeated small bowel resections may lead to short bowel syndrome and significant nutritional compromise.

Small Bowel Crohn's Disease

As previously mentioned, the small bowel is the most common site of CD activity. Nearly 40% of patients will have terminal ileal disease [2]. Common symptoms are abdominal discomfort, persistent diarrhea, or obstructive symptoms. Radiographic or endoscopic evaluation often will establish the diagnosis (Fig. 45.1). Fortunately, there are multiple medical therapies for CD. The algorithms used to determine the optimal therapy for an individual patient are beyond the scope of this chapter. However, all CD medical therapies are directed at reducing the local or systemic inflammatory process. The traditional approach to CD therapy was to start with agents directed at reducing local intestinal inflammation to avoid the use of agents with systemic effects, the so-called bottom-up approach. However, there is some evidence suggesting that early initiation of agents directed at mediators of systemic inflammation may alter the natural history of CD (top down approach) [4].

Surgery for small bowel CD is most commonly performed for medically refractory disease or obstructive symptoms related to terminal ileal disease (Fig. 45.2). The extent of the ileal resection was once a topic of debate. Previously, there was a belief that achieving microscopically negative margins would reduce CD recurrence. However, extended resections lead to excessive loss of small bowel increasing the risk of nutritional compromise in patients that required re-resection. In a prospective randomized, controlled trial, Fazio and colleagues evaluated the extent of resection for focal small bowel CD and found no statistical difference in clinical recurrences between extended versus limited resection [5]. Based upon this study and clinical experience, the standard is to limit resection of only grossly involved bowel.

Fig. 45.1 CT image of isolated small bowel Crohn's disease (*arrows*). This image clearly demonstrates the segmental nature of CD the hyper-enhancement of mesenteric blood vessels and the thickened bowel wall characteristic of CD



Fig. 45.2 Terminal ileal CD which demonstrates thickening of the mesentery, creeping fat along the bowel, and the increased vascularity of the intestinal serosa which is characteristic of CD



Despite medical therapies, recurrence of CD is common after resection. Upwards of 50% of CD patients who undergo terminal ileal resection will experience endoscopic recurrence of disease in the region of the anastomosis within 1–2 years [6]. However, endoscopic evidence of recurrence does not always translate into recurrent symptoms. McLeod et al. showed that nearly 75% of patients have endoscopic or radiologic recurrence by three years after surgery but only 40% had symptoms [7]. The role of medical prophylaxis to minimize CD recurrence is unclear. A meta-analysis of available trials of postoperative chemo-prophylaxis demonstrated that mesalamine might slightly decrease symptomatic recurrences [8].

Strictureplasty

Another common presentation of small bowel CD is focal stricturing disease which leads to obstructive symptoms. There might be a single area or multiple areas along the length of the small bowel (Fig. 45.3). The strictures are the result of chronic inflammation in which the transmural scarring leads to compromise of the lumen. Previously, areas of multiple strictures were resected or by-passed. Both led to loss of functional bowel length which resulted in nutritional compromise. Furthermore, long segments of by-passed small bowel are associated with bacterial overgrowth and potential increased risk of malignancies in the by-passed segment. The use of strictureplasty in CD was first reported in 1982 [9]. The advantage of this technique is it can be repeated along the length of the bowel without the need for resection. The most commonly used technique is the Heineke–Mikulicz strictureplasty. This technique is useful for strictures up to 4–5 cm in length. The stricture is divided by making a longitudinal enterotomy along the antimesenteric border (Fig. 45.2). The enterotomy is then closed in a transverse fashion which reestablishes and widens the lumen of the bowel. Different types of strictureplasties have been developed for specific situations. For longer strictures (approximately 6–15 cm), the stricture can be folded upon itself and a side-to-side strictureplasty can be performed. In rare cases, a patient will have a long segment of bowel with multiple strictures or a single long stricture. Traditionally, this situation would have required a resection of the entire involved segment. To address this complex problem, Michellassi developed the long segment side-to-side isoperistaltic strictureplasty [10]. The segment of involved bowel is divided in the middle and then opened along the antimesenteric border. The bowel segments are overlapped in an isoperistaltic fashion and a side-to-side anastomosis is performed.

Strictureplasty is successful in relieving obstructive symptoms while preserving small bowel length. The Cleveland Clinic reported a large series of 698 strictureplasties performed in 162 patients without any septic complications or deaths [11]. The reoperation rate at five years was similar to patients who underwent formal bowel resections. For unclear reasons, in patients requiring reoperation for recurrent strictures, the majority of new strictures occurred at sites remote from the previous strictureplasties [12]. In a recent meta-analysis, strictureplasty is associated with an overall complication rate of 11% with major complications occurring in 5% of cases [13]. The median surgical recurrence rate was 24% after a median follow-up of 46 months.

Treatment of Enteric Fistulas in CD

The transmural inflammation associated with CD results in the fistula formation. Fistulas can occur between different regions of the small and large bowel, adjacent organs, or the abdominal wall. Often, only one side of the fistula is involved with active CD and many entero-enteric



Fig. 45.3 Multiple CD small bowel strictures

fistulas may be asymptomatic. However, if a large segment of bowel is by-passed by the fistula either by connecting to a distal segment of bowel or to the abdominal wall, it may compromise nutrient and fluid absorption to such an extent that surgical division is required.

The initial treatment of a CD enteric fistula is similar to the management of any enteric fistula. Any septic focus needs to be controlled and the anatomy of the fistula delineated. An abdominal CT scan with oral water-soluble contrast is the test of choice because it is both diagnostic and possibly therapeutic. Ideally, any intra-abdominal fluid collection should be drained percutaneously. If the patient has any evidence of systemic infection, broad spectrum antibiotics should be started. Any fluid and electrolyte abnormalities need to be corrected and the patient should be placed on bowel rest. In patients with enterocutaneous fistulas, prompt consultation with a wound or enterostomal therapist is essential in order to avoid skin breakdown and to aid in determining the amount of fistula output with the bowel at rest. Early initiation of intravenous hyper-alimentation is important to prevent further nutritional deterioration. If the patient can be fed orally without significantly increasing the fistula output while maintaining their nutritional state that is preferred to chronic hyperalimentation. An extended period of intense medical therapy and nutritional support should be attempted prior to surgery. The patient's CD medication management needs to be optimized prior to surgery including the use of biologic therapies. The addition of infliximab to standard medical therapy for enterocutaneous fistulas has resulted in a 68% closure rate compared to 13% for placebo [14].

Surgery for CD fistulizing disease requires detailed counseling and operative planning. The patient should be seen and marked by a certified enterostomal therapist for a possible ostomy. At the time of surgery, it is important to distinguish between areas of active CD and "innocent bystander" bowel. The fistula always originates from the active disease site and extends to nondiseased bowel. The principle in internal fistula surgery is to resect the active disease and repair the nondiseased bowel. If both ends of the fistula are involved with active disease then both segments of bowel are removed. The same strategy is used for fistulas to other organs or the skin. A recent review of the surgical treatment of enterocutaneous fistulas at the Cleveland Clinic reported a 90-day mortality of 3.5% and a fistula recurrence rate of nearly 21% over three months [15]. However, using the treatment algorithm of controlling sepsis, maximal nutritional and medical therapy, and resection of active disease, they achieved an 80% closure rate.

Crohn's Colitis

In some CD patients, the disease is limited to the colon. However, CD is a disease of the entire bowel and patients may present with disease limited to the colon but later develop small bowel disease [16]. The colitis may demonstrate a chronic relapsing course or occur as a fulminant episode. Indications for surgery in Crohn's colitis include intractable symptoms, fistula formation, stricture formation, or severe perianal disease. Fulminant Crohn's colitis may present with massive hemorrhage, free perforation, or systemic toxicity. The surgical management of these patients is similar to patients with fulminant ulcerative colitis. A subtotal colectomy with ileostomy and oversewing of the rectum is the preferred operation. Once the patient's overall medical condition improves and there is no longer evidence of active disease, intestinal continuity can be reestablished by performing an ileorectostomy if the rectum is not involved with Crohn's disease.

In CD patients with segmental involvement of the colon, limited resection is the preferred approach. The Mayo Clinic experience demonstrates that resection of the focal Crohn's colitis leads to 86% of patients remaining stoma free for more than ten years [17]. However, nearly 50% will experience a recurrence in the colon and nearly a third require a second operation for that recurrence. Due to the high recurrence rate, some prefer to offer total proctocolectomy with a permanent end ileostomy as the treatment of choice for segmental Crohn's colitis. Certainly for patients with severe perianal fistulizing CD and segmental colitis a total proctocolectomy and ileostomy should be performed.

Another consideration in Crohn's colitis is the possibility of colon cancer. A meta-analysis by Jess et al. reported nearly a doubling of the standardized incidence ratio for colorectal cancer in Crohn's patients compared to the general public [18]. Any colonic stricture needs to be thoroughly evaluated for a potential malignancy. Furthermore, 10% of CD patients with a colonic malignancy will have synchronous tumors. In this setting, the preferred operation is a total abdominal colectomy with ileorectostomy.

Perianal Crohn's Disease

Perianal CD is a difficult management problem. Eleven percent of patients will present with perianal disease [16]. There are numerous manifestations of perianal disease, including hypertrophic anal skin tags, ulceration, fissures, anal stenosis, abscesses, and fistulas. A patient may have one or multiple manifestations of perianal CD. Common presenting symptoms include severe pain, mucus discharge, bleeding, fecal urgency, or incontinence. Surgery for perianal CD is directed at symptom management. Extensive anal operations should be avoided as scarring and recurrent disease may cause irreversible damage to the anal sphincter mechanism resulting in fecal incontinence. Procedures should be limited to controlling local sepsis and preventing recurrent sepsis.

Perianal abscesses need to be drained. Antibiotics alone are not adequate treatment. At the time of abscess drainage if an associated anal fistula is discovered, a loose draining Seton should be placed to provide long-term drainage. If no internal anal opening of a fistula is found, the abscess cavity can be packed as one would a routine perianal abscess. For larger abscess cavities, a mushroom tip rubber catheter can be secured into the cavity to provide long-term drainage.

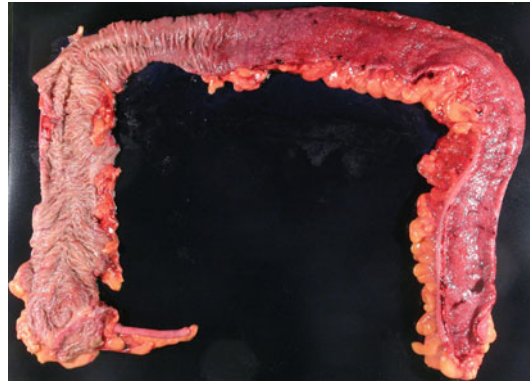
In patients with complex perianal CD disease, especially fistula disease, imaging is very helpful in guiding management. The combination of endoanal ultrasounds, pelvic MRI, and examination under anesthesia will correctly identify 100% of pathology when at least two of the modalities are used [19]. While surgery is directed at controlling symptoms, newer medical therapies are directed at reducing the initiating inflammatory process. Biologic agents, such as infliximab, are very effective in treating perianal CD [20]. Once CD medical therapy is optimized and all local sepsis has resolved, an attempt at surgical treatment for any chronic anal fistulas may be considered. Complex dissections and flap procedures should be avoided in CD patients with anal fistulas to minimize any unnecessary disruption of the sphincter mechanism. A surgical option for these CD fistulas is to fill the fistula track with a slowly absorbable material. Fibrin glue injected into the tract has been modestly effective in healing perianal fistula tracts [21, 22]. Another option is to place a plug of bioabsorbable material in the tract which permits slow scarring of the tract and eventual closure [23].

In patients with extensive medically refractory perianal CD, the sphincter mechanism may become impaired leading to fecal incontinence or severe pain such that proctectomy is the only treatment option. Up to 25% of patients with persistent perianal CD will require eventually proctectomy [24]. Fecal diversion often temporarily improves perianal disease but when reversed, active disease frequently returns. Surgery cannot cure perianal CD. However, conservative surgical management of perianal CD can greatly improve a patient's quality of life.

Surgery for Chronic Ulcerative Colitis

Chronic ulcerative colitis (CUC) is a recurring inflammatory condition limited to the colon and rectal mucosa. Since the disease is limited to colon, surgery is curative for the intestinal manifestations of the disease. By definition, the inflammation begins in the rectum and progresses uninterrupted for a variable distance into the colon (Fig. 45.4). The disease course is notable for low level chronic

Fig. 45.4 The typical mucosal pattern of CUC which demonstrates contiguous involvement from the low rectum to the transverse colon



intestinal inflammation with intermittent worsening of the inflammation. These flares of disease activity result in worsening symptoms frequently manifested as by abdominal pain and bloody diarrhea. In a minority of patients, the initial presentation of CUC is a severe acute illness associated with high fever, tachycardia, abdominal pain, distension, and bloody diarrhea. Toxic, or fulminant, colitis is a medical emergency and requires rapid assessment and treatment [25]. Ideally, these patients should be managed by both the gastroenterologist and the surgeon experienced in the care of CUC.

Surgery in CUC is divided into two categories, emergent and elective. The operation performed is influenced by the setting in which it is performed. Emergency operations are performed for life-threatening complications such as massive hemorrhage or toxic colitis or severe medically refractory disease requiring hospitalization. In this emergency setting, a definitive operation is not recommended. Elective surgery is undertaken to treat intractable symptoms of the disease or for dysplasia or malignancy in the setting of CUC. In appropriate patients, a definitive operation can be performed. A definitive operation removes the entire colon and rectum. In many patients, a restorative operation can be performed avoiding the need for a permanent ileostomy.

In appropriately selected patients, the best surgical option is total proctocolectomy with ileal pouch-anal anastomosis (IPAA). IPAA avoids the need for a permanent stoma and maintains the normal route of defecation. This operation is technically demanding and should be performed by surgeons comfortable with the procedure. Long-term follow-up of IPAA patients has demonstrated durable functional results with a high degree of patient satisfaction.

Emergency Surgery for CUC

Emergency surgery is performed in patients with fulminant disease, or toxic megacolon or rarely massive hemorrhage. In the emergency setting, the goal of the operation is to remove the abdominal colon, construct an ileostomy, and leave the rectum undisturbed. Preservation of the rectum maintains the option of a future restorative procedure, such as an IPAA. Using this “damage control” approach allows the patient to recover from their acute illness, discontinue any immunosuppressive medication, and improve their nutritional state before a definitive operation is undertaken.

Fulminant colitis is the initial presentation in 10% of CUC patients [26, 27]. Truelove and Witts defined the clinical syndrome of fulminant colitis [28]. It is characterized by the sudden onset of severe bloody diarrhea (more than ten per day), abdominal pain, dehydration, and anemia. In addition, the patient must have at least two of the following: tachycardia, temperature greater than 38.6°C,

leukocytosis, and hypoalbuminemia. These patients are extremely ill and require immediate medical attention. Initial therapy involves aggressive intravenous fluid resuscitation, correction of electrolyte abnormalities, and anemia. If the patient has abdominal distension a nasogastric tube should be placed. Stool cultures should be obtained to rule out *C. difficile* or hemorrhagic infectious enteritis. A patient with known CUC may be started on intravenous steroids. In patients without a diagnosis of CUC, an endoscopic evaluation of the colon needs to be performed to help establish the diagnosis. A complete endoscopic evaluation of the colon is not required and may be unsafe in this setting. If the patient is clinically stable, there is no need for antibiotic therapy. However, in the presence of a fever or leukocytosis, broad-spectrum antibiotics should be initiated after cultures are obtained. Close observation for 24–48 h while on maximal medical therapy is crucial. If there is no improvement, or if the patient's condition deteriorates, then surgery is advised. During this observation period, the development of peritonitis or hemodynamic instability requires immediate operation.

Other complications requiring immediate surgical evaluation are toxic megacolon and massive hemorrhage. Toxic megacolon may be seen in the setting of fulminant colitis or in isolation. Transverse colon dilatation greater than 5.5 cm defines the radiographic criteria of toxic megacolon. The entire colon or an isolated segment of the colon (usually transverse) is involved with gaseous distension. Clinically, the patients have significant abdominal distension and pain. They may also have fever and a leukocytosis. These patients are treated in a fashion similar to those with fulminant colitis. Operation is indicated if the patient's clinical or radiographic status worsens, or if there is no improvement after 24–36 h of medical therapy. Profound intestinal hemorrhage is a rare complication of CUC. Aggressive fluid and blood-product resuscitation is required as well as correction of any electrolyte or clotting deficiencies. Ideally, upper intestinal endoscopy needs to be performed to rule out a bleeding gastric or duodenal ulcer since these patients are often on steroids at the time of presentation. If the patient remains hemodynamically unstable even after resuscitation, then operation is indicated; medical therapy is too slow to reverse the mucosal inflammation responsible for the bleeding. If the patient responds to resuscitation, then a trial of intravenous steroids may be instituted. Persistent bleeding requiring transfusion after 48–72 h of therapy is an indication for surgery. Perforation outside the setting of toxic megacolon rarely occurs. A patient presenting with a perforation without megacolon should raise concern that the actual diagnosis is CD, or that there is another cause for the perforation, such as a gastric or duodenal ulcer. Whatever the cause, there is no role for conservative therapy, and the patient should immediately undergo exploration.

Regardless of the indication for an emergency CUC surgery, the operation of choice is a subtotal colectomy with an end ileostomy. A pelvic dissection is avoided so the planes in the pelvis are not disturbed, making future surgery easier. The rectum should be retained even in patients who are not candidate for a future restorative procedure. Performing the rectal dissection during emergency surgery increases the complexity of the case, lengthens the operation, and increases the risk of bleeding. After the patient recovers and their health status improves, the retained rectum can be addressed at a future elective definitive procedure.

Elective Surgery in CUC

The most common indication for elective surgery is intractability, despite medical therapy. Other indications include: colonic dysplasia, a dysplasia associated lesion or mass (DALM), malignancy, or side effects of the medications. In children, stunting of normal growth is also an indication for surgery. Intractability is a clinical definition. In the chronic disease setting, it refers the inability to discontinue oral steroids completely or the development of severe drug-related side effects.

The presence of colonic dysplasia is an important consideration in CUC patients especially in those with long-standing disease. CUC patients are at high risk of developing colorectal cancer.

The cancer risk increases with both duration and extent of the disease. The lifelong risk of colorectal cancer is estimated to be anywhere from 2% at 20 years after onset of CUC to 43% at 35 years [29]. The presence of colonic dysplasia on endoscopic biopsies is evidence of epithelial instability. This is considered a premalignant state. Initially reported by Taylor and colleagues and expanded upon recently by Gorfine et al., colon specimens with dysplasia of any grade were 36 times more likely to harbor a cancer [30, 31]. In the Mayo Clinic experience, 18 CUC patients with low-grade dysplasia were observed with serial colonoscopies for a median of 32 months [30]. Nine of the 18 patients developed advanced neoplastic lesions including a cancer. Ullman and colleagues reported a similar high rate of malignancy in the setting of low grade dysplasia without any associated masses [32]. While some recommend increased endoscopic surveillance for low grade dysplasia others consider its presence an indication for surgery. The presence of a polyp not associated with any surrounding dysplasia usually can be managed with endoscopic removal without any increased risk for malignancy [33]. While increased frequency of endoscopic surveillance for low grade dysplasia may be warranted, evidence of recurrent low grade dysplasia or any evidence of moderate or high grade dysplasia is an indication for surgery.

The two primary definitive operations for CUC are total proctocolectomy with end ileostomy (TPC) or total proctocolectomy with IPAA. The TPC can be performed in a single stage with a relatively low morbidity. However, the patient is left with a permanent ileostomy. The IPAA is often a multiple stage procedure and is associated with a higher complication rate. The advantage of IPAA is that it avoids the need for a permanent ileostomy and preserves the normal route of defecation albeit with a higher number of daily bowel movements.

IPAA is the procedure of choice in appropriately selected patients who wish to avoid a permanent ostomy. Due to technical considerations, obese patients and extremely tall patients may not be good IPAA candidates. Our experience is that IPAA needs to be abandoned in less than 4% of patients intraoperatively due to technical or anatomic problems [34]. Furthermore, advanced age which was once considered a contraindication to IPAA is now considered a relative contraindication.

Whether IPAA is performed through a traditional open or a minimally invasive approach, the operation involves four steps: (1) removal of the intra-abdominal colon, (2) dissection and removal of the rectum sparing the pelvic nerves and the anal sphincter mechanism, (3) construction of an ileal reservoir, (4) anastomosis of the ileal reservoir to the anal canal. We have previously described our IPAA technique [35]. At our institution, nearly all patients also receive a diverting loop ileostomy at the time of IPAA.

Construction of the ileal pouch requires that the small bowel mesentery be completely mobilized from the retroperitoneum up to the inferior border of the pancreas to ensure there is adequate mesenteric length to reach the anal canal. To achieve adequate length, it may be necessary to divide either the ileocolic vessel or one of the branches of the superior mesenteric artery. Once the mesentery has been mobilized, the pouch is fashioned. A J-shaped reservoir is constructed from the terminal 30–35 cm of the ileum. The pouch is constructed by folding the terminal ileum into a J-shape. The common wall between the two limbs is divided by repeated firings of a linear cutting stapling device from the apex of the pouch. The pouch is anastomosed to the anal canal by using a circular stapler or alternatively by a hand-sewing the pouch to the anal canal. As mentioned previously, our practice is to construct a proximal diverting loop ileostomy at the time of the pouch operation. However, as will be discussed later, in some select patients omitting the ileostomy can be considered. Eight to twelve weeks after the operation if there is no evidence of a leak from the pouch on a contrast study performed through the anus, the ileostomy is reversed.

While the majority of the literature regarding IPAA is based upon traditional laparotomy, increasingly, a minimally invasive approach is the preferred operative technique. In a case matched series reported by Dunker and colleagues, laparoscopic-assisted IPAA as compared to open IPAA resulted in similar functional results and quality of life outcome measurements [36]. A report from the Mayo Clinic on their initial experience with laparoscopic IPAA reported similar findings [37].

Functional Outcomes

A number of surgeons and institutions have reported their IPAA experience. Across these many studies, the functional results are quite similar [38–40]. The majority of patients report good to excellent function with their IPAA. In the Mayo Clinic IPAA experience, the average number of daytime bowel movements after ileostomy closure was six and one at night [38]. Incontinence was an unusual occurrence during the day with 79% of patients reporting complete continence, 19% occasional incontinence, and 2% frequent incontinence episodes. Nocturnal incontinence was more common with 59% reporting no incontinence episodes and 49% occasional incontinence episodes.

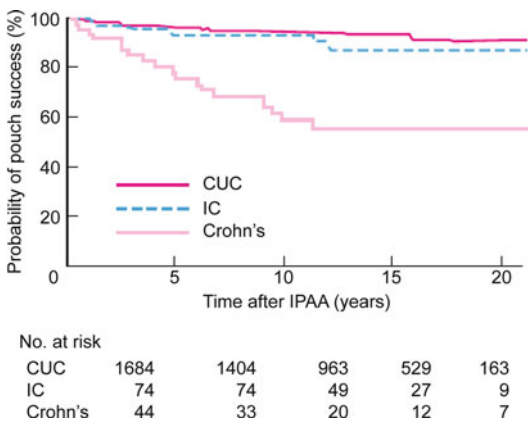
Thirty years after the introduction of IPAA, the long-term durability of the pouch has been assessed. Hahnloser et al. reported on the functional outcomes of IPAA patients who have had their pouch in place for up to 20 years [41]. Pouch failure is rare even in those who suffer postoperative complications. The overall pouch success rate is 92% (Fig. 45.5). Most commonly, permanent diversion or pouch excision was performed for poor pouch function. The strongest association with pouch failure was with postoperative pelvic sepsis. Other reasons for pouch failure were chronic pouchitis and subsequent development of CD.

Complications

IPAA is associated with a number of early and late complications. Small bowel obstruction and pelvic abscesses are the most common early complications. Late complications include anastomotic stricture, pouch fistulas, pouchitis, and cuffitis. Delayed presentation (>1 year after reversal of the ileostomy) of a pelvic abscess or fistula raises the concern that the underlying diagnosis is not CUC but rather CD.

Numerous reports of IPAA experience have had similar postoperative complication rates [42–44]. The overall postoperative morbidity rate ranges between 25 and 30%. The most worrisome postoperative complication is a pouch leak and associated pelvic sepsis which occurs with a rate between 5 and 24%. As will be discussed later, this rate of pelvis leak does not seem to be influenced by proximal diversion with an ileostomy at the time of pouch construction. Patients with a pelvic phlegmon CT scan respond to broad-spectrum antibiotics. If there is drainable fluid collection, percutaneous CT-guided drainage is the preferred approach. Rarely laparotomy with abdominal washout and drainage is required.

Fig. 45.5 The probability of long-term pouch success over an extended nearly 20-year follow-up period at a single institution, Mayo Clinic, Rochester. [Reprinted from Hahnloser D, et al. Results at up to 20 years after ileal pouch-anal anastomosis for chronic ulcerative colitis. *Br J Surg* 2007;94(3):333–340. with permission from John Wiley & Sons LTD]



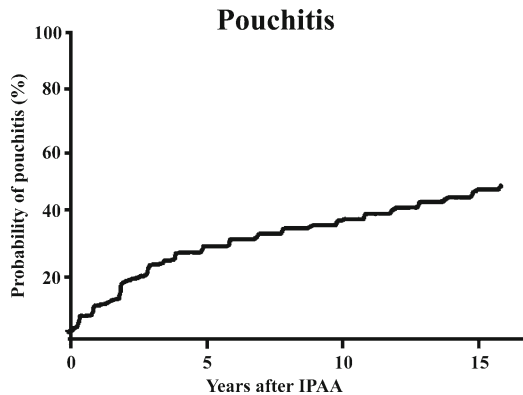


Fig. 45.6 The probability of developing an episode of pouchitis after IPAA for CUC during a 15-year follow-up period as reported from a single center, Mayo Clinic, Rochester, in over 400 patients. [Reprinted from Hahnloser D, et al. The Effect of Aging on Function & Quality of Life in Ileal Pouch Patients: A Single Cohort Experience of 409 Patients with Chronic Ulcerative Colitis. *Ann Surg.* 2004;240(4):615–621. with permission from Kluwer/Lippincott, Williams & Wilkins]

Small bowel obstruction is both a short- and long-term complication after IPAA. In the Mayo Clinic experience, perioperative small bowel obstruction was 15% with nearly a quarter requiring operation [45]. The most common site of obstruction was in the pelvis. MacLean reviewed the literature and reported an average incidence of bowel obstruction as 18% at one year, 27% at 5 years, and 31% at 10 years [46]. The impact of minimally invasive IPAA on the incidence of bowel obstruction has not been reported. However, in one small series where pelvic adhesions were assessed at the time of ileostomy closure there were significantly reduced [47]. Pouch-anal anastomotic stricture is another common complication after IPAA [45]. There is no correlation between stricture formation and anastomotic technique, hand sewn versus stapled. Fortunately, these strictures are easily dilated either in the operating room or by the patient using soft dilators.

The most common long-term IPAA complication is pouchitis [48]. The reported incidence of pouchitis is heavily influenced by the diagnostic criteria used. Patients report symptoms similar to colitis including fever, anemia, and diarrhea [49]. An increased number of pouch leakage episodes or fecal incontinence is a common complaint. There are few reliable pre- or postoperative risk factors that have been associated with development of pouchitis. Fortunately, chronic pouchitis is infrequent and often responds to therapy. The most common treatment is a 2-week course of oral antibiotics either metronidazole or ciprofloxacin [50]. Patients who suffer frequent recurrent episodes or develop chronic pouchitis may require prolonged antibiotic therapy or even immunomodulator therapy. Rarely, chronic pouchitis requires permanent diversion or pouch excision. In the long-term follow-up of IPAA patients, nearly 50% of patients reported at least one episode at 10 years but it rose to 78% after 20 years [41] (Fig. 45.6). In this cohort, chronic pouchitis developed in less than 5% of patients and only 2% required diversion or pouch removal.

Although the IPAA experience spans three decades, there are a number of technical and outcome issues that continue to be discussed. These include the use of a defunctioning ileostomy, IPAA in older patients, fertility in women after IPAA, and the impact of newer biologic therapies on postoperative IPAA outcomes.

Role of Proximal Diversion

Early in the IPAA experience, the role a proximal ileostomy was to protect the pouch from fecal content while the anastomosis healed. This was deemed to be essential in minimizing the risk of a pelvic leak and sepsis. Over the last decade a number of authors have reported their experience with IPAA without the “protecting” ileostomy [51–53]. Advocates of this approach believe IPAA can be performed without an increased risk of pelvic sepsis while avoiding the inconvenience of a temporary ileostomy and the need for a second operation. A large single institution report from Sugerman et al. detailed results in 201 patients who underwent a stapled IPAA; 196 were done without a diverting ileostomy [51]. The majority of these procedures were performed for CUC and nearly all the patients were on steroids at the time of surgery. Anastomotic leaks developed in 12% of patients with only nine patients requiring operations to construct a proximal ileostomy. In this study, there was no impact on the long-term IPAA function. Others have reported similar outcomes [52]. A small randomized control trial has been performed which found no difference between the standard use of a proximal ileostomy or no ileostomy [53]. Despite some evidence that there is no need for a proximal ileostomy at the time of IPAA, most surgeons prefer constructing one. This is based upon the concern that the severity of complications in patients without ileostomy is greater than those with a proximal ileostomy. This belief is supported by a comparative study between one- and two-stage IPAA which demonstrated while the complication rates were similar the rate of life-threatening complications were significantly higher in the patients without a proximal ileostomy [54]. Overall, the current evidence suggests that in highly selected patients who have technically uncomplicated procedures performed by experienced IPAA surgeons, a proximal ileostomy may be omitted. However, the surgeon needs to closely monitor the patients for early signs of pelvic sepsis related to a pouch or anastomotic leak.

IPAA and Age

Traditionally, IPAA was offered only to younger CUC patients who required surgery. CUC patients older than 50 who required surgery were not considered candidates for IPAA, because of over concerns about poor functional outcomes. However, reports from multiple institutions have demonstrated no difference in functional outcomes or quality of life even in their 1980s [55]. Overall, the most important consideration is the general health of the patient rather than their chronologic age.

CUC and Fertility

Many CUC patients are diagnosed during their young adulthood years; therefore, the impact of both medical and surgical treatment on fertility needs to be considered. Recent investigations have demonstrated that IPAA has a significant negative impact on fertility in young women [56–58]. It is estimated that IPAA reduces a woman’s ability to become pregnant by nearly 50%. Prior to considering the surgery, this issue needs to be thoroughly discussed with the young woman and her family. A detailed analysis of pregnancy rates in Sweden after IPAA revealed a significant reduction in postoperative fertility [56]. Their birth rate was compared to the expected pregnancy and birth rate for age-matched Swedish women. There was a no difference in the expected birth rate from the onset of their CUC to the time of surgery. However, after IPAA there was a significant reduction in birth rate ($P < 0.001$). Also, women with IPAA required a much higher rate of in vitro fertilization,

29% compared to 1%, respectively. In subsequent studies, women who had an ileorectal anastomosis instead of IPAA did not appear to experience a reduction in fertility [59, 60]. While the exact cause of reduced fertility is not known, most assume altered pelvic anatomy and pelvic adhesions play a major role. As an option for young women with CUC who desire to start a family or who are in a stable relationship and might start a family within a few years, total abdominal colectomy and ileostomy is a very reasonable option. This approach removes the majority of the disease, allows medication to be withdrawn, and does not disturb the pelvic anatomy. IPAA can be subsequently performed after child-bearing is complete.

After an IPAA, the mode of delivery, either vaginal or cesarean section, should be based upon obstetrical concerns rather than the presence of the pouch [61, 62]. Specific obstetrical issues favoring a cesarean section include: breech position, above gestational age size, prior need for episiotomy, and possible need for an instrument-assisted vaginal delivery. However, given the uncertainty of how a labor will progress, many IPAA patients and their obstetricians elect an elective cesarean section to avoid possible injury to the pouch and anal sphincter complex.

Biologic Therapies and IPAA

In a number of studies, steroid use prior to IPAA has been associated with increased risk of postoperative complications [51, 63, 64]. With the recent introduction of anti-TNF biologic therapies for CUC, a number of authors have evaluated its impact on surgical outcomes [64–67]. The Mayo Clinic was the first to report a possible association between biologic therapy and adverse outcomes in IPAA patients [65]. The outcomes of 47 CUC patients who received preoperative biologic therapy prior to IPAA were compared to 254 who were on therapies other than biologic agents including steroids. The patients who had biologic therapy were statistically more likely to have postoperative infectious complications and pelvic abscesses. Multivariate analysis, including disease severity and steroid use, demonstrated that anti-TNF medication remained independently associated with an increased risk of IPAA pouch related and overall infectious complications. A subsequent study from the Cleveland Clinic found a similar association between preoperative biologic therapy and postoperative complications [66]. However, in much smaller studies, the association has not been demonstrated [64, 67]. All the studies to date are retrospective making it hard to draw any conclusions regarding the exact role of biologic therapies on postoperative adverse events. Further prospective studies that evaluate the extent and severity of disease, duration of disease activity, and medical therapy need to be performed to clarify this important issue. Given the uncertainty about the role of biologic therapy and postoperative IPAA complications, a conservative approach to avoid pouch complications is to perform a three-stage procedure starting with a subtotal colectomy and end ileostomy. This allows the patient to recover their health and resolve the possible effect of the biologic agent and other immunosuppressive agents prior to IPAA.

Conclusion

Successful surgical outcomes for patients with either CD or CUC require a thorough understanding of both the pathophysiology the specific disease-related complications unique to them. Furthermore, close collaboration with a gastroenterologist experienced in caring for IBD patients will improve the coordination of care for these complex patients. Surgical therapy for CD is directed at treating symptoms and complications of the disease as surgical cure is impossible. The nature of CD transmural inflammation leads to a number of complex problems including intra-abdominal abscesses,

perforations, fistulas, and strictures. The operative approach to all of these problems is to minimize the resection of nondiseased bowel in order to preserve bowel length and intestinal function. Unlike CD, surgery for CUC cures the patient of the intestinal manifestations of the disease by removing the colon and rectum. During emergent CUC operations, the goal is to remove the abdominal colon leaving the rectum in situ. Leaving the rectum facilitates a future restorative procedure after the patient's health has improved. Ileal-pouch anal anastomosis (IPAA) is a restorative operation that preserves the normal route of defecation, albeit with a different frequency, and avoids a permanent stoma. This is a complex procedure which is associated with a number of short- and long-term complications with which the surgeon needs to be familiar and capable of addressing. For both CD and CUC, minimally invasive surgery is technically feasible in many circumstances and provides the patients with short-term recovery benefits.

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Chapter 46

Extraintestinal Manifestations of Inflammatory Bowel Disease: Autoimmune Hepatitis

Nora Schweitzer and Michael P. Manns

Keywords Hepatitis • Autoimmune hepatitis • Transplantation • Liver biopsy • Liver • Autoantibodies

Definition

The most common liver disease in inflammatory bowel disease (IBD) is primary sclerosing cholangitis (PSC). The main diagnostic tool to confirm PSC is a cholangiography. In case of a normal cholangiography, a small-duct PSC should be considered and can be ruled out by liver biopsy. One important differential diagnosis of liver diseases in IBD is autoimmune hepatitis (AIH) and in some series, 17% of the AIH-patients had IBD [1].

AIH is a chronic progressive inflammation of the liver of unknown origin. It is characterized by elevated aminotransferases, bilirubin and γ -globulins, the presence of autoantibodies and a typical histological picture with interface hepatitis and plasma cell infiltration. Untreated, the prognosis of AIH is poor and studies in the 1970s revealed a 5 year overall survival of 50%. Notably, these data are limited by the lacking ability to test for HCV at that time. At the time of diagnosis, cirrhosis is present in 30% of the patients [2]. Albeit pathogenesis is not entirely understood, genetic predisposition, loss of immune tolerance and environmental factors play important roles in the development of AIH. According to the antibody-pattern, two types of AIH are differentiated. In type 1 AIH anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) are predominant, whereas in type 2 AIH, antibodies to liver/kidney microsome 1 (anti-LKM-1) are characteristic. The latter is mainly seen in children and adolescents. In Norway and Sweden, the point prevalence is 11–17 per 100,000 persons per year and the incidence 1–2 per 100,000 persons per year [3]. Similar data are supposed to be found for the Caucasians in North America.

N. Schweitzer, MD • M.P. Manns, MD (✉)
Department of Gastroenterology, Hepatology, and Endocrinology, Medical School of Hannover,
Carl – Neuberg Street 1, 30625 Hannover, Germany
e-mail: manns.michael@mh-hannover.de

Clinical Presentation

The onset of AIH is usually insidious and up to 34–45% of patients are asymptomatic. Symptomatic patients often present with non-specific symptoms as fatigue, jaundice, abdominal pain, weight loss or arthralgias. In general, the complaints are not specific for AIH and range from asymptomatic individuals to individuals with severe and acute threatening disease with severe liver dysfunction. Other autoimmune disorders, e.g. autoimmune thyroiditis, rheumatoid arthritis, or IBD are common in the medical history of the patient or first-degree family members. AIH can occur at every age and in all ethnical groups. Women are more often affected than men (3.6:1). The main complication of AIH is the progression to liver cirrhosis with severe consequences, e.g. the development of ascites, hepatic encephalopathy and variceal bleeding. In the case of cirrhosis, patients are at risk for developing hepatocellular carcinoma. Studies in the past revealed that patients with IBD and AIH without signs of cholangitis have a better outcome compared to those with an abnormal cholangiography [1].

Diagnosis

Diagnostic Scores

As there exists no single AIH-specific diagnostic criterion, diagnosis is established by a combination of a typical autoantibody-pattern, certain histological findings and elevated immunoglobulins and aminotransferases. Confirmation of the diagnosis requires the exclusion of other hepatopathies such as viral hepatitis, cholestatic liver diseases, or metabolic disorders. In 1993, the International Autoimmune Hepatitis Group (IAIHG) formulated descriptive criteria and a scoring system for diagnosing definite or probable AIH. A review of the scoring system was undertaken in 1999 (Table 46.1) [4, 5]. In clinical practice, the descriptive criteria are sufficient to diagnose AIH in most cases. For more challenging cases, the revised original scoring system, which was originally made for clinical trials, can be applied. This score includes the patient's gender, the ratio between alkaline phosphatase (AP) and AST, the γ -globulin or IgG-level, antibodies, viral markers, drug or alcohol intake, HLA type, other immune diseases, histological features and the response to treatment. A pretreatment score of 10–15 points or a posttreatment score of 12–17 points are required for the diagnosis “probable AIH,” whereas a pretreatment score of 16 or higher and a posttreatment score of more than 17 leads to the diagnosis “definite AIH.” The sensitivity of the pretreatment score of 10 points (probable AIH) and pretreatment score of 15 (definite AIH) is 100 and 95%, the specificity 73 and 97% and the diagnostic accuracy 67 and 94%, respectively. In 2006, simplified diagnostic criteria containing only four parameters (autoantibodies, IgG-level, histology, absences of viral hepatitis) were published with 88% sensitivity and 97% specificity for probable AIH (≥ 6 points) and 81% sensitivity and 99% specificity for definite AIH (≥ 7 points) (Table 46.2) [6]. Until now, the simplified scoring system was tested only in retrospective studies [7–9].

Biochemical Findings

One characteristic biochemical abnormality of AIH is hypergammaglobulinemia in the absence of cirrhosis with selective elevation of serum IgG. Elevation of liver enzymes shows a hepatic pattern

Table 46.1 Revised original scoring system of the International Autoimmune Hepatitis Group

Gender	Female	+2	HLA	DR3 or DR4	+1
AP:AST (or ALT) ratio	>3	−2	Immune disease	Thyroiditis, colitis, others	+2
	<1.5	+2			
γ-globulin or IgG level	>2.0	+3	Other markers	Anti-SLA, actin, LC1,	+2
above normal	1.5–2.0	+2		pANCA	
	1.0–1.5	+1			
	<1.0	0			
ANA, SMA, or anti-LKM1 titers	>1:80	+3	Histological features	Interface hepatitis	+3
	1:80	+2		Plasmacytic	+1
	1:40	+1		Rosettes	+1
	<1:40	0		None of above	−5
				Biliary changes	−3
				Other features	−3
AMA	Positive	−4	Treatment response	Complete	+2
				Relapse	+3
Viral markers	Positive	−3			
	Negative	+3			
Drugs	Yes	−4	Pretreatment aggregate score:		
	No	+1	Definite diagnosis >15		
			Probable diagnosis 10–15		

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Table 46.2 Simplified diagnostic criteria for autoimmune hepatitis, according to Hennes et al. Hepatology 2008^a

Variable	Cutoff	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	
or LKM	≥1:40	2 ^a
or SLA	Positive	
IgG	>Upper normal limit	1
	>1.10 times upper normal limit	2
Liver histology (evidence of hepatitis is a necessary condition)	Compatibel with AIH	1
	Typical AIH	2
Absence of viral hepatitis	Yes	2
		≥6: probable AIH
		≥7 definite AIH

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^aAddition of points achieved for all autoantibodies (maximum, 2 points)

in most cases. However, aminotransferase activity (ALT, AST) and bilirubinemia may range from just above the upper limit of normal (ULN) to 50-fold ULN, but this level does not correlate to histological inflammatory activity [10]. AP and γ-glutamyl transferase (γGT) usually are only moderately elevated or normal.

Autoantibodies

The serological assessment of the diagnosis includes testing ANA, SMA, anti-LKM-1 and anti-liver cytosol type 1-antibodies (anti-LC1). The vast majority of adult patients show significant titers ($>1:40$) of ANA, SMA or both, 3–4% are positive for anti-LKM1 and up to 20% present with no antibodies. None of these antibodies is specific for AIH and their presence is not sufficient for affirming the diagnosis nor does their absence preclude AIH. The expression of antibodies can vary during the course of disease, but in contrast to children, the titer level does not correlate to disease activity in adults. In case of seronegativity for ANA, SMA and anti-LKM-1, testing for atypical perinuclear antineutrophil cytoplasmatic antibodies (pANCA) and antibodies to soluble liver antigen (SLA/LP) should be performed. Atypical pANCA were originally considered to be specific for IBD and PSC [11, 12] but are also found in AIH, sometimes as the only positive antibody. Anti-SLA have a limited sensitivity but a high specificity for AIH. Moreover, the presence of anti-SLA is associated with a more severe course of disease and a worse outcome [13, 14]. Serological evaluation should include AMA to preclude PBC. For further investigation of seronegative patients, following antibodies may be of interest: LKM-2 and -3 antibodies, and LM antibodies (Table 46.3). For a valid diagnosis, the exclusion of other liver diseases is essential. In particular, hereditary disorders as Wilson disease and alpha 1 antitrypsin deficiency, viral hepatitis, steatohepatitis and drug induced hepatitis should be ruled out. The differentiation between AIH and the autoimmune cholestatic liver diseases PBC or PSC may be difficult, but the effort should be undertaken given the implications for treatment regimen and prognosis.

Histology

A liver biopsy is recommended for establishing the diagnosis and for evaluation of the response to treatment. In patients with coexisting IBD, it is also useful to rule out small-duct PSC. Although the histological appearance of AIH is characteristic, there are no pathognomonic features. Typical findings are mononuclear cell infiltrates with infiltration of the limiting plate, also called piece meal necrosis or interface hepatitis, which can progress to lobular hepatitis or central–portal bridging necrosis. Plasma cell infiltrates are regularly seen. Biliary lesions as ductopenia or destructive cholangitis or granulomas are indicative for a different diagnosis. Fibrosis is seen in all but the mildest forms and the degree ranges from mild fibrosis to bridging fibrosis or cirrhosis (Fig. 46.1).

Diagnostic Difficulties: Overlap Syndrome

Diagnosing AIH is especially delicate in autoantibody-negative patients. If AIH is suspected in an autoantibody-negative patient, a liver biopsy may become of immense importance. Otherwise, the patients are diagnosed and treated late.

Also the setting of autoantibodies or other results matching to more than one autoimmune liver disease may cause confusion. Overlapping features of AIH and PSC or AIH and PBC are not uncommon. Practically that means for example that a patient with AIH can be AMA-positive, what is highly specific for PBC. Or a patient with AIH presents with an abnormal cholangiography, which is indicative for PSC. Some authors also count AMA-negative patients with otherwise typical PBC to AIH–PBC overlap or “Autoimmune Cholangitis.” The overlapping diseases can appear simultaneously or sequentially in one individual patient. However, criteria for diagnosing an overlap

Table 46.3 Antibodies in autoimmune hepatitis

Antibody	Target antigen (s)	Liver disease	Value in AIH
ANA*	Multiple targets including: • Chromatin • Ribonucleoproteins • Ribonucleoprotein complexes	AIH PBC PSC Drug-induced Chronic hepatitis C Chronic hepatitis B Non-alcoholic fatty liver disease	Diagnosis of type 1 AIH
SMA*	Microfilaments (filamentous actin) and intermediate filaments (vimentin, desmin)	Same as ANA	Diagnosis of type 1 AIH
LKM-1*	Cytochrome P450 2D6 (CYP2D6)	Type 2 AIH Chronic hepatitis C	Diagnosis of type 2 AIH
LC-1*	Formiminotransferase cyclodeaminase (FTCD)	Type 2 AIH Chronic hepatitis C	Diagnosis of type 2 AIH Prognostic implications Severe disease
pANCA (atypical)	Nuclear lamina proteins	AIH PSC	Diagnosis of type 1 AIH Re-classification of cryptogenic chronic hepatitis as type 1 AIH
SLA	Soluble liver antigen	AIH Chronic hepatitis C	Diagnosis of AIH Prognostic implications Severe disease Relapse Treatment dependence
LKM3	UDP-glucuronosyl-transferases type 1 (UGT1A)	Chronic hepatitis D Type 2 AIH	Diagnosis of type 2 AIH
ASGPR	Asialoglycoprotein receptor	AIH PBC Drug-induced hepatitis Chronic hepatitis B, C, D	Prognostic implications Severe disease Histological activity Relapse
LKM2	Cytochrome P450 2C9	Ticrynafen-induced hepatitis	None, does not occur after withdrawal of ticrynafen
LM	Cytochrome P450 1A2	Dihydralazine-induced hepatitis APECED hepatitis	Diagnosis of APECED hepatitis

*This antibody belongs to the conventional serological repertoire for the diagnosis of AIH

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are lacking and there are ongoing discussions about the terminology and diagnostic criteria. This is why the estimated prevalence of AIH–PSC overlap ranges from 7.6 to 53.8% in different studies [15]. Overlaps of AIH and PBC can be found in 5–10% of the patients with AIH. In patients with IBD and AIH, an overlap with PSC should always be considered. Particularly children present overlapping signs of AIH and PSC very often (30–50%) [16]. The AIH–PSC overlap in children is also called “Autoimmune Sclerosing Cholangitis.” In clinical practice, patients often require therapy both with anti-inflammatory agents and ursodeoxycholic acid. The prognosis of the AIH–PSC overlap is worse than of AIH alone, mainly because of the risk of developing a malignancy, which is highly elevated in PSC [17]. The outcome of the AIH–PBC overlap is better than in AIH alone [18].

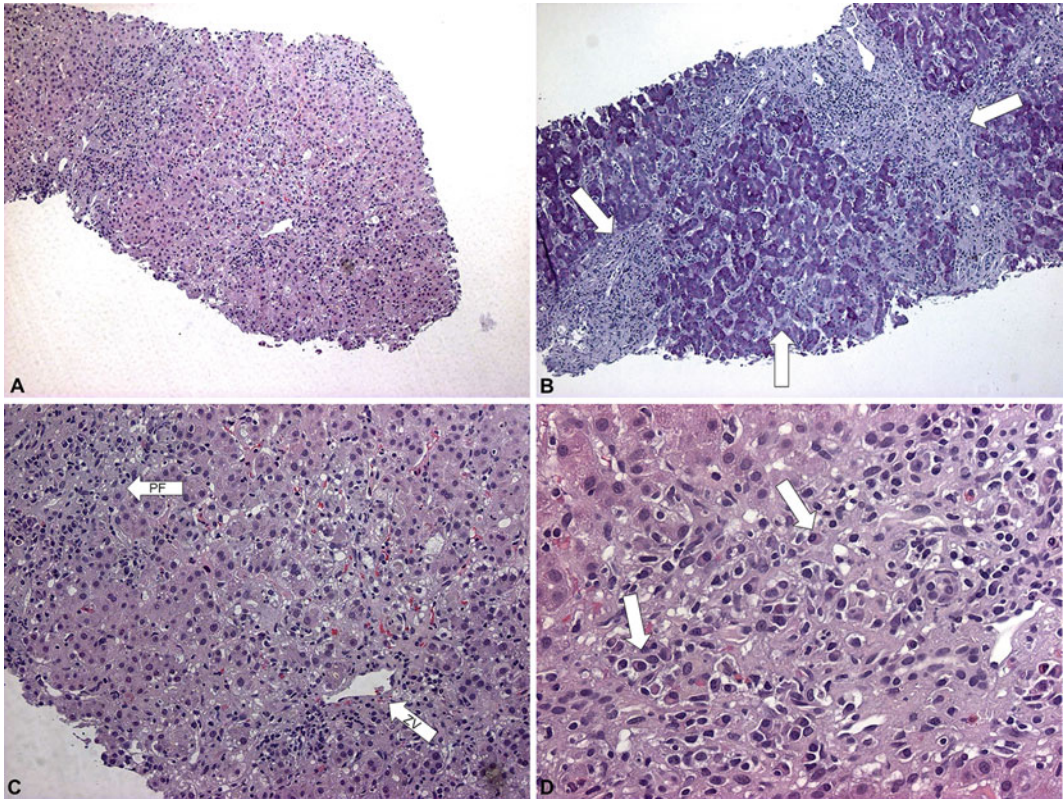


Fig. 46.1 (a–d) Histological findings in Autoimmune hepatitis (a) Broadened periportal fields and lobules with lymphocytic infiltration (HE $\times 100$) (b) Bridging fibrosis (arrows) between remaining parts of lobuli (lower arrow) (PAS $\times 100$) (c) Lymphoplasmacytic infiltration of periportal field (PF) and lobuli (ZV=central vein) with piecemeal necrosis and single cell necrosis (HE $\times 200$) (d) Wide plasmacellular infiltration in a periportal field beside lymphocytes and eosinophilic granulocytes (HE $\times 400$). (All figures kindly provided by Prof. H.-P. Kreipe, Medical School Hannover)

Similar to AIH patients, sera of HCV-infected patients are frequently positive for ANA, SMA and LKM. LKM-1 antibodies are found in 5–10% of patients with chronic HCV infection. The clinical relevance of these autoantibodies for HCV patients remains elusive. LKM are regarded as autoimmune phenomena associated with HCV infection, only high titer antibodies are considered to be a sign for a relevant autoimmune reaction. In individual patients, hepatic flares can occur under interferon based treatment for HCV.

Pathogenesis

Cellular Autoimmunity

The pathogenesis of AIH is not entirely understood. One concept is that in a genetically predisposed individual, environmental factors (e.g. viruses, drugs) can provoke AIH by initiating immune processes. The histological hallmark of AIH is a portal mononuclear cell infiltrate with T and B lymphocytes, macrophages and plasma cells. This massive inflammatory infiltration enables acute

and ongoing liver damage. Among the T cells, the majority are CD4 positive. There is evidence for an alteration in T and B cell function in AIH. In particular, regulatory T cells (CD4+CD25+ Treg cells) are reduced both in number and function [5, 19] in patients with AIH. Tregs are important modulators of CD8+ cells. They control the innate and adaptive immune reaction by inhibition of autoreactive T cells. They go in direct contact with the target cells, reduce interferon production and increase the secretion of IL-4, IL-10 and TGF β . Studies of Treg function in family members of AIH patients suggest a genetic relation [20].

Autoantigens

Beside self-reactive B and T cells, autoantigens that are presented by MHC class II molecules are required for an autoimmune process in the liver. For a number of autoantibodies found in AIH, the target antigen is known. In AIH type 2, the antigen of anti-LKM-1 antibodies is the enzyme cytochrome P450 2D6 (CYP2D6). Mouse models that express the human antigenic region of CYP2D6 produce antibodies, and develop hepatitis [21]. Anti-LKM-3 antibodies react with UGT [22]. The substrate of anti-SLA is the transfer ribonucleoprotein complex tRNP^{(Ser)Sec}, renamed SEPSECS (Sep [O-phosphoserine] tRNA:Sec[selenocysteine] tRNA synthase) [23–25] and anti-LC1 recognizes formiminotransferase cyclodeaminase [26, 27]. The target antigen of anti-LM antibodies is CYP1A2 and was first described in patients with a dihydralazine induced AIH. Furthermore, they are found in patients with APECED (see below). Thus, several specific autoantigens in AIH are known but their role in pathogenesis remains unclear.

Molecular Mimicry

There is growing evidence suggesting that molecular mimicry plays a key role in the generation of liver specific autoantibodies. Molecular mimicry relies on the similarity of infectious agents with host antigens. Such similarity may lead to an inability of the host immune system to recognize the foreign antigen or it may lead to an autoreactive immune response by cross-reactivity. One well described example for postinfectious autoimmunity is the acute rheumatic fever, which occurs after contact to antigenic epitopes of streptococcus pyogenes. Sequence homologies between CYP2D6 and HCV, the common viruses herpes simplex virus type 1 (HSV1), cytomegalovirus (CMV), Epstein-Barr-Virus (EBV) and human adenovirus [28, 29] have been discovered. Also cross-reactivity between HCV, SMA and ANA were described [30]. According to the “multiple hit-theory,” in genetically predisposed patients multiple contacts to viruses might induce a cross-reactive subset of T-cells and permit a loss of immunological self-tolerance.

Genetic Influences

AIH is a complex polygenetic disorder and does not follow a Mendelian pattern. Multiple genetic associations with the major histocompatibility complex (MHC) locus have been described. The MCH region is located on the short arm of chromosome 6 and encodes the human leukocyte antigens (HLA). In Caucasian Europeans and North Americans, HLA DRB1*0301 and DRB1*0401 are associated with a susceptibility to AIH [30, 31]. The significance of this relation lies in the observation that most autoimmune diseases are T cell dependent and that T cell response is MCH

restricted [32, 33]. HLA alleles not only cause susceptibility to AIH but also seem to have influence on the course of the disease: patients with DRB1*0301 are younger at diagnosis and have a higher frequency of treatment failure. HLA B8 is associated with a more severe disease and HLA DRB1*0401 develop other autoimmune diseases more often [31, 34]. Beyond that, genes outside the MCH might also contribute to autoimmunity, e.g. the cytotoxic T lymphocyte antigen 4 (CTL4) and a number of SNPs of various genes including cytokines, vitamin D receptor, CD45 and Fas receptor.

One well defined exception is AIH in the setting of the rare autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). APECED is caused by a single-gene mutation on chromosome 21q22.3 which results in a defect autoimmune regulator (AIRE) protein. Patients with this autosomal recessive inherited disease suffer from multiple endocrine organ failure, mucocutaneous candidiasis and ectodermal dystrophy.

Treatment

Treatment Regimens

As mentioned above, the outcome of untreated AIH can be fatal. Immunosuppression is the treatment of choice for AIH [35–38]. It is eminently important to diagnose AIH in early stages of the disease to prevent the progression to severe fibrosis or cirrhosis. In fact, cirrhosis at presentation is a predictor for a poor outcome [2]. Around one-third of adult patients already presents with histological features of cirrhosis at diagnosis [2]. However, consequent treatment can lead to a certain regression of fibrosis [39] and only in a minority, a progress of fibrosis under treatment occurs. Those are mainly patients with treatment failure of corticosteroids.

Two strategies are equally effective in the initial treatment of AIH:

1. Prednisone alone in a dose of 60 mg per day, or
2. Prednisone in a lower dose (30 mg daily) in combination with azathioprine in a daily dose of 50 mg (or 1–2 mg/kg body weight) [38].

For both regimens, improvements in biochemical and histological signs of inflammation and an amelioration of symptoms were shown [35–37]. It is evident that immunosuppressive treatment of chronic active hepatitis improves the outcome and the 20-year life expectancy can be enhanced to 80% [39]. After remission, prednisone can be tapered down to an individual dosage sufficient to maintain remission. It is important not to reduce doses before the goal of treatment is reached. An early titration is associated with a delayed histological improvement and with a prolonged duration of therapy [38]. Until the dose of 20 mg, weekly reduction of 10 mg is adequate, below the dose of 20 mg prednisone, reduction should not exceed 5 mg per week and at a daily dose of 10 mg, 2.5 mg should be reduced weekly until a daily dose of 5 mg. The advantage of the combined therapy regimen is the lower occurrence of steroid related side effects. Therefore, the combination of prednisone and azathioprine is the preferred treatment [38]. In Europe, prednisolone is preferred over prednisone. A single therapy with prednisone is appropriate for patients who are at high risk for azathioprine related adverse events. Those are patients with cytopenia, pregnant women or those planning a pregnancy, patients with malignancies and individuals with complete thiopurine methyltransferase deficiency. Furthermore, a sole therapy with prednisone is suggested when a short time of treatment is probable. Patients with increased risk for steroid related side effects should obtain the combined treatment. Those are postmenopausal females, patients with osteoporosis, diabetes or brittle diabetes, obesity or hypertension and last but not least emotional unstable individuals.

An alternative and interesting approach to reduce steroid side effect has been investigated recently in a multicenter study. Budesonide, a steroid with a low systemic effect due to a 90% first pass effect,

was in adjunction to azathioprine capable to induce complete remission in non-cirrhotic patients while the rate of steroid related side effects was much lower than in the group treated with prednisone and azathioprine [40]. Thus, Budesonide is a promising option for the treatment of AIH.

Independently from the used agent(s), treatment should be continued until remission, treatment failure, incomplete response or drug toxicity [38]. There is no scheduled minimum or maximum duration of immunosuppressive treatment in AIH. It should rather be adapted to the individual course of disease.

Over the last decades it has become evident that an individualized therapy is necessary for optimal treatment.

1. First, it is unclear how to manage asymptomatic patients best. Asymptomatic patients are most often identified by incidental abnormal liver tests. They tend to be older than symptomatic patients, have a higher frequency of “probable AIH” vs. “definite AIH” but show cirrhosis as often as symptomatic patients. Spontaneous remission is seldom in this group. It is not proven that the outcome of asymptomatic patients ameliorates when treated with corticosteroids and/or azathioprine. For instance, Fedl et al. reported similar prognosis in treated and in untreated asymptomatic patients [2].
2. Also in elderlies, higher stages of fibrosis before treatment than in younger individuals were reported, but this does not result in a higher frequency of definite cirrhosis. Elderly patients have a high percentage of remission [41] but, however, in one study, untreated elder patients had the same prognosis as younger and treated individuals. Thus, it is a matter of debate how to treat elderly patients properly.
3. Children generally do respond well to treatment but have cirrhosis in almost 50% at presentation. They need long-term or even lifelong immunosuppressive treatment in most cases and despite that, they require liver transplantation in 15% before the age of 18 [42].
4. In patients with end-stage disease without inflammatory activity, meaning inactive or “burned out” cirrhosis, a treatment is not indicated and even can be harmful because of higher drug toxicity.
5. Finally, the clinical picture, treatment response and outcome vary between ethnic groups. Black North Americans are younger and present more often with cirrhosis than white North Americans. Also, patients from South America, Africa and Asia have an earlier onset of the disease and are icteric in many cases.

Adjunctive Therapies

In awareness of the patient’s individual risk, adjunctive therapies should be applied to reduce treatment related toxicity. Besides regular weight bearing exercise program, a supplementation of vitamin D and calcium should be standard under therapy with steroids. For individual patients at high risk, the administration of bisphosphonates may be appropriate. In general, people on long-term, corticosteroid treatment should be monitored for bone disease [38].

Vaccination against HAV and HBV should be done in every patient with AIH, and if possible, before the initiation of immunosuppression.

Treatment Related Side Effects

During corticosteroid therapy, in up to 80%, distracting cosmetic changes occur: weight gain, facial rounding, striae distensae, acne, alopecia and facial hirsutism. More severe adverse effects include opportunistic infections, osteopenia and pathological fractures, brittle diabetes, labile hypertension

and psychosis. Thirteen percent of premature drug withdrawal is attributed to steroid related side effects and nearly half results from intolerable cosmetic changes and obesity [43]. Azathioprine-related toxicity includes nausea and emesis, rash, cholestatic hepatitis, pancreatitis, cytopenia and consecutive opportunistic infections, and malignancies (1.4-fold greater risk than normal) [43, 44]. In total, 10% of all azathioprine-treated patients suffer from side effects, and in 5% early adverse events lead to cessation of treatment. In many cases, a reduction of the dose is sufficient to manage the side effects. A rare complication is a diarrheal syndrome with malabsorption and small intestine villus atrophy that improves after discontinuation of azathioprine therapy [45].

At higher risk for adverse events in the treatment of AIH are

1. Patients with cirrhosis. They are more often affected than patients without cirrhosis. Moreover, pretreatment cytopenia due to hepatomegaly is common and thus, patients with cirrhosis are more susceptible for severe cytopenia due to azathioprine.
2. Pregnant women. Congenital malformations after treatment with azathioprine have been described in mice but not in humans. The major risk is prematurity and associated with this a higher mortality of the newborn. Fetal loss in women with AIH is higher than in healthy mothers but no greater than in women with other chronic illness. Nevertheless, only anecdotal experience is made with azathioprine during pregnancy, so it is advised to reduce or terminate immunosuppression where possible and to attempt preconception counseling [38].
3. Patients with very low thiopurine methyltransferase activity (0.3–0.5% of the population) have a higher risk for azathioprine related myelosuppression. Patients with a moderate reduction in thiopurine methyltransferase activity commonly tolerate daily doses of 50 mg fairly well and the enzyme activity may increase during treatment [46]. Laboratory testing of the thiopurine methyltransferase activity is only recommended in case of pretreatment cytopenia, new developed cytopenia under treatment or high dose treatment (>150 mg/d) [43].

Treatment Endpoints

Following treatment endpoints are distinguished: remission, treatment failure, incomplete response, drug toxicity.

Remission

The goal of immunosuppressive treatment in AIH is the normalization of liver transaminases, bilirubin and immunoglobulins, as well as the resolution of histological signs of inflammation. The improvement of the objective parameters is accompanied by an amelioration of the symptoms. In adults, a decline in biochemical abnormalities is commonly reached in the first 2 weeks. Normalization of the named values can be expected in the first 12–24 months. Beyond that period, a complete remission is not likely anymore. Preferably, a discontinuation of treatment should be considered after at least 24 months and at complete remission. Eighty-seven percent of patients with long-term remission have normal liver tests and immunoglobulins. On the other hand, 60% relapse despite of resolution of laboratory indices [47]. Indeed, histologically, interface hepatitis is described in more than half of the patients with normal laboratory results. These patients should not terminate treatment because of a high risk for relapse. In general, a liver biopsy is recommended before immunosuppression is stopped.

Treatment Failure

A treatment failure is assumed if under an adequate treatment with corticosteroids with or without azathioprine (and under the precondition that the patient is compliant) the symptoms and the laboratory and histological features worsen. Unfortunately, this happens in around 9% and can be noticed after 3–6 weeks of treatment. In that case, a high dose therapy with prednisone (60 mg) or prednisone (30 mg) in combination with azathioprine 150 mg should be established and maintained for at least 1 month [38]. This strategy results in an improvement of disease activity in 70% but in a histological remission of only 20% [48]. Patients with treatment failure must be followed closely to recognize the development of cirrhosis respectively the complications of cirrhosis. Once a patient presents with hepatic encephalopathy, ascites or variceal bleeding, a liver transplantation must be considered.

Incomplete Response

Incomplete response means that the patient experiences an improvement but no resolution of the elevated biochemical indices and the histology features under treatment. In these individuals the treatment dose of prednisone may be reduced until the lowest possible level where the aminotransferases remain stable. Azathioprine can also be used to establish stable inflammation parameters.

Drug Toxicity

If intolerable adverse effects occur, the treatment must be discontinued or better reduced in dose until side effects diminish or are tolerable.

Relapse

Relapse means a recurrence of inflammatory activity after complete remission and consecutive cessation of treatment. About 80% of all patients in remission will experience a relapse, so it is a very prevalent event. The management of the relapse should be in first line to re-establish the treatment with prednisone with or without azathioprine. The goal is to gradually reduce and finally to eliminate prednisone and to increase the dose of azathioprine to 2 mg/kg daily as an indefinite treatment. With this regimen 83% stay in remission [49].

Salvage Therapies

If treatment failure occurs under high dose prednisone therapy or 30 mg prednisone in conjunction with 150 mg azathioprine, alternative treatment regimens have to be considered. Most alternative drugs have been used anecdotally and thus, experience is small. The most promising agent is mycophenolate mofetil [50]. The following agents have also been used for salvage therapy: Cyclosporine, tacrolimus, ursodeoxycholic acid, budesonide, 6 mercaptopirine, methotrexate and cyclophosphamide.

Budesonide and ursodeoxycholic acid were tested in randomized controlled clinic trials and failed to give an option as a salvage therapy [51, 52] which is no surprise.

Last but not least, liver transplantation is a very effective salvage therapy. Liver transplantation comes into question for patients with (1) acute liver failure, (2) decompensated liver cirrhosis and a MELD-score ≥ 15 and (3) HCC (within the Milano-criteria). The 5-year survival-rates after liver transplantation are approximately 78% [53]. Importantly, a recurrence of AIH in the transplanted organ happens in approximately one-third of the patients [53, 54].

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Chapter 47

Extraintestinal Manifestations of Inflammatory Bowel Disease: Primary Sclerosing Cholangitis

John Halliday and Roger W. Chapman

Keywords Primary sclerosing cholangitis • Secondary sclerosing cholangitis • Small duct sclerosing cholangitis • Cholangiocarcinoma • Liver transplantation

Introduction

Primary sclerosing cholangitis (PSC) is a chronic and progressive disease of the biliary tree characterised by concentric, obliterative fibrosis leading to bile duct stricturing and eventually cirrhosis in the majority of cases. The disease course is highly variable between individuals but most patients reach the combined end-point of death or liver transplantation 12–17 years following their diagnosis [1]. One-third of the patients PSC will develop cholangiocarcinoma. Although the underlying aetio-pathogenesis of PSC is not yet fully elucidated it is generally accepted to be a condition of immune dysregulation.

Approximately three quarters of the Northern European PSC population have concomitant inflammatory bowel disease (IBD), with the predominant form of IBD being ulcerative colitis (UC). In 1874, only 7 years after PSC was defined, CH Thomas first recognised this association when he described a man who died of a “much enlarged, fatty liver in the presence of ulceration of the colon” [2].

Caring for the patients PSC presents a number of challenges – Unfortunately medical therapy remains controversial and the only intervention with proven survival benefit is liver transplantation. Not only is the diagnostic work-up and management of PSC complex, but also treatment of associated conditions such as IBD and cholangiocarcinoma requires expertise that bridges the ever growing divide between hepatology and luminal gastroenterology.

J. Halliday, MBBS (Hons.), FRACP • R.W. Chapman, MD, FRCP (✉)
Department of Hepatology/Translational Gastroenterology, John Radcliffe Hospital,
Headley Way, University of Oxford, Oxford, Oxfordshire OX3 9DU, UK
e-mail: roger.chapman@ndm.ox.ac.uk

Epidemiology and Clinical Features

Epidemiology

The insidious and slow progression of PSC has made it difficult to obtain accurate data for the true incidence and prevalence of this condition.

A recent study of a population of approximately 1.5 million adults from the Vastra Gotaland region in southern Sweden found a prevalence of 16.2/100,000 and incidence of 1.22/100,000 [3]. This is significantly higher than earlier studies in Northern European descendants that demonstrated the point prevalence and annual incidence of PSC to be approximately 9/100,000 and 0.9–1.3/100,000 years, respectively. Similar figures to these lower rates have been demonstrated in a number of population-based studies across Canada, Norway and Great Britain [4–7]. In the United States, the prevalence has been extrapolated to be 2–7 cases per 100,000 using an assumption that 2.5–7.5% of the 40–225/100,000 UC patients have PSC. However this result is an underestimate of the true prevalence, as 20–30% of patients with PSC have no associated IBD. Indeed, a study from a population in Olmstead County, MN in the United States estimated a prevalence of 20.9 per 100,000 men and 6.3 per 100,000 women [8].

In addition to the recent Swedish study, data from Spain has also suggested that the incidence of PSC may be increasing [9]. Additionally, a change in disease presentation has been observed over time based on a recent study by Berquist et al. [10]. This study compared the presentation of patients diagnosed with PSC prior to 1998 ($n=185$) with those diagnosed after 1998 ($n=61$). Patients diagnosed after 1998 were significantly older (41 versus 37 years), had fewer symptoms at presentation (47% versus 63%), and had a lower frequency of concurrent IBD (69% versus 82%). It is likely these apparent historical changes reflect a higher capture of incident and prevalent cases through improved clinician awareness and greater use of diagnostic imaging modalities such as MRCP. Higher rates of liver transplantation among prevalent cases (ascertainment bias) may have also contributed to this change.

For as yet unexplained reasons, the reported prevalence of PSC in South East Asia and Southern Europe is 10–100-fold less than that of Europe and America [11].

Risk Factors

The median age of onset of PSC is 30–40 years but the disease can present at any age. Interestingly, unlike most immune mediated diseases, two-thirds of PSC patients are male. Siblings of PSC patients have a 1.5% risk of developing PSC and there is a 0.7% risk in first-degree relatives [12].

Cigarette smoking has been recognised as a protective factor against the development of UC. Additionally, smoking may also protect against the development of PSC [13–15]. This protective effect is even more marked in patients with PSC than UC and has also been observed in PSC patients who do not have concomitant IBD. The mechanism by which smoking protects against both disorders is unknown and trials of nicotine therapy have shown no benefit in altering disease progression.

Etiopathogenesis

In 1991, a PSC review article in GUT hypothesised that PSC is an “Immunologically mediated disease, probably triggered by acquired toxic or infectious agents that may gain access through the colon” [1]. Although current understanding continues to support this, the etiology and pathogenesis

Table 47.1 Key HLA haplotypes associated with primary sclerosing cholangitis (PSC)

Haplotype	Significance in PSC
B8-TNF*2-DRB3*0101-DRB1*0301-DQA1*0501-DQB1*0201	Strong association with disease susceptibility
DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603	Strong association with disease susceptibility
DRB5*0101-DRB1*1501-DQA1*0102-DQB1*0602	Weak association with disease susceptibility
DRB4*0103-DRB1*0401-DQA1*03-DQB1*0302	Strong association with protection against disease
MICA*008	Strong association with disease susceptibility

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of PSC are not yet fully elucidated. Indeed it is likely that a combination of mechanisms result in the development of PSC. Autoimmunity is very likely to play a major role as supported by; the strong human leukocyte antigen (HLA) haplotype association in PSC, high frequency of other autoimmune diseases such as rheumatoid arthritis, high prevalence of autoantibodies in patient sera, and the link with IBD [16].

Genetics Factors Predisposing to PSC

The importance of genetic risk in the development of PSC is highlighted by the fact that siblings of patients PSC are 9–39 times more likely to develop PSC than the general population [17]. Additionally, irrespective of whether they develop PSC, siblings have three times the incidence of IBD compared with the general population, suggesting a shared genetic susceptibility for PSC and IBD.

The major histocompatibility complex on the short arm of chromosome 6 encodes the human leukocyte antigen (HLA) molecules. These molecules are highly polymorphic and play a central role in T cell response. As early as 1982 the HLA complex was demonstrated to be a risk locus for PSC [18]. This study demonstrated an association between PSC and HLA-B8 and DR3. HLA-DR2 and DR6 are also associated with PSC, whereas HLA DR4 may be protective (Table 47.1). Considering their role in antigen presentation, these HLA associations support the hypothesis that specific (auto-) antigens may be pathogenetically important in PSC.

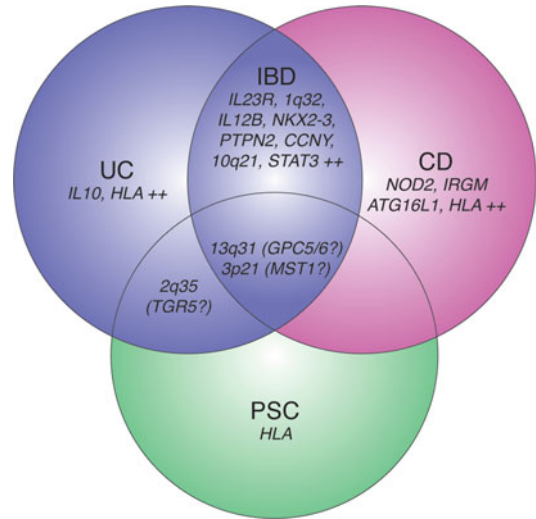
HLA-B and -C play an additional role as ligands for killer immunoglobulin-like receptors on natural killer cells and some T cell subtypes. More recent data suggests that gene variants may impair these functions and subsequently protect against PSC [19]. This is supported by the fact that these protective variants are less commonly found in Northern European populations where PSC is most prevalent.

Not surprisingly, a recent Genome Wide Association Study (GWAS) of 285 Norwegian PSC patients found strong associations in the HLA complex [20]. There are also other genes outside the HLA region that may play a role in the pathogenesis of PSC. Fifteen previously established susceptibility loci for UC were evaluated in this GWAS study but only two of these (chromosome 3p21, chromosome 2q35) showed any significant association in PSC (Fig. 47.1). This limited overlap in genetic susceptibility supports data from clinical trials that suggests IBD in association with PSC may represent its own disease phenotype. Further GWASs are currently in progress to help identify possible susceptibility genes for PSC.

Autoantibodies

Although autoantigens such as smooth muscle antibodies, antinuclear antibodies and antineutrophil cytoplasmic antibodies (p-ANCA) are often detected in patient serum, none of these are specific to

Fig. 47.1 The genetic architecture of Primary Sclerosing Cholangitis. [Reprinted from Dig Liver Dis. 42(6). Karlsen, TH. Schrumpf E. Boberg KM. *Update on primary sclerosing cholangitis*. p. 390–400. ©2010. With permission from Elsevier]



PSC. The prevalence of p-ANCA approaches 88% in some studies but it is also found in patients with UC alone (60–87%), in patients with type I autoimmune hepatitis (50–96%) and primary biliary cirrhosis (PBC) [16, 21, 22]. Given this lack of specificity it is unlikely pANCA is involved directly in the pathogenesis of PSC and it is not a useful screening test.

Pathogenesis

One hypothesis to explain the association between IBD and liver disease is that PSC is mediated by long-lived memory T cells derived from the inflamed gut that enter the enterohepatic circulation [23]. Aberrant expression of chemokines and adhesion molecules on liver endothelial cells may cause recruitment of these T cells in turn leading to biliary inflammation, fibrosis and bile duct stricturing.

In support of this, patients with PSC have been demonstrated to aberrantly express adhesion molecules including vascular adhesion protein-1 (VAP-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM1) on biliary epithelium [24]. Additionally, the chemokine CCL25, ordinarily confined to the gut, is up-regulated in the liver in PSC, helping to recruit CCR9+ T cells. The mechanisms that lead to aberrant expression of adhesion molecules remain unknown but it may be that in genetically susceptible individuals, bacterial antigens, arising from a “leaky gut” from the inflamed colon, act as molecular mimics and cause an immune reaction responsible for initiating PSC.

Fickert et al. recently proposed that a process similar to arteriosclerosis may also play a role in the pathogenesis of PSC [25]. Work with multidrug resistance knockout mice (Mdr2^{−/−}) that are unable to produce phospholipids, suggests a subsequent inability to form mixed micelles (bile acids/phospholipids/cholesterol) results in accumulation of hepatotoxic bile acids and cholesterol-supersaturated bile. Support for this theory in humans is, however, lacking. Genetic studies of the human ortholog of Mdr2 (MDR3) have not demonstrated any association between MDR3 genetic variants and susceptibility to PSC.

Clinical Features

The clinical presentation of PSC is variable and typical symptoms are non-specific including right upper quadrant abdominal pain, fatigue and pruritis. Up to 55% of patients are asymptomatic at the time of presentation [7]. It is rare for patients with PSC to present with cholangitis unless they have previously had biliary intervention or have a dominant stricture [26, 27]. Similarly, presentation with jaundice is uncommon and may herald the development of cholangiocarcinoma. Osteopenic bone disease is both a complication of advanced PSC and IBD. Steatorrhea and malabsorption of fat soluble vitamins only occurs after prolonged cholestasis with jaundice.

Few patients present with features of decompensated cirrhosis and portal hypertension such as ascites and variceal hemorrhage. Nevertheless, hepatomegaly and splenomegaly are the most frequent abnormal physical findings at clinical examination at the time of diagnosis in PSC.

Diagnosis of PSC

Laboratory Investigations

Discovery of elevated cholestatic liver biochemistries (alkaline phosphatase and gamma GT) in an asymptomatic patient IBD should always prompt consideration of the diagnosis of PSC. Blood tests typically fluctuate over time and at times may even return completely to normal. Autoantibody tests are of little diagnostic significance. IgM concentrations are increased in about 50% of patients with advanced PSC.

Serum IgG4 levels should be measured in all patients with suspected PSC. They are elevated in about 9% of patients and are associated with a worse outcome [28] (See Histology).

Radiological Features

The first imaging modality recommended in the work-up for a patient with cholestatic LFTS is a transabdominal ultrasound but in the vast majority of patients PSC, this will be non-diagnostic (Fig. 47.2). Usually, a diagnosis of PSC is made when cholangiography (MRCP or endoscopic retrograde pancreatography (ERCP)) demonstrates characteristic bile duct changes of multifocal

Fig. 47.2 Diagnostic work-up for cholestatic liver biochemistry. [Reprinted from Chapman, R., et al., *Diagnosis and management of primary sclerosing cholangitis*. Hepatology, 2010. 51(2): p. 660–78. With permission from John Wiley & Sons, Inc.]

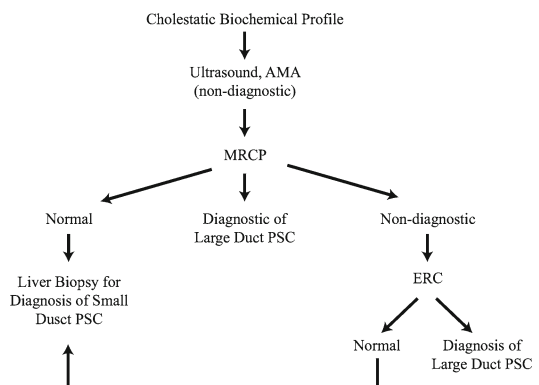
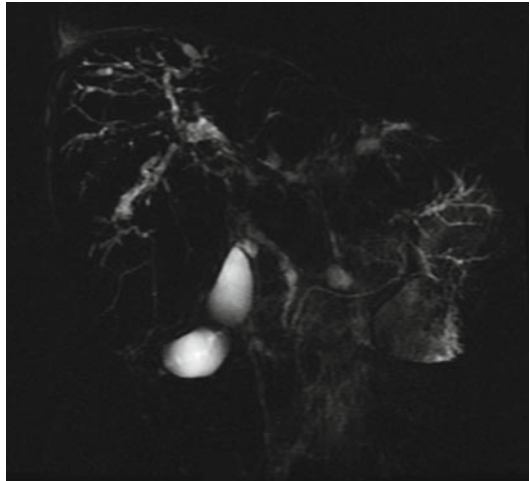


Fig. 47.3 MRCP appearance of PSC



stricturing and segmental dilatations, causing a “beaded appearance,” in the absence of a secondary cause (Fig. 47.3).

Patients with small duct PSC have normal cholangiographic findings – this subgroup, who share similar biochemical and clinical features to large duct PSC, is instead diagnosed when histological changes of PSC are demonstrated on liver biopsy [29] (See Laboratory Investigations).

Magnetic resonance cholangiopancreatography (MRCP) is now the investigation of choice over endoscopic retrograde cholangiopancreatography (ERCP). MRCP is non-invasive, does not involve radiation, avoids ERCP complications such as pancreatitis and is comparable to ERCP for diagnosis of PSC with good inter-observer agreement [30, 31]. ERCP may still have a place in patients where the diagnosis remains uncertain after MRCP and is most useful for imaging subtle abnormalities in the intrahepatic biliary tree. As yet, there is no data on the utility of CT cholangiography for PSC diagnosis.

Histology

When radiological findings support the diagnosis of PSC, histological examination of the liver is not required to confirm this and only exposes patients to unnecessary morbidity.

Histology is diagnostic in only one-third of patients PSC, although in another third there may be findings suggestive of biliary disease. The characteristic early biopsy findings of PSC are inflammation with periductal “onion-skin” fibrosis, portal oedema and bile ductular proliferation resulting in expansion of portal tracts (Fig. 47.4). With disease progression, bridging fibrosis eventually leads to cirrhosis. The focal nature of both early and late changes in PSC can make “staging” liver biopsies unreliable [32].

Secondary Sclerosing Cholangitis

When a diagnosis of PSC is suggested by imaging and histology, there are a number of causes of secondary sclerosing cholangitis (SSC) that must be considered (Table 47.2). At times, it can be very difficult to distinguish these from PSC, particularly in patients PSC who have co-existing pathology such as choledocolithiasis. In these patients, the clinical history, presence of IBD, and distribution of cholangiographic abnormalities are most helpful in identifying the predominant disease process [33].

Fig. 47.4 Typical liver histological changes in PSC

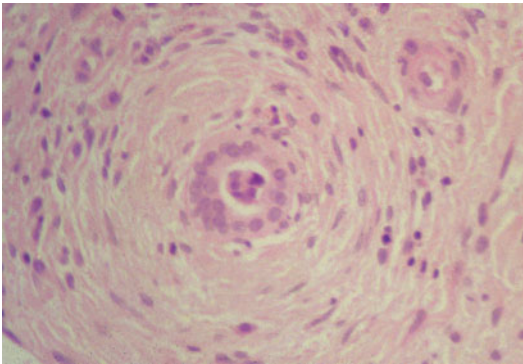


Table 47.2 Secondary causes of sclerosing cholangitis

Cholangiocarcinoma
Choledocholithiasis (with sepsis)
Diffuse intrahepatic metastasis
Chemotherapy (e.g. FUDR)
Biliary infections – CMV and immunodeficiency
Cryptosporidium and immunodeficiency
Ascariasis
Ascending cholangitis
Eosinophilic cholangitis
Hepatic inflammatory pseudotumour
Histocytosis X
IgG4-associated cholangitis
Ischemic cholangitis
Mast cell cholangiopathy
Portal hypertensive biliopathy
Recurrent pancreatitis
Surgical biliary trauma
AIDS cholangiopathy

Special Patient Populations

Small Duct PSC

Small duct PSC is normally diagnosed in the patients with IBD who have cholestatic serum biochemistry with a normal cholangiogram, but it may occur in patients without IBD. It is characterised by histological changes on liver biopsy characteristic of PSC. It occurs in approximately 10% of the PSC population [34–36].

Small duct PSC typically runs a milder course than large duct disease with a reduced likelihood of progression to cirrhosis and with a significantly improved survival compared with large duct disease. To date there have been no reports of cholangiocarcinoma in the small duct patients PSC population [37, 38]. Approximately one-quarter of small duct patients PSC will subsequently develop large duct disease over a period of 10 years [37–41]. A recent study from the Calgary health region in Canada has shown an incidence of small duct PSC as 0.15/100,000. In children the incidence rate was 0.23/100,000 compared with 1.11/100,000 in adults [7].

Autoimmune Hepatitis and PSC

Various studies suggest that between 1.4 and 8% of patients PSC have co-existing autoimmune liver disease (AIH) – recently defined as PSC-AIH syndrome [42–45]. PSC-AIH is more commonly found in children and young adults and characterised by clinical, biochemical and histological features of AIH in the presence of cholangiographic findings identical to PSC [38, 40, 46–49]. PSC-AIH should be considered if the aminotransferase level is elevated more than twice the upper limit of normal and the serum IgG is elevated. Rarely, AIH features can develop in patients with established PSC. A liver biopsy should always be performed in these patients to confirm the diagnosis before treating with immunosuppressants. Immunosuppression is helpful in improving disease progression in this group [50–53].

IgG4 Associated Cholangitis

Elevated IgG4 serum levels were first reported more than 10 years ago in patients with autoimmune pancreatitis (AIP) [54]. These patients commonly have associated intra- and extrahepatic biliary stricturing that may mimic PSC. In 2004, a Japanese case series found a subset of patients PSC had a significant infiltrate of IgG4 positive plasma cells isolated to the biliary tree (in the absence of any pancreatic abnormality) [55]. This group is now recognised to represent a distinct clinical entity that like AIP may be responsive to immunosuppression. The term IgG4 associated with cholangitis is used to encompass both this patient group and those patients who have biliary pathology in association with AIP [56].

Recent retrospective studies have found elevated serum IgG4 levels in 9–12% of patients PSC [28, 57]. IgG4 positive patients have a reduced incidence of IBD and a more severe disease course in PSC patients when compared with patients who are negative [28].

A recent re-evaluation of 98 consecutive liver transplants for patients originally diagnosed with PSC, found that 23 (23%) of explanted livers stained positive for IgG4. Serum IgG4 levels were elevated in 18 of these patients. Tissue IgG4 positivity was associated with a more aggressive clinical course manifested by shorter time to transplant and a higher likelihood of disease recurrence post-transplant [58].

The important clinical implications and potential benefit of immunosuppression in this patient group make it imperative that IgG4 cholangitis is recognised and serum IgG4 levels should be checked in all patients PSC.

Inflammatory Bowel Disease and PSC

Epidemiology

There is a strong association between IBD and PSC. In patient series from Northern Europe and North America, the prevalence of IBD in people with PSC ranges between 60 and 80% [59–61]. There are, however, significant geographical variations in the reported prevalence of IBD in PSC with a much weaker association found in certain countries (Table 47.3). This discrepancy may partly be explained because studies reporting a weaker association often only used sigmoidoscopy (and/or no biopsies) subsequently missing a significant proportion of colitis, which may be right sided and microscopic in patients PSC.

Table 47.3 Prevalence of IBD in PSC patients

Country	Number of pts	IBD (%)	UC	Crohns
Norway [153]	77	96	74	14
Sweden [59]	305	81	72	
UK [132]	126	73	71	
US [131]	174	71		
Italy [110]	117	54	36	10
Spain [9]	43	46	44	2
India [154]	18	50		
Japan [155]	388	37		

Table 47.4 Clinical characteristics of IBD associated with PSC

Characteristic	UC	UC/PSC
Extend of colitis	54% (pancolitis)	87% Pancolitis [62]
Rectal sparing	6%	52% [62]
Backwash ileitis	7%	51% [62]
Pouchitis (following colectomy and ileo-anal pouch formation)	15%	60% [156]
Disease course		Typically mild, quiescent [65]
Dysplasia and cancer		RR 4 [74, 75, 157] Particularly right sided cancer
Other		Increased risk of peristomal varices in pts undergoing proctocolectomy with ileostomy

IBD in patients PSC

The predominant form of IBD is UC with approximately 85% of patients PSC/IBD affected [61, 62]. IBD can be diagnosed at any time in patients with PSC but most commonly the diagnosis predates PSC by several years [60, 62, 63]. Interestingly, even patients who have received liver transplantation for PSC continue to be at increased risk of developing IBD [64].

All patients with a new diagnosis of PSC should have a full colonoscopy with biopsies, regardless of the presence of gastrointestinal symptoms to evaluate for IBD [56]. Because asymptomatic colitis and rectosigmoid sparing are common features in IBD/PSC, a flexible sigmoidoscopy is insufficient for screening. It is unclear whether interval endoscopies should be performed if a patient remains symptom free following a colonoscopy with normal colonic histology. Some clinicians advocate repeat endoscopy every 5 years.

It is likely that PSC/UC represents a distinct UC phenotype [62]. The natural history of IBD in patients with PSC has a more benign course than in those patients with IBD alone despite the fact that it usually involves the whole colon [65]. Common features in the PSC/IBD group include: rectal sparing (52% versus 6%), backwash ileitis (51% versus 7%) and an increased prevalence of pouchitis (following colectomy and ileo-anal pouch formation). The reason for increased rates of pouchitis is unknown (Table 47.4).

There have been no case-controlled studies to date specifically examining the course of Crohns disease in patients with PSC/Crohns. Small, uncontrolled series suggest that Crohns/PSC typically manifests as extensive colitis and also that isolated small bowel Crohns is not associated with PSC [61, 66].

Table 47.5 Prevalence of PSC in patients with UC

Country	Percentage of UC pts with PSC	Number of UC pts evaluated
Sweden [4]	3.7	1,500
UK [158]	2.9	681
Norway [159]	4	336

PSC in patients IBD

The true prevalence of PSC within the IBD patient population is unknown because until recently, accurate data have required invasive cholangiography to be carried out on unselected patient groups and patients PSC may have normal liver biochemistry. Work underway using non-invasive MRCP will hopefully provide this data in the near future. Available evidence suggests that approximately 5% of UC patients have co-existing PSC although this is likely to be an underestimate [67]. This data comes from three major trials (Table 47.5). The largest of these, found that 5% of a cohort of 1,500 patients UC had elevated alkaline phosphatase levels, and in those who subsequently underwent ERCP, 85% had evidence of PSC [4].

The prevalence of PSC in Crohns disease is significantly less than that of UC, with an estimated percentage of 3.4% of patients affected [66]. Typically these patients have either ileo-colonic or extensive colonic Crohns disease.

Both the development of PSC and its outcome are independent of the activity of colitis. It may even occur after proctocolectomy. Interestingly, however, colectomy prior to transplantation for PSC is protective against the development of PSC in the transplanted liver [68].

PSC and Malignancy

Patients with PSC have a high rate of malignancy and currently more patients die of malignancy than end-stage liver failure (Table 47.6) [69]. The reason for the high rate of malignancy is probably explained by chronic inflammation in the biliary system and the colon, although whether PSC patients have a particular genetic susceptibility to develop cancer is unclear.

Colorectal Cancer

The increased risk of colorectal cancer (CRC) in UC compared with the general population is well established [70–74]. Based on a meta-analysis of 11 studies, in UC patients with co-existing PSC this risk is elevated five times higher again (OR of 4.79 (95% CI 3.58–6.41). This risk increases with time and continues even after liver transplantation [75, 76] (Fig. 47.5).

Although CRC can present at any time in the disease course, the median time from diagnosis of colitis to development of CRC is 17 years [77]. Interestingly, the majority of these cancers (76%) are right sided [74]. It has been proposed that this right-sided predominance may result from a carcinogenic effect of secondary bile acids such as lithocholic acid.

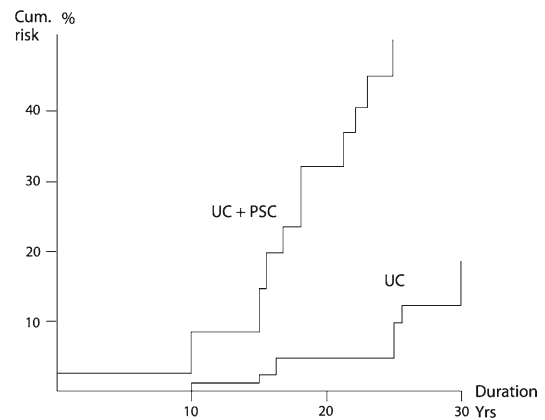
Considering the absolute risk for colonic dysplasia or cancer in PSC/UC approaches 31% after 20 years of colitis [78] it is understandable that guidelines recommend 1–2 year interval colonoscopies with biopsies from the time of diagnosis of PSC/IBD. Patients with Crohns/PSC are included in this recommendation.

Table 47.6 Standard incidence ratio for first cancer after diagnosis of PSC including and excluding first year after diagnosis of PSC

Site of cancer	Observed	Expected	Standard incidence ratio	95% confidence interval	Excluding
All sites	87	14.3	6.1	(4.9–7.5)	
All sites excluding colorectal and hepatobiliary carcinoma	16	11.8	1.4	(0.8–2.2)	
All gastrointestinal cancers	71	2.5	28.6	(22.4–36.1)	
Oesophagus	0	0.1	0.0	(0–30.5)	
Stomach	1	0.4	2.2	(0.1–12.5)	
Colon-rectum	12	1.2	10.3	(5.3–18.1)	
Hepatobiliary tract	53	0.3	160.6	(120.3–210.1)	
Pancreas	5	0.3	14.3	(4.7–33.4)	
Oesophagus	0	0.1	0	(0–34.2)	1st year
Stomach	1	0.4	2.5	(0.1–14.1)	1st year
Small intestine	0	0.1	0.0	(0–56.8)	1st year
Colon-rectum	7	1.0	6.8	(2.7–14.0)	1st year
Hepatobiliary tract	31	0.3	106.9	(72.6–151.7)	1st year
Pancreas	3	0.3	9.7	(2.0–28.4)	1st year

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Fig. 47.5 Risk of colonic neoplasia in PSC. [Reprinted from Broome, U., et al., *Primary sclerosing cholangitis and ulcerative colitis: evidence for increased neoplastic potential*. Hepatology, 1995. 22(5): p. 1404–8. With permission from John Wiley & Sons, Inc.]



UDCA treatment in patients with PSC and UC may decrease the risk of colorectal dysplasia and CRC [79, 80] (see Cholangitis).

In patients who develop PSC-associated colorectal malignancy, proctocolectomy with ileo-anal pouch formation is the preferred surgical management as it avoids the complication of peristomal varices in patients with an ileal stoma.

Cholangiocarcinoma

Cholangiocarcinoma complicates the clinical course of PSC in 10–20% of patients, with an annual incidence (starting 1 year after diagnosis of PSC) of 0.5–1.5% [59, 81–84]. Male gender, smoking and a long history of IBD were identified as risk factors for CCA in a case-controlled review of 39 patients PSC presenting with CCA [85].

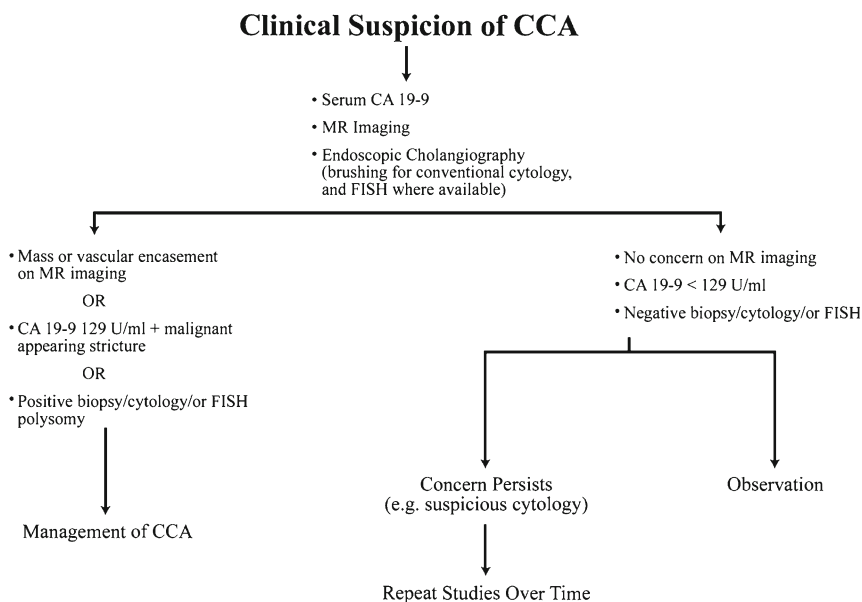


Fig. 47.6 Work-up for clinical suspicion of cholangiocarcinoma. [Reprinted from Chapman, R., et al., *Diagnosis and management of primary sclerosing cholangitis*. Hepatology, 2010. 51(2): p. 660–78. With permission from John Wiley & Sons, Inc.]

One-third of the patients who develop CCA are diagnosed within 1 year of the diagnosis of their PSC. The likely explanation for this is that the development of symptomatic cholangiocarcinoma brings a number of patients with previously unrecognised PSC to medical attention.

The diagnosis of CCA can be challenging in patients PSC and early detection is difficult. Often patients with CCA are asymptomatic and when symptoms develop they are non-specific, typically indicate metastatic disease and mimic PSC disease progression [86]. Consequently, worsening jaundice/bilirubin levels, pruritis, weight loss and abdominal pain in any patient PSC should always prompt evaluation for CCA. Unfortunately, computed tomography (CT), ultrasonography (US) and MRCP have poor sensitivity for detection of CCA [87].

Tumour markers play a limited role in the early detection of CCA [56, 84, 88–91]. Using a cut-off level for Carbohydrate Antigen 19-9 (CA19-9) of 130 U/ml (normal <55), the sensitivity and specificity is 79 and 98%, respectively [89]. CA19-9 can be elevated in benign biliary disease as well as other malignancies including pancreas, colon, stomach and gynaecological cancers. It is important to note that 7% of the general population (those negative for Lewis antigen) will not produce CA 19-9 regardless of their CCA status. Generally, CA 19-9 has only been useful to identify patients with advanced, unresectable CCA and has no role in cancer surveillance in PSC. Elevated levels of serum trypsinogen-2 were reported to represent an early marker of CCA, but this needs confirmation in a larger study [92].

CCA can develop in any part of the biliary tree. Perihilar CCAs (so-called Klatskin tumours) are the most common tumour site. These are typically infiltrating, desmoplastic tumours. Mass lesions in the liver make up about 20% of cancers and distal CBD tumours are uncommon (<10%) [93].

The discovery of a dominant perihilar stricture on imaging presents a significant diagnostic challenge (Fig. 47.6). The majority of these strictures will be benign. It is uncommon for a co-existing mass to be identified, but if found, this has a virtually 100% sensitivity and specificity for CCA [84]. In a recent retrospective study of 89 patients with dominant strictures, MRCP was found to be

superior to ERCP for the diagnosis of CCA with a sensitivity, specificity and accuracy of 96, 85, 81% compared with 80, 75 and 78%, respectively [94]. One advantage of ERCP, however, is that it does allow acquisition of cytological specimens using brushings.

Conventional brush cytology specimens obtained through ERCP have poor sensitivity, 18–40% [84, 91, 95–97]. However, when positive, the specificity approaches 100%. New diagnostic modalities such as direct image analysis (DIA) and fluorescent in situ hybridisation (FISH) have been employed in attempts to increase diagnostic yield on cytological specimens. Early studies indicated a significant improvement in sensitivity [96] but more recently this has been brought into question with the most recent study using FISH polysomy demonstrating a sensitivity of 46%, only slightly higher than cytology alone [98].

Distinguishing PSC and CCA by direct visualisation of the biliary tree with techniques such as endoscopic intraductal US and cholangioscopy is promising. A recent study reported a sensitivity of 92% and specificity of 93% for diagnosing malignant strictures compared to 66 and 51%, respectively, using ERCP alone [99]. However, these techniques have not yet been tested in large patient populations. PET is not helpful to distinguish PSC from CCA [100].

Unfortunately, the prognosis for CCA is poor. The median survival is 5–11 months [82, 101, 102]. Most patients are treated palliatively. Even with surgical resection, the 3-year survival is <30% [103, 104]. The Mayo and Omaha clinics in the USA use neoadjuvant chemoradiotherapy, followed by liver transplantation in highly selected patients with non-metastatic cancers of less than 3 cm in diameter. The Mayo clinic has published 5-year survival rates of up to 82% in this highly selected group [105, 106].

Gallbladder and Pancreatic Cancer

PSC is recognised as a major risk factor for gallbladder carcinoma. In a study examining 286 patients with PSC, 18 (6%) of patients were found to have gallbladder mass lesions of which 10 were gallbladder cancer [107]. Guidelines recommend annual US surveillance, and cholecystectomy for any mass lesion identified regardless of size.

One study has suggested that the risk of pancreatic carcinomas may also be increased in PSC. This remains to be confirmed [69].

Non-malignant Complications of PSC and Their Management

Dominant Strictures

Due to an absence of population-based studies, the exact prevalence of dominant strictures is unknown but estimated to occur in approximately 45–58% of patients PSC during follow-up [108–110]. The majority of these strictures are benign but their discovery should always prompt consideration of a CCA (see IBD in PSC patients). Dominant strictures are associated with bacterial cholangitis as well as biliary stones.

Patients with symptoms from dominant strictures such as cholangitis, jaundice, pruritus, right upper quadrant pain or worsening biochemical indices are appropriate candidates for therapy. ERCP with sphincterotomy and balloon dilatation (with prophylactic pre-procedural antibiotics) has the best evidence of benefit [111–116]. Stenting should be reserved for strictures that are refractory to

repeat dilatation. Large retrospective studies suggest that endoscopic therapy results in clinical improvement and prolonged survival. To date, however, there has not been a randomised controlled trial to confirm this.

Bacterial Cholangitis

It is very unusual for patients PSC to develop cholangitis in the absence of a previous history of instrumentation of the biliary tree or a dominant stricture [117, 118]. The presence of a dominant stricture is thought to predispose to cholangitis through bacterial colonisation of stagnant bile acids.

A recent study demonstrated that 40.5% of patients with a dominant stricture had bacterial colonisation of their biliary tree (in the absence of a stricture, patient bile samples were all sterile) [119]. Most infections are caused by aerobic, enteric organisms such as *E. Coli*, *Klebsiella* and *E. Faecalis* [120]. Unfortunately, short-course antibiotics are poorly effective in eradicating bacteria. However, biliary drainage procedures, combined with antibiotics are usually effective. Patients with recurrent bacterial cholangitis may benefit from cyclical prophylactic long-term antibiotics usually quinolones. In patients who fail antibiotic therapy, recurrent cholangitis may be the primary indication for OLTx.

Fatigue and Pruritus

Fatigue is a common problem and has a major impact on the quality of life in patients PSC [120]. It occurs independently of the severity of liver disease. No medical therapy is of proven benefit although the antinarcology agent modafinil has been tried off-label with varying degrees of success.

Pruritus is also common in patients with PSC. Eighty to ninety percent of patients will respond to first line therapy with cholestyramine [121].

The antibiotic rifampicin is used as a second line agent for patients who have not responded to cholestyramine. It has shown to be effective treatment for pruritus in cholestatic liver disease [122–124]. The mechanism of action is unknown but it probably induces the hepatic microsomal drug-metabolising system causing metabolism of endogenous pruritogenic compounds. It may alternatively have a direct antimicrobial effect in the intestinal lumen, causing a change in synthesis of secondary bile acids. It is also a nuclear receptor agonist of PXR. Long term administration is limited by its potential hepatotoxicity.

There is an up regulation of opioid receptors in patients with chronic cholestasis as well as an increased central opioidergic tone and opioid antagonists (nalmefene, naloxone and naltrexone) have been shown to be effective in pruritus. These drugs can cause a withdrawal-like reaction by preventing binding of endogenous opioids but this complication is transient and settles within the first couple of days [125–127].

A variety of fourth line medications are useful including sertraline. UDCA has not been shown to benefit pruritus though its effect in this regard has not been directly studied. Occasionally, intractable pruritus may be an indication for OLTx [26].

Metabolic Bone Disease

The possibility of Hepatic osteodystrophy should be evaluated in all patients with a new diagnosis of PSC, and every 2–3 years thereafter using bone mineral density scanning [128]. The incidence of osteoporosis is 4–10% in PSC, with a particularly increased risk in older patients with a long

duration of disease, decreased BMI and possibly, worse disease [129, 130]. Calcium and Vitamin D should be prescribed in all patients PSC with osteopaenia, and bisphosphonate therapy considered when osteoporosis develops.

Gallstones

Patients PSC are predisposed to gallstone disease. In a retrospective review of 286 patients PSC, 25% of patients had gallstones that were diagnosed at a mean of 5 years following the diagnosis of PSC. Characteristically these stones are small brown bile pigment stones. Ursodeoxycholic acid (UDCA) does not influence the frequency of gallstones [107].

Prognosis and Treatment

Prognosis

Once a patient is diagnosed with PSC, the median time to death or liver transplantation in all-comers is 9.6–18 years [40, 108, 131–133]. The significant differences in survival estimation in these studies may reflect the variation in the definition of onset between studies (symptom onset versus diagnosis at imaging) and also the absence of a reliable marker for early disease. There have been no prospective studies examining prognosis due to the impracticality of studying such a rare and slowly progressive disease.

Although the majority of patients who are asymptomatic at the time of diagnosis will eventually develop symptoms, not surprisingly these asymptomatic patients have a better prognosis than the overall PSC population with a survival rate population with a survival rate of 88% at 5 years and greater than 70% at 16 years [131, 132]. A study from Germany in 2007 demonstrated that a persistently raised serum bilirubin (>3 months) following initial diagnosis is an independent risk factor correlating with poor outcome and high risk of CCA.

Several prognostic models have been developed to help predict outcomes in PSC. Unfortunately, due to the highly variable disease course between individuals, these models are generally only useful for examining patient cohorts. The Mayo score provides the most valid survival information [56]. It uses age, bilirubin, serum AST and albumin along with a history of variceal bleeding as prognostic parameters to divide patients into low, intermediate and high risk.

The Child–Pugh classification has also been demonstrated to be a satisfactory alternative to disease-specific models in both research studies and clinical decision-making [78].

Medical Therapy

Unfortunately, no medical therapy has been proven to improve prognosis in PSC. Advocates of medical therapy appropriately point to a number of reasons why conclusive trial data is lacking. The scarcity of patients PSC, lack of reliable surrogate markers for disease progression, long time until primary end-points (such as transplantation/death) and high individual disease variability all make it very difficult to design and carry out trials that are appropriately selective and sufficiently powered to demonstrate a significant benefit.

UDCA, a naturally occurring bile acid has been extensively studied for the treatment of PSC over the past 20 years. Surprisingly, a recent trial using high dose UDCA 28–30 mg/kg day was terminated

prematurely because of an increased risk of serious adverse events (death or liver transplantation) compared with placebo [134]. The likely explanation for this unexpected result is that high doses of UDCA result in the conversion of unabsorbed colonic UDCA into lithocholic acid, a hepatotoxic bile acid. This is supported by data from the original trial that demonstrates markedly elevated serum lithocholic acid levels in the patients treated with high dose UDCA compared with those who received only placebo [135].

Despite the controversy that stems from the high-dose trial, it remains European practice to prescribe 15–23 mg/kg/day to patients with large duct PSC and co-existing IBD, in keeping with the EASL clinical practice guidelines [56]. This recommendation is made for several reasons:

1. Multiple trials have shown this dose to be safe and effective in improving liver biochemistry [95, 136–138].
2. UDCA has been demonstrated in three clinical studies to reduce rates of colonic dysplasia and CRC, in patients with co-existing IBD [79, 80, 139].
3. A randomised, double blind, controlled trial evaluating 219 patients over 5 years found a strong trend towards increased survival using UDCA (Unfortunately due to few endpoints, this trial was insufficiently powered to reach statistical significance.) [138].
4. The use of UDCA may reduce the incidence of biliary tract cancers but this needs further study [134, 140].

A number of other medical therapies have been trialled in PSC (including azathioprine, cyclosporine, budesonide and etanercept), none of which have any proven efficacy and these are not recommended in the routine treatment of PSC. In the context of PSC/AIH overlap syndrome, immunosuppressive medications, particularly corticosteroids may have a therapeutic role and a trial of therapy in those circumstances is sometimes indicated [141].

Transplantation

Liver transplantation (LTx) is the only proven lifesaving therapy for end-stage liver disease secondary to PSC. The 5-year survival rate is excellent at approximately 85% [142–144]. Although in some Scandinavian countries, PSC is the leading indication for liver transplantation, in the USA, it is the 5th leading cause of liver transplantation [145].

Appropriate timing of LTx for PSC is particularly challenging due to the highly variable disease course and unpredictable risk of cholangiocarcinoma. The decision to list a patient for LTx should be made on the basis of clinical signs, biochemical parameters and cholangiographic findings. There is no justification for performing pre-emptive LTx to reduce the risk of developing CCA. Traditionally, CCA is regarded as a contraindication to LTx although recent data in highly selected patient groups has been promising (see IBD in PSC patients).

The recurrence rate of PSC in a transplanted liver is estimated to be between 20 and 25% after 5–10 years although it can be very difficult to distinguish recurrent disease from secondary causes of biliary structuring such as peritransplant ductal ischaemia [142, 146, 147]. Interestingly the risk of recurrent PSC is reduced in PSC/IBD patients who have had a colectomy prior to, or at the time of LTx, compared with those who have not [68, 148]. High-dose post-transplant prednisolone therapy is also associated with an increased risk of recurrent disease.

In patients PSC/IBD, the risk of colorectal dysplasia and cancer persists post-transplantation and ongoing colonic surveillance is necessary. Some studies suggest this risk increases even further after liver transplantation and annual colonoscopy is recommended.

IBD rarely improves post-transplantation and up to 30% of patients will have increased and worsening activity. UC may even develop de novo in patients with PSC post-transplant [149, 150].

Conclusion

Over recent years PSC has been increasingly recognised as an important clinical entity, particularly amongst the IBD population where it is found in up to 10% of patients. The use of MRCP has simplified the diagnostic process with avoidance of the complications attached to ERCP, without the need for liver biopsy. Indeed, in conjunction with increased clinician awareness, greater application of MRCP has likely led to an increase in the capture of incident and prevalent PSC cases. This may in part explain the changing epidemiology of PSC with an increase in reported prevalence and greater number of patients PSC being asymptomatic at the time of diagnosis.

While better insight into the pathogenetic mechanisms underlying the development of PSC has been made, its exact etiology is not yet elucidated and it is likely a number of mechanisms play a role in disease pathogenesis. GWASs currently underway will hopefully shed further light on this complex process.

No medical treatment has been established to halt the progression of this insidious disease and recent trials on the use of UDCA have raised more questions than they have answered. Advances in endoscopic, radiological and molecular techniques have improved therapeutic options and allowed clinicians to better differentiate benign from malignant biliary disease. Nevertheless, screening for and confirming a suspected diagnosis of cholangiocarcinoma still remains one of the significant challenges of managing this complex disease.

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Chapter 48

Extraintestinal Manifestations of Inflammatory Bowel Disease: Autoimmune Pancreatitis and Other IgG4-Related Conditions

Terumi Kamisawa, Taku Tabata, Go Kuwata, and Koichi Koizumi

Keywords Autoimmune pancreatitis • IgG4 • Ulcerative colitis • Crohn's disease

Introduction

In 1961, Sarles et al. (1) first reported pancreatitis associated with hypergammaglobulinemia and suggested autoimmunity as a pathogenetic mechanism. Since Yoshida et al. (2) proposed the term “autoimmune pancreatitis” (AIP) in 1995, many cases of AIP have been reported in Western countries as well as in Japan. AIP is defined as a unique pancreatitis with a presumed autoimmune etiology (3). AIP is frequently associated with various sclerosing extrapancreatic lesions, but histopathological features for these extrapancreatic lesions are similar to the dense infiltration of IgG4-positive plasma cells and lymphocytes, and fibrosis detected in the pancreas. Therefore, we proposed a new clinicopathological entity of “IgG4-related sclerosing disease” and suggested that AIP represents a pancreatic lesion of this systemic disease (4–7). This paper focuses on the clinicopathological features of AIP and other IgG4-related conditions, especially bowel conditions, based on our 62 AIP cases.

Autoimmune Pancreatitis

Clinical Features

A recent nationwide Japanese survey reported that there were about 3,000 individuals with AIP (8). There are several hypotheses about the pathogenesis of AIP, but the precise pathogenic mechanism is unclear. AIP occurs predominantly in elderly males. In our series ($n=62$), the mean age of patients was 64.3 years (range, 25–83 years) and the male-to-female ratio was 3:1. The major initial

T. Kamisawa, MD, PhD (✉) • T. Tabata, MD • G. Kuwata, MD • K. Koizumi, MD
Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital,
3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, Japan
e-mail: kamisawa@cick.jp

Table 48.1 Clinical diagnostic criteria for AIP (2006) in Japan

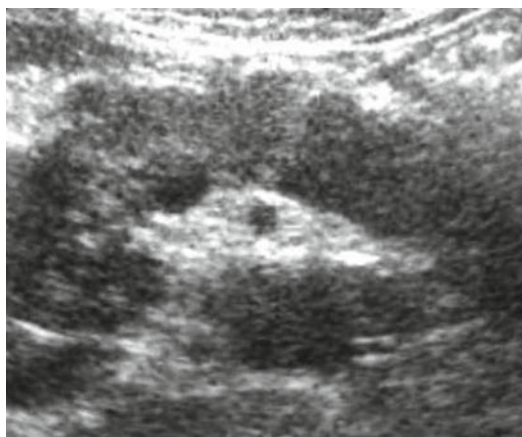
1. Diffuse or segmental narrowing of the main pancreatic duct with irregular wall and diffuse or localized enlargement of the pancreas by imaging studies, such as abdominal ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI)
2. High serum γ -globulin, IgG or IgG4, or the presence of autoantibodies, such as antinuclear antibodies and rheumatoid factor
3. Marked interlobular fibrosis and prominent infiltration of lymphocytes and plasma cells in the periductal area, occasionally with lymphoid follicles in the pancreas

Diagnosis of AIP is established when criterion 1, together with criterion 2 and/or 3, are fulfilled

However, it is necessary to exclude malignant diseases such as pancreatic or biliary cancers

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Fig. 48.1 Ultrasonography of a patient with autoimmune pancreatitis showing diffuse hypoechoic enlargement of the pancreas



symptom is obstructive jaundice induced by associated sclerosing cholangitis. Diabetes mellitus, usually type 2, is often observed. In many cases, the diagnoses of diabetes mellitus and AIP are made simultaneously; some cases show exacerbation of preexisting diabetes mellitus with the onset of AIP (9).

Diagnosis and Diagnostic Criteria

As it is difficult to take specimens from the pancreas, AIP is diagnosed using clinical, radiological, laboratory, and histopathological findings. It is most important to distinguish AIP from pancreatic cancer. According to the Japanese “Diagnostic Criteria for Autoimmune Pancreatitis, 2006” (3), the diagnosis of AIP requires the presence of radiological imaging criterion as well as either laboratory or histopathological criterion (Table 48.1).

Radiological imaging criterion consists of enlargement of the pancreas and irregular narrowing of the main pancreatic duct. Diffuse enlargement of the pancreas, the so-called “sausage-like” appearance, is typical and rather specific to AIP. On dynamic CT and MRI, there is delayed enhancement of the enlarged pancreatic parenchyma. A capsule-like low-density rim surrounds the pancreas on CT; this rim might be induced by inflammatory and fibrous changes involving the peripancreatic adipose tissue and is another finding that is specific to AIP. Ultrasonography shows an enlarged hypoechoic pancreas with hyperechoic spots (Fig. 48.1). It is difficult to differentiate

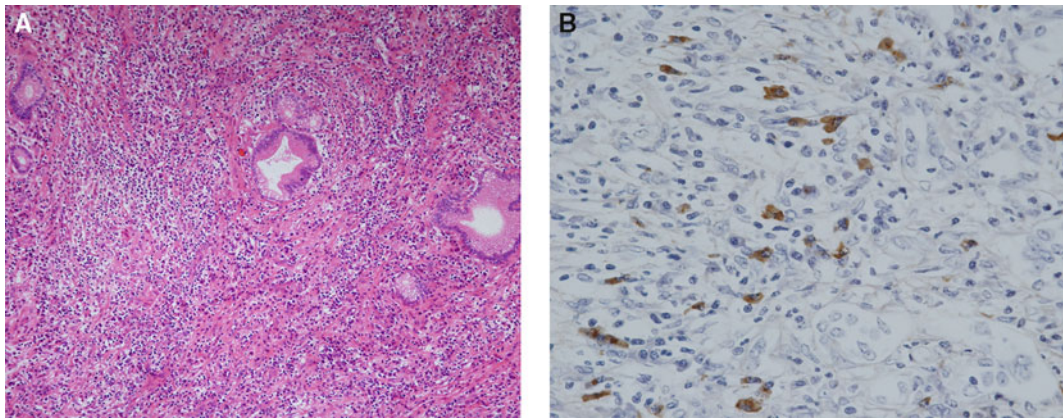


Fig. 48.2 Histology of the pancreas of a patient with autoimmune pancreatitis. (a) Dense lymphoplasmacytic infiltration and fibrosis called LPSP (HE). (b) Abundant infiltration of IgG4-positive plasma cells in the pancreas (IgG4-immunostaining)

segmental AIP from pancreatic cancer, but the upstream dilatation of the distal pancreatic duct is less than that in cases of pancreatic cancer (10).

Laboratory criterion consists of elevated levels of serum gammaglobulin and/or IgG, and/or IgG4, or the presence of autoantibodies. Elevation of serum gammaglobulin or IgG levels and the presence of autoantibodies, such as antinuclear antigen (ANA) and rheumatoid factor, are detected in almost half of AIP patients. Serum IgG4 levels are significantly and frequently elevated in AIP patients. The sensitivity of elevated serum IgG4 levels to identify AIP was 79% (48/61) in our series, 81% (39/48) in the United States (11), and 68% (25/37) in Korea (12). However, among patients with pancreatic cancer, elevation of serum IgG4 levels was detected in 4% (5/116) in our series (13) and 7% (5/71) in the United States (14); thus elevation of serum IgG4 levels alone cannot differentiate AIP from pancreatic cancer.

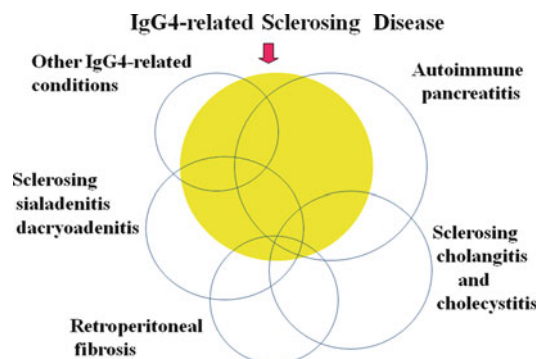
Histopathological criterion for AIP consists of lymphoplasmacytic infiltration and fibrosis in the pancreas, referred to as lymphoplasmacytic sclerosing pancreatitis (LPSP) (Fig. 48.2a) (15). Obliterative phlebitis is frequently detected. Immunohistochemically, infiltrating cells consist mostly of T lymphocytes and IgG4-positive plasma cells (Fig. 48.2b) (4–7).

New diagnostic criteria proposed from the United States (16) and Korea (17) in 2006 contained two additional diagnostic factors: response to steroid therapy and other organ involvement. In 2008, Japanese and Korean pancreatologists developed Asian diagnostic criteria including response to steroid as an optional criterion (18).

Treatment and Prognosis

Oral steroid therapy is the standard treatment for AIP. Indications for steroid therapy in AIP patients are symptomatic such as obstructive jaundice. Before steroid therapy is started, blood glucose levels and jaundice should be controlled. The most commonly used initial dose of prednisolone is 0.6 mg/kg/day, and it is tapered by 5 mg every 1–2 weeks while monitoring the patient's symptoms as well as biochemical, serological, and imaging findings. To prevent relapse, maintenance therapy (5 mg/day) is recommended for 6–12 months. In relapsed cases, re-administration or increased doses of steroids are effective (19, 20).

Fig. 48.3 Schematic illustration showing the relationship between IgG4-related sclerosing disease and other IgG4-related conditions



Most AIP patients treated with steroid therapy have good short- and long-term clinical, morphological and functional outcomes. However, as some patients develop pancreatic stones or malignancy during or after steroid therapy, AIP patients should be rigorously followed up (20).

Other IgG4-Related Conditions

IgG4-Related Sclerosing Disease

AIP is frequently associated with sclerosing extrapancreatic lesions such as sclerosing cholangitis, sclerosing cholecystitis, sclerosing sialadenitis, sclerosing dacryoadenitis, retroperitoneal fibrosis, and pseudotumors. As histological findings for these extrapancreatic lesions are similar to pancreatic findings in AIP patients, we proposed the existence of a novel clinicopathological entity, an “IgG4-related sclerosing disease.” This IgG4-related sclerosing disease represents a systemic disease characterized by extensive IgG4-positive plasma cells and T lymphocyte infiltration of various organs. Clinical manifestations are apparent in organs such as the pancreas, bile duct, gallbladder, salivary glands, and retroperitoneum, where tissue fibrosis with obliterative phlebitis is pathologically induced. AIP is not simply a pancreatitis, but rather a pancreatic lesion reflecting an IgG4-related sclerosing disease. In some cases, only one or two organs are clinically involved, whereas others show effects on three or four organs (Fig. 48.3) (4–7).

IgG4-Related Sclerosing Cholangitis

Sclerosing cholangitis is the most frequent extrapancreatic lesion of AIP (79% in our series), and is thought to be an IgG4-related sclerosing cholangitis. Although IgG4-related sclerosing cholangitis is commonly associated with AIP, some cases show slight or no pancreatic lesions. Because IgG4-related sclerosing cholangitis responds well to steroid therapy, discriminating it from primary sclerosing cholangitis (PSC) and cholangiocarcinoma is important. The histological findings of IgG4-related sclerosing cholangitis include transmural fibrosis and dense infiltration of IgG4-positive plasma cells and lymphocytes in the bile duct wall (21, 22).

Patient age at clinical onset is around two decades older in IgG4-related SC than in PSC. Obstructive jaundice is more frequently the chief complaint in patients with IgG4-related sclerosing cholangitis than PSC. Inflammatory bowel disease (IBD) and cholangiocarcinoma, which are occasionally associated with PSC, are rarely associated with IgG4-related sclerosing cholangitis.

Elevation of serum IgG4 levels is rarely seen in PSC patients. On cholangiography, IgG4-related sclerosing cholangitis shows lower bile duct stenosis and relatively long stricture from hilar to intrahepatic biliary systems with simple distal dilatation, whereas PSC shows characteristic findings of band-like stricture, beaded appearance, pruned tree appearance, and diverticulum-like out-pouching. In IgG4-related sclerosing cholangitis, wall thickening is frequently extensive, and is sometimes observed where cholangiography shows normal findings. Oral prednisolone is the first line of therapy for IgG4-related sclerosing cholangitis (21, 22).

IgG4-Related Sclerosing Cholecystitis

Thickening of the gallbladder was detected on ultrasonography and/or CT in 32% of our AIP patients. The gallbladder displayed transmural or mucosal-based infiltration of lymphocytes and IgG4-positive plasma cells and fibrosis (23). IgG4-related sclerosing cholecystitis is most frequently associated with AIP patients with extensive bile duct involvement (24).

IgG4-Related Sclerosing Sialadenitis and Dacryoadenitis

Swelling of the salivary glands and lacrimal glands was detected in 24 and 3% of our AIP patients, respectively. Salivary and lacrimal gland lesions usually involve bilateral swelling, are frequently associated with cervical lymph node swelling, and respond well to steroid therapy. Histologically, they are IgG4-related sclerosing sialadenitis and dacryoadenitis, which consist of dense infiltration of IgG4-positive plasma cells and lymphocytes and fibrosis. These swellings sometimes precede AIP (25, 26). Mikulicz's disease has been recently recognized as the same lesion as IgG4-related sclerosing sialadenitis and dacryoadenitis (27).

IgG4-Related Retroperitoneal Fibrosis

In our series, retroperitoneal fibrosis occurred preceding AIP ($n=1$), simultaneous with AIP ($n=2$), and subsequent to AIP ($n=1$). Retroperitoneal masses consisted of fibrosis with infiltration of IgG4-positive plasma cells and obliterative phlebitis and responded well to steroid therapy (28).

Other IgG4-Related Conditions

Other reported IgG4-related conditions include tubulointerstitial nephritis (29), interstitial pneumonia (30), inflammatory pseudotumors of the liver (31), lung (32) and breast (33), prostatitis (34), inflammatory aortic aneurysm (35), and lymphadenopathy (36).

Inflammatory Bowel Disease and AIP

Chronic pancreatitis in the setting of IBD has been reported (37), but relationship between AIP and IBD is unknown. In our series, two AIP patients (3%) also experienced ulcerative colitis (UC). One was a 45-year-old male who had suffered total colon-type UC since the age of 14 and

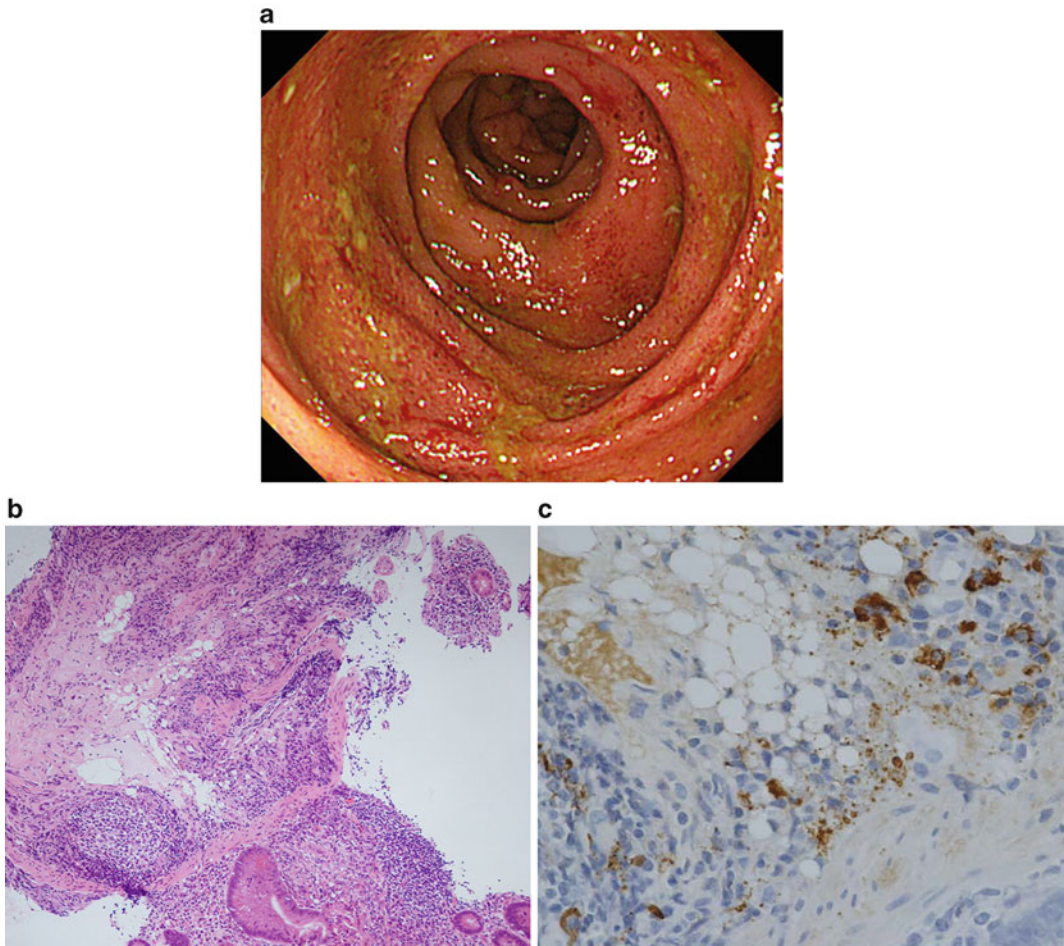


Fig. 48.4 A patient associated with both autoimmune pancreatitis and ulcerative colitis. (a) Colonoscopy showing mild inflammation with diffuse erythema, granularity and exudate, and patchy subepithelial hemorrhage in the transverse colon. (b) Histology of the biopsy specimen taken from the lesion showing colitis with neutrophilic infiltration (HE). (c) Immunohistochemically, infiltration of many IgG4-positive plasma cells were detected (IgG4-immunostaining)

developed diffuse-type AIP. His serum IgG4 level was 180 mg/dl and both AIP and UC responded well to steroid therapy. The other patient was a 32-year-old male who developed both segmental-type AIP of the pancreatic body and tail and total colon-type UC simultaneously (Fig. 48.4a). His initial symptoms were upper abdominal pain and bloody diarrhea, and AIP occurred as acute pancreatitis. His serum IgG4 level was normal (45 mg/dl), but infiltration of many IgG4-positive plasma cells was detected in the colonic mucosa (Fig. 48.4b, c). Both AIP and UC improved after steroid therapy. In the Mayo Clinic (38), 4 (6%) of 71 AIP patients had IBD (3 UC and 1 Crohn's disease). They were all males and had a median age at diagnosis of AIP of 54 years (range, 44–70 years). UC preceded AIP in all patients, whereas diagnosis of AIP and Crohn's disease was simultaneous. Serum IgG4 levels were elevated in one of two patients. Infiltration of many IgG4-positive cells was detected on the biopsy sample from one UC patient, whereas no IgG4-positive cells were seen on the sample from the patient with Crohn's disease. Infiltration of many IgG4-positive cells can be detected in the colonic mucosa of AIP patients without the presence of colonic disease (39, 40). Matsui et al. (41) reported on an AIP patient with a colonic polyp

(ascending colon) containing many IgG4-positive plasma cells who developed colonic polyposis (descending colon) containing many IgG4-positive plasma cells 1 year after complete remission of AIP with steroid therapy. The polyposis was markedly reduced by re-administration of steroid. They suggested that overreactivity to antigens derived from intestinal microflora underlies the immunopathogenesis of AIP involving the colonic mucosa. In our series, AIP developed in three patients after surgery for colonic cancer.

The other type of AIP has been reported mainly in the United States and Europe (42, 43). This AIP is histologically characterized by neutrophilic infiltration in the pancreas, which is not seen in LPSP, and is called idiopathic duct-centric pancreatitis (IDCP) (42) or AIP with granulocytic epithelial lesion (GEL) (43). IDCP is sometimes detected in Western countries, but is uncommon in Japan and Korea (44). It is reported that IDCP affects younger patients than LPSP, may not have a male preponderance and does not have sclerosing extrapancreatic lesions, but is sometimes associated with IBD and acute pancreatitis (7, 44). In the series by Notohara et al. (42), one case of UC and one case of Crohn's disease was noted in 13 IDCP patients. Zamboni et al. (43) reported five cases of UC and two cases of Crohn's disease in 24 patients with AIP with GEL. In an Italian series (45), UC occurred in 26 (30%) of 87 AIP patients, Crohn's disease occurred in 3 (3%), and acute pancreatitis occurred in 28 (32%). IBD is rare in LPSP patients but is occasionally associated with IDCP.

Conclusion

AIP is a unique pancreatitis with a presumed autoimmune etiology, it is characteristically responsive to steroid therapy. AIP is diagnosed on the basis of a combination of clinical, radiological, serological, and histopathological features. AIP and its various extrapancreatic lesions represent manifestations of IgG4-related systemic sclerosing disease. Although malignant tumors are frequently suspected on initial presentation, IgG4-related sclerosing disease should be considered in the differential diagnosis to avoid unnecessary surgery. IBD is rare in LPSP patients but is occasionally associated with IDCP.

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Chapter 49

Extraintestinal Manifestations of Inflammatory Bowel Disease: Cutaneous and Oral Manifestations of Inflammatory Bowel Disease

Caroline P. Allen and Susan M. Burge

Keywords Skin • Oral • Erythema nodosum • Neutrophilic • Pyoderma gangrenosum • Sweet's • Peri-stomal • Immunobullous • Toxic epidermal necrolysis

Introduction

Cutaneous and oral manifestations occur in 6–30% of patients with ulcerative colitis and up to 60% of patients with Crohn's disease, but may precede the diagnosis of inflammatory bowel disease (IBD) by several years. The skin or oral mucosa may be affected directly by IBD or indirectly by its treatment, but the prevalence of some inflammatory dermatoses is also increased in IBD (Table 49.1) [1]. Psoriasis is approximately five times more common in Crohn's disease patients, and genes in the IL-12/IL-23 pathway have been associated with both diseases. T-helper 17 cells may play a role in the development of psoriasis as well as Crohn's disease [2]. Conditions such as aphthous ulcers, erythema nodosum, and some neutrophilic dermatoses (pyoderma gangrenosum, Sweet's syndrome, bowel-associated dermatosis-arthritis syndrome) are associated with IBD, but the pathogenesis of the relationship is uncertain. In this chapter we discuss the presentation and management of the more common and important cutaneous and oral problems that may be seen in IBD.

Nutritional Deficiencies

Malnutrition is a well-documented complication of IBD caused by problems such as inadequate diet, malabsorption and chronic inflammation of the gastrointestinal tract with bleeding, diarrhea, or bowel fistulae [3]. Patients may be deficient in protein, carbohydrate, vitamins, minerals, and/or

C.P. Allen, MA, MBBS, MRCP (✉)

Department of Dermatology, The Churchill Hospital, University of Oxford, Headington, OX3 0NH, UK

e-mail: caroline.allen@gmail.com

S.M. Burge, BSc, DM, FRCP

Department of Dermatology, The Churchill Hospital, Old Road, University of Oxford, Headington, Oxford OX3 7LJ, UK

Table 49.1 Cutaneous manifestations in inflammatory bowel disease

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- Nail clubbing and palmar erythema
 - Erythema nodosum
 - Aphthous ulcers and glossitis
 - Thrombophlebitis
 - Perianal fissures, fistulae, and abscesses – more severe in Crohn’s disease than ulcerative colitis
 - Genital or orofacial Crohn’s disease
 - Peri-stomal dermatitis or folliculitis
 - Cutaneous adverse drug reactions
 - Manifestations of nutritional deficiency
 - Psoriasis (increased prevalence in Crohn’s disease)
 - Neutrophilic dermatoses such as pyoderma gangrenosum, Sweet’s syndrome, and Bowel Associated-Dermatosis-Arthritis Syndrome
 - Vitiligo
 - Autoimmune bullous diseases (a rare association): bullous pemphigoid, linear IgA disease, and epidermolysis bullosa acquisita
-

essential trace elements, but iron deficiency caused by chronic blood loss is particularly common. Cutaneous features of nutritional deficiency include:

- Itching (iron deficiency may cause itching)
- Pallor (iron deficiency anemia)
- Dryness of the skin with scaling
- Dry hair and brittle nails (fatty acid deficiency)
- Hyperpigmentation (generalized or localized)
- Hair loss (iron deficiency)
- Bruising, petechiae, oral bleeding (Vitamin K or Vitamin C deficiency)
- Angular stomatitis, cheilitis, glossitis (smooth sore red tongue), mucosal erosions (deficiency of Vitamin B complex or folic acid)
- Perifollicular hemorrhage and follicular keratoses on upper arms, back, buttocks, and lower extremities containing “corkscrew” coiled hairs (Vitamin C deficiency, i.e. scurvy)
- Delayed wound healing
- Follicular hyperkeratosis, mainly affecting the extensor surfaces of extremities (Vitamin A deficiency)

Dry, itchy skin, in the absence of an alternative explanation such as eczema, raises the possibility of nutritional deficiency. Exclude other causes of itch (Table 49.2). Practical steps that may help to relieve itchy dry skin are outlined in Table 49.3.

Zinc Deficiency

Zinc deficiency may be secondary to malabsorption or to parenteral nutrition without zinc. Typical features of deficiency include:

- Scaly erythematous rash resembling psoriasis on hands and feet, but often with the addition of vesicles or pustules
- Pustular paronychia, i.e. pustules and boggy swelling of the nail fold
- Periorificial (perioral, periorbital, and perianal) crusted papules and plaques which may be vesicular or pustular

Table 49.2 Causes of itch

Primary skin disease, e.g. eczema, psoriasis, urticaria
Infestation (scabies, fleas, lice)
Drugs including statins (also cause dryness), ACE inhibitors, opiates, barbiturates, antidepressants
Systemic disease
• Iron deficiency
• Thyroid disease
• Cholestatic jaundice
• Chronic renal failure
• Polycythaemia
• Lymphoma
• HIV infection

Table 49.3 Management of itchy skin

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- Emollients – prescribe 500 g tubs and apply at least x2/day. Creams are preferable for day-time use and greasier oil-based emollients for the evening
 - Avoid soap, instead recommend a soap substitute
 - Aqueous cream with 1% menthol may relieve itch if emollients are not effective
 - Moderate or potent topical corticosteroid ointments applied x1 or x2/day may be helpful if skin is inflamed
 - Sedating antihistamines may be helpful at night
 - Trim nails to reduce damage from scratching
-

- Photophobia with blepharitis and conjunctivitis
- Slow hair growth or generalized alopecia
- Nail dystrophy with Beau's lines in chronic zinc deficiency
- Delayed healing of wounds
- Secondary bacterial and fungal infections

Niacin Deficiency

Pellagra caused by niacin deficiency has been reported in Crohn's disease. Pellagra is characterized by a symmetrical dermatitis on light-exposed skin (the only sign in one-third of patients), diarrhea, and dementia. A shiny edematous erythema develops on the exposed skin of nose and cheekbones (malar erythema), anterior neck (Casal's necklace), and dorsa of the hands. The rash may blister and the skin becomes scaly with time. Heat, friction, or pressure triggers the rash. The acute erythema and superficial scaling fade to leave reddish-brown pigmentation. Typically the lips are dry and cracked, the tongue swollen, and the buccal mucosa dry and smooth.

Cutaneous and Oral Crohn's Disease

Definitions

1. Cutaneous Crohn's disease: Crohn's disease affecting the skin at sites in direct continuity with the gastrointestinal tract, particularly perianal, perineal, vulval, and peri-stomal sites.
2. Orofacial Crohn's disease: Crohn's disease affecting the lips and oral mucosa.
3. Metastatic Crohn's disease: Crohn's disease affecting the skin at sites that are separated from the gastrointestinal tract by normal tissue [4].

Epidemiology and Associations

Cutaneous, orofacial, and metastatic Crohn's disease are rare. They usually present after the onset of intestinal Crohn's disease, but may precede the onset of intestinal disease by months to years. Mean age at presentation is 34 years and cutaneous disease is more common in woman [4, 5].

Pathogenesis

The pathogenesis of Crohn's disease is discussed in the epidemiology and immunobiology chapters of this book.

Clinical Features

The morphology of cutaneous Crohn's disease is variable. Patients may have a single lesion or widespread disease, but perianal disease is often an early feature. Signs such as perianal erythema, polypoid tags, moist vegetating plaques, perianal fissures, and sinus tracts occur in around one-third of patients. Aphthous ulcers may involve the anal canal and anal sphincter. Severe disease is associated with abscesses and fistulae that may result in scarring with deformity. Crohn's disease may also involve surgical sites including laparotomy scars.

Metastatic Crohn's disease affects the genitalia, lower extremities, and flexures. Patients present with dusky erythematous papules, plaques, nodules, or ulcers exuding pus. Genitalia may be erythematous, swollen and indurated, particularly in children.

Orofacial involvement causes persistent lip or cheek swelling with angular cheilitis, indurated fissuring of the lower lip, and gingival erythematous papules. The edematous infiltrated buccal mucosa develops a "cobblestone"-like appearance. Aphthous ulcers are common [4–6].

Diagnosis and Histopathology

The differential diagnosis in cutaneous or metastatic Crohn's disease is broad and includes granulomatous infections (mycobacterial, spirochaetal, deep fungal), foreign body reactions, and cutaneous sarcoidosis. Genital or inguinal disease may simulate hidradenitis suppurativa (Fig. 49.1), but the grouped open comedones (blackheads) that are a feature of hidradenitis are not present in Crohn's disease. Genital swelling may be caused by obstructive lymphedema. Ulcers in Crohn's disease may resemble pyoderma gangrenosum.

Orofacial Crohn's disease simulates granulomatous cheilitis (orofacial granulomatosis), a poorly understood condition that causes persistent non-tender swelling of the lips. Rarely these patients develop facial nerve palsy and a fissured tongue (Melkersson-Rosenthal syndrome). In some of these patients lip swelling is triggered by an allergic reaction to foods, food additives, or flavorings such as cinnamates, but this is not common.

Skin biopsy reveals superficial and deep non-caseating granulomas that may be difficult to differentiate from cutaneous sarcoidosis, but the presence of a lymphocyte-rich infiltrate, ulceration, and edema favor Crohn's disease [4]. Tissue should be cultured to exclude infection.

Chest radiology should be performed to exclude sarcoidosis and patch testing considered in patients with lip swelling.

Fig. 49.1 Chronic fistulae, abscesses, and scarring in hidradenitis suppurativa may be difficult to differentiate from cutaneous Crohn's disease. Grouped open comedones (blackheads) in affected skin suggest hidradenitis and patients with hidradenitis may have a long history of recurrent boils in axillae, breasts, groins, vulva, or perianal skin



Fig. 49.2 Irritant contact dermatitis is a common problem around stomas



Treatment

Treatment is difficult and the course tends to be chronic. Medical options include oral metronidazole (250 mg x3/day), corticosteroids (very potent topical, intralesional, or systemic), sulfasalazine, dapsone, azathioprine, and TNF-alpha inhibitors (infliximab, adalimumab) [5].

Low-dose antibiotics (e.g. penicillin V 250 mg x2/day for 6 months) may have a role in patients with persistent lip swelling and fissuring to prevent streptococcal infection and lymphatic damage.

Peri-stomal Skin Problems

Skin disorders are reported in >70% of patients with an abdominal stoma, despite the use of hydrocolloids to secure appliances and protect the skin [7]. Irritant contact dermatitis is common (Fig. 49.2), often with secondary infection, and chronic irritation causes over-granulation or erosions at the mucocutaneous junction. Potent topical corticosteroids settle inflammation, but may impair adhesion of the bag. Betamethasone valerate (0.1%) aqueous lotion can be applied to the adhesive surface of the stoma appliance and any alcohol allowed to evaporate before placing the appliance on the skin. Sucralfate powder prevents irritation and may promote healing of erosions [7].

Allergic contact dermatitis is uncommon, but patch testing is sensible to exclude allergy if dermatitis is difficult to control.

Shaving the skin to help the bag to adhere may cause a bacterial folliculitis. Take skin swabs for microbiological culture and treat with antibiotics as well as an antiseptic wash [7].

Peri-stomal psoriasis can usually be controlled with topical corticosteroids. Peri-stomal pyoderma gangrenosum is a rare problem that is discussed below.

Erythema Nodosum

See Table 49.4.

Definition

Erythema nodosum is a septal panniculitis most commonly affecting the shins.

Epidemiology and Associations

Erythema nodosum is the commonest form of panniculitis. It presents most often in Northern European women. Most cases appear between the second and fourth decades, with a peak incidence between the ages of 20 and 30 years. In the Northern hemisphere the incidence peaks in late winter and early spring suggesting an environmental trigger, perhaps streptococcal infection [8].

Erythema nodosum is common in IBD; studies suggest it is present in up to 15% of patients with Crohn's disease and 10% of patients with ulcerative colitis. The presence of erythema nodosum may correlate with underlying disease activity. Sarcoidosis is one of the commonest causes in the Western world [9]. Erythema nodosum usually occurs in the acute phase in association with bilateral hilar lymphadenopathy [8]. A strong relationship exists to upper respiratory infections with Group A hemolytic streptococcus – the nodules develop 2–3 weeks after the infection. Tuberculosis (now an uncommon cause in Europe), atypical mycobacterial infections, HIV, and hepatitis are among other infectious triggers [9, 10]. Numerous medications have been implicated including the oral contraceptive pill, bromides, sulphonamides, penicillin, granulocyte colony stimulating factor, and codeine [9]. Other associations include coeliac disease, rheumatoid arthritis, Behçet's disease and Still's disease. Erythema nodosum has also been reported in association with malignancies, both hematological and solid organ [11]. No underlying cause is found in 40–60% of patients [9].

Table 49.4 Erythema nodosum: key features

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- Bilateral tender warm plaques and nodules usually affecting the anterior shins
 - Nodules resolve spontaneously without ulceration or scarring
 - Histopathology reveals a septal panniculitis without vasculitis
 - Associations – numerous underlying conditions
 - Infections particularly streptococcal throat infections and tuberculosis
 - Sarcoidosis
 - Inflammatory bowel disease
 - Pregnancy
 - Drugs including the oral contraceptive pill, bromides, and sulphonamides
-

Fig. 49.3 Tender erythematous nodules on the shins in erythema nodosum



Etiology and Pathogenesis

Despite the numerous well-defined provocations, the pathogenesis of erythema nodosum is unclear. The disease may be a hypersensitivity response involving deposition of immune complexes around venules in subcutaneous fat or a type IV delayed hypersensitivity reaction [9].

Clinical Features

Patients develop tender, erythematous, warm nodules, measuring 1–5 cm or more in diameter in a symmetrical distribution on the shins, ankles, and knees (Fig. 49.3). Less frequently, nodules appear on the arms or trunk. Fever, malaise, and headache are common. Some patients complain of abdominal pain, vomiting, or diarrhea.

Nodules do not ulcerate, but fade over 2–6 weeks without loss of fat or scarring, leaving a bruise-like discoloration that resolves more slowly. Resolution is usually more rapid in children.

A chronic migratory variant (subacute nodular migratory panniculitis, erythema nodosum migrans) is much less common; the indurated erythematous plaques are tender and may be asymmetrical.

Diagnosis and Histopathology

Investigations should ensure there is no other underlying cause in patients with IBD. FBC, ESR, urinalysis, and chest radiography should always be performed, but further investigations should be guided by the clinical history and examination. Local prevalence of etiological factors such as bacterial, viral, fungal, or protozoal infections should be considered and investigation directed accordingly.

A deep elliptical biopsy including fat is required to demonstrate the typical changes, but is rarely necessary. Histopathology reveals a septal panniculitis, generally with a superficial and deep perivascular lymphocytic infiltrate in the overlying dermis, but no vasculitis [9].

Differential diagnosis includes trauma, cellulitis, insect bites, and superficial thrombophlebitis. Nodular vasculitis is another panniculitis, sometimes associated with tuberculosis, that causes symmetrical nodules on the legs; however, these nodules tend to be present on the calves rather than the shins and the nodules ulcerate and heal with a depressed scar caused by loss of fat. Histopathology reveals a lobular panniculitis with vasculitis and fat necrosis in contrast to erythema nodosum [10].

Treatment

Most cases resolve spontaneously within six weeks. Relapses are more common in patients with idiopathic disease or in those with preceding upper respiratory tract infection than in patients with an underlying problem such as IBD [9].

Pain should be managed with regular non-steroidal anti-inflammatory drugs. Elevation of the legs and support stockings may help to control swelling and speed resolution. Generally oral corticosteroids are not required. Potassium iodide 200–600 mg/day has been recommended in persistent disease; however, this is contraindicated in pregnancy, and has been associated with hypothyroidism [9].

Pyoderma Gangrenosum

See Table 49.5.

Definition

Pyoderma gangrenosum is an uncommon neutrophilic dermatosis characterized by rapidly enlarging ulcers that are usually extremely painful. The condition was described in 1916 by Brocq, and further characterized by Brunstung, Goeckerman, and O’Leary in 1930, who coined the term pyoderma gangrenosum.

Epidemiology and Associations

The annual incidence of pyoderma gangrenosum is estimated at 3–10 patients per million. Pyoderma gangrenosum is most common in middle-aged adults and has an equal sex distribution; however, it has been described in children and in the elderly. Fifty percent of patients with pyoderma gangrenosum have an associated systemic condition and in approximately one-third this is IBD, more often ulcerative colitis than Crohn’s disease. Other associations include rheumatoid arthritis, seronegative arthritis, and hematological malignancy (particularly myeloid leukemia). Twenty percent of patients have a monoclonal gammopathy of uncertain significance, most commonly an IgA gammopathy [12, 13].

Table 49.5 Pyoderma gangrenosum: key features

• Painful ulcer with irregular violaceous undermined border • Slough or hemorrhagic base • Purulent discharge • Rapid enlargement (usually) • History of minor trauma preceding ulcer • Sterile pustule preceding ulcer • Pain out of proportion to the ulcer • Healing with cribriform scarring • Responds to systemic corticosteroids

Fig. 49.4 Classical pyoderma gangrenosum. The deep painful ulcer has an irregular, undermined inflammatory border and a necrotic base



Etiology and Pathogenesis

The etiology and pathogenesis of pyoderma gangrenosum are not well understood, but may be linked to abnormalities in neutrophil function. IgA gammopathies that impair neutrophil function in vitro are associated with pyoderma gangrenosum. Interleukin-8 (IL-8), a leukocyte chemotactant, is over-expressed in pyoderma gangrenosum and ulceration can be induced in xenografts transfected with recombinant human IL-8 [13]. Another clue to the role of neutrophil dysfunction is provided by the dominantly inherited autoinflammatory disorder, PAPA (pyogenic sterile arthritis, pyoderma gangrenosum and acne). PAPA is caused by mutations in a gene that encodes proline serine threonine phosphatase-interacting protein 1 (PSTPIP-1). PSTPIP-1 co-localizes with pyrin in neutrophils. Normally pyrin down-regulates inflammation, but in PAPA, the altered PSTPIP-1 binds more avidly than normal to pyrin, inhibiting its action and leading to activation of IL-1 β and the accumulation of neutrophils [14].

Clinical Variants of Pyoderma Gangrenosum

A number of variants have been described, but the classical and pustular forms of pyoderma gangrenosum are those most often associated with IBD [13].

Classical pyoderma gangrenosum is characterized by a painful ulcer with an irregular, undermined violaceous (bluish-red) border and a necrotic base producing a purulent or hemorrhagic exudate (Fig. 49.4). Patients may have a fever and considerable pain. Typically the ulcers enlarge rapidly. The border may become serpiginous if ulceration extends at different rates in different directions. Healing occurs from the edge resulting in a characteristic pattern of scarring with perforations known as “cribriform” (sieve-like) scarring. Pyoderma gangrenosum triggers neither lymphadenopathy nor lymphangitis, despite the inflammatory appearance. Approximately 20% of patients demonstrate pathergy (new lesions are initiated by minimal trauma). Pyoderma gangrenosum is most common on the lower limbs, the trunk is affected in 10% of patients and lesions on the head, groin, genitalia, or upper extremities are less common. A sterile neutrophilic infiltrate may be found in other organs, e.g. lungs, heart, liver, spleen, gastrointestinal tract, and lymph nodes.

Rarely pyoderma gangrenosum develops around the stoma of patients with IBD (Fig. 49.5). Peristomal pyoderma gangrenosum may present from a few months to many years after the formation of the stoma. Pathergy is thought to play a role in the pathogenesis. Skin may be irritated by leakage of stomal contents or by adhesives used to attach the stoma bag.

Fig. 49.5 Peri-stomal pyoderma gangrenosum in this patient responded promptly to treatment with a very potent topical corticosteroid



A pustular form of pyoderma gangrenosum has been described in association with active IBD. Discrete sterile pustules, 0.5–2 cm in diameter, with a surrounding erythematous halo occur in association with joint pains and fever. Pustules may heal without scarring or progress to the ulcerated lesions of classical pyoderma gangrenosum. The activity of pustular pyoderma gangrenosum tends to follow the activity of the bowel disease and may improve when the underlying disease is treated. Pyostomatitis vegetans may be the mucosal form of pustular pyoderma gangrenosum. This rare condition is associated with ulcerative colitis more often than Crohn's disease. Friable grey–yellow pustules may involve oral, vaginal, nasal and, rarely, periocular mucosa. Ruptured pustules leave vegetating erosions or ulcers. Even extensive oral involvement may be relatively painless [15].

Bullous pyoderma gangrenosum has been recorded in association with hematological malignancy rather than IBD. Painful vesicles and bullae lead to superficial erosions. Sweet's syndrome (see below) may have a similar appearance. Superficial granulomatous pyoderma gangrenosum is a localized, less aggressive variant that tends to involve the head and neck. Underlying disease is unusual and some cases may be an atypical form of Wegner's granulomatosis [11, 13].

Differential Diagnosis

Pyoderma gangrenosum may simulate a variety of conditions and the diagnosis can be difficult if the clinical signs are atypical. Infection and malignancy are the most important conditions to exclude (see Table 49.6).

Table 49.6 Pyoderma gangrenosum: differential diagnosis

Vascular	Venous or arterial insufficiency, occlusive vasculopathy including antiphospholipid antibody syndrome
Infectious	Bacterial including mycobacterial, viral, deep fungal, parasite
Malignancy	Primary cutaneous, lymphoma, metastatic
Medications	Hydroxyurea
Inflammatory	Sweet's syndrome, Wegner's granulomatosis, panniculitis, Autoimmune bullous diseases
Trauma	Bites-insect, spider, snake; Dermatitis artefacta

Investigations

- Take a deep cutaneous biopsy from the edge of the ulcer. Tissue should be sent for both histopathological examination and for culture (including atypical mycobacterial and fungal culture). Histopathological findings of follicular or perifollicular inflammation, intradermal abscesses, and/or sterile dermal neutrophilia without vasculitis are supportive of the diagnosis, but are not diagnostic. Clinicopathological correlation is vital.
- Surface swabs are of limited value as bacterial colonization is inevitable, but blood cultures should be performed if systemic infection is suspected.
- Blood tests should include FBC, ESR, CRP, protein electrophoresis, and immunoglobulins.

Course and Prognosis

The course of pyoderma gangrenosum is unpredictable; the disease may progress rapidly or be slowly progressive. Lesions may resolve spontaneously and can return for no obvious reason after being quiescent for many years. It was believed that the activity of pyoderma gangrenosum followed that of the underlying bowel disease, but flares of pyoderma gangrenosum have been reported in patients after bowel resection and in patients with inactive ileitis. Severe pyoderma gangrenosum has a mortality of 30%. Poor prognostic factors include older age at first onset, male sex, and bullous disease associated with underlying hematological malignancy [13].

Treatment

Treatment is challenging. Treatment of underlying bowel disease should be optimized, but does not always lead to improvement in pyoderma gangrenosum. It is helpful to monitor the response to treatment objectively by measuring ulcers and by clinical photography [16].

General measures for wound management are important, but surgical debridement may trigger extension of pyoderma gangrenosum and should be avoided. Soaks or wet dressings with 0.01% potassium permanganate solution may help to reduce bacterial colonization. Moisture retaining wound dressings facilitate wound healing and help with pain control. Alginate dressings absorb exudate and prevent maceration.

Localized pustules, bullae, or small ulcers may be managed with topical treatments such as a very potent topical corticosteroid ointment or 0.1% tacrolimus ointment. Peri-stomal pyoderma gangrenosum may also respond to a very potent topical corticosteroid or 0.1% tacrolimus in carmellose sodium paste [7].

Most pyoderma gangrenosum requires systemic treatment. Systemic corticosteroids (prednisolone 1–2 mg/kg/day or pulses of intravenous methylprednisolone) should be started promptly to prevent progression. Response is usually rapid, but prolonged treatment may be required with combinations of drugs. Steroid-sparing agents such as ciclosporin, methotrexate, or dapsone may have a role. Recent data suggest that TNF alpha-blocking agents (infliximab, adalimumab, etanercept) may be very effective and these agents may also be indicated to treat underlying IBD [16].

Pain can be severe and require systemic opiates. Pain levels should be documented and can be used as a marker of treatment efficacy. Increasing pain or exudate may indicate secondary bacterial infection.

Sweet's Syndrome

See Table 49.7.

Definition

Sweet's syndrome is a neutrophilic dermatosis characterized by erythematous papules, nodules and plaques, fever and neutrophilia. The syndrome was first described by Robert Sweet in 1964 [17].

Epidemiology and Associations

The disease is more common in women, and classically presents between the ages of 30 and 50 years, with no racial predilection [18].

Sweet's syndrome may be precipitated by infection of the upper respiratory tract or gastrointestinal tract. In other cases the syndrome is associated with an underlying systemic disease such as IBD, chronic arthritis, or malignancy, particularly hematological malignancies, e.g. acute myelogenous leukemia and less commonly myeloproliferative disease or myelodysplastic syndromes, lymphoma, or paraproteinaemias. Solid organ tumors, generally those of the breast, genitourinary system, and gastrointestinal tract, have also been linked to Sweet's syndrome. Pregnancy, vaccination, and drugs, including granulocyte colony-stimulating factor (G-CSF), may also trigger the syndrome [17, 18].

Etiology and Pathogenesis

The etiology is unclear. An antigen of infectious or tumor origin may trigger a hypersensitivity reaction. Granulocyte-colony stimulating factor (G-CSF) and interleukin-6 appear to play a role.

Table 49.7 Sweet's syndrome: key features

-
- Tender erythematous plaques and nodules on the head, neck, and upper extremities
 - Fever >38°C
 - Leukocytosis with neutrophilia and raised ESR
 - Associations: preceding infection, IBD, malignancy, pregnancy, drugs
-

Fig. 49.6 The tender, edematous erythematous plaques of Sweet's syndrome are often misdiagnosed as cellulitis



G-CSF is a known precipitant; tumors that produce G-CSF have been isolated in patients with Sweet's syndrome and patients with active Sweet's syndrome have been found to have raised levels of serum G-CSF compared to patients in whom the disease was quiescent [18].

Clinical Features

Sweet's syndrome is characterized by the abrupt onset of painful erythematous nodules or plaques associated with a high fever ($>38^{\circ}\text{C}$). Patients appear unwell and may complain of headache, arthralgia, and myalgia. Other extracutaneous features include iritis, episcleritis, and/or conjunctivitis, polyarthrititis, and oral mucosal ulcers which mimic aphthae.

The juicy papules and plaques, which present most often on the face and upper trunk, are tender, well-demarcated, and edematous (Fig. 49.6). The surface of plaques may appear to be vesicular (pseudo-vesicles), reflecting the intense dermal edema, but it is rare to find discrete vesicles or bullae that can be ruptured. Occasionally plaques become pustular. Like pyoderma gangrenosum, Sweet's syndrome demonstrates pathergy, i.e. cutaneous lesions may develop at sites of trauma such as skin biopsies or venipuncture. Patients may also have pyoderma gangrenosum.

Diagnosis and Histopathology

The diagnosis is suggested by the presence of the typical clinical features, in association with a raised ESR, a raised CRP, and a leukocytosis, which is predominantly a neutrophilia. Blood cultures are sterile. See Tables 49.8 and 49.9 for differential diagnoses and investigations.

Treatment

Very potent topical corticosteroids (clobetasol propionate 0.05%) x2/day may control localized disease. Systemic corticosteroids are the first-line treatment for more widespread disease. A typical regime consists of prednisolone 30 mg/day in conjunction with a very potent topical corticosteroid x2/day. Recurrences are common. Steroid-sparing agents that may be helpful include dapsone, colchicine, and ciclosporin [18].

Table 49.8 Sweet's syndrome: differential diagnosis

-
- Infection such as streptococcal cellulitis, bacterial septicaemia, herpes simplex infection, deep fungal infection
 - Cutaneous vasculitis
 - Pustular pyoderma gangrenosum
 - Malignancy- primary cutaneous or metastatic
 - Urticaria or urticarial vasculitis
 - Erythema nodosum
 - Insect bites
-

Table 49.9 Investigations in Sweet's syndrome

-
- FBC and ESR; a neutrophilia and raised ESR are present in Sweet's syndrome
 - Blood cultures and skin swabs from pustular lesions should be performed to exclude infection
 - Take a skin biopsy from a well-developed plaque. Histopathology reveals a dense dermal neutrophilic infiltrate with leukocytoclasia but without vasculitis. Gram stain is negative
 - Generally underlying malignancy or inflammatory disease should be excluded, but these are unlikely associations in a patient with IBD
-

Table 49.10 Bowel-associated dermatosis-arthritis syndrome: key features

-
- Serum sickness like constitutional signs and symptoms of fever and general malaise
 - Crops of erythematous macules, purpuric papules, and small vesicopustules on upper trunk and extremities
 - Migratory polyarthralgia, non-erosive polyarthritis, or tenosynovitis
 - Histopathology reveals a perivascular neutrophilic infiltrate
-

Bowel-Associated Dermatositis-Arthritis Syndrome

See Table 49.10.

Definition

The bowel-associated dermatosis-arthritis syndrome is a neutrophilic dermatosis that is associated with an underlying condition causing an overgrowth of bowel flora. The syndrome comprises cutaneous lesions, non-erosive arthritis, arthralgia, and fever.

Epidemiology and Associations

Early cases were associated with bowel surgery that created a “blind loop,” allowing bacterial overgrowth, but subsequently the condition was observed in patients who had not had this type of surgery. Now it is accepted that any condition that predisposes to bowel stasis, including IBD, can precipitate bowel-associated dermatosis-arthritis syndrome [19, 20].

Etiology and Pathogenesis

The overgrowth of bacteria in the bowel with subsequent deposition of immune complexes is thought to trigger disease. *Escherichia coli* and other bacteria are believed to release peptidoglycans that lead to the formation of immune complexes [21].

Clinical Features

Cutaneous lesions generally affect the upper trunk and extremities, and consist of crops of erythematous macules, purpuric papules, and crusted vesicopustules. Patients also have a migratory polyarthralgia or a non-erosive polyarthritis in association with fever and malaise.

Diagnosis and Histopathology

Diagnosis is based on the characteristic clinical findings and the history of a predisposing bowel condition. Skin biopsy should be performed to confirm the diagnosis. The histopathological findings resemble Sweet's syndrome with dermal edema and a dense neutrophilic infiltrate.

Treatment

The underlying cause should be corrected when possible. Oral antibiotics have been advocated to eradicate bacteria, e.g. oxytetracycline, erythromycin, clindamycin, tetracycline-sulphamethoxazole, or metronidazole. A short course of oral prednisolone may provide symptomatic relief.

Immunobullous Diseases

Definition

The immunobullous diseases are caused by autoantibodies directed against structural proteins in the skin such as keratins, desmosomal proteins, hemidesmosomal proteins, or basement membrane zone proteins. Blisters form because adhesion fails between the keratinocytes (intraepidermal blisters) or within or below the dermo-epidermal junction (subepidermal blisters). Three subepidermal immunobullous diseases have been described in association with IBD: bullous pemphigoid, linear IgA disease (LAD), and epidermolysis bullosa acquisita (EBA).

Etiology and Pathogenesis

The target antigens in bullous pemphigoid, LAD, and EBA are in components of the basement membrane zone. In bullous pemphigoid autoantibodies to collagen XVII, a transmembrane component of

Fig. 49.7 Bullous pemphigoid with urticated papules, tense blisters, and crusted erosions on an erythematous background



the hemidesmosomes, appear to initiate disease. The IgA class autoantibodies in LAD are also directed against epitopes in collagen XVII. Type VII collagen, a component of the anchoring fibrils that fasten the lamina densa of the basement membrane to the underlying papillary dermis, is the antigen in EBA [22].

Epidemiology and Associations

Autoimmune blistering diseases are not common and even less common in patients with IBD. Even bullous pemphigoid, the most common immunobullous disease, is rarely reported in IBD. However, the association between IBD and immunobullous diseases appears to be genuine and may reflect a genetically determined susceptibility to develop autoimmune diseases.

Clinical Features

Bullous pemphigoid presents with itchy urticated papules or plaques and tense blisters, 1–3 cm in diameter, on inflamed or normal-appearing skin (Fig. 49.7). Blistering tends to predominate on the lower trunk, flexural surfaces of limbs, axillae, and groins. Blisters heal without scarring. Oral blisters are present in about one-third of patients.

Blisters in LAD may resemble those in bullous pemphigoid or patients may have small itchy vesicles on extensor surfaces that suggest dermatitis herpetiformis. Patients may have mucosal involvement.

Patients with EBA notice skin fragility or blistering in areas exposed to trauma, e.g. knuckles, elbows, knees, toes. Blisters may be hemorrhagic and underlying skin inflamed or scarred. Patients may develop a nail dystrophy or scarring alopecia. Mucosal lesions are unusual. The signs may suggest porphyria cutanea tarda.

Diagnosis and Histopathology

More common causes of blistering such as infection, acute eczema, or a drug eruption should be excluded before considering an autoimmune blistering disease (see Table 49.11).

Table 49.11 Causes of widespread blistering

• Acute eczema
• Cutaneous infections
• Viral – herpes simplex (may generalize in conditions such as atopic eczema) or varicella-zoster (chickenpox)
• Bacterial – widespread staphylococcal infection (bullous impetigo)
• Burns
• Staphylococcal scalded skin syndrome caused by a circulating exotoxin produced by staphylococcus aureus
• Drug reactions
• Stevens-Johnson syndrome
• Toxic epidermal necrolysis (TEN)
• Autoimmune blistering diseases
• Cutaneous porphyria
• Epidermolysis bullosa (EB) group of diseases (genetic)

To confirm the diagnosis of an immunobullous disease, take blood for indirect immunofluorescence microscopy to look for circulating autoantibodies to antigens in the basement membrane zone and biopsy a pre-bullous urticated lesion or a small blister for histology (blisters are subepidermal). Also snap freeze a biopsy from perilesional skin for direct immunofluorescence microscopy to look for deposits of immunoglobulin and complement.

Treatment

The course of these diseases is punctuated by exacerbations and partial remissions. Blistering in bullous pemphigoid is usually controlled by combinations of very potent topical corticosteroids and systemic corticosteroids (30–60 mg/day). Tetracycline (500–2,000 mg/day) in combination with nicotinamide (500–2,500 mg/day) has been advocated in mild to moderate disease [23]. Systemic corticosteroids, dapsone, intravenous immunoglobulins, plasmapheresis, and rituximab are used with variable success in EBA and LAD.

Cutaneous Adverse Effects of Treatment

Cutaneous adverse drug reactions are common (2–3% of hospitalized patients), the signs diverse, and time courses variable. The predisposition to idiosyncratic drug reactions probably involves both genetic and environmental factors. Reactions may not settle immediately when the drug is withdrawn and some persist for months [1].

Corticosteroids

Cutaneous adverse effects of corticosteroids include cutaneous atrophy, telangiectasia, acne-like pustular rashes and cutaneous infections, both bacterial and candidiasis. Recurrent staphylococcal folliculitis may be a problem in some patients. The nostrils should be swabbed for bacterial culture and nasal carriage of staphylococcus aureus eradicated with mupirocin 2% ointment. Antiseptic skin cleansers or bath additives containing triclosan may be helpful.

Sulfasalazine

Sulfasalazine is a combination of 5-aminosalicylic acid and sulfapyridine. Photosensitivity may be troublesome, but skin failure caused by reactions such as erythroderma, drug rash with eosinophilia and systemic symptoms (DRESS), or toxic epidermal necrolysis is life-threatening.

Photosensitivity

Patients who are photosensitive develop a scaly erythematous rash on exposed skin. The rash tends to involve the prominences of the forehead, the nose, the cheeks, particularly over the cheekbones, the back of the neck, if not covered by hair, and the upper chest where the shirt sits open. The extensor surfaces of forearms and the backs of the hands may also be affected. Involved skin may pigment. Covered skin is spared. Sparing is usually apparent around the orbit (ask the patient to close his eyes to see the eyelids), on the upper lip, under the nose, below the chin, behind the ears and under the hair on the forehead, or at the back of the neck.

Ideally sulfasalazine should be withdrawn, but it may be possible to reduce the impact of photosensitivity with hats, clothing, and regular application of a high-factor sunblock to all exposed skin. A moderate or potent topical corticosteroid ointment will reduce erythema and itch.

Erythroderma

Erythroderma (exfoliative dermatitis) is defined as erythema, with variable amounts of scale, affecting 90% or more of the body surface. Numerous drugs including sulfasalazine have been reported to cause erythroderma, but erythroderma may also be secondary to primary skin diseases such as eczema or psoriasis and, rarely, to cutaneous T-cell lymphoma.

Most of the skin is itchy, erythematous, and scaly. Sometimes the skin is edematous or blisters and oozes serous fluid. Hair and nails may be lost. Fever, malaise, and lymphadenopathy are common.

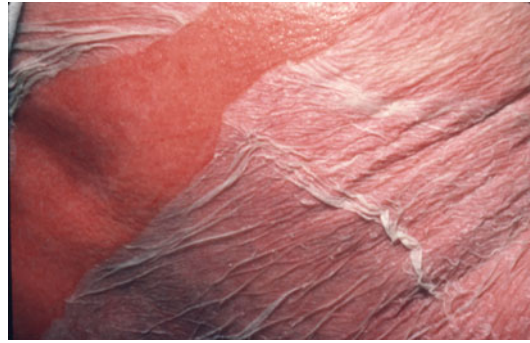
Any potentially causative drug should be withdrawn. Patients lose heat and may become hypothermic, so temperature should be monitored. The skin barrier is inadequate, increasing the risk of infection as well as loss of fluid and electrolytes. Cardiac failure is another complication, particularly in the elderly. Bland emollients applied x3–4/day will soothe itchy inflamed skin and soap should be avoided. A sedating antihistamine will reduce irritation. Potent topical corticosteroids or oral corticosteroids are helpful in erythroderma secondary to drugs or eczema.

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

These life-threatening conditions usually develop 2–3 weeks after starting drugs such as sulfonamides, aminopenicillins, anti-epileptics, or allopurinol. Vague flu-like symptoms (fever, cough, headache, sore throat, rhinorrhea, and malaise) may precede the onset. Epidermal involvement is more severe and widespread in TEN (>30% body-surface area) than in SJS (<10% body surface area).

SJS: Poorly defined, painful erythematous macules evolve rapidly into papules and target lesions, with two or three concentric zones of color change and dark purpuric centers. Large bullae rupture leaving denuded skin. The rash is usually maximal by the fourth day. SJS is characterized by severe mucosal ulceration with involvement of at least two mucosal surfaces (lip, oral cavity, conjunctiva, nasal, urethra, vagina, gastrointestinal tract, respiratory tract). The lips become crusted and hemorrhagic. Painful stomatitis interferes with eating and drinking. Purulent conjunctivitis is associated with photophobia. Late ocular complications include dry eye, scarring, and loss of vision.

Fig. 49.8 Toxic epidermal necrolysis. Large sheets of necrotic epidermis detach leaving a painful, denuded, oozing dermis



TEN: Painful skin or a burning discomfort may herald the onset. The tender dusky erythema progresses to widespread blistering involving <30% of body-surface area. The thin-walled blisters rupture easily and large sheets of necrotic epidermis detach leaving a painful denuded oozing dermis (Fig. 49.8). Even gentle pressure extends blisters and inadvertent shearing pressure when handling the patient may cause further detachment. Mucosal ulceration may cause dysphagia, photophobia, or painful micturition. Epithelia of trachea, bronchi, and gastrointestinal tract may also be involved. Sequelae include scarring, irregular pigmentation, dry eye, corneal scarring, photophobia, visual impairment, phimosis, and vaginal synechiae.

All systemic drugs started within the preceding four weeks should be withdrawn and patients should be admitted to an intensive therapy unit or burn unit for supportive care. IV immunoglobulins, 1 g/kg for 4 days, have been recommended but their role is unproven. Systemic corticosteroids increase the risk of infection and should be avoided [24].

Drug Rash, Eosinophilia, Systemic Symptoms

DRESS (also known as Hypersensitivity Syndrome) is a life-threatening cutaneous reaction. Typically DRESS presents 2–6 weeks after the drug is started with a morbilliform erythematous rash, facial edema simulating angioedema, lymphadenopathy, a high fever, leukocytosis (often with an eosinophilia), and abnormal liver function tests. Some patients progress to liver failure. Kidneys, lungs, or heart may be affected. Any suspected drugs should be withdrawn. Early treatment with oral prednisolone (0.8–1 mg/kg body weight) or IV methyl prednisolone has been recommended to prevent progression [25].

Tumor Necrosis Factor-Alpha (TNF- α) Antagonists

Although these drugs are used in the treatment of psoriasis, paradoxically TNF- α antagonists induce psoriatic-like rashes in some individuals. Plaque, guttate, and pustular psoriasis have been reported as well as pustular reactions on palms and soles (palmoplantar pustulosis). Other skin problems such as eczema, skin infections, and acneiform rashes have also been observed. In one study, 62% of 50 patients with IBD developed a cutaneous reaction within 12 months of starting adalimumab [26]. In many patients it is possible to continue treatment with the TNF- α antagonist or a substitute from the same class while controlling the skin problem with topical treatments, sometimes in combination with systemic agents, under the guidance of a dermatologist [27].

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Chapter 50

Extraintestinal Manifestations of Inflammatory Bowel Disease: Arthritis, Arthropathy and Osteoporosis

Roger Sturrock and Jagtar Singh Nijjar

Keywords Arthritis • Spondylitis • Enthesitis and osteoporosis

Introduction

Musculoskeletal manifestations make up the commonest extraintestinal complaints in the inflammatory bowel diseases (IBDs) [1]. Problems range from peripheral arthritis and ankylosing spondylitis to fibromyalgia and osteoporosis. Although certain musculoskeletal complaints follow the course of gut disease, others do not and therefore require specific rheumatological input. Occasionally, joint disease can precede bowel involvement and in these cases colonoscopy carried out at the time of diagnosis of joint disease shows inflammatory bowel lesions. We will compare Crohn's disease (CD) and ulcerative colitis (UC) in terms of epidemiology, clinical features and treatment options. Furthermore, osteoporosis is a problem in IBD and there is evidence that this may be related to steroid use or the disease process itself.

IBD-related arthropathies are included with other spondyloarthropathies in the ESSG classification outlined in Table 50.1.

As shown above, the spondyloarthropathies have common features and as well as joint disease being an extraintestinal manifestation of IBD, up to 70% of SpA patients have gut involvement in the long term with between 7 and 12% of patients going on to diagnosis of a formal IBD [2].

R. Sturrock, BD, MD, FRCP (✉)

Division of Immunology, Infection and Inflammation, University of Glasgow, Glasgow, UK

Centre for Rheumatic Diseases, Glasgow Royal Infirmary, University of Glasgow,
Castle St, Glasgow G4 2SP, UK

e-mail: rogersturrock@mac.com

J.S. Nijjar, MBChB (Hons.), BSc (Med. Sci.)

Centre for Rheumatic Diseases, Glasgow Royal Infirmary, University of Glasgow,
Castle St, Glasgow G4 2SP, UK

e-mail: jagtar.nijjar@glasgow.ac.uk

Table 50.1	SpA diagnostic criteria according to ESSG
Inflammatory spinal pain or asymmetric and predominantly lower limb synovitis	
Any one of the following of:	
• Positive family history • Psoriasis • Inflammatory bowel disease • Alternate buttock pain • Enthesopathy • Sacroiliitis	

Fig. 50.1 Venn diagram showing inter-relationships between the spondyloarthropathies

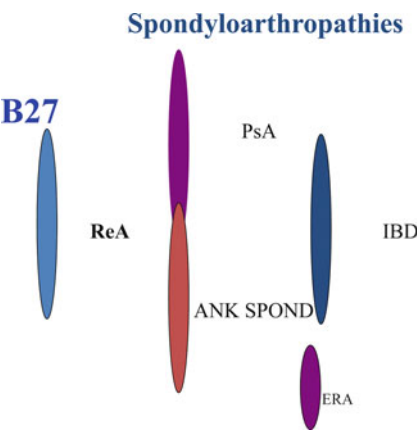


Table 50.2 Clinical manifestations of musculoskeletal disease in Crohn’s disease

Inflammatory back pain
Asymmetrical peripheral arthritis
Symmetrical peripheral arthritis
Enthesitis

Crohn’s Disease

CD is characterized by extra-bowel manifestations one of which is involvement of the musculo-skeletal system. Approximately 20% of CD patients will develop a musculoskeletal problem [3] with 6% presenting with symptoms prior to the clinical manifestation of the IBD [4].

The inflammatory joint disease accompanying Crohn’s is typically seronegative for anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor antibodies and is classified under the general heading “Seronegative spondyloarthritides”. The members of this group include ankylosing spondylitis, reactive arthritis, psoriatic arthritis and the arthritis associated with CD and UC (Fig. 50.1). The typical *clinical* musculoskeletal manifestations seen in CD are listed in Table 50.2.

Inflammatory Back Pain

This is characterized by low back pain and stiffness of more than 3 months duration, exacerbated by inactivity and relieved by NSAIDs. It is characteristically worse in the morning and may wake

Fig. 50.2 X-ray of pelvis showing bilateral sacroiliitis in classical ankylosing spondylitis

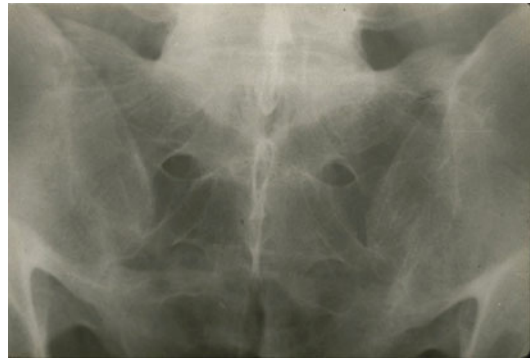
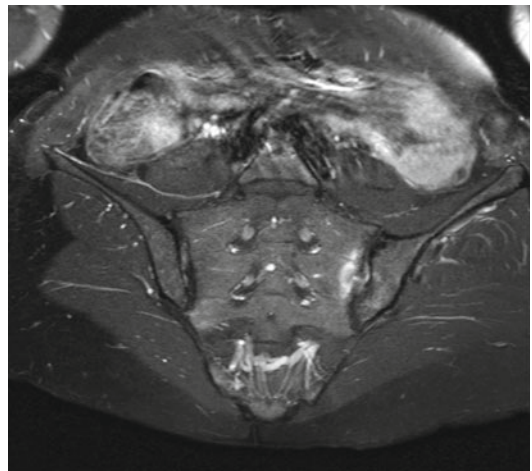


Fig. 50.3 MRI of the sacroiliac joints showing bone oedema of the L sacroiliac joint in unilateral sacroiliitis



up the patient in the early hours of the morning. The pain often alternates from one buttock to the other. Inflammatory back pain is a key feature in making the diagnosis of ankylosing spondylitis where it is associated with bilateral sacroiliitis (Fig. 50.2). However, sacroiliitis is often asymmetrical in CD and may be asymptomatic and only detected by MRI (Fig. 50.3) [5].

Back pain associated with inflammatory spinal disease may also occur independently of the level of bowel-related inflammation [6].

Classical ankylosing spondylitis (AS) is characterized by bilateral sacroiliitis, bony fusion of the intervertebral bodies resulting in spinal stiffness and a typical “question mark” posture in severe cases of the disease (Fig. 50.4).

Although CD may occur in association with full blown AS, it usually features sacroiliitis without progressive spinal disease [7].

Asymmetrical Peripheral Arthritis

The most commonly involved joints are the knees, ankles and the small joints of the hand such as the wrist and the proximal interphalangeal joints but any synovial joint may be involved [8]. Typically, the patient presents with a swollen and painful knee or ankle, and the joint inflammation may be a harbinger of a flare of the CD. Involvement of the digits presents as a dactylitis. X-rays show joint swelling but no bone erosion.

Fig. 50.4 Classical posture in ankylosing spondylitis



Symmetrical Peripheral Arthritis

Occasionally there may be a symmetrical arthritis involving the small joints of the hand with swelling of the metacarpophalangeal and proximal interphalangeal joints in a pattern similar to that seen in rheumatoid arthritis. Radiographs of affected joints may show soft tissue swelling but not deforming or erosive features; however, they may reveal other causes of joint pain such as osteoarthritis or gout.

Enthesitis

The enthesitis is the junction of ligament to bone and is often a site of inflammation in the seronegative spondyloarthropathies such as AS. Common sites that are associated with local pain and tenderness are shown in Fig. 50.5.

Enthesitis can be difficult to detect on clinical examination but ultrasound imaging will reveal inflammation at classical sites such as the insertion of the achilles tendon to the calcaneus. (Fig. 50.6)

The patient with CD will complain of what appears to be non-specific pains around the shoulder, chest and heels but on clinical examination is found to be tender at the enthesitis sites. Enthesitis occurs in 5–10% of UC patients and dactylitis in 2–4%, whereas the prevalence in CD is 15.3% although dactylitis rates are similar in both conditions [9].

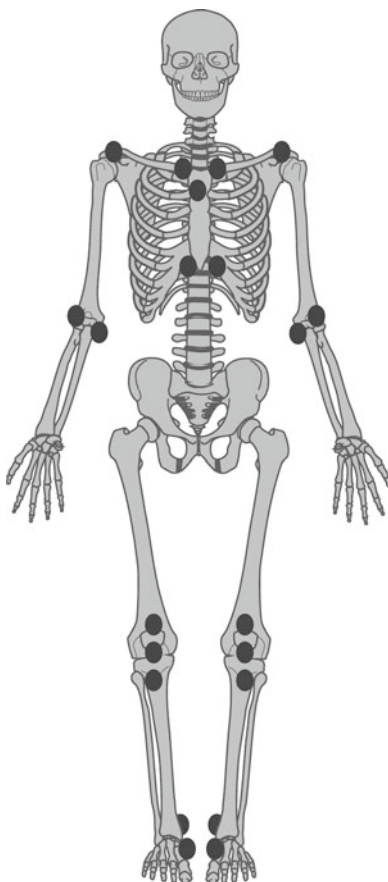


Fig. 50.5 Dark spots show common enthesitis sites

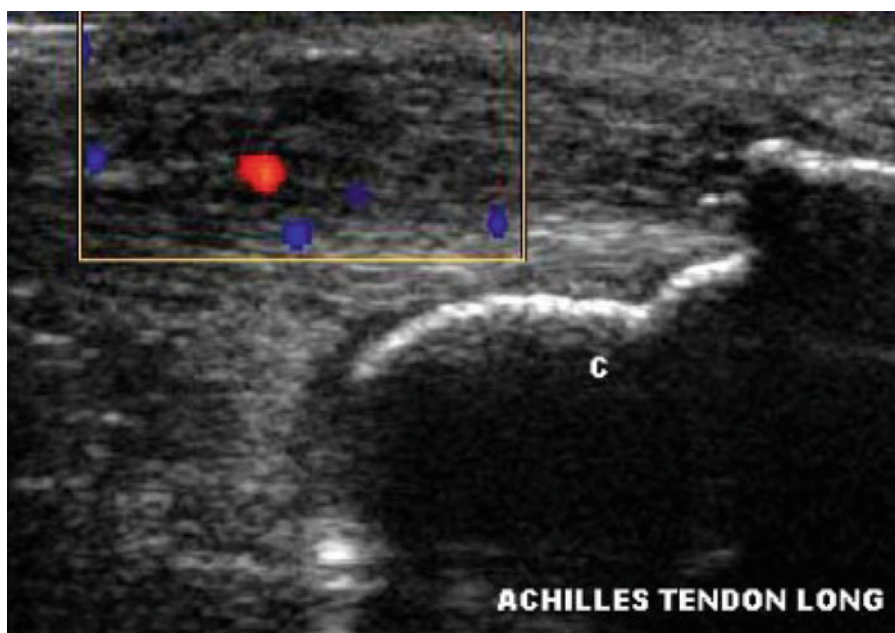


Fig. 50.6 Power Doppler signal and thickening of the achilles tendon in enthesopathy

Ulcerative Colitis

Peripheral arthritis as well as axial involvement occurs in UC to varying degrees. Incidence of musculoskeletal manifestations varies between studies (15–30%) and reasons for this include study population, setting of the study for example, tertiary referral centre or district general hospital and the study design. A further reason for this wide range is that often manifestations, especially peripheral arthritis, coincide with the activity of bowel disease meaning that the point prevalence for active disease is low but will occur during disease flares.

Peripheral Arthritis

The peripheral arthritis associated with UC like that of CD is often acute, self-limiting, non-deforming and seronegative. It can take the form of a polyarticular disease or oligoarticular with common joints involved being hands, knees and ankles. Rates vary but a recent study in central European and Middle Eastern patients described a rate of 13.5% in patients with UC [8], and of these patients up to half had a symmetrical joint distribution. There are similar rates of joint disease in both UC and CD for peripheral disease although axial disease is more common in CD [10]. It often coincides with a disease flare and therefore remits with treatment of the joint disease. Occasionally, the disease may become persistent and often involve a greater number of joints. Studies of extraintestinal manifestations have shown that erythema nodosum [6], pyoderma gangrenosum and anterior uveitis are associated with peripheral arthritis [3].

Investigation of peripheral arthritis should include measurement of both rheumatoid factor and antibodies to CCPs. CCP has a high sensitivity for diagnosis in rheumatoid arthritis but has also been raised in psoriatic arthropathy and reactive arthritis. CCP levels in patients with IBD have been shown to be similar to those in healthy controls [11].

Genetics

HLA B27 is associated with many of the spondyloarthropathies and may be involved in the pathogenesis of disease by facilitating presentation of arthritogenic peptides to CD8-positive T cells [12]. In IBD-related SpA, HLA B27 is only strongly associated with ankylosing spondylitis and therefore is often negative in peripheral disease and in CD patients with isolated sacroiliac disease [6].

Peripheral arthritis often improves with treatment of a UC flare and this should be the first stage in management. Symptomatic management includes simple analgesia and non-steroidal anti-inflammatory drugs. In the past, NSAIDS were thought to be associated with flares of IBD especially small bowel Crohn's; however they can be used in disease flares with caution at lower doses [13] and the newer COX-2 inhibitors have also been evaluated [14]. Intra-articular steroid injections can be particularly useful to control acute joint swelling and gives rapid relief from articular symptoms while gut disease is controlled. Sulphasalazine and methotrexate are used as treatment options for peripheral disease and doses may need to be increased in excess of what is required to control gut disease.

TNF- α blockers and biologic drugs in general have become an important treatment option in rheumatic diseases. These agents can be used in arthritis associated with IBD in the absence of active bowel disease if joint symptoms are persistent. There is now a considerable bank of safety data from the various rheumatology registers of biologic drugs; however, there is still an increased risk of tuberculosis and vigilance for infections is important.

As well as the treatment options outlined above, the multidisciplinary team remains the backbone of rheumatology treatment and disease management. Teams of physiotherapists, occupational

Table 50.3 Inflammatory back pain

Calin et al.	Berlin criteria
• Insidious onset • Age at onset <40 years • Back pain for ≥3 months • Associated with morning stiffness • Improvement with exercise	• Morning stiffness of >30 min • Improvement in back pain with exercise but not with rest • Alternating buttock pain • Awakening because of back pain during the second half of the night only

Table 50.4 Modified New York criteria for diagnosis of AS

Unilateral sacroiliitis grade 3 or 4 or bilateral sacroiliitis grade 2
Low back pain for 3 months improved by exercise and not relieved by rest
Limited motion of lumbar spine in sagittal and frontal planes
Decreased chest expansion compared to age- and sex-matched controls

Table 50.5 Radiological scoring of sacroiliac joint radiographs

Grade 0	Normal
Grade 1	Suspicious changes
Grade 2	Minimal abnormality – small localized areas with erosion or sclerosis without alteration in joint width
Grade 3	Unequivocal abnormality – moderate or advanced sacroiliitis with one or more of the following: erosions, sclerosis, widening, narrowing or partial ankylosis
Grade 4	Severe abnormality – total ankylosis

Table 50.6 Comparison between classical ankylosing spondylitis and IBD-related spondyloarthritis

	Ankylosing spondylitis	IBD-related SpA
Age of onset	<40 years	Any age
Male:Female	2.5:1	1:1

therapists and specialist nurses allow patients to feel safe on new treatments, have a familiar point of contact and enable them to rehabilitate themselves with dignity.

It is interesting to note that peripheral joint disease in UC has been shown to remit following colectomy [15]. This lends further evidence to the hypothesis that the gut is a crucial component in the development of auto-reactive adaptive immune cells [16].

Axial Disease

The diagnosis of axial arthropathy in the context of IBD is difficult due to the many causes of back pain which can include inflammatory back pain, osteoporotic fractures and herniated discs. Furthermore, axial disease comprises a complete spectrum from asymptomatic sacroiliitis to IBD associated SpA.

Screening tools for inflammatory back pain include Calin's criteria [17] and the Berlin criteria [18]. In the diagnosis of Inflammatory back pain, if 4/5 Calin criteria are fulfilled, it results in a sensitivity of 82% and specificity of 73%. If the 2/4 Berlin criteria are used the result is a sensitivity of 83.6% and specificity of 62.5%. It should be noted that these criteria are used for the diagnosis of inflammatory back pain (Table 50.3), which is one of the components of ankylosing spondylitis. Modified New York diagnostic criteria [19] for ankylosing spondylitis are outlined in Table 50.4 with radiological criteria for sacroiliitis shown in Table 50.5. There are some demographic differences between patients with AS and IBD related SpA, which are outlined in Table 50.6.

Treatment involves use of simple analgesia as well as NSAIDs. Physical therapy in an intensive program is also a mainstay of therapy and can include hydrotherapy. If patients continue to have symptoms and there is evidence of active inflammatory back pain on radiographic imaging, anti-TNF therapies such as infliximab or adalimumab are indicated.

Enthesitis

Enthesitis especially of the achilles tendon, plantar fascia and tibial tubercle can be a source of further musculoskeletal problems and occurs where the tendon inserts into bone. Clinical examination may also reveal a dactylitis or “sausage digit” which is caused by tenosynovitis and inflammation of the adjacent subcutaneous tissues.

Osteoporosis in IBD

Osteoporosis and osteopenia are common in IBD. They are diagnosed based on the number of standard deviations below the T-score, which is the bone mineral density of a healthy 30 year old which is thought to be the peak lifetime bone mass. Osteopenia is defined as 1.0–2.49 standard deviations below peak bone mass, and osteoporosis is defined as 2.5 standard deviations and greater below peak bone mass.

The cause of osteoporosis in IBD is a complex topic and studies have shown that glucocorticoid use [20], disease duration [21] and increased disease activity [22] are associated with decreased Bone Mineral Density (BMD). However, there are other studies showing that neither length of disease nor its activity has influence on BMD.

Diagnosis is based on DEXA scanning and this is advocated in patients over 60, those who have already suffered an insufficiency fracture and also patients on long-term steroid therapy.

Vitamin D and calcium supplementation should be prescribed for patients on long-term steroid therapy. Bisphosphonates act by decreasing osteoclastic resorption and therefore increasing BMD and decreasing risk of fractures. Weight bearing exercise also increases bone mineral density as well as conferring other benefits such as in cardiovascular disease.

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Part V

Nutrition

Chapter 51

Nutrition in Inflammatory Bowel Disease

Alan L. Buchman

Keywords Crohn's disease • Ulcerative colitis • Diet • Nutritional assessment • Malnutrition • Enteral nutrition • Parenteral nutrition • Vitamin deficiency • Nutrient deficiency

Malnutrition and Nutrient Deficiencies

It is estimated that as many as 75% of hospitalized patients with Crohn's diseases are malnourished [1]. In general, the likelihood of nutritional deficiencies is greater in patients with Crohn's disease than in those with ulcerative colitis. Reduced intake of food because of abdominal cramping, nausea and nutrient loss in diarrhea are prominent causes of weight loss in patients with IBD. Intestinal malabsorption also contributes to malnutrition in patients with active IBD, primarily those with Crohn's disease involving the small intestine in whom entero-enteric fistulas that bypass large segments of the proximal intestine may also result in substantial nutrient malabsorption. Extensive mucosal disease, bacterial overgrowth proximal to strictures, and surgical resection all contribute to malabsorption and subsequent weight loss. Increased energy expenditure, as seen with fever, abscess or sepsis, or systemic inflammation, can also result in weight loss. Nutrient deficiency can result in altered cellular immunity with increased risk of infection, delayed wound healing, and in children, growth retardation. Therefore it is important to identify those patients that are at potential risk of malnutrition. Medical and surgical management plans should then include prevention of, and correction of nutritional deficits.

History and physical examination are the probably the best tools currently available to evaluate the gross nutritional status of an individual patient. Those who have lost significant weight (defined as greater than 10%) and have had reduced oral caloric intake over a 2–24 week period are at risk of both macronutrient and micronutrient deficiencies. The important findings on physical examination besides an accurate weight include loss of subcutaneous fat, muscle wasting, dependent edema, and development of ascites. The subjective global assessment (SGA) is a clinical method for the evaluation of nutritional status, and includes historical, symptomatic and physical parameters of patients [2].

A.L. Buchman, MD, MSPH (✉)
Department of Gastroenterology, Feinberg School of Medicine, Northwestern University,
676 N. St. Clair St., Suite 1400, Chicago, IL 60611, USA
e-mail: a-buchman@northwestern.edu

Table 51.1 SGA score (subjective global assessment (SGA) of nutritional status)**A. History**

1. Weight change

Overall loss in past 6 months: amount=# _____ kg

Change in past 2 weeks: _____ increase
_____ no change
_____ decrease

2. Dietary intake change (relative to normal)

_____ no change

_____ Change: duration=# _____ weeks

Type: _____ suboptimal solid diet _____ full liquid diet

_____ hypocaloric liquids _____ starvation

3. Gastrointestinal symptoms that persisted >2 weeks)

_____ None _____ Anorexia _____ Nausea _____ Vomiting _____ Diarrhea

4. Functional capacity

_____ No dysfunction (e.g. full capacity)

_____ Dysfunction: duration=# _____ weeks

_____ working suboptimally

_____ ambulatory

_____ bedridden

5. Disease and its relation to nutritional requirements

Primary diagnosis (specify) _____

Metabolic demand (stress) _____ None _____ Low _____ Moderate _____ High

B. Physical (for each trait specify 0=normal, 1+=mild, 2+=moderate, 3+=severe)

_____ Loss of subcutaneous fat (triceps, chest)

_____ Muscle wasting (quadriceps, deltoids, temporals)

_____ Ankle edema, sacral edema

_____ Ascites

_____ Tongue or skin lesions suggesting nutrient deficiency

C. SGA rating (select one)

_____ A=Well nourished (minimal or no restriction of food intake or absorption, minimal change in function, weight stable or increasing)

_____ B=Moderately malnourished (food restriction, some function changes, little or no change in body mass)

_____ C=Severely malnourished (definitely decreased intake, function, and body mass)

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The findings from a history and physical examination are subjectively weighted to rank patients as well nourished (A), moderately malnourished (B), or severely malnourished (C) (Table 51.1). The SGA provides reproducible results with more than 80% agreement [2].

Vitamin, Mineral, and Trace Metal Deficiencies

Deficiencies of vitamins, minerals, and trace elements may result from either inadequate intake or increased intestinal losses. These deficiencies are more common in Crohn's disease than ulcerative colitis given the majority of micronutrients are absorbed in the small intestine. History and physical examination are useful tools in the diagnosis of specific nutrient deficiencies.

Folic acid and vitamin B-12 are the two most common water-soluble vitamin deficiencies that can occur. Deficiency of other water-soluble vitamins is rare. Folate deficiency may result from

intestinal malabsorption when proximal jejunal disease is present, as well as interaction with sulfasalazine which inhibits folate uptake. Approximately 30% of Crohn's patients may have low serum folate [3]. Replacement can be given with oral folic acid at a dose of 1.0 mg daily. Vitamin B-12 absorption can be impaired if the distal 60 cm of the ileum is diseased or resected, which can occur with Crohn's disease [4]. Bacterial overgrowth that occurs proximal to strictures in the small intestine can also reduce vitamin B-12 absorption. In patients at risk 1,000 µg monthly of intramuscular subcutaneous vitamin B-12 should be given. Vitamin B-12 can also be absorbed sublingually and intranasally.

Vitamin D is the most common fat-soluble vitamin (vitamins A, D, E, and K) deficiency reported in patients with IBD. Fat-soluble vitamin deficiency results from malabsorption secondary to a reduced bile salt pool resulting from terminal ileal disease or resection. This results in an inability to form sufficient micelles necessary for fat and fat-soluble vitamin assimilation. The combination of vitamin D and calcium malabsorption, as well as corticosteroids (which inhibit calcium absorption) may result in significant metabolic bone disease, including both osteomalacia and osteoporosis. Corticosteroids cause both decreased intestinal absorption and increased urinary excretion of calcium. Patients at risk should receive 1,000–1,500 mg of elemental calcium daily. Measurement of bone density using dual-energy X-ray absorptiometry (DEXA) should be performed early after the diagnosis of IBD. Supplementation with 1,000 IU of daily vitamin D has been reported to prevent bone loss in patients with Crohn's disease [5]. Sixteen percent of patients with IBD may also have low serum vitamin A and E concentrations [6]. One study reported a consistent relationship between low vitamin A and E concentrations and disease activity [6].

Iron deficiency is common in both active Crohn's disease and ulcerative colitis, and has been reported in 20–40% of IBD patients; it usually results from gastrointestinal blood loss. Low serum ferritin concentration is the most reliable marker of reduced iron stores, although as an acute phase reactant, serum ferritin may be elevated in the presence of systemic inflammation. Anemia in IBD however is often a result of the chronic disease rather than iron deficiency.

Calcium deficiency may develop in part as a result of vitamin D deficiency and deficient calcium absorption, although the diet of many individuals (even those without IBD) may be deficient in calcium to begin with. Magnesium and potassium are electrolytes that may require replaced especially in those patients who have had partial small bowel resections or who have significant diarrhea. Because oral magnesium supplements act as a cathartic, intramuscular, or intravenous replacement is often necessary.

Zinc deficiency (40% of patients with Crohn's disease) may also occur especially in patients with significant diarrhea and small bowel fistula losses [7]. A combination of low serum and urinary zinc concentrations is highly suggestive of zinc deficiency. Zinc deficiency can be corrected with oral zinc sulfate 220 mg twice daily. Selenium deficiency is rare.

Dietary Contributions to the Pathogenesis of IBD

It has been suggested that IBD may at least in part be related to dietary antigens, the interaction of diet with commensal bacteria (which may differ between individuals or between those with and without IBD), or the interaction of diet with genetics – specifically the presence of single or multiple single nucleotide polymorphisms (SNPs), which alone, or in combination, could be a risk factor for the development of IBD in the correct setting with the “wrong” bacteria and/or the “wrong” dietary macronutrient. Further, diet may impact bacterial colonization, which itself may be in part dependent upon genetics. Indeed, the role of diet in the development of IBD has never been clarified, and has often been dismissed in recent years. However, diet may play a very important role in the pathogenesis of IBD, albeit indirectly. Gene expression may be modified based on nutrient–gene interactions [8]. It is also potentially possible the intestinal immune response against gut flora may

be regulated in part by diet. The fields of nutrigenomics and nutrigenetics are in their development, but may eventually result in “personalized” diets to prevent or treat IBD. Otherwise, dietary risk assessment for development of IBD is largely based on large epidemiological studies, although the effects of diet within a population may be highly among individuals variable [9, 10]. For example, the EPIC study that included 260,686 adults at centers in the United Kingdom, Sweden, Denmark, Germany, and Italy, found few specific dietary risk factors for development of ulcerative colitis although a possible association with increased polyunsaturated fat diet intake [11], and more specifically, linoleic fatty acid was found [12].

A link between increasing rates of obesity and IBD has been suggested, although it is not clear whether this observation reflects that fat and adipokines produce inflammatory cytokines [13] which may have a role in the pathogenesis of IBD, or whether the observation of obese patients with Crohn’s disease or ulcerative colitis merely represents the increasing rate of obesity in society as a whole [14]. Small studies to date have not shown an increased risk for relapse or disease severity in patients with elevated BMI, although one retrospective study suggested an increased risk of anorectal complications [15].

Although consumption of a diet with significant intake of refined sugars is not a risk factor for development of IBD [16, 17]. Alun Jones found patients often report similar specific foods that tended to trigger the symptoms of their Crohn’s disease [18]. These foods included wheat, dairy products, and some vegetables such as mustard greens and various cruciferous vegetables. In one study, induction of remission was obtained in 20 patients with active Crohn’s disease with either total parenteral nutrition (PN) or an elemental enteral formula. Subjects were then randomized to receive an unrefined carbohydrate diet or nil per os (npo), with re-introduction of specific foods each day, starting with foods unlikely to induce Crohn’s-like symptoms as described by the patients (presumably including diarrhea and abdominal discomfort). The most frequently described food intolerances were wheat, dairy products, and brassicas in descending order of prevalence, all of which contain significant amounts of sulfur. Egg and red meat intolerance were also frequent. During the first month the subjects were also provided with an elemental enteral formula to drink and maintain their nutritional status. Eight of ten subjects in the unrefined carbohydrate diet group relapsed in the first 2 months (all within the first 6 months), while at 6 months 7/10 subjects in the exclusionary diet group remained in remission [19]. It must be noted, however, that these foods may have triggered symptoms that were consistent with IBD, but nonetheless, were not actual manifestations of IBD. For example, lactose intolerance in individuals with lactase deficiency may result in “gas,” bloating and diarrhea; wheat bran is an excellent stool bulking agent, and consumption may result in increased fecal excretion. A small study evaluated the continued consumption of an “exclusionary” diet on the maintenance of remission in patients with Crohn’s disease [20]. Although median remission time was longer and the likelihood of relapse greater in a group that received corticosteroids, the results were still suboptimal and drop-out was significant. Nevertheless, the study suggested a potential role for specific dietary components in either relapse, or the maintenance of remission in Crohn’s disease. There have been studies of no elimination diets in ulcerative colitis, although similar to Crohn’s disease diets high in sulfur have been associated with disease development.

Andresen suggested in 1925 that ulcerative colitis was due to a “food allergy.” [21] He reported in 1942 that cow’s milk, wheat, tomatoes, oranges, potatoes, and eggs appeared to be the primary factor in the development of ulcerative colitis in two-thirds of his patients [22]. It is noted that milk, wheat, and eggs all have high sulfur content. Truelove reported the exclusion of milk successfully maintained remission in five subjects, all of whom relapsed within 2 days to 6 weeks following the re-introduction of milk into their diet; lactose intolerance was not investigated [23]. He then prescribed a gluten-free, milk-free diet to 77 subjects and reported a fewer number of relapses [3] in 26 subjects that received that diet and could tolerate it, when compared with those subjects that received no special diet (8 of 24) [24].

Major dietary sources of sulfur and sulfate include cow's milk, cheeses, eggs, red meat (beef, lamb, sausage), pork, salmon, herring, shellfish, cruciferous vegetables, white beans, soy, lentils, corn, white rice, dried fruits and vegetables, tomato juice, tree nuts, sulfited wines, dried fruit, cordial, and San Pellegrino® brand water [25–29]. These were many of the same foods that were associated with symptoms consistent with Crohn's disease in the Alun Jones studies. There is some inconsistency, however, in the available data concerning sulfate content in food although commercial breads, dried fruit, and red meat appear consistently to contain significant amounts of sulfate. Sulfate is also produced from methionine transsulfuration to cysteine followed by oxidation of that substrate to pyruvate and inorganic sulfate. Inorganic sulfur (sulfate and sulfite) is then reduced, and sulfur-containing amino acids are fermented by colonic bacteria to sulfide [30], although inorganic sulfur that is oxidized to sulfate is excreted in the urine.

Colono-toxic effects of sulfur have been observed with the development of acute colitis in rodents treated with dextran sodium sulfate [31, 32]. Sulfide compounds appear to be the most toxic to isolated colonocytes [33]. Sulfate-reducing bacteria produce hydrogen sulfide [34], which is an acid at a pH of 6.0, although this in turn is converted to anionic sulfide by colonic bicarbonate [35]. Hydrogen sulfide then impairs colonocyte butyrate utilization [33], the preferred metabolic fuel for colonocytes. Fecal sulfide concentration increased in normal volunteers after they ate red meat [36]. Some studies have reported increased fecal sulfide concentration in patients with active ulcerative colitis [37], and normal sulfide concentration is normal in patients whose disease is in remission [36, 37]. In vitro studies of rat and human colonic tissue have shown that perfusion with sulfide leads to apoptosis of epithelial cells, goblet cell depletion, and increased cellular proliferation [30]. Hydrogen sulfide is rapidly absorbed by colonocytes and oxidized to sulfate or methylated by mucosal thiol S-methyltransferase [38, 39]. This essentially detoxifies the sulfide, although only once it has entered the colonocyte. In addition, 5-ASA drugs inhibit sulfide formation by colonic bacteria [36, 40, 41], and in fact, that may represent one of the mechanisms of action of the 5-aminosalicylate compounds.

There may also be an additional mechanism by which a high sulfur diet promotes endothelial inflammation. A (1.5 g/day vs. >4.5–6 g/day) high methionine diet leads to increased plasma homocysteine [42, 43]. High dietary methionine intake leads to an increase in the serum homocysteine concentration [44], and increased cellular homocysteine has been associated with an increase in vitro in the adhesion of monocytes to endothelial cells [45]. Surface expression of vascular cell adhesion molecule (VCAM-1) is triggered and secretion of monocyte chemo-attractant (MCP-1) is stimulated. Increased plasma homocysteine concentration may also increase monocyte adhesion to endothelial cells [46], and is associated with increased urinary neopterin, a marker of inflammation [47]. Increased plasma and mucosal (colonic) homocysteine have been observed in patients with IBD [45, 47, 48].

Magee et al. observed that patients with ulcerative colitis who consumed diets that contained “high intakes of sulfite containing foods” were likely to have an increased endoscopic disease severity using a novel food-sigmoidoscopy score [49]. In this study from the United Kingdom, bitter and lager beer, red and white wine, burgers and sausage, and soft drinks were the foods most frequently associated with increased endoscopic disease severity, although the investigators did not have data on dried fruit, dehydrated potatoes, and seafood because of the few number of subjects that had consumed these food items. Jowett et al. reported much lower mean daily sulfur and sulfate intakes in patients with ulcerative colitis, but still found those that remained in remission had significantly lower intakes than those that relapsed (396 and 668 mg/day, respectively, versus 350 and 636 mg/day; $p < 0.05$) [25]. In a preliminary study in four subjects with ulcerative colitis in whom medical remission had recently been induced, Roediger found elimination of sulfur-containing foods (eggs, cheese, whole milk, ice cream, mayonnaise, soy milk, mineral water, wine, cordials, nuts, cabbage, broccoli, cauliflower, and brussel sprouts) as well as decreased intake of red meat lead to prolonged remission (0 relapses over 56 patient months with an expected rate of 22.6% in four subjects) [50].

For most non-hospitalized patients the most important advice is for patients to consume a diet liberal in protein, with sufficient calories to maintain weight, even with the understanding that dietary intake of sulfur may increase. It is important to recognize that the serum albumin concentration will not normalize in the presence of a protein-losing enteropathy and/or a significant acute phase response, during which acute phase proteins are synthesized in the liver at the expense of visceral proteins. Oral intake of 25–35 kcal/kg of ideal body weight per day (40 kcal/kg for weight gain) and 1.0–1.5 grams per kilogram/day of protein will meet the requirements of most adults who are normally nourished to begin with. In regard to the specifics of a diet, controlled studies have not shown benefit of low-residue diets except for those patients with intestinal obstruction. There is some, albeit limited data to support the use high soluble fiber diets to maintain remission in patients with ulcerative colitis [51]. Soluble fiber such as pectin is fermented by colonic bacteria to short chain fatty acids, the preferred fuel for the colonocyte.

Lactose intolerance is not commonly associated with IBD unless the individual was lactose intolerant antedating their IBD or they have Crohn's disease that involves the proximal jejunum, where lactase is located. Dietary lactose should therefore only be restricted if patients have symptoms associated with dairy intake and in whom lactose intolerance can be demonstrated by breath hydrogen testing; many patients with symptoms of lactose intolerance are not actually lactose intolerant [52]. Lactose-containing foods are the primary source of dietary calcium. Furthermore, there is no consistent epidemiological data supporting the role of milk as a cause of IBD.

A low oxalate diet may be required in those patients who have had their terminal ileum resected or who have significant fat malabsorption in the presence of residual colon anastomosed in continuity with the small bowel. These patients have a propensity for development of oxalate kidney stones.

Nutritional Therapy in IBD

Specific Nutritional Supplements

Initial studies with fish oil supplements (n-3 fatty acids) in ulcerative colitis showed decreased disease activity in patients that received these formulas, but larger randomized trials in Crohn's disease have failed to show consistent results [53–55]. Fish oil may have anti-inflammatory activity because n-3 fatty acids are thought to compete in the substrate pool of the lipoxygenase pathway, thus reducing the production of inflammatory leukotrienes [56]. A study by Belluzzi et al. found 2.7 g/day of n-3 fatty acids administered as an enteric-coated fish oil preparation maintained 59% of Crohn's patients in remission after 1 year compared to 26% in the placebo group, $p < 0.05$ [53]. Another study by Lorenz-Meyer failed to show a difference in remission rates compared to placebo [54]. In each study large amounts were given which is unpalatable for most people. Feagan et al. reported on the results of two double-blinded, randomized placebo-controlled trials which utilized the same fish oil preparation used in the earlier study of Belluzzi. They found that although fish oil was safe, the rate of relapse at 1 year was virtually identical between the fish oil group and those treated with placebo [55]. It is unclear whether a study of longer duration would have resulted in different results because most studies of maintenance therapy have used the 1 year threshold. It appears the door on fish oils in IBD has been closed.

Studies have not shown any benefit of glutamine supplementation in either patients with Crohn's disease or ulcerative colitis [57–60]. Animal studies have actually suggested glutamine supplementation may worsen IBD [60, 61].

When Is Nutritional Support Necessary?

Nutritional support refers to the use of either intravenous/parenteral (PN) or enteral tube feeding (EN) and is usually administered to hospitalized patients, although selected patients (usually those with Crohn's disease and short bowel syndrome or patients with short bowel syndrome resulting from mesenteric infarction in the presence of IBD) may require short- or long-term PN at home. Nutritional support of the hospitalized patient should be instituted promptly when it has been determined from daily calorie counts that a patient is not taking sufficient oral intake of food for ≥ 7 days. After approximately 7–10 days of npo negative nitrogen balance may develop; this increases the risk of infection and interferes with wound healing. Nutritional support may also be considered an adjunctive therapy in malnourished patients in whom sufficient oral intake to promote nutritional repletion is not immediately achievable. For both active Crohn's disease and ulcerative colitis, nutritional therapy therefore has a significant supportive role. The role for nutritional support as *primary therapy* for inflammatory bowel disease is limited as discussed below. The use of preoperative PN has been suggested to improve surgical outcome and limited bowel resection in Crohn's patients undergoing small bowel resections, but not in large bowel resections [62]. Most of the reports are retrospective and uncontrolled. An analysis of the data shows generally improved indices of nutritional status, but that were not accompanied by reduced postoperative complications. Therefore, routine use of nutritional support in the preoperative patient should be restricted to seriously malnourished patients (SGA "C") who are not candidates for enteral feeding usually because of bowel obstruction. For patients who are significantly nutritionally depleted, longer term nutritional support may be required in order to improve postoperative morbidity. However, surgery should not be delayed in order to administer nutritional support in the majority of patients. Delayed surgery often leads to a further decline in the nutritional reserve of a patient. Nutritional support should be continued, or initiated postoperatively if the patient is considered moderately (SGA "B") or severely malnourished (SGA "C") preoperatively.

Indications for parenteral feeding usually include small bowel obstruction, which may develop in Crohn's disease because of adhesions related to prior surgery, severe edema with luminal compromise during an acute flare, or chronic, fibrotic scar tissue; severe diarrhea and malabsorption during active disease; small bowel ileus; gastrointestinal hemorrhage; treatment for enterocutaneous or entero-enteric fistulae; and as supportive care in patients that are severely malnourished (SGA "C") or who have active disease with compromised absorptive surface. PN may also be indicated in a patient with ulcerative colitis and toxic mega colon in which enteral nutrition is contraindicated. PN is not generally indicated in patients that have a non-obstructive gastrointestinal tract or when the duration of nutritional support is expected to be < 7 days.

It is dogma that the gut "atrophies," in the absence of enteral nutrition. While this may be the case in animal studies, the data in humans fail to support this concept. It is commonly thought that in the absence of enteral nutrition, bacteria will translocate across the intestinal epithelium, to the mesenteric lymph nodes, and into the systemic circulation, resulting in sepsis and multi-organ failure. Although this has been reported in the rat model, it rarely occurs in humans [63]. When bacterial translocation does occur in humans, it is usually in the setting of small bowel obstruction, and unrelated to the route of feeding, and is usually clinically inconsequential [63].

Nutritional Support as Therapy for IBD

Whether the combination of complete bowel rest and PN can be used successfully as *primary therapy* in patients with acute inflammatory bowel disease with or without the addition of other medical therapy including diet is controversial. The consensus of the literature would suggest that

patients with Crohn's enteritis can achieve clinical remission with the combination of bowel rest and parenteral nutrition alone [64–69]. The composite results suggest npo and PN for 3–6 weeks will achieve a clinical response rate of 64% in patients with acute Crohn's disease [69]. However, in most studies prednisone was administered simultaneously with PN, which makes it difficult to discern whether the positive effects observed were the result of bowel rest and PN or the combined effects with prednisone. The consensus of the literature would also suggest that patients with Crohn's colitis and idiopathic ulcerative colitis do not respond any better to PN and bowel rest (with or without prednisone) than patients treated with prednisone and diet [70–73]. It must also be recognized that 10% of the patients in these studies developed complications from PN; these included pneumothorax from central catheter placement, catheter sepsis, and various metabolic complications. Therefore, prior to the administration of a therapy with questionable benefit, the potential risk of therapy should be considered. PN is generally reserved for supportive therapy to maintain nutritional reserve rather than as primary treatment.

Intestinal fistula is one circumstance in which npo and bowel rest may serve as primary treatment. A 38% of fistula closure rate has been reported in Crohn's patients [74]. However, the reported studies lacked a non-PN control group and there no long-term follow-up was generally reported. Surgery is usually required if fistula closure is not complete after 3 months of nutritional support. For Medicare reimbursement, 3 months or longer of PN is usually required and it must be documented that enteral feeding distal to the fistula is either not possible or would result in significant malabsorption. With newer medications such as infliximab and adalimumab, PN and bowel rest may serve less of a role in the treatment of fistulas. A randomized study comparing PN with bowel rest to anti-tumor necrosis antibody (TNF) therapy, with to an oral diet is needed.

With regard to EN, products may be provided either orally or via a feeding tube, and may have potential benefit as primary treatment in Crohn's patients [75–80]. The composite data suggest that the administration of either an elemental, peptide-based, or polymeric diet for 3–6 weeks will achieve a remission rate of approximately 68%, which is similar to the remission rate reported with PN and bowel rest. There is no advantage of an elemental or semi-elemental formula, which are often more expensive, over a formula that contains intact protein [80]. Remission rates achieved are comparable to that with corticosteroids [80]. The reason patients with active Crohn's disease may respond to polymeric enteral formulas, but not an ad-lib regular oral diet is unclear, but may be related to the lipid composition of the enteral formula or the fact the formula diets are all sterile. Formulas in addition increased concentrations of long chain triglycerides and polyunsaturated fats may be risk factors for the relapse of Crohn's disease [79, 80], although other data is contradictory [81]. A systematic review of the studies in which EN was used to maintain remission in patients with Crohn's disease is difficult given the limited number (two) of controlled trials, and different study designs [82].

In summary, nutrition plays a prominent role in the treatment of IBD, both directly and indirectly, and may also play an important role in the pathogenesis of IBD.

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Part VI
Pregnancy, Fertility, and Pediatric
Aspects of Inflammatory Bowel Disease

Chapter 52

Fertility and Pregnancy in Inflammatory Bowel Disease

Sharmeel K. Wasan and Sunanda Kane

Keywords Fertility • Pregnancy • Breastfeeding • Birth outcomes

Introduction

The peak incidence of inflammatory bowel disease (IBD) is late adolescence and early adulthood. This simple fact highlights the importance of fertility and pregnancy in the management of the ulcerative colitis (UC) and Crohn's disease (CD) patient. In fact, about 25% of females with IBD conceive for the first time after their disease is diagnosed [1]. During pregnancy, the patient's focus often shifts from her own health to the consequences of her disease and medications on her pregnancy and unborn child. This chapter aims to summarize our current understanding of the issues surrounding fertility and pregnancy in UC and CD patients.

Fertility

Many women with IBD harbor fears about a potential relationship between their disease and infertility (defined as the failure to conceive after one year of regular intercourse without conception). Although prior studies have demonstrated high rates of infertility in CD and UC patients in the time period preceding the use of immunomodulators and biologic agents, more current data suggests that the overall rates of infertility in women with IBD do not differ from that of the general population [2, 3]. Unfortunately, many women with IBD have not been appropriately educated and voluntary childlessness is still prevalent with unjustified fears about adverse outcomes including IBD heritability, risk of congenital abnormalities, and teratogenicity of medications [4].

In the post-surgical IBD population, however, infertility rates are higher than that in the general population. Women with UC who have undergone ileal pouch anal anastomosis (IPAA) have been shown to have a significant decrease in fecundability, with evidence of decreased probabilities of

S.K. Wasan, MD • S. Kane, MD, MSPH (✉)
Department of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
e-mail: kane.sunanda@mayo.edu

conception rather than complete infertility [5–9]. This reduction in fertility has been attributed to postoperative changes in the pelvis with adhesions, scar tissue, and damage to the reproductive organs during the extensive dissection to create the IPAA [10, 11]. Prior reports of the effects of IPAA in patients with familial adenomatous polyposis (FAP) also described a reduction in fertility [12, 13]. Similar to UC, fertility rates have also shown to be lower in women with severe active CD requiring surgery [3, 14].

Fertility in men is also affected by IBD and the medications used to treat it. The most commonly cited IBD medication to affect semen quality is sulphasalazine. It has been shown to cause oligospermia and impair sperm motility – effects that have been shown to recover after three months of stopping the medication [15]. Poor nutritional status has also been linked to sperm abnormalities [16], while a slightly increased risk of sexual dysfunction has been shown to be associated with the depression that can be associated with IBD [17, 18] and after proctocolectomy [19].

Effects of IBD on Pregnancy

There are diverse opinions regarding the potential risk of adverse events in the pregnancy of an IBD patient. A large, Swedish population study found that IBD (both UC and CD) was an independent risk factor for low birth weight (LBW) and premature infants [20]. IBD patients from California with predominantly mild disease were also found to be at an increased risk for adverse conception outcomes (spontaneous abortions), adverse pregnancy outcomes (preterm births, SGA, and still births), and a complication of labor compared to women without IBD [21]. Similarly, a meta-analysis of 12 studies from 1986 to 2005 demonstrated an increased incidence of both premature birth and LBW in IBD patients [7]. Conversely, a large Danish cohort study of UC patients found no difference in rates of LBW, preterm labor, or small for gestational age (SGA) infants compared with controls [22, 23]. Other studies have also noted the rate of healthy babies in mothers with UC [1, 23]. However, the same adverse events of preterm birth, SGA, and LBW have been found to be higher in other studies of CD patients but not in UC patients [24, 25].

Discordant data have also been published regarding the rate of congenital malformations. Women with UC specifically delivered babies with a higher rate of congenital anomalies in some studies [7, 24, 26], while there was no difference in the rate of congenital malformations among UC patients compared to controls or to CD patients in the California-based study [21]. Given these mixed results, it remains unclear whether the medications used, disease activity, or other possible contributing factors may be playing a role in the development of congenital anomalies.

Predictors for adverse events have included ileal CD [27], previous bowel resection [21], and the presence of active disease. Severe disease activity at the time of conception and during gestation has been shown to increase the likelihood of delivering a premature or LBW infant, especially in CD patients [28–31]. The rates of miscarriage were also found to be increased in patients with active disease [32]. Two population-based studies, however, demonstrated an increased disease activity did not predict increased adverse events above that expected for IBD patients in general [21, 33].

Effects of Pregnancy on IBD

Disease activity at conception appears to influence the course of IBD activity during pregnancy. Patients in remission during conception are more likely to remain in remission during pregnancy [34]. If the disease is active at the time of conception, it is likely to remain active, or even worsen;

consequently up to two-thirds of patients will have to contend with active disease during the course of their pregnancy [35, 36]. This is particularly important because increased disease activity during pregnancy may be associated with adverse outcomes. This commonly cited “rule of one thirds” describes the likelihood of a disease flare during pregnancy – one-third of patients will improve, one-third will stay the same, and one-third will flare. Nielsen et al. reported an exacerbation rate of 34% per year during pregnancy and 32% per year when not pregnant in UC patients [23]. Similar rates of exacerbation were also found among pregnant CD patients [37]. Other studies have also demonstrated the rates of disease flare during pregnancy to be anywhere from 26 to 34%, which are similar to the rates of flare described in non-pregnant IBD patients [38, 39].

Within individuals, disease activity can vary from pregnancy to pregnancy despite inactivity of IBD at conception, which may be explained by the maternal immune response to paternal HLA antigens that results in immunosuppression. This hypothesis was validated in a study of 50 pregnancies in 38 women with IBD in which HLA disparity predicted a lower postpartum disease activity score [40].

Pregnancy may also play a role in future disease course. The rate of relapse was found to be decreased in the 3 years following pregnancy [41]. A European cohort of 580 pregnancies also found a similar decreased rate of relapse in the years following pregnancy in both UC and CD patients [42]. Despite these striking observations, there are no clear reasons for why this may be true.

Mode of Delivery

There is significant controversy regarding the best mode of delivery for IBD patients. In general, the obstetrical indication should dictate the mode of delivery. Women with both UC and CD have higher rates of caesarean sections compared to the general population, although no data exists on the justification behind the choice of delivery method [7]. The risk of anal sphincter damage with possible associated incontinence following vaginal delivery has been well described [43, 44] and is the rationale quoted in multiple articles to explain the increased use of caesarean sections. In patients with active perineal CD, there is concern that the vaginal delivery and episiotomy may exacerbate the perineal disease [45]. However, in patients with inactive perianal disease, vaginal delivery does not appear to aggravate the perianal disease [46–48]. Given the possible risk of injuring the anal sphincter or worsening the perianal disease, patients with active perianal disease should be considered for caesarean section. In CD patients without active perianal disease, the obstetrical needs should dictate the mode of delivery. It is therefore important for the patient, the gastroenterologist, the obstetrician, and the colorectal surgeon, if relevant, to have a frank and tailored discussion about the risks and benefits of the possible delivery routes in an individual patient.

Pregnant women with an IPAA also require unique attention due to the potential compromise of anal sphincter function after a vaginal delivery. Several studies have demonstrated that although pouch function may deteriorate during the third trimester and in the immediate postpartum period, it often returns to the pre-pregnancy state within 3 months after delivery [49–51]. Vaginal delivery is not contraindicated in IBD patients with pouches. This was demonstrated in a survey study of 232 patients in whom no difference in outcomes was observed between patients who underwent vaginal delivery compared to caesarean section [52]. For pregnant UC patients who might be candidates for an IPAA in the future, there are no data regarding the role that the possible damage to the anal sphincter during vaginal delivery may have on future pouch function. Thus, as with the patient with perianal CD, a joint decision regarding the most suitable method of delivery should be determined after considering the intricacies of each patient’s case.

Breastfeeding

Breastfeeding has been shown to have many beneficial effects on both infants and mothers, with average rates of breastfeeding ranging from 74% at birth to 43% at 6 months in the general population in the United States [53]. However, how one should advise an IBD patient regarding breastfeeding is affected by several factors. These include a possible role of breastfeeding in postpartum disease activity and a concern that particular medications may pass through the breast milk. One study at a tertiary care center in 2005 demonstrated that only 44% of 122 IBD patients initiate breastfeeding in their infants, citing a fear of medication effects on the infant, physician recommendations, and personal choice as reasons for not breastfeeding [54]. This rate of breastfeeding was significantly lower than that seen in the general population. A more recent population-based study in Canada, however, demonstrated that 83.3% of women with IBD initiated breastfeeding, a rate similar to that in the general population in Canada [55]. Moffatt et al. thus suggest that the different rates of breastfeeding might be due to geographic and cultural differences between patient populations, as well as possibly different degrees of IBD activity.

There has also been a concern about whether breastfeeding may worsen disease activity in the nursing IBD patient. In the study by Kane et al. [54] described above, after adjusting for medication cessation, no relationship between breastfeeding and disease activity was found. Similarly, breastfeeding was not associated with an increased risk of disease flare in the Canada-based study [55].

Finally, breast milk, which protects infants against other immune-mediated diseases, may protect against their future development of IBD [56]. Other studies, however, have found no association [57, 58], while yet others have demonstrated that breastfeeding might be a risk factor for IBD [59, 60], and particularly for CD [60]. In the face of such conflicting data, one cannot draw conclusions about the effects of breastfeeding on the child of a mother with IBD.

Medications

One of the most controversial topics regarding the management of the conception, pregnancy, and postpartum period of the IBD patient is the use of medications. Despite the concern of the patient and her treating physicians, the majority of medications used to treat IBD are not associated with significant adverse effects or congenital anomalies. In fact, it is important to remember that the greatest risk to the pregnancy is not the medications, but the presence of active disease. The safety of the various IBD medications for pregnancy and breastfeeding are described in Table 52.1. It is important to keep in mind that the FDA has recently decided to abandon this rating system for new drugs coming to market given that the ratings can be confusing and potentially irrelevant for any specific individual.

Surgery

Obstruction, perforation, uncontrollable bleeding, fulminant disease refractory to medical therapy, and intra-abdominal abscesses that cannot be drained by other methods are all indications for surgery in the pregnant patient. While maximizing medical therapy prior to potential surgery is often the recommended algorithm, it is important to avoid delaying an inevitable surgery as the delay may place the mother and fetus at an even greater risk than the surgical procedure itself. Data regarding surgery in pregnant patients without IBD have demonstrated that there is no strong evidence that

Table 52.1 Summary of the medications used to treat IBD

Drug	FDA class	Recommendations for pregnancy	Recommendations for nursing	Comments
<i>Aminosalicylates</i>				
Sulphasalazine	B	Low risk – large studies show no adverse effects even though it readily crosses placenta [31, 56, 57] Supplement with 2 mg folic acid daily [58, 59]	Probably low risk. Unlike other sulfonamides, no bilirubin displacement occurs in the infant [60, 61]	Can cause diarrhea in infant [62–64]. If this occurs, either stopping medication or nursing should be considered
Balsalazide	B	Low risk	Probably low risk	
Mesalamine (mesalazine)	B	Low risk – large studies show no adverse effects [65, 66]. Likely safe in doses up to 4.8 g/day, though recommend decreasing dose when feasible. Safe to use topical therapy [67]	Probably low risk. Studies have shown very low or undetectable levels in breast milk [68]	
Olsalazine	C	Low risk	Probably low risk. Studies have shown very low or undetectable levels in breastmilk [69]	
<i>Corticosteroids</i>				
Prednisone/prednisolone	C	Possible small risk of cleft palate/lip [70, 71], adrenal insufficiency and premature rupture of membranes [72]	Compatible – minimally crosses into breast milk. Could minimize exposure to infant by waiting 4 h after ingestion before nursing [31, 73]	Adverse maternal outcomes include hypertension, edema, glucose-intolerance, and pre-eclampsia
Oral budesonide	C	No increased risk of adverse outcomes in small study of 8 CD patients	No evidence of harm	
<i>Immunosuppressants</i>				
Azathioprine/6-mercaptopurine	D	Low risk – multiple case series in IBD, do not show a clear association with congenital anomalies [56]. Thought to be safer to stay on these drugs than to risk a flare and a potential pregnancy complication Contraindicated – teratogen	Traditionally discouraged. However, recent studies show minimal to no levels in breast milk, suggesting it may be safe	The early fetal liver lacks the enzyme inosinate pyrophosphorylase needed to convert AZA to 6MP
Methotrexate	X	Contraindicated	Contraindicated due to danger of accumulation in infant [64]	
Cyclosporine	C	Low risk – several case reports to control steroid-refractory UC without adverse effects	Contraindicated – secreted into the breastmilk [64]	May be a better option than colectomy

(continued)

Table 52.1 (continued)

Drug	FDA class	Recommendations for pregnancy	Recommendations for nursing	Comments
Tacrolimus	C	Increased incidence of perinatal hyperkalemia and prematurity	Limited data. Potential toxicity	
<i>Antibiotics</i>				
Ciprofloxacin	C	Avoid – may increase risk of arthropathies in children	Limited data; compatible based on American Academy of Pediatrics guidelines [64]	Consider alternative therapies given minimal efficacy in IBD
Metronidazole	B	Low risk – possible increased rates of cleft lip when exposed in the second or third trimesters	Limited data	Consider alternative therapies given limited efficacy in IBD
Rifaximin	C	Avoid – no human data	Safety unknown	
<i>Biological agents</i>				
Adalimumab	B	Low risk. Several reports of successful use	Probably compatible. No assay to measure levels in breast milk	Crosses placenta in third trimester. Discontinue 6–8 weeks prior to delivery decrease levels in newborn
Infliximab	B	Low risk. Several reports of successful use	Likely compatible. No evidence of transfer into breast milk	and restart immediately after delivery. If not, would avoid live rotavirus in the newborn
Certolizumab	B	Low risk. Case reports of successful use	Probably compatible. No assay to measure levels in breast milk	
Natalizumab	C	Low risk. Data from controlled trials does not suggest teratogenicity	Unknown	

surgery during pregnancy increases the risk of miscarriage beyond the background rates of miscarriage [61], and that even surgery in the first trimester does not appear to increase major birth defects and should not be delayed when indicated [62]. In general, however, surgery during the second trimester avoids the issue of delivering potentially harmful drugs during organogenesis that occurs during the first trimester and is technically less complicated than surgery in the third trimester due to the large size of the uterus [63–66]. A case series of 6 CD patients with intraperitoneal sepsis requiring surgery from 11 to 33 weeks gestation reported five successful deliveries and one miscarriage [67]. A literature review of 37 cases of pregnant patients undergoing surgery for severe UC revealed an overall fetal mortality rate of 49% and maternal mortality rate of 22% [68]. However, this same study noted that in the 5 patients in their own series at the Mayo Clinic, the maternal and fetal mortality was zero after a subtotal colectomy and Brooke ileostomy, suggesting that an experienced multi-disciplinary team may be necessary for an optimal outcome [68]. There are also several case reports of successful outcomes following colectomy in the third trimester for severe colitis [69–71]. In a report of 2 cases of fulminant colitis at 16 and 18 weeks of gestation, use of the Turnbull-Blowhole colostomy and ileostomy for acute management resulted in successful outcomes in both the mother and the neonate, with subsequent completion proctocolectomy and IPAA performed at a later date [72]. A treatment algorithm suggested by Haq et al. [73] recommends that patients less than 28 weeks should undergo a Turnbull-Blowhole colostomy and ileostomy, while patients over 28 weeks gestation should undergo a combined subtotal colectomy and caesarean section.

Conclusions

In general, women with IBD can carry out successful pregnancies. Conceiving while in remission and remaining on IBD medications are key features to optimizing outcomes for both mother and child. It is important to stress to the patient that disease activity has a far greater impact on pregnancy than do the medications used to treat the IBD, recognizing that the majority of medications used are generally safe and should be continued during pregnancy. Incorporating a multidisciplinary team with an experienced gastroenterologist, high-risk obstetrician, and if necessary, a surgeon can maximize successful outcomes.

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Chapter 53

Pediatric Aspects in Inflammatory Bowel Disease

Jeffrey S. Hyams

Keywords Children • Adolescents • Crohn's disease • Ulcerative colitis • Thiopurines • Methotrexate • Infliximab • Adalimumab • Hepatosplenic T cell lymphoma

Introduction

A significant percentage of all newly diagnosed individuals with inflammatory bowel disease (IBD) fall within the pediatric age range (≤ 16 years old). These patients, ranging from infancy to adolescence, often present a set of issues that differ from adults with IBD and require a good understanding of how the effects of disease and therapy on growth and development must influence care. This chapter will describe the important differences in the presentation, behavior, overall natural history, methods of measuring disease activity, and treatment of children with IBD as compared to adults.

Pediatric Aspects of IBD

Demographics. Contemporary demographic data from children newly diagnosed with IBD in North America are available from the Pediatric Inflammatory Bowel Disease Collaborative Research Group Registry, a natural history study started in 2002. In the Registry's database of 1669 subjects diagnosed before their 16th birthday the age distribution at diagnosis was 4% <5 years, 21% from 5 to 9 years, and 75% from 10 to 16 years (unpublished data). There was a slight predominance of males over females (57% versus 43%). In a population-based epidemiologic study in Wisconsin, USA, the incidence of IBD in the pediatric population was 7.5 per 100,000 with that of Crohn's disease (4.6 per 100,000) being twice that of ulcerative colitis (2.1 per 100,000). Eighty-nine per cent of new cases were non-familial [1].

Location of disease. In Crohn's disease in children location or extent of disease differs according to age of disease onset. Children under the age of 10 years, and in particular children under the age of

J.S. Hyams, MD (✉)

Division of Digestive Diseases, Hepatology, and Nutrition, Departments of Digestive Diseases, Hepatology, and Nutrition, Connecticut Children's Medical Center, University of Connecticut School of Medicine, 282 Washington Street, Hartford, CT 06106, USA
e-mail: jhyams@ccmckids.org

5 years, characteristically present with isolated colitis, whereas ileal disease (with or without accompanying colitis) begins to occur more often in children whose disease is diagnosed after the age of 9–10 years [2, 3]. Upper tract involvement (proximal to the ligament of Treitz) is often noted at presentation in children with Crohn's disease and is present most commonly in those children with ileocolonic disease (up to 50–60%). In a recent well-defined inception cohort of children with Crohn's disease 745/932 (80%) of children had upper GI endoscopy of whom only five had UGI involvement only. Esophageal involvement was seen in 203 patients (27%) (macroscopic in 18%) and gastroduodenal involvement in 413 (56%) (endoscopic 42%) [4]. It has also become increasingly clear that newer imaging modalities such as video capsule endoscopy, CT enterography, and MR enterography continue to detect small bowel disease hitherto unrevealed by standard barium contrast radiography. Pediatric onset ulcerative colitis is characterized by extensive colitis or pancolitis in the majority of cases and isolated proctitis is unusual [5, 6].

Serology. As in adults antibodies to a variety of microbial antigens have been found in children with Crohn's disease. However, in children there appears to be an age-related expression of these antibodies [7]. In a cohort of 705 children from three prospectively characterized cohorts in North America, the rate of ASCA (both IgG and IgA) positivity was <20% for children <7 years of age compared to 40% for those 8–15 years of age, likely representing the preponderance of isolated colonic disease in young children. Anti-CBIR1 was detected in 66% and 54%, respectively, from the two age groups.

Clinical behavior at diagnosis. In a population-based study of 404 pediatric subjects from France (age at diagnosis 0–17 years), disease behavior at diagnosis was inflammatory (B1) in 72%, stricturing (B2) in 22%, and penetrating (B3) in <5% [8]. In a North American cohort of 796 pediatric subjects with Crohn's disease, 70% had inflammatory disease and 30% stricturing or penetrating disease at diagnosis [9]. In a population-based study of children with ulcerative colitis from France, 26% had proctitis, 35% left-sided disease, and 37% extensive colitis at diagnosis [6]. This disease distribution is quite different than reported previously from North America where extensive disease at diagnosis has been observed in up to 80% of children at presentation [1, 10]. Lastly, there is some evidence to subdivide a separate group of children diagnosed with IBD at a very early age (0–2 years, infantile IBD) [11]. This subgroup is highly likely to have a first degree relative with IBD (44%), more severe disease course and a high rate of resistance to immunosuppressive treatment. The recent discovery of mutations in the genes coding for one of the two IL10 receptors causing impaired IL10 signalling in some young patients with enterocolitis resembling Crohn's disease suggests a heretofore undescribed important genetic association [12].

Measuring disease activity. While measuring the severity of gastrointestinal symptoms and laboratory abnormalities is similar in children and adults, the profound effect of IBD on growth is clearly a pediatric-only problem. As noted below, growth abnormalities are quite common in pediatric Crohn's disease and may at times be the major clinical manifestation. Thus, growth data have been incorporated into the most widely used instrument to measure disease activity in children with Crohn's disease, the Pediatric Crohn's Disease Activity Index (PCDAI) [13]. As measuring changes in growth velocity can only be done accurately over periods of 6 months or more this has led to concerns that the PCDAI might not be sensitive enough to discern changes in clinical activity over the short-term (e.g. 3 months or less); however, this has not been borne out by additional studies [14]. The PCDAI can range in score from 0 to 100 with <10 denoting remission, 10–30 mild disease, and >30 moderate to severe disease. The Pediatric Ulcerative Colitis Activity Index (PUCAI) has been developed and validated to measure the activity of pediatric ulcerative colitis [15]. It ranges from 0 to 85 points with <10 signifying inactive disease, 10–34 mild disease, 35–64 moderate disease, and 65 or greater severe disease.

Growth. Without question the single greatest difference between IBD in children and adults is the effect of disease on growth. It has been estimated that up to 88% of children with Crohn's disease

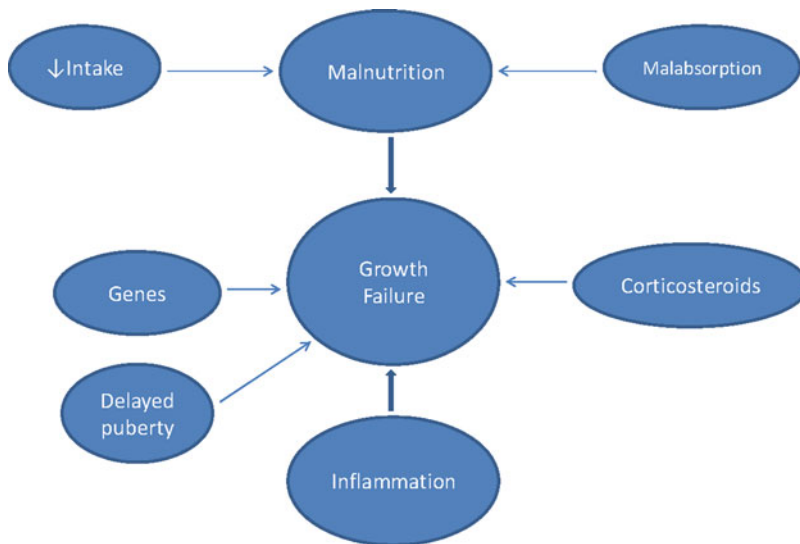


Fig. 53.1 Factors contributing to growth failure in children with inflammatory bowel disease

have abnormal growth velocity at the time of diagnosis while in pediatric ulcerative colitis this number is <10% [16]. In this author's experience the numbers for patients with Crohn's disease are not quite so stark, but certainly still involves well over 30–40% of newly diagnosed patients. Two excellent review articles on this topic have been recently published and should be consulted for further detail [17, 18].

Many mechanisms of disturbed growth have been suggested for pediatric IBD (Fig. 53.1). It had long been thought that the primary cause of growth failure was chronic caloric deprivation largely secondary to poor intake [19]. Children with Crohn's disease often have profound anorexia, and when coupled with a fear of exacerbation of gastrointestinal symptoms after eating, lead to sub-optimal energy intake. While excessive losses of fat, protein, and micronutrients can be seen in pediatric IBD it is not a major contributing factor in most patients. Additionally, most studies of energy requirements in children with IBD have shown no difference from controls except in the presence of fever [20]. It has also been proposed that genetic stature associated influences as well as Crohn's disease risk alleles may play a role in growth failure in some children [21].

More recently it has been appreciated that the inflammatory process itself in affected bowel indirectly leads to many of the growth abnormalities noted. In a proof of principle model, Ballinger et al initially used a rat model of colitis to discriminate between the effects of nutrition and inflammation on IGF-1 levels, a key mediator of growth in rat pups [22]. Using three experimental groups, the first being healthy pups with unrestricted food intake, the second being pups with chemically induced colitis that suffered from anorexia and reduced their chow intake, and the third a group of healthy pups whose diet was made similar to the colitic rats (i.e. food restricted). The most profound IGF-1 deficit was found in the colitic rats (with their decreased food intake) and this was almost twice the deficit shown in the control group whose food intake had been restricted to the level of the colitic rats. This experiment showed the independent effects of both undernutrition and inflammation on the IGF-1 axis.

IGF-1 levels are often low in children with IBD [23] and historically this has been attributed to poor nutrition. As growth hormone stimulates IGF-1 production, and most growth impaired children with IBD have growth hormone levels that are normal, it has been postulated that there is a degree of growth hormone resistance. While final explanation of this phenomenon is not totally clear, there are data to suggest downregulation of the growth hormone receptor, upregulation of post-receptor inhibitory proteins, and diminished protein synthesis or increased protein degradation [24, 25].

Inflammatory cytokines also impair growth through direct actions on growing bone [26] as well as decreasing end-organ responsiveness to testosterone [27].

Corticosteroids, often used in the management of pediatric IBD, may have a profound impact on growth and even small daily doses of prednisone (5 mg/m²) can impair growth [28, 29]. The crucial factor in the effect of corticosteroids on growth appears not to be the dose used short-term, but rather whether they are used long-term [30]. There is little evidence that an isolated short course of prednisone (<1–2 months) has long-term effect on growth. Overall therapeutic management of the child with IBD and growth failure will be discussed below.

In addition to abnormalities in linear growth, altered body composition has also been noted in children with Crohn's disease both at diagnosis and in long-term follow-up [31]. While improvement in body mass index (BMI) is commonly noted following therapeutic interventions, recent data suggest that this largely results in an increase in fat-mass, and not lean body mass. Moreover, deficits in volumetric bone density, cortical bone geometry and muscle noted at diagnosis improve incompletely following therapy, and cortical deficits may even progress [32].

Diagnosis of Pediatric IBD

The diagnosis of IBD in children is usually straightforward and the techniques employed are similar to those used in adults. One area of special concern, however, is the use of imaging tests involving ionizing radiation. Ionizing radiation is a cause of DNA damage and computed tomography (CT) scans have become frequent in the evaluation of children and adults with IBD. The potentially increased likelihood of malignancy following exposure to ionizing radiation from diagnostic imaging studies, as well as the particular sensitivity of pediatric patients has been the subject of increasing concern [33–35]. Given the long life expectancy of pediatric patients and their high risk of requiring multiple imaging exams during their lifetime it is important to minimize radiation exposure. Magnetic resonance enterography (MRI-E) is replacing standard barium small bowel series and CT scans in most instances.

Management of Pediatric IBD

There are few controlled trials of medications in the treatment of pediatric IBD; most experience is extrapolated from adult studies. This may be changing as a result of regulatory requirements mandating a demonstration of safety and efficacy of therapeutic agents in children.

Aminosalicylates: Though data to support the use of aminosalicylates for the treatment of pediatric Crohn's disease or ulcerative colitis are scant, they are commonly used for both. Specific dosing guidelines for children have not been established and in practice there is large variation. The most common dose used is 50 mg/kg/day of mesalamine though anecdotally some clinicians use up to 100 mg/kg/day (maximum 4 g). No pediatric specific side effects have been identified.

Corticosteroids: Corticosteroids have been the historical mainstay of the treatment of moderate to severe Crohn's disease and ulcerative colitis though no placebo controlled randomized trials have been published. Nonetheless the ability of corticosteroids to induce remission in most children has been well-established [36, 37]. A recent natural history study of the effect of corticosteroids in pediatric Crohn's disease showed complete remission of symptoms in 60% by 3 months, partial remission in 24%, and no response in 17%. At 1 year 61% were corticosteroid responsive, 31% corticosteroid dependent, and 8% had gone on to surgery [36]. A similar natural history study was published on the use of corticosteroids in newly diagnosed ulcerative colitis disease in children.

At 3 months following initiation of corticosteroid therapy 60% were in remission, 27% had mild disease, and 11% still moderate to severe disease. However, by 1 year 45% of children were corticosteroid dependent and 5% of the corticosteroid treated patients had come to colectomy [37]. In both reports the use of immunomodulators was common (50–75% of patients) as was that for infliximab (17–25%) in patients with corticosteroid dependence. Two reports have compared budesonide with prednisolone in the treatment of Crohn's disease in children and shown similar efficacy [38, 39], though bias in patient selection (i.e. milder patients) may have influenced the results. Pediatric specific side effects of corticosteroids are important, particularly effects on growth, bone metabolism, and the cosmetic issues such as Cushingoid appearance and acne that may significantly hamper medication adherence.

Immunomodulators. Multiple publications have looked at the use of immunomodulators in the treatment of pediatric Crohn's disease though only one was a randomized placebo controlled study [40]. In this latter study of 55 newly diagnosed children with moderate to severe CD who all received an initial course of prednisone and then either 6-mercaptopurine or placebo, initial remission rates at 3 months were similar, but by 1 year the 6-mercaptopurine treated group had received significantly less corticosteroid and had a much lower relapse rate. Largely based on this study thiopurines are now standard of care in the treatment of moderate to severe CD in children and are customarily introduced quite soon after diagnosis. In those patients unable to take thiopurines (low TPMT enzyme activity) or in those intolerant to this class of drug, methotrexate has been increasingly utilized [41]. No head-to-head comparisons of thiopurines and methotrexate in pediatric CD have been published. There are increasing data suggesting that maintaining adequate 6-thioguanine levels in thiopurine treated patients is associated with better outcomes [42, 43]. Until recently the data on thiopurine treatment of pediatric UC has been scant. One report has now documented the course of 133 children with UC treated with thiopurines and shown corticosteroid free remission at 1 year following initiation of thiopurine without the need for rescue therapy with infliximab, calcineurin inhibitors, or colectomy in 49% of subjects [44]. Small case series have been published on the use of cyclosporine and tacrolimus for severe colitis in children [45, 46]. Though the results of these studies have suggested a delay in the need for colectomy following calcineurin inhibitor treatment, the overall long-term prognosis for avoiding colectomy is low. Potential complications of immunomodulators are similar in children and adults.

Biological therapy. The REACH study, published in 2007, was the first prospective clinical trial of infliximab for use in children. Of 112 initial study subjects (mean age 13.3 years, disease duration 2 years, 59% male) with moderate/severe disease despite therapy with corticosteroids and immunomodulators who were treated with 5 mg/kg at 0, 2, and 6 weeks, 88% were in response and 59% in remission at 10 weeks. Patients in response or remission at 10 weeks were then randomized to receive either 5 mg/kg every 8 weeks or every 12 weeks, with an opportunity to step up dose to 10 mg/kg or decrease interval (for those in the 12 week group) in case of loss of response. At week 54, 33 of 52 (64%) and 29 of 52 (56%) patients receiving infliximab every 8 weeks did not require dose adjustment and were in clinical response and clinical remission, respectively, compared with 17 of 51 (33%) and 12 of 51 (24%) patients receiving treatment every 12 weeks ($P=0.002$ and $P<0.001$, respectively). These data demonstrated efficacy in maintaining remission through 1 year, with every 8 week dosing superior to every 12 week dosing.

Longer-term follow-up following infliximab therapy in children has also been examined [47, 48]. A multicenter study involving 66 patients in the Netherlands had a mean follow-up of 41 months with 15% having a prolonged response following episodic therapy, 56% were infliximab dependent (requiring repeated infusions to maintain efficacy), and 29% lost response [47]. A second multicenter study examined the long-term course of 202 children with follow-up periods ranging up to 3 years [48]. One hundred twenty eight of the study cohort were treated with maintenance therapy and had follow-up of ≥ 1 year. The likelihood of continuing infliximab was 93, 78, and 67% at 1, 2, and 3 years, respectively (Kaplan Meier analysis). A step-up in therapy (either increased dose or

decreased interval) was needed in about half the patients. Corticosteroid-free clinical remission for the periods from 0 to 1, 1 to 2, and 2 to 3 years after starting infliximab was 26, 44, and 33%. The use of infliximab in children with ulcerative colitis from a large multicenter pediatric IBD Registry was recently reported [49]. Corticosteroid-free inactive disease by physician global assessment was noted in 12/44 at 6 months (27%), 15/39 at 12 months (38%), and 6/28 (21%) at 24 months. Kaplan–Meier analysis showed the likelihood of remaining colectomy-free following infliximab was 75% at 6 months, 72% at 12 months, and 61% at 2 years.

The use of adalimumab in pediatric Crohn's disease has been reported in multiple small single center series and one larger retrospective report of 115 patients [50]. Standard dosing regimens were not employed though the majority of children received an induction of 80 and 40 mg separated by 2 weeks followed by 40 mg every other week. Almost all the patients had previously been treated with infliximab and were not using adalimumab as primary biologic therapy. Clinical response (improvement in physician global assessment to either inactive or mild) was noted in 65, 71, and 70% of patients at 3, 6, and 12 months, respectively. Corticosteroid-free remission was noted in 22, 33, and 42% of patients at 3, 6, and 12 months, respectively.

The largest issue concerning the use of anti-TNF α therapy in the pediatric population has been whether the use of concomitant thiopurines is contraindicated. This dilemma stems from the now multiple reports of hepatosplenic T cell lymphoma occurring in young patients (primarily males) treated with concomitant therapy [51]. No cases of HSTCL have been noted in adult or pediatric patients treated with infliximab monotherapy while HSTCL has been reported following monotherapy with thiopurines. In light of the recent SONIC study in adults demonstrating significantly improved corticosteroid free remission rates in thiopurine and anti-TNF α naive patients treated with azathioprine plus infliximab versus infliximab alone or azathioprine alone [52], sentiment has swung toward dual therapy in this population. However, there are also convincing data that adult patients who have failed thiopurines and move on to infliximab do not have additional success by maintaining the thiopurine [53]. Moreover, the COMMIT study in adults with Crohn's disease showed no added efficacy when comparing infliximab alone with infliximab plus methotrexate. Given the predilection of this invariably fatal lymphoma for young males, most pediatric gastroenterologists will not use combined therapy in young male patients. If there is a need to use combined therapy methotrexate has become the immunomodulator of choice in low dosage to decrease anti-infliximab antibody production. No consensus exists as to whether young females can or cannot be treated with combined therapy with thiopurines and anti-TNF α agents as HSTCL has rarely been reported in females as well [51].

Despite these concerns, the impressive remission rates and potential promise of mucosal healing have prompted early consideration for biological therapy in sick children. For pediatric patients in particular the promise of a rapid corticosteroid free remission prior to or during the years of rapid linear growth and sexual development are very important. I now consider using an anti-TNF α agent as primary therapy in the setting of extensive disease, complicated early disease, severe fistula, or disease onset in an adolescent with growth failure already showing signs of puberty in whom the window for growth is short. These latter subjects also often have evidence of skeletal demineralization further complicating the potential role of corticosteroids. Recent data have also suggested that antibody titers to specific microbial antigens measured at the time of diagnosis may predict the development of complicated disease requiring surgery (obstruction, perforation) and may potentially play a role in helping identify subjects more likely to benefit from biological therapy at the time of diagnosis [54].

There is one published report on the use of natalizumab in adolescents with Crohn's disease [55]. An open-label dosing schedule of 3 mg/kg at weeks 0, 4, and 8 was administered to 31 adolescent subjects, 55% of whom had a clinical response and 29% of whom were in remission at 10 weeks. Alpha-4 integrin saturation was <40% at 4 weeks after the first and third infusions suggesting that higher doses might have resulted in improved efficacy. As the use of natalizumab in the pediatric

population has been small it is not known whether there are increased risks of therapy in the pediatric population.

Enteral nutritional support. There is considerable variation in the frequency with which primary enteral nutrition is used as an induction strategy in children newly diagnosed with Crohn's disease. While commonly used in Europe it is utilized much less frequently in most of North America where prednisone is commonly the cornerstone of initial management. There are several published trials comparing the relative efficacy of enteral nutrition versus corticosteroids and in whole there is fairly equivalent efficacy with perhaps a slight edge to corticosteroids [56–58]. There are situations in which enteral nutrition should be the initial intervention of choice. For children with primarily small bowel disease and growth failure primary enteral nutritional therapy can not only induce remission but can reverse many of the nutritional deficiencies present and promote improved linear growth. The challenge in primary enteral therapy is motivating the child and family to pursue this route by either drinking the liquid preparation or using a nasogastric tube or percutaneous gastrostomy. In some patients it has been used for several months initially to be followed by scheduled periods of readministration after allowing a regular diet [59]. For most patients who start primary enteral nutrition therapy an immunomodulator is also started and given time to become effective as a more long-term maintenance strategy.

Severe/Fulminant Ulcerative Colitis. The management of fulminant colitis in children presents a particular challenge. Frequently these are children at or shortly following diagnosis when understanding of the disease itself may be limited, and a willingness to proceed to colectomy if necessary has not been established. A recent prospective study of children with fulminant ulcerative colitis has given additional insight into disease management in this situation [60]. Traditional therapy has included intravenous corticosteroids, nutritional support, and limiting oral intake. In this study, 126 children hospitalized with severe ulcerative colitis were followed for up to 1 year. A little over one-third failed therapy with intravenous steroids and required rescue with either infliximab, cyclosporine, or colectomy. The most sensitive predictor of the failure of intravenous corticosteroids was a Pediatric Ulcerative Colitis Active Index score of 45 or higher on Day 3 of hospitalization. Overall 9% and 19% of children hospitalized with severe/fulminant colitis required colectomy by initial discharge or 1 year, respectively. Most pediatric gastroenterologists will use infliximab as their preferred rescue medication for corticosteroid failures, though some still prefer calcineurin inhibitors. Failure of one of these agents should not lead to use of the other because of concerns of increased risk of serious infection. In this author's view saving a life is more important than saving a colon. Results of colectomy and ileal pouch anal anastomosis (IPAA) in children are similar to those of adults. The issue of possible impaired fecundity later in life for adolescent girls who may be subject to IPAA should be raised before this procedure is done.

Summary

It is crucial that clinicians caring for children and adolescents with IBD be fully informed about the relationship of disease activity and therapy to growth and development. This population also has its own intrinsic psychological and behavioral issues that need to be considered when planning various medical therapies as well as surgery.

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Part VII

Surveillance

Chapter 54

Management and Prevention of Infectious Diseases in Inflammatory Bowel Disease Patients

Kristine Macartney and Nigel Crawford

Keywords Infectious diseases • Inflammatory bowel disease • Crohn's disease • Ulcerative colitis • Infection • Immunosuppression • Immunization • Vaccines

Introduction

One of the most challenging aspects of the care of IBD patients is the management of infectious diseases and their associated morbidity and mortality. Although there are a number of factors that contribute to the prevalence of infectious diseases in IBD patients, it has become clear that those taking multiple immunomodulatory drugs are at greatest risk. There is also a temporal relationship between infection and the onset of IBD that has been discussed elsewhere and will not be reviewed in detail in this chapter. Relapse of IBD can be associated with infection, particularly from enteric pathogens, and treatment of coexistent infection in patients with active IBD can facilitate resolution of an IBD flare. This chapter will review the increased risk of infection in IBD patients, the most common infections encountered, and the approach to management and prevention, with a particular focus on vaccination of IBD patients.

Risk of Infection in IBD Patients

Mortality and morbidity from infections is over-represented in those with IBD compared with other patient populations [1, 2]. The greatest risk is associated with higher degrees of immunosuppression and disease severity [3, 4]. Although there is evidence for defective innate immunity in IBD, in particular aberrations in mucosal immunity [5], it is as yet unclear to what extent this contributes to the risk of infectious diseases overall. Patients with IBD are most significantly at increased risk of

K. Macartney, BMedSci, MBBS (Hons), DipPaeds, MD, FRACP (✉)
National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead,
Cnr Hawkesbury Road and Hainsworth Street, Westmead 2145, Australia
e-mail: KristinM@chw.edu.au

N. Crawford, MBBS, MPH, FRACP
Saefvic/General Medicine, Royal Childrens Hospital, Parkville, VIC, Australia

infection due to treatment with immunomodulatory medications [6]. Other factors also contribute and are described below. It is important to note that there is no clear correlate or measure to determine the extent of immunosuppression in IBD or other immune-mediated inflammatory diseases. Patients should generally not be considered to have a systemic immune deficit in the absence of immunotherapeutic medications or malnutrition [3].

The Disease Process

Patients with Crohn's disease (CD) have a higher risk of bowel-related infectious diseases due to the transmural and extensive nature of their disease, particularly the formation of fistulae and abscesses, than patients with ulcerative colitis (UC) [4, 7]. The need for frequent outpatient visits, hospitalization, parenteral nutrition and surgery also add to the overall infection risk for IBD patients, including from nosocomial infections [8, 9]. Patients can also be at increased infection risk because of chronic conditions related to IBD, including chronic airways disease, nephrolithiasis and primary sclerosing cholangitis [4].

Malnutrition

The presence of malnutrition is a key factor that has been shown to correlate with increased morbidity and mortality in IBD. Malnutrition is common in patients with IBD, especially in active CD in whom 70–80% of hospitalized and 20–40% of outpatients have weight loss [10]. Malnutrition is less common in UC patients but can develop rapidly in periods of active disease. Malnutrition appears to impair various components of the immune system and has, in turn, been associated with increased risk of infection, cancer and suboptimal responses to vaccination [3, 4, 11, 12].

Infection and Immunomodulatory Therapy

Drug therapy used for IBD has been identified as one of the most significant factors influencing the risk of developing infection and its severity, whether from a common infectious agent, an opportunistic pathogen, or reactivation of latent infection. The two terms “immunomodulatory” and “immunosuppressive” therapy have been used somewhat interchangeably in the literature, perhaps reflecting the uncertain extent to which these agents, or combinations thereof, are understood to impact systemically on immune function and risk of infectious diseases. However, the risks for single agent and combination therapy have been more closely studied since the introduction of anti-TNF monoclonal antibodies over a decade ago [4]. Each of the sources of data regarding infection rates needs to be carefully assessed for inherent biases and limitations, particularly in applying risk estimates to individual settings [13].

Large multicenter observational cohort registries such as TREAT (a prospective, multicenter, long-term registry of the treatment of Crohn's disease) [14] and ENCORE have not found infliximab to be an independent cause of increased infection. These studies, however, may be subject to reporting bias [15]. In contrast, pooled data from company-sponsored clinical trials found a 36% risk of infection for infliximab-treated patients, compared with 28% for noninfliximab-treated patients [4, 16–18]. Where data on the use of these medications in IBD patients have been lacking, studies of their use in patients with other autoimmune diseases, such as rheumatoid arthritis, have

Table 54.1 Risk of infection for certain immunomodulatory agents used to treat IBD

Drug	Risk of infection	Comments
Corticosteroids	Serious infections OR 2.21 (1.46–3.34) from TREAT registry of Crohn's disease patients [14] Independently associated with serious infections [103] Infections reported: fungal [7], TB reactivation, postsurgical [104]	Risk of infection particularly with prolonged high doses, e.g. daily doses >20 mg (or 1–2 mg/kg/day in pediatric patients) for >1–2 weeks
Thiopurines	Myelosuppression: predictable in thiopurine-S-methyltransferase (TPMT)-deficient patients, unpredictable in ~5% of patients [105] Infections reported: viral, fungal, bacterial [106]. Precise infection risk unclear. Infections in 7.4%, serious infection in 1.8% of 386 patients over 18 years [107] No increased risk in TREAT registry [14]	TPMT testing prior to treatment recommended Routine FBC monitoring recommended
Methotrexate	Increase in pneumonia in rheumatoid arthritis population (RR: 1.16; 95% CI: 1.02–1.33) [20]	Infection risk in IBD patients not well defined
Cyclosporin	Association with opportunistic infections (<i>Aspergillus</i> and <i>Pneumocystis</i>) and catheter-related sepsis [108]. Infection risk in IBD patients not well studied	Monitor levels and FBC Pneumocystis prophylaxis recommended with lymphopenia
Biologics	Increased risk of TB reactivation (OR 4.68, 95% CI 1.18–18.60; NNTH=681, 95% CI 143–14,706) for nine biologics reviewed in Cochrane analysis, compared to control [21]	Pretreatment screening for latent TB hepatitis B and hepatitis C Natalizumab: strict restrictions on use
<i>Anti-TNF monoclonal antibodies</i> infliximab adalimumab certolizumab pegol	Risk of serious infections: Certolizumab pegol associated with significantly higher risk compared to control treatment and other biologics (OR 3.51, 95% CI 1.59–7.79; NNTH=17, 95% CI 7–68). Trend toward increased risk of serious infections for all biologics (OR 1.19, 95% CI 0.94–1.52) [21]	
<i>Anti-adhesion molecules</i> natalizumab	Reactivation of latent hepatitis B infection [35] Other granulomatous infections [19, 22] No independent increased risk of infection reported from TREAT registry [14] Natalizumab (anti-CD20): risk of JC virus reactivation [109]	

been of value [19, 20]. A Cochrane systematic review of the biologic therapies in all diseases (other than HIV) found an overall increased risk of tuberculosis for patients treated with biologics. One particular therapy (certolizumab pegol-interleukin (IL) 1 antagonist) was identified as associated with a higher risk of infections than other biologics [21].

Although certain types of drugs have been associated with different types of infections, strict correlations between a specific agent and pathogen should not be relied upon in clinical practice [3]. The tendency for one drug therapy to predispose to certain infections is related to the drug's mechanism of action [12]. Table 54.1 summarizes data regarding individual therapies. For example, azathioprine and 6-mercaptopurine impact upon T-cell function and are more likely to be associated with viral infections (cytomegalovirus [CMV], varicella-zoster virus [VZV], herpes simplex virus [HSV]), whereas anti-TNF therapy appears to increase the risk for granulomatous infections from mycobacterial or fungal pathogens, such as tuberculosis (TB) or histoplasmosis [7, 12]. Other granulomatous infections have also been reported to occur with anti-TNF therapy [19, 22].

One retrospective review of drug therapy and infection in IBD patients found that corticosteroid use was more commonly associated with fungal infections, especially with *Candida* species [7].

Combinations of immunomodulatory therapy are associated with an increased risk of infection [7]. In a retrospective study of IBD patients from the Mayo Clinic, treatment with a single immunomodulatory drug increased the risk of infection almost threefold (odds ratio [OR] 2.9; 95% confidence interval [CI] 1.5–7.3). A more substantial increase in the likelihood of infection occurred in patients receiving two or more drugs in combination (OR 14.5; 95% CI 4.9–43). Cumulative doses of corticosteroids, but not other drugs, were associated with an increased risk of infection.

Other Factors

Increasing age is a risk factor for infection in general, and the age at diagnosis of IBD is a factor related to increased morbidity in IBD patients, even after adjusting for use of immunosuppressive therapy [7, 23]. Longer IBD disease duration has also been identified as a risk factor [24]. Pre-existing comorbidities, such as chronic lung disease, have been identified as contributing factors in studies of patients with rheumatologic disease, and, although such conditions have not been widely studied in IBD, they still should be noted. It is important to view the infectious risk of an IBD patient as that related to their lifetime exposures including consideration of infectious agents endemic for their places of residence [12] and exposures due to occupation and lifestyle [12].

Prevention, Diagnosis, and Management

Prevention of infection in IBD patients is best managed through a range of activities, including: (a) thorough clinical and laboratory investigations before starting immunomodulatory therapy, preferably at the time of IBD diagnosis; (b) vaccination and chemoprophylaxis when appropriate; (c) treatment of pre-existing viral infections, such as chronic active hepatitis B infection; (d) investigation for concomitant infection during episodes of disease relapse; (e) adjustment of immunotherapy when infection occurs; and (f) patient education. Other factors inherent in the overall good clinical management of IBD patients, such as minimizing risk of malnutrition through institution of nutritional therapies, have also been shown to impact on the prevention of infection.

Patient Assessment Prior to Immunomodulatory Therapy

The ideal time to fully evaluate IBD patients for their history and risk of infectious diseases is at diagnosis, prior to commencement of significant immunomodulatory therapy. As described in Table 54.2, a thorough and specific clinical history, physical examination, laboratory tests and other investigations, where warranted, are indicated [3, 12, 17, 18]. This assessment should be followed by a plan for vaccination (see detailed section below) and treatment, if indicated, for any pre-existing infections such as tuberculosis or hepatitis B (also discussed below). Local guidelines for the use of directed antimicrobial therapy should generally be observed, as they take into account drug availability and local antimicrobial resistance patterns. Detailed recommendations for the management of infections in IBD patients have been incorporated in European consensus guidelines [3] and detailed clinical practice reviews [12, 17], which are recommended reading for all specialists treating IBD patients.

Table 54.2 Recommended review of IBD patients prior to initiation of immunomodulatory therapy

Detailed interview

- History of travel and/or living in tropical areas or countries with endemic infections
- History of previous infections
 - Bacterial
 - VZV infection: age, reactivation to zoster?
 - HSV infection: sites, frequency and severity of recurrences
 - Fungal infections: oral and vaginal candidosis, intertrigo, nail infections
- Risk of latent or active tuberculosis
 - Country of origin, prolonged stay in countries where TB is endemic, history of contact with TB patients
 - Prior BCG vaccination? (date)
 - History of latent or active tuberculosis and treatment given
- Immunisation status and documentation of vaccination (dates, number of doses, postvaccination serology – if done)
 - Tetanus, diphtheria, poliomyelitis, pneumococcus, meningococcus, BCG
 - Rubella, measles and mumps, hepatitis B^a, HPV
 - Others?
- Future plans to travel abroad to endemic areas

Clinical examination

- Identification of systemic and/or local current infections
- Evaluation of dental status (dentist review)
- Gynecological visit and Pap smear (then regularly as per local screening guidelines)

Routine laboratory and other investigations

- Neutrophil count, lymphocyte count and, in the case of lymphopenia, CD4 lymphocyte count
- Urine analysis in patients with a history of urinary tract infection and urinary symptoms
- Serology
 - VZV in patients without a clear history of varicella immunization
 - HBV^a, HCV, CMV, and HIV serology
- In patients with chronic HCV or HBV infection, alanine aminotransferase (ALT) assay, and assessment of liver disease
- For patients having lived in tropical/endemic areas: *Strongyloides* serology, eosinophil count, and stool examination
- Tuberculin skin test (TST) or Interferon Gamma Release Assay (IGRA) – according to each country's specific guidelines
- Chest X-ray

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^aPatients with a history of hepatitis B vaccination should be tested for the presence of hepatitis B antibodies

Identification and Treatment of Acute Infection

Relapse of IBD is clearly associated with intercurrent infection. A number of studies suggest that infection, most commonly gastrointestinal infection, is present in approximately 10% of disease relapses [25–27]. With acute presentations, a high index of suspicion is important as the symptoms and signs of typical infection may be attenuated due to the blunted immune response [12]. For example, stool testing for enteric pathogens should be a routine undertaking in patients who present with a relapse of IBD [3, 4]. Treatment of active infection might avoid inappropriate or unnecessary use of steroids or other immunomodulatory therapy [4].

A multidisciplinary approach to management from the patient's gastroenterologist, infectious diseases specialist, and organ specific specialist(s) is ideal [12]. Decisions regarding the need for reduction or withdrawal of immunosuppressive therapy will vary, depending on the type of infection and available evidence [3]. While it is optimal to withdraw certain therapies, particularly corticosteroids,

in the setting of most types of infections, in certain situations, such as nonserious bacterial infections, a response to appropriate treatment without change in immunomodulatory therapy may occur.

Patient Education

Patient education is essential to minimizing the risk of infectious diseases [12]. Patients should be counseled regarding early recognition of symptoms and presentation for medical care. Advice regarding the avoidance of high-risk foods, such as unpasteurized milk, soft cheeses and cold meats (for *Listeria*), and raw or undercooked poultry/eggs (for *Salmonella*), is also important. As discussed in detail below, the risk of vaccine-preventable diseases (VPD) and the benefits of vaccination need to be explained to the patient and their family. When possible, vaccination of close household contacts should also be undertaken for the protection of the patient. It is very important that IBD patients are provided with travel advice, around both infection risk and specific and appropriate preventative measures for their intended destinations, as well as vaccination.

Specific Infections

Site-Specific Infections

Skin, respiratory tract, and urinary tract infections are all more likely in those with IBD. For example, patients with CD appear to be at increased risk of urinary tract infections [8, 28], possibly related to the transmural and fistulating nature of their disease. There is a risk of postoperative infections, such as surgical site infection, pneumonia or catheter-related infection, which is particularly high in patients on concurrent and high-dose corticosteroids [3, 9]. Other specific sites for more occult bacterial infections, such as bone/joint and dental sites, need to be considered in ill patients [15].

Viral Infections

Herpes viruses (cytomegalovirus, herpes simplex viruses, varicella-zoster virus, and Epstein–Barr virus).

These human herpes viruses are all unique for their ability to exist in a state of latency in human tissues following primary infection, and the potential to reactivate, with or without disease sequelae. In immunocompromised patients, including those with IBD, severe and disseminated herpes virus infections have been reported. For example, primary infection or reactivation of cytomegalovirus (CMV) can cause disease in almost any organ in immunosuppressed patients. CMV mimicking an acute exacerbation of IBD has been associated with increased morbidity, mortality, and surgical intervention [29, 30]. Not all CMV reactivation results in clinical disease; however, the use of antiviral therapy (ganciclovir) is recommended for severe or systemic disease, together with a reduction or discontinuation of immunomodulatory therapy [3].

More frequent and severe reactivation of herpes simplex virus (HSV) infection is reported in IBD patients, particularly those on multiple immunosuppressives [3, 7]. Although serological screening for past infection is not necessary because of the high prevalence, antiviral treatment of acute disease (using acyclovir, famcyclovir, and valacyclovir) in high-risk patients should occur, and pre-emptive or prophylactic therapy can also be considered in patients with frequent or severe recurrences.

Varicella-zoster virus (VZV) is characterized by its ability to cause more severe primary infection (chickenpox), or to reactivate to cause herpes zoster with greater frequency or morbidity, in immunocompromised hosts [15]. Morbidity and mortality from VZV disease has been reported in IBD patients on immunosuppressive agents, particularly anti-TNF therapy [31–34]. Although antiviral therapy can be helpful in ameliorating disease, prevention via vaccination is recommended (see vaccination section below).

Severe clinical Epstein–Barr virus (EBV) infection has been reported in patients with IBD, but is more notably associated with neoplasia, particularly lymphoma, in immunocompromised patients (this has been reviewed elsewhere). Screening and prophylaxis is not recommended because of high seroprevalence and frequent, usually self-limited or subclinical, reactivation [16].

Viral Hepatitis and Human Immunodeficiency Virus Infection

The course of pre-existing viral hepatitis in IBD patients on drug therapy has not been examined systematically, although there are case reports and small series of patients in whom reactivation or worsening of chronic hepatitis B infection and fulminant hepatitis, particularly associated with TNF therapy, has been reported [35]. The majority of cases of severe hepatitis B disease have been associated with withdrawal of high-dose immunosuppressive medication, presumably due to the immune reconstitution resulting in a symptomatic response (destruction of hepatocytes) toward the active viral replication [3, 35]. Screening for pre-existing hepatitis B infection is now considered essential in IBD patients; guidelines have been published for the treatment of chronic hepatitis B using antiviral therapy with advice on management of immunosuppressive therapy in IBD patients [3, 17].

In patients with pre-existing hepatitis C infection, use of TNF inhibitors appears relatively safe; however, more data are needed [15]. Similarly, patients with pre-existing HIV infection and IBD have not been well studied, and management of HIV should probably be commenced according to usual protocols initially [12]. Patients stabilized on highly active antiretroviral therapy (HAART) can probably be treated with TNF inhibitors if required [3]. These viruses have not been identified as opportunistic infections in IBD patients; however, patients should take every step to prevent their acquisition.

Influenza and Other Viruses (Human Papillomavirus, JC Virus)

There is a lack of specific data on the incidence and severity of influenza in IBD patients. However, immunomodulatory therapy is generally considered to increase the risk of influenza complications, including secondary bacterial infection [3]. IBD patients are therefore recommended to have annual influenza vaccination [16]. In addition, IBD patients should be offered diagnostic testing and early treatment with antiviral agents (oseltamivir or zanamivir) for suspected influenza [16].

There is evidence of a higher incidence of HPV-related cervical dysplasia in female IBD patients, which emphasizes the need for regular cervical screening and vaccination (discussed below) [36, 37]. Reactivation of latent JC virus causing fatal progressive multifocal leukoencephalopathy (PML) has been described in immunosuppressed patients treated with biologics, including the monoclonal antibody against alpha-4 integrin (natalizumab) which has been used to treat refractory Crohn's disease. There are no screening tests, treatment or vaccination for this virus, which, although rare, highlights the serious consequences of immunosuppressive therapy.

Bacterial Infections

As discussed earlier, enteric bacterial infections are associated with relapse of IBD, most commonly *Clostridium difficile*, *Campylobacter jejuni*, and *Salmonella* species. *C. difficile* particularly affects those with pre-existing extensive colonic involvement, with UC patients infected more commonly than those with CD [4, 38]. Asymptomatic carriage rates in IBD patients also appear higher than in the general population, and up to 79% of affected IBD cases have been community rather than hospital-acquired, as is more usual in the general population [38]. There are high rates of detection at presentation, and recurrence rates range from 9% to 57% [38]. *C. difficile* infections are recognized to cause increased morbidity and mortality in IBD patients, including a need for surgical treatment; however, more data on risk factors, including IBD medications, are needed, particularly since the emergence of the hypervirulent strain B1NNAPI/027 [38].

IBD patients are also considered to be at increased risk of invasive pneumococcal disease, and should be offered vaccination (discussed below). There is evidence for a strong relationship between TNF inhibitors and an increased risk for listeriosis, with age and concomitant use of other immunomodulatory medications playing a role. *Nocardia* species have also caused infections (skin, soft tissue, pulmonary, and central nervous system) in patients on immunomodulatory therapy. Severe infection with other bacterial pathogens, such as *Legionella pneumophila* (causing atypical pneumonia or “Legionnaires’ disease”), has also been reported in IBD patients on anti-TNF medications [16].

Tuberculosis and Other Mycobacterial Infections

Tuberculosis (TB) affects nearly one-third of the world’s population. The lifetime reactivation risk of latent infection (LTBI) is approximately 10%, but is almost certainly higher in immunocompromised persons [15]. IBD patients on standard immunomodulatory therapy appear to be at increased risk of reactivation of LTBI, with risk increased even further on anti-TNF therapy [12]. A Cochrane review of over 200 studies of the use of biologics given for any disease found that, overall, there was an almost fivefold increased risk of TB reactivation compared to controls (OR 4.68, 95% CI 1.18–18.60) [21]. TB reactivation has been seen particularly with infliximab, although other biologics including adalimumab may carry a similar risk [15, 21]. The likelihood of extrapulmonary and disseminated TB appears to be higher in patients on TNF inhibitors [3]. Two studies, in Spain and Japan, have demonstrated that screening for, and treating, LTBI prior to commencement of infliximab decreased the incidence of TB in patients with autoimmune diseases [3, 39, 40]. A thorough clinical history and examination, chest X-ray and screening test for LTBI (either by a tuberculin skin test [TST] or “Mantoux,” or a blood sample for interferon gamma release assay [IGRA]) are now considered essential prior to starting immunotherapy in IBD patients (see Table 54.2). Both of these methods of screening have limitations, however, and should be interpreted with caution and expert input, particularly in high-risk patients. Guidelines for treatment of LTBI, and of suspected or confirmed TB disease in immunocompromised patients, including those with IBD, have been published, and should be considered in consultation with specialist advice.

Parasitic and Fungal Infections

IBD patients also appear to be at increased risk of certain fungal and parasitic infections. Histoplasmosis may lead to life-threatening illness in patients undergoing TNF therapy, but has mainly been identified in those who have lived in endemic areas [15]. Coccidioidomycosis also

appears to occur more commonly in patients living in endemic areas who are on TNF therapy, but mostly as acute infection rather than reactivation [15]. *Cryptococcus neoformans* is another opportunistic fungal pathogen that can cause serious infections in immunocompromised patients. Infections with *Candida* species were among the most common identified in IBD patients on corticosteroids alone [7]. Invasive aspergillosis, due to this ubiquitous fungus, is predominantly seen in severely immunocompromised hosts. Aspergillosis has been reported in IBD patients using TNF inhibitors, although the exact risk has not been well defined [16].

Pneumocystis carinii (*jiroveci*) can cause severe pneumonia (PCP) in immunocompromised patients, with risk factors including older age, coexisting pulmonary disease and lymphopenia. In a review of over 5,500 patients in Australia and New Zealand, PCP occurred most commonly within the first few months of taking combination therapy [24]. Antibiotic prophylaxis to prevent PCP should be strongly considered for patients with lymphopenia, and in those taking TNF inhibitors, high-dose glucocorticoids or cyclosporine [4, 41].

The risks for parasitic infections, such as *Toxoplasma gondii* and *Strongyloides stercoralis*, have not been well quantified, but both these organisms are known to cause severe disease in immunocompromised hosts. Toxoplasmosis most commonly presents as a focal encephalitis, and has been reported in IBD patients on immunomodulatory therapy [16]. Infection with the nematode *Strongyloides stercoralis* is common in developing countries with poor sanitation. Reactivation of infection in immunocompromised hosts can cause disseminated disease, with a high-case fatality rate. Screening and pre-emptive therapy are recommended for those from endemic areas (see Table 54.2).

Vaccine-Preventable Diseases and Response to Immunization

A number of the infections detailed above are vaccine-preventable diseases (VPD). The key factors that contribute to increased risk of VPD in IBD patients are described above and are also depicted in Fig. 54.1. This section will review the utility, immunogenicity and safety of vaccines in IBD patients, and present strategies for optimizing immunization in these patients. This includes reviewing and documenting vaccines already received, giving “booster” doses of vaccines that may have been previously administered, and the use of additional vaccines to be considered in IBD patients.

Vaccines are categorized as inactivated (a killed or nonreplicating whole or part of the organism or “antigen”) or live attenuated (containing a modified or weakened form of the virus or bacteria, which actively replicates in the host to generate an immune response). Inactivated vaccines can be given to patients with IBD, even those on immunosuppressive therapy. Although there are some studies which suggest that the immune response to vaccination may be less optimal in patients with IBD than in healthy individuals, in other studies vaccine responses in IBD patients are not different to healthy controls. Overall, a response to vaccination is still very likely to provide significant protection in this vulnerable population.

Live attenuated viral vaccines are generally considered to be contraindicated in patients on immunosuppressive therapy, although there are some exceptions. For example, measles-mumps-rubella (MMR) vaccine should not be administered to IBD patients on immunosuppressive therapies due to the risk of unchecked vaccine virus replication causing disease. There have been case reports of measles vaccine virus causing severe morbidity and mortality in patients taking immunosuppressive therapy. Other live attenuated vaccines, such as varicella, herpes zoster and yellow fever vaccines, although generally not recommended, can be considered on a case-by-case basis, taking account of the specific vaccine and the patient characteristics detailed below. IBD patients, and their household contacts, – should not be administered the live attenuated oral polio vaccine (OPV), but can receive inactivated polio vaccine (IPV). Table 54.3 provides a summary of broad recommendations from current guidelines and expert reviews for many vaccines in IBD patients.

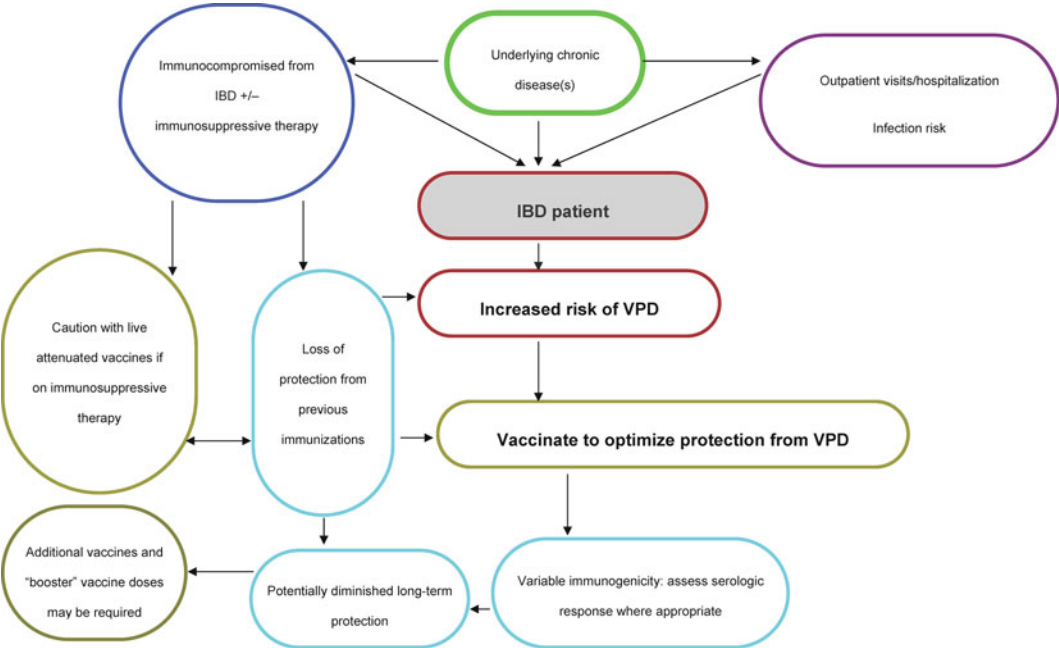


Fig. 54.1 Key factors contributing to increased risk of vaccine-preventable diseases (VPD) in IBD patients [Reproduced from Crawford N. et al. Optimizing Immunization in Pediatric special risk groups. Expert Review of Vaccines 2011; 10(2):175–186. With permission from Expert Reviews Ltd.]

Table 54.3 Recommendations for immunization of IBD patients

Vaccine preventable disease		Recommendations for use in IBD patients Based on USA, European and Australian guidelines and selected expert reviews [3, 53–55, 110, 111]
Vaccine type		
<i>Routine</i>		
Diphtheria tetanus pertussis	Inactivated (also separate tetanus, and diphtheria/tetanus vaccines)	Recommended in childhood, with boosters in adulthood (frequency depending on local guidelines)
Hepatitis B	Inactivated	Routinely recommended in childhood in some countries. Check serology, and consider vaccination if nonimmune (evidence for fulminant hepatitis B disease in immunosuppressed IBD patients)
<i>Haemophilus influenzae</i> type b (Hib)	Inactivated	Routinely recommended in childhood in some countries. Consider single dose if not given in childhood
Human papillomavirus	Inactivated	Routinely recommended in childhood in some countries. Serology not useful in clinical setting. Consider vaccination if age-eligible and not previously vaccinated
Influenza	Inactivated or live attenuated intranasal	Recommended annually in IBD patients. Use inactivated vaccine

(continued)

Table 54.3 (continued)

Vaccine preventable disease	Vaccine type	Recommendations for use in IBD patients Based on USA, European and Australian guidelines and selected expert reviews [3, 53–55, 110, 111]
Measles-mumps-rubella	Live attenuated (also available as single measles, rubella and measles/rubella vaccines)	Routinely recommended in childhood. Two doses needed for adequate protection. Do not use in immunocompromised IBD patients
Meningococcal	Inactivated (conjugated and polysaccharide formulations with different serotypes)	Conjugate vaccines recommended routinely in some countries. Consider use of conjugate vaccine (4-valent) followed by booster of polysaccharide vaccine (4-valent) if available
Pneumococcal	Inactivated (conjugated and polysaccharide formulations with different serotypes)	Routinely recommended in childhood in some countries. Additional doses and/or primary vaccination recommended in IBD patients. Recommendations for use and number of doses of conjugate (7-valent, 10-valent or 13-valent) or polysaccharide (23-valent) vary by country
Poliomyelitis	Inactivated trivalent (IPV) or oral live attenuated (OPV)	Routinely recommended in childhood. Serology not required. If booster indicated use IPV. OPV contraindicated in immunosuppressed patients and their household contacts
Rotavirus	Oral live attenuated	Only indicated for infants. Not applicable for IBD patients
Varicella	Live attenuated	Both vaccines contraindicated in immunosuppressed patients. Check serology for previous natural VZV infection. If negative, and not previously immunized, varicella vaccine can be considered if not immunocompromised (adolescents and adults need 2-dose schedule). Zoster vaccine for use in those aged >50 years
Herpes zoster	Live attenuated (high titer VZV)	
<i>Travel-related and other selected vaccines</i>		
Hepatitis A	Inactivated	Routinely recommended in childhood in some countries. Recommended for travelers to hepatitis A endemic countries. Consider for IBD patients
Typhoid fever	Oral live attenuated or inactivated	For travel/residence in endemic areas. Use inactivated (Vi polysaccharide vaccine), not oral live attenuated
Yellow fever	Live attenuated	Contraindicated in immunocompromised. Waiver for certificate of vaccination for travel is available for medically ineligible persons. Need alternative measures to protect against mosquito bites or consider not travelling to YF endemic areas
Cholera	Inactivated	Rarely indicated. Efficacy modest
Japanese encephalitis	Inactivated	Recommended for travel/prolonged stay in endemic areas
Rabies	Inactivated	Not generally indicated in IBD patients
BCG (Bacillus–Calmette–Guérin)	Live attenuated	Contraindicated in immunocompromised. BCG efficacious for protection against severe TB when given in infancy. Not indicated in IBD patients

Vaccine Safety in IBD Patients

Inactivated vaccines in IBD patients are generally considered to have a similar safety profile to that in non-IBD patients [42]. Although there have been case reports of flares of disease activity post vaccination, including UC flares post influenza vaccine [43], overwhelmingly the data available from well-designed controlled studies does not support a causal relationship between vaccination and disease flares. Some of this evidence comes from vaccine immunogenicity and safety trials in the IBD population [44–46]. Although no large observational studies in IBD have been performed, studies of vaccination and disease relapse in patients with rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis have not shown any evidence for an association [47, 48]. It is important that exacerbations of chronic diseases are included as conditions of interest for vaccine postmarketing surveillance.

The suggestion of a potential temporal association between vaccines and the onset of autoimmune diseases is long-standing, but the weight of evidence does not support a causative effect [49]. In addition, a number of studies have investigated the temporal link between immunization and the onset of autoimmune conditions, such as multiple sclerosis and rheumatoid arthritis, with no link found [50].

Strategies to Promote Immunization

Although there is increasing awareness among gastroenterologists of the risk of VPD [13], low levels of vaccine coverage have been found in outpatient surveys of IBD patients. This emphasizes the important role of gastroenterologists in recommending and, when possible, planning for and delivering vaccines to the IBD population [51]. One survey of adult gastroenterologists found that 39% (17 out of 44) of respondents did not routinely vaccinate IBD patients [52]. Many respondents only reviewed whether routine childhood vaccinations were up-to-date, and only 11% clarified vaccination status prior to TNF inhibitor use. Even if vaccination is a shared care responsibility between the patient's primary care doctor and gastroenterologist, close collaboration and communication between both physicians is essential. The use of electronic reminder systems and "flags" has been identified as one method to clarify the immunization status and make a vaccination plan [13, 52]. Individual patient vaccination plans should take into account existing guidelines relevant to the patient's location, and also account for any pre-existing risk factors [42].

Assessment of Immunization Status in IBD Patients

All immunization guidelines have in common the recommendation that IBD patients should be reviewed at diagnosis to ensure that all routine vaccinations have been received according to their local immunization schedule [42]. Consideration should also be given to additional vaccines, such as pneumococcal, influenza (annual), varicella and HPV vaccines, and other immunizations depending on the availability and national schedule [42, 53–55]. The few published studies on compliance with immunization guidelines in IBD patients suggest that vaccine coverage among IBD patients is low and not well documented. A United States survey of 169 adolescents and adults diagnosed with IBD attending an IBD specialty clinic identified that 86% were on immunosuppressive therapies [51]. On recall of immunization status, only 28% of patients reported having received influenza vaccine and 9% pneumococcal vaccine. Serological assessment for hepatitis B virus indicated that only 47 (28%) had been vaccinated. This study also found most patients (81%) had a history of varicella (chickenpox) but 31 patients were uncertain and only 12 reported varicella vaccination,

leaving 19 (11%) potentially “at risk” of varicella infection [51]. In these 19 patients, seven had varicella serology performed and 57% (4 out of 7) were confirmed to be seronegative. This low vaccine coverage and the risk for VPDs highlights the need to develop immunization plans for all patients with IBD.

One of the difficulties in obtaining a good vaccination history at IBD diagnosis is relying on recall as a measure of vaccination status. In a number of studies self-reporting of vaccination status has been found to be a relatively sensitive tool, but of low specificity. A cross-sectional survey of patients of a United States Veteran Affairs Medical Center found self-reported influenza vaccination had a sensitivity of 1.0 and specificity of 0.79. Self-reported pneumococcal vaccination status had a sensitivity of 0.97 and a specificity of 0.53. This may reflect the fact that influenza vaccine is recommended annually and recalled more readily, whereas other vaccines, such as pneumococcal polysaccharide vaccine, may have been administered a number of years previously and forgotten [56]. Similarly, an Australian hospital-based study of nearly 5,000 subjects aged ≥ 65 years had high sensitivity (98%) for self-reported influenza vaccination status but low specificity (56%) [57]. This highlights that vaccine history needs to be carefully clarified and should be verified whenever possible with the patient’s primary care provider. While there are some population-based immunization registers, they exist variably in most regions of the world, and are often for children rather than adults [58].

Role of Serologic Testing

In certain instances, serology can be used to measure the response to vaccination; however, the tests performed are laboratory-dependent and may only be suitable for use as a research tool, or for detecting antibody from prior natural infection (not vaccination). Serology can be helpful when there is a clear correlate of protection, such as the presence of hepatitis B surface antibody 6 weeks post hepatitis B vaccination. Other vaccine antibody responses for which routine serology is generally considered reliable as a test of seroconversion and likely protection, include measles, mumps, and tetanus. However, for most VPD, even the best serologic marker does not provide a strict correlate of protection, and so, if doubt exists as to whether a patient has previously had the appropriate number of vaccine doses, re-vaccination is generally recommended. Individual physicians will consider the use of serology to guide their decision making with IBD patients about previous clinical exposure to VPD such as varicella [51].

Vaccines for IBD Patients

Hepatitis B Vaccine

There is a risk of reactivation of hepatitis B infection with the use of immunosuppressive therapies in IBD as detailed above [59–62]. Excluding latent infection and ensuring satisfactory response to hepatitis B vaccination in patients with IBD is crucial and should be conducted at diagnosis. Populations vary widely in the seroprevalence of hepatitis B, which is impacted by both disease epidemiology in the country of residence and vaccination policy. In one IBD outpatient serosurvey, only 28% had serological evidence of hepatitis B protection [51]. Similarly, a detailed review of 315 IBD patients (252 Crohn’s disease and 63 ulcerative colitis) who had been vaccinated as part of the routine immunization schedule in France years earlier revealed an adequate postvaccination anti-Hep B surface antibody (anti-hepBsAb of >10 IU/mL) in 49% of patients [60]. In a multivariate analysis, age at diagnosis of >31 years ($p=0.005$) and disease duration of >7 years ($p=0.005$) were

associated with a lack of antibody [60]. This was similar to a cross-sectional multicenter study in Spain [63].

As a nonlive vaccine, hepatitis B vaccine can be given to IBD patients irrespective of whether they are receiving immunosuppressive therapy. Vaccination is generally in a three-dose course over a 6-month period [53–55]. If a previously fully immunized person is shown to be nonimmune, an additional “booster” dose should be administered, and confirmation of serological response (>10 mIU/mL) should be undertaken 4–6 weeks after the dose. If the patient continues to show nonresponse to a booster dose, strategies such as high-dose vaccination, repeat boosting and/or intradermal administration can be considered, as these approaches have been successful in other high-risk populations such as hemodialysis patients [64].

Influenza Vaccine

As the risk of morbidity and mortality from influenza infection appears higher in IBD patients, it is recommended they receive inactivated seasonal trivalent influenza vaccine annually [53–55]. If the diagnosis of IBD is made in childhood (<10 years of age) and the child has not previously received at least two doses of influenza vaccine (<10 years of age), it is recommended that two doses are given in the first year they receive the vaccine (minimum 1 month apart) with single dose annual vaccination thereafter [53–55].

The response to influenza vaccine in patients receiving immunomodulatory therapy is variable. One immunogenicity study of the trivalent influenza vaccine (TIV) in 146 IBD patients found better seroprotection against A/H1N1 and A/H3N2 than the B strain ($P<0.02$), independent of immunosuppression status, although anti-TNF therapy diminished the response to one of the three vaccine strains [44]. Other studies have found immunosuppressant treatment modalities impact on influenza vaccine immunogenicity response in IBD patients [45]. For example, in one study, 79 subjects (29 healthy controls, 50 IBD patients) as one patient did not complete the study. (a) TNF inhibitor (infliximab) and immunomodulatory (corticosteroids, 6-mercaptopurine, or methotrexate) (16 subjects); (b) immunomodulatory alone (20 subjects); and (c) anti-inflammatory therapy (14 subjects). The 16 patients receiving infliximab and immunomodulatory therapy were less likely to respond to two influenza vaccine antigens (A/New Caledonia/20/99 and B/Hong Kong/330/2001; $p=0.018$ and $p=0.0002$, respectively). This decreased immunogenicity may potentially be overcome in selected circumstances by administering an additional dose of vaccine, as seen in some special risk group studies of H1N1 vaccination [65].

Studies to specifically assess efficacy of the trivalent influenza vaccine (TIV) in preventing influenza disease are generally lacking in special risk groups, including patients with IBD [66]. One small randomized trial in HIV ($N=103$) patients reported high efficacy in those receiving TIV [67]. This study, as with many immunogenicity trials in high-risk groups such as IBD patients, was limited by a small sample size. Overall, there is need for further research in this area, including of new vaccine delivery methods, such as intradermal vaccination, that could potentially be more immunogenic and hence produce better clinical protection [68, 69]. New vaccine adjuvants may also prove to be more immunogenic in immunocompromised patients, such as those with IBD [68, 69].

Pneumococcal Vaccines

Vaccination against pneumococcal disease, with either conjugate or polysaccharide vaccines, is generally recommended for patients with IBD, depending on their age and past history of immunization. Many developed countries now incorporate pneumococcal conjugate vaccines (PCVs) in their infant

immunization schedule [53, 55]. The schedule is usually three doses in infancy, followed by a “booster” fourth dose after 12 months of age. There are 90 pneumococcal serotypes and the conjugate vaccine links the individual serotype capsular pneumococcal antigen with a carrier protein. The first conjugate vaccine available was the 7-valent PCV (against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) linked to CRM197, a nontoxic mutant of diphtheria toxin. Conjugate vaccines have been shown to be immunogenic and efficacious in preventing childhood invasive pneumococcal disease (IPD) in numerous international settings [70, 71]. The vaccines have also been administered and shown to be efficacious in high-risk groups such as indigenous Americans [72].

The next generation of conjugate pneumococcal vaccines came into widespread international use in 2010, with the 13-valent PCV (containing five additional serotypes – 1, 3, 5, 6A, 7F, and 19A – linked to the same carrier protein) replacing the 7vPCV in the United States, and the United Kingdom [73, 74]. There is also a 10-valent PCV (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F), which uses a different carrier, “protein D,” a highly conserved surface protein from nontypeable *Haemophilus influenzae* (NTHi). Eight of the 10 pneumococcal antigens are conjugated to protein D from a NTHi, one is conjugated to a diphtheria toxoid carrier protein and the other is conjugated to a *Clostridium tetani* (tetanus) toxoid carrier protein.

There are limited data specifically examining the use of these conjugate pneumococcal vaccines in IBD patients, but a study of juvenile idiopathic arthritis (JIA) patients on biologic therapies showed excellent immunogenicity [75]. Children receiving anti-TNFs produced mean antibody titers above the threshold level, but had significantly lower titers against serotypes 4, 14, and 23F ($p < 0.05$) than controls [75].

The 23-valent polysaccharide vaccine (23vPPV) is routinely recommended for use in older persons, or those with an increased risk of invasive pneumococcal disease. This vaccine, which is not conjugated, contains more serotypes but is not immunogenic in infants and children <2 years of age, and does not induce strong memory responses. In adults known to be at high risk of IPD, including IBD patients on immunosuppressive therapies, some guidelines recommend a 23vPPV vaccine [53, 76]. However, the immunogenic response to the 23vPPV in patients on immunosuppressive therapies appears to be reduced. A study of 23vPPV immunogenicity in adult IBD patients measured five pneumococcal serotypes, with the patients divided into three patient groups: (a) combination TNF-blockers plus immunomodulators (64 subjects); (b) IBD patients not on immunosuppressive therapy (25 subjects); and (c) age-matched healthy controls (19 subjects). There was no difference in pre-vaccination titers among the three groups. Vaccine responses were lower in Group A than in Group B ($P < 0.01$ for four out of five antigens) and Group C ($P < 0.01$ for all five antigens), strongly suggesting decreased immunogenicity in patients on combination therapies [77]. Variable immunogenicity of 23vPPV has also been seen in other high risk groups such as patients with rheumatoid arthritis, again particularly those on combination therapies [78].

Further studies of the best combination of the available pneumococcal vaccines are still needed to maximize protection from IBD in different age groups [79]. The use of higher valency pneumococcal vaccines, including the 10- and 13-valent PCVs, will be part of evolving strategies to improve IPD protection. Trials of pneumococcal vaccines for the prevention of invasive pneumococcal disease and community acquired pneumonia in adults are currently ongoing which may change recommendations for use of these vaccines in older persons [80].

Varicella Vaccine

Live attenuated vaccines such as varicella must be considered with caution in patients who are immunosuppressed due to risk of infective complications [81]. This needs to be balanced with the risk of wild-type varicella infection; in an adult IBD study using a detailed history and serological assessment, approximately 10% were found not to be protected from clinical varicella [51]. The best

approach to preventing varicella in IBD patients is to review the clinical history and immunization status at diagnosis and undertake varicella serology, if unclear, for evidence of past infection. Varicella vaccination should ideally be given as two doses (minimum 1 month apart), at least 4 weeks before commencing immunosuppressive therapy. This “window of opportunity” is becoming smaller with the earlier use of immunomodulatory therapy in IBD and may not always be possible [81].

A small case series of six children with IBD safely received varicella vaccination while on infliximab therapy [46]. The small numbers of patients means universal recommendations cannot be made. Larger prospective studies are required before this can be recommended as there are case reports of disease from disseminated vaccine virus infection in immunocompromised patients [82]. Larger studies of live attenuated varicella vaccine have been conducted in other special risk groups, such as children with leukemia on chemotherapy. However, although the incidence of varicella and later herpes zoster was reduced, vaccine associated rash was common (40%), and required antiviral therapy in 4% of patients [83]. In this study of 437 children, 98% responded after one or two doses. Of those on maintenance chemotherapy when immunized, 40% (149/372) had a nonfatal rash which was treated with acyclovir in 4%. These patients were found to have a decreased incidence of herpes zoster on follow-up ($p < 0.05$) [83, 84], however, these results have not lead to routine recommendations for varicella immunization in children on chemotherapy. In summary, the administration of live vaccines needs to be made on a case-by-case basis with input from gastroenterologists, and immunization specialists as required.

Protecting IBD patients from VPD such as varicella can also be maximized through “cocooning,” that is, ensuring immediate close household contacts are protected against varicella. The feasibility of this approach has been shown via varicella vaccination of household contacts of children with leukemia [85].

Zoster Vaccine

A high-titer varicella-zoster virus (VZV) vaccine for the prevention of herpes zoster is now registered in some countries for persons >50 years of age who have previously had varicella infection. It is also a live attenuated vaccine with an approximately 14-fold higher varicella titer than the varicella vaccine. This booster dose has the effect of suppressing virus reactivation and hence the development of a “shingles” rash and associated neuralgia. In a single placebo-controlled trial of >38,000 healthy adults aged >60 years followed up with a median of 3.1 years, vaccination reduced the burden of illness due to herpes zoster by 61% ($P < 0.001$) [86]. It also reduced the incidence of postherpetic neuralgia by 67% ($P < 0.001$) [86]. This vaccine should therefore be considered in all adults aged >50 years, including those with a chronic illness such as IBD. The same provisos described above with regards to safety with a live attenuated virus apply and, ideally, the vaccine should be administered at least 4 weeks before immunosuppressive medication is commenced [87].

Human Papillomavirus Vaccine

Female IBD patients are at increased risk of HPV-related cervical Pap screen changes [36, 37] so optimizing their protection is essential. Approximately 70% of cervical cancers are associated with HPV serotypes 16 and 18. Two other types, 6 and 11, are the major cause of anogenital warts [88]. There are two inactivated vaccines available internationally: both offer protection against the oncogenic types 16 and 18, with one also containing types 6 and 11. Both are inactivated vaccines, based on virus like particle (VLP) technology. In healthy females HPV vaccines have been shown to be

highly immunogenic and efficacious against cervical carcinoma-in-situ (CIN) [89, 90]. Vaccination has also been shown to be immunogenic in males, and to protect against external genital lesions in males (4-valent HPV vaccine) [91, 92].

The vaccines are registered for females aged 9–45 years and males aged 9–26 years. As HPV vaccines are prophylactic, they will not clear infection (i.e. are not therapeutic) and ideally should be administered before exposure to HPV through sexual contact. Most national HPV vaccination programs administer the vaccines between 9 and 13 years of age [53–55]. It is safe to administer in patients with IBD. Vaccination should be considered in all females up to 45 years of age, as the individual risk of future potential HPV exposure and level of immunosuppression is unknown. Regular screening for cervical changes is still recommended for all women (including those with IBD) who have been vaccinated as vaccination does not protect against all cervical cancer causing strains.

HPV vaccines are not currently funded for widespread use in males, despite being recently registered for use in adolescent males in many countries. The reasons for vaccinating males, particularly those with IBD, include reducing the burden of anogenital warts and the risk of HPV-associated anogenital and oropharyngeal cancers in males, and reducing HPV infection of nonimmunized females [93, 94].

There is currently no serological correlate of protection for HPV vaccines and the follow-up has not been long enough to measure the impact on cervical cancer rates. HPV vaccines appear to be more immunogenic than natural infection and high antibody titers have persisted for greater than 6 years of follow-up of original trial patients [90, 95, 96]. There are also a number of ongoing studies reviewing the immunogenicity of HPV vaccination among IBD patients on immunosuppressive therapy which will help determine whether additional doses will be required in the future [97, 98].

Meningococcal Vaccines

Up to 25% of IBD patients are diagnosed in adolescence or early adulthood [99] and the incidence in the pediatric population is increasing [100]. This is one of the age groups at highest risk of meningococcal disease, and the United States now recommend two doses of 4-valent (A, C, W135, Y) conjugate meningococcal vaccine for adolescents [101]. Depending on the local meningococcal disease epidemiology, some countries, such as Australia and the United Kingdom, currently administer a conjugate meningococcal C vaccine alone in early childhood [53].

It is important to consider whether immunosuppressive therapies will affect the response to meningococcal vaccines. Although there are no studies of the immunogenicity of meningococcal conjugate vaccines in IBD patients, a multicenter cohort study of JIA patients (aged 1–19 years) found the overwhelming majority were able to mount a satisfactory immune response, including patients on high-dose immunosuppressive medication [102]. Importantly, there was no increase in JIA disease activity or relapse frequency. A meningococcal vaccine “booster” vaccination, preferably using conjugate rather than polysaccharide vaccine, should therefore be considered in adolescence or early adulthood as part of standard programs in both males and females with IBD.

Future Studies of VPD and Vaccines

There is a requirement to improve the data on the immunogenicity, efficacy and safety of vaccination in individuals with IBD, particularly those on immunosuppressive therapies. These data need to be compared with that from with other special risk groups on similar therapeutic regimens, such as patients with arthritis on biologic therapies. Studies of immune persistence will also help to

determine whether “booster” doses are required for certain patient groups. More trials into novel vaccine delivery methods (e.g. intradermal) [69], new adjuvants, and of vaccines with greater strain coverage than currently available vaccines (e.g. pneumococcal conjugate vaccines) are required to optimize protection in vulnerable IBD patients.

Conclusions

Patients with IBD disease, particularly those on immunosuppressive therapies, are at increased risk of infection, including VPDs. The increased risk of infection in IBD patients impacts on their associated morbidity and mortality. A Preventative medicine approach for IBD patients is crucial to improving their quality of life [16]. This should include a comprehensive and ongoing risk assessment for infectious disease risk in all IBD patients that incorporates screening for and treatment of underlying infection(s), patient education, vaccination, chemoprophylaxis, and careful monitoring while on immunosuppressive therapy. Implementation of these preventative strategies will have an appreciable impact on the organization of care and on wellbeing of all IBD patients.

Conflict of Interest

Murdoch Childrens Research Institute research fund has received honoraria for NC sitting on a Wyeth/Pfizer advisory board for pneumococcal vaccines and presentations at conferences and NC investigator-led research project support from CSL for active surveillance of Guillain-Barré syndrome following H1N1 vaccination.

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Chapter 55

Diagnosis and Management of Colorectal Neoplasia in Patients with Inflammatory Bowel Disease

Aarti Kakkar and Francis A. Farraye

Keywords Colorectal cancer • Primary sclerosing cholangitis • Endoscopic surveillance • Dysplasia • High grade dysplasia • Low grade dysplasia • Dysplasia associated lesion or mass • Chromoendoscopy • Colectomy • Chemoprevention

Introduction

Colorectal cancer (CRC) is a feared complication of longstanding ulcerative colitis (UC) and Crohn's colitis. Though several risk factors for the development of CRC have been recognized, the identification of colitis-related dysplasia confers the greatest risk. Consequently, enrollment in a colonoscopic surveillance program to detect dysplasia has been advocated by many gastrointestinal societies. The goal of colonoscopic surveillance is to minimize the morbidity and mortality from CRC with appropriate and timely referral for colectomy. This chapter will review the epidemiology and risk factors for CRC in patients with IBD, outline the most recent surveillance guidelines from the American Gastroenterology Association (AGA) and British Society of Gastroenterology (BSG), discuss management of dysplasia, and briefly summarize chemoprevention.

Epidemiology

For over 50 years, the association between inflammatory bowel disease (IBD) and CRC has been recognized though it remains incompletely understood. Features that distinguish colitis-related CRC from sporadic CRC include:

1. A known increased risk of CRC in patients with chronic colitis compared to the general population
2. The rate of synchronous tumors in IBD is higher than in sporadic CRC (12% to 3–5%) [1]
3. Absence of a preceding adenomatous lesion

A. Kakkar, MD • F.A. Farraye, MD, MSc (✉)
Section of Gastroenterology, Boston Medical Center, Boston University School of Medicine,
85 East Concord Street, Boston, MA, USA
e-mail: francis.farraye@bmc.org

4. The presence of diffuse clonal molecular and genetic changes that place the entire colorectal mucosa at risk for malignancy
5. A more rapid rate of progression to dysplasia

The exact risk of developing CRC is difficult to quantify due to significant variations in clinical studies. Importantly, two population based studies of patients with CRC and UC and CRC and CD suggest that the prognosis of CRC is worse for patients with IBD [2, 3].

Evidence to support the link between UC and CRC is well established. A large meta-analysis by Eaden et al. assessed the risk of cancer in patients with UC at 2% after 10 years, 8% after 20 years, and 18% after 30 years of disease [4]. More recent data from a 30-year prospective surveillance program in the UK by Rutter et al. reports the cumulative incidence of developing CRC at 2.5% at 20 years, 7.6% at 30 years, and 10.8% at 40 years [5]. Subsequent population based studies have suggested that the risk may not be this high and actually may have decreased over time. The incidence of CRC in IBD in more recent series suggests a decrease in risk [6]. This may be due to more widespread use of medications to control inflammation or colectomy, or reflect the efficacy of surveillance programs.

The data in CD is less clear. Many early studies failed to adjust for variables such as disease location, history of colonic resection, or duration of disease. However, in those patients with Crohn's colitis who did not have a colectomy, an increased incidence of CRC has been noted [7–10]. A large population based study by Bernstein et al. confirmed an increased incidence of CRC in CD patients that was equal to the risk for UC patients [11]. A meta-analysis of 12 studies by Canavan et al. reported an overall CRC relative risk (RR) in CD patients of 2.5 (95% CI, 1.3–4.7). The RR increased to 4.5 (95% CI, 1.3–14.9) in patients with colonic Crohn's [12]. Therefore, the risk of developing CRC appears to be equivalent in patients with Crohn's colitis and UC.

Risk Factors

Several risk factors are associated with the development of colorectal neoplasia. These include disease specific features as well as family history and primary sclerosing cholangitis (PSC).

Disease duration and extent of colitis in UC or CD are well known risk factors for the development of CRC. The extent of disease should be defined by the most extensive disease histologically or endoscopically at any time during the patient's illness. Patients with subtotal colitis and pancolitis have the highest risk of developing CRC; whereas those with proctitis or distal proctosigmoiditis alone are at no increased risk compared with the general population [13]. Patients with left-sided colitis carry an intermediate risk; however, their risk approaches that of patients with pancolitis as disease duration increases [13–16]. There is insufficient evidence to support the presence of backwash ileitis as an independent risk factor for developing CRC [17]. Duration of disease has been observed to increase the risk of CRC in IBD. Specifically, the RR rises after 8–10 years of disease and this is the rationale behind the initiation of surveillance colonoscopy [4, 5].

A concurrent diagnosis of PSC is an undisputable risk factor for the development of CRC. A meta-analysis of 11 trials by Soetikno et al. reported a fourfold increased risk of developing colonic neoplasia in patients with PSC and UC compared to those with UC alone [18]. In addition, several clinical trials have demonstrated a persistently elevated risk of CRC despite undergoing orthotopic liver transplantation [19–22]. For these reasons, those patients with IBD and PSC should undergo surveillance colonoscopy annually beginning at the time PSC is diagnosed.

Other identified risk factors for CRC in IBD include family history, age of onset of IBD, gender, and severity of histologic and endoscopic inflammation. A case control study of 297 patients from the Mayo Clinic found that a family history of CRC was twice as common in patients with UC and

CRC compared to those patients with UC alone [23]. In a study by Askling et al. a family history of CRC was associated with a greater than twofold increased risk of CRC in patients with IBD [24]. Though disease duration has been proven to increase the risk of developing CRC, studies examining a link between early age of onset of IBD and CRC are conflicting. Thus, according to the American Gastroenterology Association Guidelines, surveillance should be based on duration of illness, not chronological age [17]. A recent population based cohort study of 7,607 patients diagnosed with IBD assessed the sex-specific incidence of CRC and found that IBD confers a lower risk of CRC to females than to males [25].

The degree of inflammation determined by endoscopy and histology has also been examined as a factor that affects the risk of CRC. A case control study of 68 patients with UC found that the degree of histologic inflammation was positively correlated with an increased risk of neoplasia [26]. A cohort study of 418 patients with UC found a positive association between degree of microscopic inflammation and advanced neoplasia [15, 27]. Endoscopic features, such as colonic strictures and pseudopolyps, have been studied as well. Colonic strictures in UC are considered a risk factor for CRC, whereas this is not the case for colonic strictures in CD [28, 29]. Data from Rutter et al. also demonstrated that a shortened, tubular colon increases CRC risk [29]. Lastly, the presence of multiple pseudopolyps increases the risk of developing CRC and may limit the ability to adequately survey the colon [29]. These clinically important associations should be considered when counseling a patient about their risk of developing CRC.

Surveillance

The purpose of endoscopic surveillance is to reduce morbidity and mortality related to CRC. This belief is based on indirect evidence that endoscopic surveillance may reduce the risk of death from CRC in the IBD population and be cost effective. Though no randomized studies have been performed, the American Gastroenterology Association, British Society of Gastroenterology, American College of Gastroenterology, and the Crohn's and Colitis Foundation of America agree that all patients with UC should undergo a screening colonoscopy between 8 and 10 years after disease onset [17, 30–32]. Patients with small bowel Crohn's disease or limited colonic Crohn's disease and patients with ulcerative proctitis or distal proctosigmoiditis are considered to be at average risk for developing CRC; however, an index screening exam should be done at 8–10 years after disease onset to assess true microscopic extent of disease [17].

There are several factors that influence the success of performing an adequate surveillance colonoscopy. Firstly, the ability of the endoscopist to detect dysplastic areas can be variable. A retrospective study found that two-thirds of dysplastic lesions in patients with UC were endoscopically visible; however, dysplasia can still be present in a normal appearing segment of colon [33]. Patients need to be informed of this limitation. If a dysplastic lesion is found, its resectability will then affect surveillance decision-making. The success of surveillance also depends on adequate tissue sampling. When random biopsies are performed surveillance guidelines advise that 33 biopsy specimens be obtained. This recommendation is based on one study in which 33 biopsies were needed to detect dysplasia with 90% probability [34]. Anatomic features such as strictures and pseudopolyps may also interfere with ability to adequately sample colonic mucosa. Patient acceptance and compliance with a surveillance program is essential for success. Lastly, the ideal surveillance interval remains unclear. In a study in which UC patients underwent colonoscopies every 2 years, interval cancers were observed to develop between 10 and 28 months after a dysplasia-free exam [35]. This has led to some variations in surveillance interval guidelines between the major societies.

Table 55.1 AGA surveillance guidelines for CRC in IBD

- All patients, regardless of extent of disease at initial diagnosis, should undergo a screening colonoscopy a maximum of 8 years after onset of symptoms, with multiple biopsy specimens obtained throughout the entire colon, to assess the true microscopic extent of inflammation
- Patients with ulcerative proctitis or ulcerative proctosigmoiditis are not considered at increased risk for IBD-related CRC and thus may be managed on the basis of average risk recommendations
- Patients with extensive left-sided colitis should begin surveillance with 1–2 years after the initial screening endoscopy
- After 2 negative exams (no dysplasia or cancer), further surveillance exams should be performed every 1–3 years
- Patients with PSC should begin surveillance colonoscopy at the time of PSC diagnosis and then yearly
- Patients with a history of CRC in a first-degree relative, ongoing active endoscopic or histologic inflammation, or anatomic abnormalities (shortened colon, multiple pseudopolyps, or stricture), may benefit from more frequent surveillance colonoscopy
- Representative biopsy specimens from each anatomic section of the colon is recommended. Though no prospective trials have determined the optimal number of biopsies to take, one study has recommended a minimum of 33 biopsy specimens
- Surveillance colonoscopy should ideally be performed when the patient is in remission
- These recommendations apply to Crohn's colitis who have disease involving at least one-third of their colon

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The most recent guidelines published by the AGA and BSG outline techniques for successful CRC surveillance. Ideally, the colonoscopic exam should take place when the patient is in clinical remission. The presence of inflammation can make pathologic discrimination of dysplasia difficult, but surveillance should not be put off if remission cannot be reached [30]. Four quadrant biopsy specimens should be obtained from every 10 cm of colon or from each anatomic segment of the colon. Specimens should be placed in separate jars in order to localize dysplastic areas.

Newer imaging techniques such as narrow band-imaging, confocal endomicroscopy and chromoendoscopy have been developed in an effort to improve the diagnostic yield of colonoscopic surveillance. Chromoendoscopy, which appears to be the most readily available method for use during colonoscopy, involves spraying the entire colonic mucosa with either methylene blue or indigo carmine. Recognition of pit patterns allows better differentiation between dysplastic and non-dysplastic tissue. In one of the first studies of chromoendoscopy by Kiesslich et al. 165 UC patients were randomized to standard colonoscopy or chromoendoscopy. There were increased dysplasia detection rates in the chromoendoscopy group compared to the standard group [36]. Several subsequent studies showed improved dysplasia and cancer detection rates when dye spraying techniques are used to allow for targeted biopsies compared to random biopsies [37, 38]. Evidence supporting the use of narrow band imaging to improve detection of dysplasia has not materialized [39, 40]. Preliminary studies using confocal endomicroscopy are promising but this technique is not easily introduced in endoscopic practice [40].

Specific recommendations from the 2010 AGA Technical Review on the Diagnosis and Management of Colorectal Neoplasia in IBD are outlined in Table 55.1 [17].

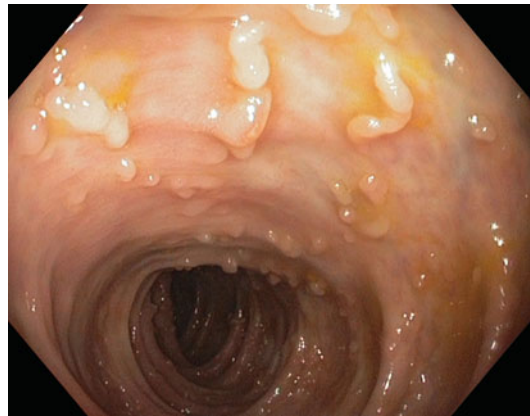
The BSG recently updated their guidelines in 2010 (Table 55.2) and incorporate risk factors and newer imaging techniques [30]. See Table 55.2 for risk stratification.

1. All patients with UC or Crohn's colitis should have a screening colonoscopy 10 years after onset of colitic symptoms.
2. Pan-colonic dye spraying with targeted biopsies of abnormal mucosa is recommended. If chromoendoscopy is not used, then 2–4 random biopsies every 10 cm should be taken.
3. Screening intervals should be yearly, 3-yearly, or 5-yearly depending on patient's risk factors.

Table 55.2 BSG surveillance colonoscopy guidelines

Low risk – 5 year colonoscopy	• Patients with extensive colitis without evidence of endoscopic/microscopic inflammation at last colonoscopy, or left-sided-colitis • Crohn's colitis affecting <50% of the colon
Intermediate risk – 3 year colonoscopy	• Patients with extensive colitis with mild endoscopic/microscopic inflammation on previous colonoscopy • Family history of CRC in a first degree relative over 50
Higher risk – yearly colonoscopy	• Patients with extensive colitis with moderate or severe endoscopic/microscopic inflammation on previous colonoscopy • Stricture within the past 5 years • Confirmed dysplasia within the past 5 years in a patient who declines surgery • PSC • Family history of CRC in a first degree relative under the age of 50

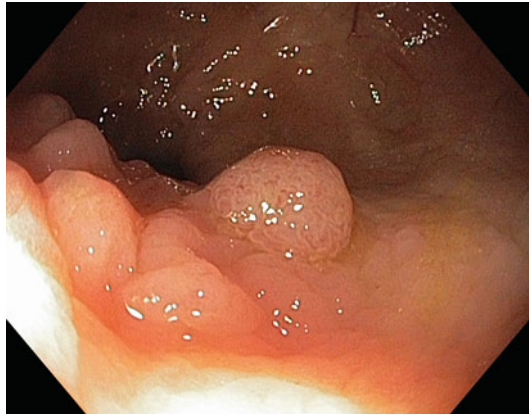
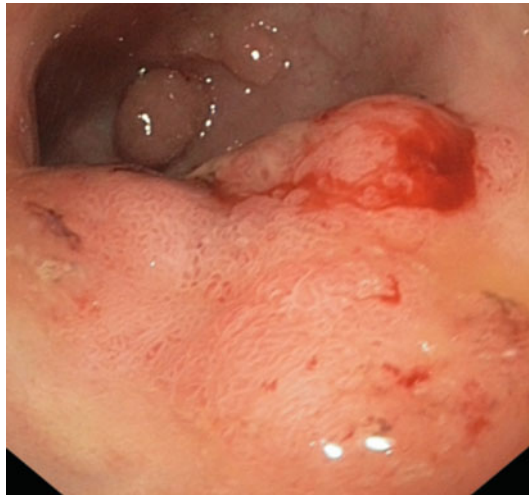
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Fig. 55.1 Pseudopolyps

Dysplasia

The purpose of surveillance is to detect dysplastic mucosa, which is the best marker of CRC risk in IBD patients. The endoscopist and pathologist must work together to accurately identify and classify dysplasia. Biopsies taken during surveillance colonoscopy must be graded as (1) positive for dysplasia, (2) negative for dysplasia, or (3) indefinite for dysplasia. Dysplastic specimens must be further classified as (1) low-grade dysplasia (LGD), (2) high-grade dysplasia (HGD), or (3) carcinoma [41]. The greatest inter-observer variability among pathologists surrounds the interpretation of low-grade dysplasia and indefinite for dysplasia categories. Therefore, once dysplasia is detected, a second pathologist with expertise in interpretation of gastrointestinal biopsies should confirm the diagnosis.

Dysplastic mucosa may be characterized as either flat or raised. Flat dysplasia is generally considered endoscopically undetectable, identified by random biopsies and can be further classified as either multifocal or unifocal. Raised or polypoid dysplasia is referred to by the acronym DALM-dysplasia associated lesion or mass. Early studies indicated that the presence of DALMs was associated with a high incidence of CRC and colectomy was recommended [42]. However, DALMs were subsequently recognized to behave differently based on their endoscopic appearance. An adenoma-like DALM has features similar to sporadic adenomas whereas those that appeared sessile, irregular, or ulcerated are classified as non-adenoma-like DALMs [43]. The distinction between adenoma and non-adenoma-like DALMs and unifocal and multifocal dysplasia is critical to patient management. Figures 55.1–55.3 are examples of pseudopolyps, adenoma-like DALMs, and non-adenoma-like DALMs.

Fig. 55.2 Adenoma-like DALM**Fig. 55.3** Non-adenoma-like DALM

Management of Dysplasia: When to Perform a Colectomy

The management of adenoma-like DALMS depends on the endoscopist's ability to accurately identify and completely remove the lesion. The colonic mucosa adjacent to the raised lesion should also be biopsied to evaluate for dysplasia. Several studies have concluded that, in the absence of dysplasia elsewhere in the colon, patients who have undergone polypectomy of an adenoma-like DALM had a low risk of developing dysplasia or carcinoma over a follow-period ranging from 49 to 82 months [43–45]. Endoscopic resection of adenoma-like DALMs is an effective and safe treatment course provided the remainder of the colon is dysplasia free and the lesion is removed in its entirety [46]. A follow-up colonoscopy 3–6 months after resection of a DALM should be performed to ensure absence of residual dysplastic tissue. In contrast, non-adenoma-like DALMs are generally endoscopically unresectable and have been shown to correlate with the presence of synchronous and metachronous carcinoma. Patients with IBD and non-adenoma-like DALMs should be treated with colectomy [17].

Colectomy is the treatment of choice when flat HGD is confirmed by a second GI pathologist or incompletely resected raised dysplasia is discovered. In a review of ten prospective surveillance trials that include 1,225 patients, the prevalence of synchronous CRC in patients with flat HGD was

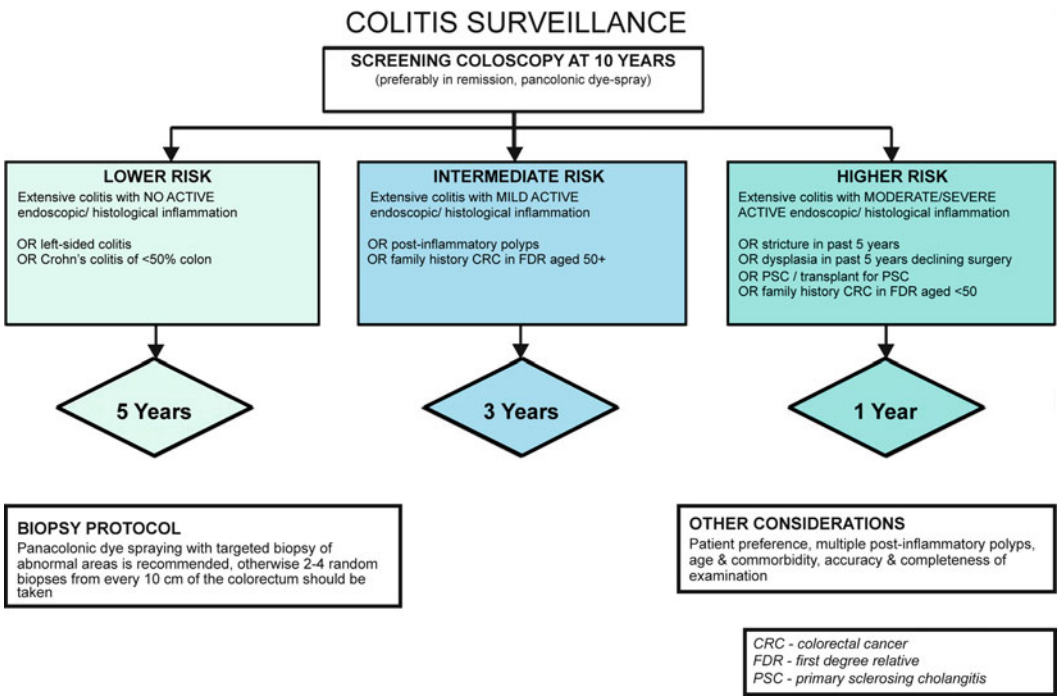


Fig. 55.5 Recommendations from the British Society of Gastroenterology (Reproduced from Gut. Cairns SR, Scholefield JH, Steele RJ, et al. 59:666-89. ©2010 with permission from BMJ Publishing Group Ltd)

Chemoprevention

Several chemopreventive agents that may help decrease the risk of CRC in IBD have been studied. These include mesalamine-based agents (5-ASAs), corticosteroids, immunomodulators (thiopurine analogues), folic acid, and ursodeoxycholic acid (UDCA). Unfortunately, most data is retrospective and unable to provide resounding proof of a significant effect.

5-ASA compounds have been shown to have a chemoprotective effect in some, but not all, studies. A systemic review of the literature concluded that mesalamine is chemoprotective; however, their results were limited by marked heterogeneity among the trials [54]. Furthermore, a chemoprotective effect from mesalamine independent of histologic inflammation has not been shown [26, 27]. Whether or not 5-ASAs decrease CRC risk, their utility in maintaining remission warrants continued use.

Folic acid, which has been noted to have a protective effect for sporadic CRC, was investigated in IBD patients by Lashner et al. Though a statistically significant protective effect was not discerned, there was a trend toward protection against CRC [55, 56]. Given the low cost, safety, and ease of administration, daily folic acid should be considered for all high-risk IBD patients [46]. The AGA concluded that there was insufficient evidence to recommend for or against the use of thiopurines, supplements, or statins as chemopreventive agents [17]. Of note, just prior to publication of the AGA position paper, Beaugerie and colleagues reported (in abstract form) that use of thiopurines was associated with a 3.5-fold reduction of advanced neoplasia (CRC or high-grade dysplasia) in a cohort of 2,841 patients with longstanding and extensive UC [57].

In patients with UC and PSC, high dose UDCA has demonstrated a chemoprotective effect with a 40% difference in neoplasia noted between the UDCA treated and the untreated group [58].

Another randomized clinical trial of high dose UDCA noted a 74% reduction in dysplasia or CRC [59]. Recent data from the Mayo Clinic however suggests that the risk of CRC may actually be increased in PSC patients on high dose UDCA [60]. In this study, patients who received 28–30mg/kg/day of UDCA were at significantly higher risk of developing colorectal neoplasia compared with those who received placebo after 235 patient years (hazard ratio: 4.44, 95% confidence interval: 1.30–20.10, $P=0.02$). Despite this recent development, some clinicians continue to recommend low-dose UDCA at 10mg/kg/day in patients with UC and PSC for chemoprevention. Further studies are needed to better ascertain the risks and benefits of UDCA in CRC prevention.

In summary, colitis-related CRC continues to be a complication of longstanding and extensive UC and Crohn's disease of the colon. All patients with IBD should undergo an initial surveillance colonoscopy 8–10 years after disease onset to assess the extent of colonic involvement. Patients with PSC should undergo yearly colonoscopy after the diagnosis of PSC is established. At least 33 random biopsies should be taken from throughout the colon in patients with pancolitis. Pan-colonic dye spraying can be used to improve dysplasia detection and is an alternative to random biopsies in endoscopists trained to perform this technique. Enrollment in a colonoscopic surveillance program reduces the morbidity and mortality of CRC while simultaneously preventing unnecessary colectomy. Flat high-grade dysplasia and multifocal flat low-grade dysplasia are indications for colectomy. Raised polypoid lesions detected at endoscopy (DALMs) may be managed conservatively if there are adenoma-like features with a complete resection. There is some evidence that mesalamine and folic acid may reduce the risk of CRC; however, the effect of high dose UDCA merits further investigation.

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Chapter 56

How to Identify High-Risk Patients in Inflammatory Bowel Disease?

Jacques Cosnes, Harry Sokol, and Philippe Seksik

Keywords Crohn's disease • Ulcerative colitis • Natural history • Prognosis

Introduction

Inflammatory bowel disease (IBD) includes two entities, Crohn's disease (CD) and ulcerative colitis (UC). Both are chronic conditions with progressive anatomical damage, frequent complications, and possible requirement of surgical procedures. Both involve young people and have a great impact on patient's quality of life. During the recent years, medical management of IBD has been revolutionized by the advent of increasingly effective medical therapies, immunosuppressants, and biologics.

These therapies when given early in the course of the disease may prevent the development of an irreversible digestive damage unresponsive to medical therapy and requiring surgery [1]. However, first the effect of these therapies is not sustained and they should be used for a very long time, perhaps indefinitely, and second their use is associated with significant side-effects, with increased risk of serious infections and cancers.

It is thus mandatory to identify patients most at risk of developing a disabling or complicated course who could be targeted for aggressive therapy. This should be discussed at different times during the disease course, in particular at presentation and after first surgery.

Relevant End Points

They may vary according to the time and type of IBD one considers.

J. Cosnes, MD (✉) • H. Sokol, MD, PhD • P. Seksik, MD, PhD
Department of Gastroenterology and Nutrition, Saint-Antoine,
184 Rue Du Faubourg St-Antoine, Paris 75571, France
e-mail: jacques.cosnes@sat.aphp.fr

Crohn's Disease at Presentation

The goal of therapy should be to reduce at maximum the burden of the disease. To achieve this, several end points should be considered: having a benign disease course, with infrequent and mild symptoms, avoiding complications, not being operated on...etc.

Disabling Disease

In a recent study from our group [2], we arbitrarily defined CD course as disabling during the 5-year period following diagnosis when at least one of the following criteria was present in this time interval: more than two steroid courses required and/or dependence on steroids; further hospitalization after diagnosis for flare-up or complication of the disease; presence of debilitating chronic symptoms; need for immunosuppressive therapy; intestinal resection or surgical operation for perianal disease. Somewhat similar criteria were used by the group of Liège to define disabling CD [3].

Occurrence of Complications

The natural history of CD is marked by the occurrence of complications: perforation (abscess, fistula, or peritonitis) and strictures. The occurrence of complications is nearly constant in such a life-long disease with progressive and irreversible anatomical damage; however, their delay of before onset is highly variable [4].

Intestinal Resection

Most of these complications are inaccessible to medical therapy and require surgery. Less commonly, medicines fail to control disease activity and removal of the diseased segment is the only alternative. In the Copenhagen cohort, the 20-year cumulative risk of intestinal resection was 82% [5]. Other series from 1970 to 2000 showed rather similar rates [6, 7].

Permanent Stoma

Another fear of CD patients is the risk of permanent stoma. Proctocolectomy for Crohn's colitis is less often performed, as segmental colonic resection gives somewhat similar results to total colectomy [8]. However, permanent fecal diversion may still be necessary in patients with disabling chronic active perianal disease. It is estimated that more than 10% of CD patients eventually require permanent fecal diversion [9].

Death

Mortality in CD is slightly elevated when compared to the general population. A recent meta-analysis using a random effects model showed the pooled estimate for standardized mortality ratio in CD was 1.52 (95% confidence interval 1.32–1.74) [10].

After Intestinal Resection for CD

CD is characterized by recurrence after surgery. Aphthous ulcers in the ileum above anastomosis are visible in up to 2 patients out of 3 at 3 months, and less than 5% of the patients have a normal endoscopy 10 years after surgery. Clinical symptoms follow usually by 2–3 years the anatomic lesions. As the anatomical damage progress, patients may require surgery again. The cumulative risk of a second operation is about 30%, 10 years after the first one [5]. Thus, recurrence may be defined through endoscopy, or development of new clinical symptoms, or need for re-operation.

Ulcerative Colitis at Presentation

The overall severity of UC may be assessed from the frequency of flares, their clinical severity, need for hospitalization, and the need for surgery (colectomy).

Colectomy

Colectomy is needed in fulminant flares or chronic active disease refractory to therapy and in case of advanced neoplasia. The cumulative risk of colectomy is highly variable from one series to another, usually about 20–30% after 25 years, sometimes more in series from referral centers [11] or less in population-based studies and in series from Southern Europe [12]. This risk is particularly elevated during the year following diagnosis, then reaches a plateau at an annual rate of 1%.

Death

Mortality is not significantly different from that of the general population, although there is an increased risk during the first attack [13]. Most of these deaths are observed in the perioperative period, in patients with severe disease [14].

After Colectomy for UC

Colectomized patients who have ileal pouch-anal anastomosis (IPAA) are exposed to pouchitis, with acute episodes observed in half the patients during the 5 years following operation. In a minority of patients, up to 10%, pouchitis has a chronic or short-relapsing course refractory to antibiotics. This figure can be regarded as a recurrence of the inflammatory disease within the pouch, requiring again immunosuppressants and biologics, and eventually pouch excision [15]. Moreover, the development of penetrating perianal lesions and of small bowel involvement mimicking CD may occur even though the examination of the colectomy specimen did confirm the diagnosis of UC. Thus, both the risks of chronic pouchitis and subsequent CD should be regarded when evaluating prognosis after IPAA.

Predictive Factors

Numerous studies have tried to recognize demographic, clinical, and anatomical characteristics associated with a worse prognosis of IBD. Some characteristics do not seem to play a significant role and will not be examined in this review. It is the case of gender, ethnicity, state of residency,

educational level. Other characteristics may be used to identify subgroups of IBD patients who differ in their natural history and complications.

Age

IBD may develop at any age, from less than 2 years to more than 90 years. In CD, it is well established that the disease course is more severe in children than in adults. Comparative studies are few, but they demonstrate that, compared to adult-onset CD, childhood-onset CD patients experience a more severe disease regarding both disease activity and medical therapy requirements [16]. This increased severity exists independently of the disease location, whereas the disease tends to be more extensive and jejunal lesions more prevalent in children [17]. These data together suggest that childhood-onset CD shares a particular severe phenotype. Moreover, due to the differences of age, complications, and surgery occur at an age 15–20 years earlier than in adult-onset CD, thus these patients are exposed early in their life to major sequelae. These findings support an aggressive therapeutic approach in most of children diagnosed with CD [18]. Similarly, UC is often severe in children with widespread localization at diagnosis, frequent occurrence of flares unresponsive to medical treatment, a high rate of disease extension and an increased need for surgery [19, 20]. In the EPIMAD study, 20% of children had their colon removed after 5 years [20].

In adults with CD, we found that age under 40 was associated with a more frequent disabling course [2]. This negative effect of young age was not confirmed in another similar study from Belgium [3]. Data regarding the need for surgery are more consistent. The risk of surgery for non-neoplastic bowel disease decreases with increasing age at diagnosis [21]. In population-based studies from Norway [22] and Maastricht [23], young age was associated with an increased risk for surgery. After surgery, the risk of post-operative recurrence does not seem dependent on age at first resection [24], but increased in young patients at disease onset [25]. On the other hand, according to European multinational population-based study, CD patients above 40 years at diagnosis have an increased mortality risk compared to a background population of same age [26]. In other terms, although as a whole CD may be less active in older people, the risk of death from CD is particularly elevated in this age group.

The effect of age on disease severity in UC is less clear. In a population-based study from Maastricht, it was found that in UC, older age at diagnosis initially increased recurrence risk but was subsequently protective [23]. The risk of surgery for non-neoplastic bowel disease is unrelated to age at diagnosis [21]. Finally, after proctocolectomy and IPAA, the risk of developing subsequent CD is increased in younger patients [27].

Another important point to consider is the fact that side-effects of immunosuppressants and biologics are increased in patients older than 65 [28], particularly regarding the risk of lymphoma [29]. This should be kept in mind when discussing such therapies in these patients.

Severity of the First Flare

In CD, the need for steroids to treat the first flare was found to be an important predictor of disabling diseases during the five following years in two independent studies including patients in the last nineties [2, 3]. Need for steroids should be regarded as a marker of a severe clinical presentation. In the IBSEN study from Norway, it was found that, in patients with ileal CD, a high C-reactive protein value was associated with an increased risk of surgery during the next 5 years [30]. In UC, the severity of the first attack is not per se an indicator of a poor prognosis. In the Copenhagen study,

patients with a severe presentation who required intensive intravenous therapy but avoided colectomy tended to have a more benign course during the following years [31]. However, there is now a subgroup of patients who do not respond to intravenous steroids but are controlled with cyclosporine or Infliximab. These patients, particularly those with severe endoscopic lesions, are at high risk of recurrence and colectomy, then an efficient maintenance treatment (thiopurines or biologics) is mandatory.

Disease Location

Development of complications in CD is highly related to disease location. Ileal involvement may be complicated at diagnosis or during the very first years by an abscess/fistula or by a stricture followed by a fistula formation [8], whereas a colonic disease may remain uncomplicated, inflammatory, for many years. Upper gastrointestinal disease at diagnosis is associated with an excess risk of flares during the first 10 years of the disease [32] and jejunal disease is more prone to strictures formation. The disease tends also to be more severe when the lesions are extensive, when compared to involvement of a short segment of small bowel or colon. This is the case in patients with diffuse jejunoileitis, or extensive ileocolitis, or pancolitis. The role of disease extent – in other terms, the length of the resection – for predicting early post-operative recurrence is more debated [33–35].

Perianal location is another important predictor in CD [36]. At presentation, about 30% of patients share perianal lesions and 20% have or have had a fistula [37]. Perianal location is associated with a disabling course [2, 3] and is recognized by most authors as having a negative impact on prognosis both through occurrence of local complications and its association with a more severe luminal course [6, 38, 39]. Its role in postoperative recurrence is less clear [33, 39]. Perianal lesions may worsen and require a permanent stoma, notably where when there is an associated rectal disease.

In UC, the initial extent of the lesions is not associated with an increased risk of flares but with a more severe clinical severity [23, 40–42]. Moreover, in both colonic CD and UC, the risk of colorectal cancer increases in proportion with the extent of the inflammatory process within the mucosa and is greatest in pancolitis. The risk of pouchitis after IPAA is also increased in case of pancolitis [43, 44].

Disease Behavior

It is not surprising that CD patients presenting at diagnosis with a stricture or a fistula are more at risk to come to surgery. Actually those patients are diagnosed too late, while the anatomical damage has become irreversible, complications respond poorly to medical therapy, and surgery cannot be avoided in most cases. Indeed in the IBSEN study stricturing and penetrating behavior at diagnosis were independent risk factors for subsequent surgery [22], and most other studies made similar conclusions [3, 23, 45, 46]. More importantly, patients operated on for a penetrating complication (abscess, fistula, or peritonitis) have an increased risk to develop a surgical recurrence from a similar penetrating complication. Conversely, patients with a stricture tend to recur again in a stricturing mode [47]. Greenstein et al. suggested that the post-operative recurrence develops earlier in the former than in the latter [47], and some studies also found an increased risk of post-operative recurrence in patients operated on for a penetrating complication [24, 48, 49]. However, some other studies did not find such a difference [33, 50, 51]. Thus, it is not clear that perforating disease is a risk factor for recurrence.

Extra-Intestinal Manifestations

There is no indication that IBD patients presenting with joint, skin, or eye involvement have a more severe luminal disease than those without extra-intestinal manifestations [52, 53]. One study found an increased risk of surgical recurrence after first surgery in CD patients with extra-intestinal manifestations [24]. In UC patients, an increased risk of pouchitis after IPAA in such patients is well documented [54–58].

Primary sclerosing cholangitis (PSC) prevalence in IBD is about 5% in UC and 2% in CD. IBD associated with PSC is more frequently a pancolitis, often with backwash ileitis and rectal sparing. The intestinal disease activity is usually low. However, there is a higher risk of colorectal neoplasia and cancer than in IBD without PSC [59]. This higher risk is unrelated to disease location or extent [60]. Moreover, after IPAA, pouchitis is more frequent. Finally, prognosis is embedded by the risk of evolution of the hepatobiliary disease which may require orthotopic liver transplantation. The risk of cholangiocarcinoma (and/or gallbladder carcinoma) is about 1% every year.

Environmental Factors

In CD, current smoking increases by more than 50% the risk of flare-up as compared to never-smokers [45, 61], and is associated with more frequent intestinal penetrating complications. The risk of being operated on at least once during disease course is increased in smokers vs. non-smokers in most studies [7, 23].

After surgery, the cumulative rates of clinical and surgical recurrence, respectively, were found consistently more elevated in smokers than in non-smokers, and actually smoking is the factor the most determinant for post-operative recurrence in CD [35, 62–64]. By contrast, in UC, smokers run a more benign disease course when compared to non-smokers: flare-up and hospitalization rates are less frequent and colectomy rate is lower [65–69]. After colectomy and IPAA, current smoking is associated with an increased risk of subsequent CD [70]. It has been claimed that current smokers have a lower incidence of pouchitis [71] but this observation was not confirmed in most recent series [54, 57, 72].

Prior appendectomy, although more frequent in CD compared to a background population, has no clear effect on evolution of the disease [73]. In UC, appendectomy is associated with a less severe course and a lower colectomy rate [74, 75].

Genetic Factors

Before the era of genome-wide association study, the role of genetic factors in IBD severity has been looked for by comparing familial to sporadic IBD. Although having a relative with IBD increases the risk for CD, the severity of CD is unaffected by family history [76], nor that of UC [65, 77]. A family history of CD increases the risk of subsequent CD after IPAA. Identifying genetic prognostic factors in IBD is very attractive as they are already present at the onset of the disease and actually even earlier. Their main advantage is their stability along time, which is not the case for many other potential predictive factors such as clinical parameters or serologic markers. However, despite a growing number of identified susceptibility loci in both CD and UC [78], only very few have been associated with disease outcome. The presence of NOD2 polymorphism has been associated with a more aggressive clinical course of CD with higher risk of intestinal stenoses and earlier need for first surgery [79, 80]. Some studies also tried to identify genetic factors associated with response to treatment.

Polymorphisms in MDR (Multi Drug Resistant) 1, TNF (tumor necrosis factor), and MIF (migration inhibitory factor) genes have been associated with corticosteroid refractory or sensitivity in CD and UC [81–83]. MDR1 polymorphism has also been associated with a higher risk of Cyclosporine failure in patients with steroid-resistant UC [84]. The efficacy of infliximab is partly due to the induction of apoptosis in activated T lymphocytes. The group of Leuven analyzed infliximab-treated patients and found polymorphisms in apoptosis genes predicting response to infliximab therapy in luminal and fistulizing CD [85].

Despite their attractiveness, genetic markers will probably never be able to fully predict IBD behavior and complications, notably because of the major role of environmental factors in the disease pathogenesis. On the other hand, they can be easily associated with other types of factors, such as clinical or microbiological data to build more powerful composite predicting tools.

Serologic Markers

The search for serologic markers in IBD has led to the discovery of specific antibodies. Perinuclear anti-neutrophil antibody (pANCA) is associated with UC or UC-like CD, whereas anti-Saccharomyces cerevisiae antibody (ASCA, glycan antibody) is mostly associated with CD [86, 87]. Three other markers linked to immune response toward bacteria have been identified; antibodies to the *Escherichia coli* (*E. coli*), outer-membrane porin C (OmpC), the *Pseudomonas fluorescens* CD-related protein [anti-CD-related bacterial sequence (I2)], and the CBir1 flagellin [88, 89].

Many studies have been performed to assess the predictive value of these serological markers. Reactivity to ASCA, OmpC, anti-I2, and CBir1 have been associated with early disease onset CD, fibrostenosing and penetrating disease, and need for early small bowel surgery [89–91]. In Pediatric CD patients, baseline ASCA reactivity has been associated with earlier complications, relapsing disease, and needs for an additional surgery [92]. The frequency of disease complications increases with reactivity to increasing numbers of antigens (ASCA, anti-I2, anti-OmpC, and anti-CBir1) [93]. pANCA has been shown to be associated with less severe disease, UC-like disease and negatively associated with small bowel complication [90, 94]. On the other hand, high pre-colectomy levels of pANCA have been prospectively associated with chronic pouchitis in UC patients undergoing IPAA [95]. Conversely, ASCA positivity has been associated with CD of the pouch after IPAA [96].

High-Risk Patients

From this review of the possible different predictors of the disease course, some emerged as more important: age at disease onset, severity of the first flare, and perianal location in CD; very young age and disease extent in UC. Smoking is an important factor in CD but it may be corrected, and quitters have a course which becomes similar to that of never smokers [63, 97]. Genetic and serologic markers are promising and interesting at the epidemiological level, but to date, none is accurate enough to guide alone the patient therapeutic strategy. The role of disease location and behavior is important; however, it is partly redundant and concerns mainly the need for surgery. Now early intestinal surgery may be associated with a non-severe disease, for example, a short stricturing ileitis, which many physicians would refer to the surgeon. Therefore, the increased risk for surgery should not be considered per se as a marker of a more severe disease. Finally, it is important to note that we are not able to predict the disease course in the long term, and that prediction should be considered only as indicative. One patient with a very quiescent disease for years may suddenly develop a severe acute flare or complication leading to surgery.

Table 56.1 Factors collected at presentation associated with a more severe course in CD

	Relative importance	References
Age <17 years	+++	[16, 99]
Perianal disease	++	[2, 3]
Need for steroids	++	[2, 3]
Ileocolonic location	+	[3, 100]
Age <40 years	+	[2]
Current smoking	+	[45, 61]
Stricture	+	[3]
Large disease extent	+	[101]

Table 56.2 Factors collected at presentation associated with earlier surgery in CD

	Relative importance	References
Fistula or abscess	+++	[22]
Stricture	++	[22]
Jejunal location	++	[102]
Ileal location	++	[7]
Younger age	++	[21–23]
Current smoking	+	[7, 23]
High C-reactive protein	+	[30]
ASCA	+	[91, 103]
NOD2 polymorphism	+	[79, 80]

Table 56.3 Factors collected at presentation associated with a higher risk of colectomy in UC

	Relative importance	References
Very young age	++	[19, 20]
Extensive disease	++	[23, 40–42]
No response to steroids	++	[104]
Severe endoscopic lesions	++	[105, 106]
Non-smoking	+	[69]
No appendectomy	+	[74, 107]

Table 56.4 Factors collected at surgery associated with a higher risk of post-operative recurrence in CD

	Relative importance	References
Current smoking	+++	[108, 35, 49, 109, 110]
Short disease duration	+	[62]
Large disease extent	+	[33, 35, 111]
NOD2 polymorphism	+	[80]
Jejunal disease	+	[102]
Fistula, abscess, or peritonitis	+	[48, 49, 112]
	–	[50, 51, 113]
Perianal disease	+	[33, 39]
	–	[114]

The most relevant predictors are indicated in Tables 56.1–56.5: at presentation, regarding the risk of a 5-year disabling course in CD (Table 56.1), the risk of early intestinal resection in CD (Table 56.2), and the risk of colectomy in UC (Table 56.3). In addition, factors associated with an increased risk of post-operative recurrence in CD and of late complications after IPAA for UC are given in Tables 56.4 and 56.5, respectively. The relative importance of each factor is indicated, based on the data of the literature and our clinical experience.

Table 56.5 Factors collected at surgery associated with late complications following IPAA

	Relative importance	References
<i>Chronic pouchitis</i>		
Pancolitis	++	[43, 44]
Associated PSC	++	[115, 116]
Younger age	+	[27]
Extra-intestinal manifestations	+	[54, 56–58, 117]
High pANCA	+	[57, 95]
Non-smoking	+	[57, 71]
	–	[72]
<i>Crohn's disease</i>		
Current smoking	++	[70]
Family history of Crohn's	++	[96, 118]
Younger age	+	[27]
ASCA	+	[96]

Conclusion

In patients most at risk to have a disabling disease or to require surgery, active treatment with immunosuppressants or biologics should be discussed in balance with the goal of therapy, which may be different in a young adult searching for work and a retired individual. For example, ileocecal resection may be the preferred option in a young nonsmoker patient with a limited stricturing disease, because if unoperated on he will have a disabling course, whereas surgery will probably give him several years of remission. On the other hand, in one CD patient with a severe clinical presentation, an ileocolonic location, a perianal disease, and current smoker, early surgery must be avoided and to achieve this, immunosuppressants and biologics should be started within the few weeks following diagnosis. In patients operated on but at high risk of post-operative recurrence, particularly in those with prior extensive or multiple resection, post-operative anti-TNF should be considered [98]. Moreover, during the disease course, high-risk patients should be checked regularly for the presence of intestinal ulcerations and thickening, using noninvasive techniques (CRP, ferritin, fecal calprotectin, videocapsule, and MRI) and treated as early as possible with the goal to heal lesions before the disease expresses clinically.

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Part VIII
Patient Perspective and Resources

Chapter 57

Patient Perspective on Inflammatory Bowel Disease

Marjorie Merrick

Keywords IBD • Crohn's • Colitis • IBD patient perspective

Approximately 1.4 million Americans have Crohn's disease and ulcerative colitis and as many as 70,000 new cases of inflammatory bowel disease (IBD) are diagnosed in the United States each year [1, 2]. Although most people are diagnosed after the age of 15, an estimated 50,000 children (under the age of 20) have IBD in the United States [3]. All are seeking answers to a variety of questions about their disease.

For the newly diagnosed patient, life has just taken a much unexpected turn. The initial reaction can be shock, fear, disbelief, or even relief to finally be able to put a name to why they or their loved one has been so sick. The realization that they will have to deal with a disease that may be managed but never cured is very difficult for most patients and their families to accept. For many patients, the next step is to gather information about the immediate aspects of their illness. Initial questions may include: What is Crohn's disease or ulcerative colitis? Is it contagious? Is there a cure? Did I do something to make me get sick? Was it something I ate (or didn't eat)? What diet should I follow? I'm under a lot of stress – did that cause me to get sick? Will my kids get this disease? What are the medicines used to treat this disease and which will I need? How long will I need to take the medicine? Are there side effects to the medicine? Will I ever need surgery? Will I get cancer? Will I be able to have children? Why am I always tired? Will I ever feel good again – in this lifetime?

Then the practical questions begin: Where is the nearest bathroom and will I have access to it? How do I tell my boss (or teacher or loved one) about my disease? What will they think about me after they know that I have a “bathroom” problem? Will I be able to keep my job? Stay in school? What can I eat that won't make me feel worse? How do I avoid embarrassing “accidents” that soil my clothes? Why am I having problems with anger and depression? Will my health insurance cover my medical costs? Can I qualify for Social Security Disability?

In 2003, CCFA conducted an internet-based survey which was completed by more than 4,000 members. The study revealed a number of interesting facts about persons newly diagnosed with IBD, including their experiences in being diagnosed, anxieties about their diseases, attitudes about treatment, and other aspects of living with IBD. In 2011, most of the issues are still as prevalent as

M. Merrick, MA (✉)

Department of Research and Scientific Programs, Crohn's & Colitis Foundation of America,
386 Park Avenue South, 17th Floor, New York, NY 10016, USA
e-mail: mmerrick@ccfa.org

they were 8 years ago, as borne out by the almost 80,000 patients who have contacted CCFA's Information Resource Center by phone or email over the past 3 years. A few of the more common trends are discussed below.

The average patient is fairly knowledgeable about their disease because the unpredictability and severity of IBD drives them to educate themselves about their diseases. This is particularly true of parents whose young child has just been diagnosed. However, they were not so knowledgeable about treatments or side effects.

Patients are very consistent in how they find educational materials. Physicians are the primary source of information. Secondary sources of information include CCFA's website, internet sites, and books. The internet has no shortage of sites purporting to be about IBD but, unfortunately, only a modest percentage of sites offer peer-reviewed, scientifically valid information. The challenge is to direct patients and families from the time of diagnosis toward reputable websites or materials for their educational needs.

Quality of life is a major concern. Patients report that they tend to spend more time at home and reduce their social activities, particularly when their disease is active. Many patients are very much afraid of not having easy access to a bathroom. They may not eat anything for several hours before a social event and consume nothing during the event to minimize the chance of embarrassing flatulence or uncontrollable diarrhea. Many persons will not venture more than a few miles (don't even think about getting on a plane!) so that they can return home in a hurry if they feel sick or have soiled their clothes. When they do go out, they may carry an extra set of clothing along, "just in case," but then are embarrassed and worry that unwelcome questions will be asked if they go into the bathroom then reappear in different clothing.

Communication between physicians and patients is a key ingredient to patient satisfaction. Patients want to feel that they and their physician are partners and that treatment decisions are being made collaboratively. It is also interesting to note that quite often the doctor's idea of a successful treatment is very different from the patient's evaluation. For instance, the doctor may feel that the treatment that reduced the number of bowel movements from 15–20 per day to 5–7 per day is not a total success, but the patient may be thinking that having only 5–7 per day is a huge improvement that allows them to have a fuller life. Open communication – with both physician and patient asking the right questions – will help create a successful doctor–patient relationship.

IBD patients tend to be up-to-date on what new drugs are in the pharmaceutical pipelines; in fact, it is not unusual for patients to know that a new drug has been released before their physician knows! They want to know about potential side effects and the costs of the drug. Those who need surgery want to know details on the procedure, how long will it take to recover, are they likely to need surgery again in the future, and what will be their functional status post-op. Here again, physicians need to be aware that the patient viewpoint may be very different from their own, should take patient concerns into account and involve them in creating the treatment plan.

One of the most frequently asked questions concerns diet and nutrition. Every IBD person has a list of foods that they do not tolerate well, especially when in an IBD flair. Patients often believe that their disease is caused by and can be cured by diet. They will investigate, and likely try, any fad diet, usually without consulting their physician. Nutrition is a common discussion among the pediatric IBD community in order to help encourage proper growth but, unfortunately, patients report that diet and nutrition are not discussed as frequently with adults. Again, a strong doctor–patient partnership should work together to insure the best possible nutritional outcomes.

Having IBD can be emotionally burdensome and IBD patients may experience a wide range of emotions in response to having this condition and to varying degrees. Anxiety and depression may be an IBD patient's daily companions but symptoms are often downplayed to not only loved ones but also to their doctor. Family friends, the patient's physician, a mental health counselor can be very helpful, as can patient support groups and community internet chat rooms like those offered by CCFA.

Research confirms that Crohn's and colitis are extremely expensive diseases. Direct medical costs include expenses for hospitalizations, physician services, prescription drugs, over-the-counter drugs, skilled nursing care, diagnostic procedures, and other healthcare services. Indirect costs are the value of lost earning or productivity. Indirect costs also include the value of leisure time lost. Total annual direct costs for all patients with IBD in the United States in the year 2004 (the last year for which data are available) were \$1.84 billion [4]. Total indirect costs in 2004 were \$328 million. Based on a national health survey in 1999, nearly 32% of symptomatic IBD patients reported being out of the work force in a 1-year period [5]. Patients and their families worry about how to pay all of their medical costs even when they have jobs and worry about losing those jobs because they have to miss significant amounts of time when in a full flare. Even if they have adequate insurance, co-pays can be very burdensome and getting Social Security Disability benefits can be very difficult.

All of these are serious concerns but there are tools available to help both doctor and patient. A wealth of materials for patients, their families, and for healthcare professionals can be found in CCFA's Web site at <http://www.ccfa.org>. These materials include brochures, fact sheets, webcasts, newsletters, and links to community chat rooms. Physicians will also find a series of template letters to use to advocate for their patients to insurance companies, Social Security Administration, and others. These templates may be found at (http://online.ccfa.org/site/PageServer?pagename=Appeal_Letters).

Patients may also speak to an education specialist at CCFA's Information Resource Center by calling 888.MY.GUT.PAIN (888-694-8872). All information and printed materials are free of charge.

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Additional Resources

Crohn's & Colitis Foundation of America (www.ccfa.org)
CCFA's Information Resource Center (888-694-8872)
CCFA Educational Brochures
Living with Ulcerative Colitis
Living with Crohn's Disease
Crohn's Disease and Ulcerative Colitis: A Guide for Parents
Crohn's Disease and Ulcerative Colitis: A Guide for Teachers and Other School Personnel
Managing Flares and Other IBD Symptoms
Understanding IBD Medications and Side Effects
Surgery for Crohn's Disease and Ulcerative Colitis
IBD Facts Booklet

Selected Books

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- Jaffe, Jennifer C. Know your rights: a handbook for patients with chronic illness. Farmington, CT: Advocacy for Patients with Chronic Illness, Inc.; 2010
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Chapter 58

Patient Resources in Inflammatory Bowel Disease

Ben Wilson and Marco Greco

Keywords Expert patients • Online patient information • Patient associations • Patient communities • Patient experience • Patient groups • Patient information leaflets • Patient information • Patient resources • Patient Web sites

Key Messages

- People with IBD have a right to clear, unbiased, high-quality knowledge about their condition, its management, and lifestyle factors, and should be assisted in accessing a range of resources applicable to us by healthcare professionals.
- People with IBD should be assisted in developing resource interpretive skills.
- A range of personal characteristics influence a person's "resource of choice."
- "Resourceful patients" are increasing in number, and can interpret medical knowledge – information for patients, and for healthcare professionals, need not be separate.
- The inclusion of the patient experience is vital for successful resources.
- People with IBD should be supported in accessing communities and groups, facilitated at specific clinics, or by individual clinicians.

Knowledge is the enemy of disease.

Sir Muir Gray

B. Wilson, BA(Hons), MSC • M. Greco, JD, PhD (✉)
EFCCA – European Federation of ulcerative Colitis and Crohn's Associations,
Rue Des Chartreux 33–35, Brussels 1000, Belgium
e-mail: ben.wilson@efcca.org; marco.greco@efcca.org

The Resourceful Patient

It is now recognized that patients need, and have a right, to knowledge about their condition and its treatment; however, this was not always the case. In the twenty-first century, the balance of power has shifted significantly from the traditional “paternalistic” role of the doctor, to a more egalitarian approach. In particular, the rise of “shared decision-making” has, in some fortunate cases, transformed the patient experience of healthcare; from the patient visiting an authoritative healthcare professional who simply issued directions which were received with faith alone, to a situation where “treatment decisions (are) made through dialogue and discussion of evidence-based treatment options, and the values and expectations of the patient” [1, 2]. This revolution depends on the provision of high-quality, reliable resources for people with IBD. Degner presents evidence that 66% (two thirds) of patients wish to assume some level of control over treatment decisions which affect them [3]. Governments across Europe have enshrined the principle of patient choice and shared decision-making in national policy and even law [4]. However, despite this commitment to approaches that have high-quality information provision as pre-requisites, according to a previous EFCCA patient survey, lack of information is still considered to be a grand challenge, and an urgent need for people with IBD, particularly those who have been recently diagnosed. The empowering principles of shared decision-making and patient choice are sound, but people with IBD are not yet being provided with the resources needed to achieve these goals.

Patient Attitudes to IBD and Knowledge

As knowledge in a person with IBD increases, fear of the unknown, and the anxiety brought about by lack of control decreases; choice, and more importantly, hope, increase; this brings about true empowerment. People with IBD are engaged in a life-long fight to overcome daily symptoms of this chronic disease, which can be disabling, embarrassing, and humiliating [5]. Despite this, people with IBD are often resilient in the face of their illness, we dislike being defined through it, and we are keen to take control over their illness through acquiring knowledge, as EFCCA’s 25 national IBD patient associations have found. EFCCA feels that people with IBD have a right to clear, unbiased, and high-quality knowledge about their condition, its management, and lifestyle factors, and we should be assisted in accessing a range of resources applicable to us by healthcare professionals.

The impact of IBD on quality of life ranges well beyond a narrow medical definition of the disease – there is a spectrum of complications, co-morbidities, and related diseases. This may be why there are differing attitudes and approaches in how people with IBD perceive and tackle their illness – desire for information about IBD is dependent on these attitudes:

- Denial – unfortunately, a long diagnostic process, or mis-diagnosis, is common in IBD. Some patients are diagnosed after a distressing emergency admission, or an acute flare-up. This may lead to intense anxiety and fear. This patient may not be interested in learning about their condition, and may seek to avoid information for a considerable time. This patient may not opt for shared decision-making processes.
- Acceptance – the search for answers. Patients who are more gradually exposed to the possibility of diagnosis with IBD, allowing them to come to terms with their situation, often have very specific questions and learning needs related to their condition, its management, or everyday life. This patient may approach the wealth of information available on a “question and answer” basis – ranging through multiple resources, seeking a specific answer to satisfy a particular need. Shared decision-making is important to this patient, but based around clinical issues of particular value to them.

- Expert patients. Many patients, after satisfying immediate information needs, may find the learning process grants control and empowerment. This patient is interested in wider background learning, and discovering new knowledge. A patient immersed in high-quality clinical knowledge may converse fluently with healthcare professionals, and may desire full shared decision-making.
- Gray discusses the phenomenon known as “le maladie de petit papier” [2]. Patients attending a consultation with a written list of questions. Rather than scorned, this technique is now encouraged as a consulting aid, and has sometimes become “le maladie de grand print-out.”

Classifying Patient Resources

There is no official classification of “patient’s resources,” neither a clear listing of them. Resources are many, and vary according to disease type, severity, and acute or chronic nature. People with IBD are familiar with several classes of resources, often depending on the time since diagnosis. Classes include: information resources, community-based resources, resources for healthcare choice, the patient medical record, and social security. Many are designed by healthcare professionals, but increasingly, patient associations (such as EFCCA’s 25 associations covering 24 European countries) are developing their own high-quality resources that are truly patient centric.

Information Resources in the Digital Age

Before the “digital age” and the emergence of the world wide web, patients derived information from their community – friends, family, home medical encyclopedias, or public libraries [2, 6]. Many prefer the sense of “ownership” that holding printed information provides, however in the digital age, printed information carries the disadvantage that it becomes out-of-date the moment it is printed. Even so, in a hospital setting where access to communications may be difficult, printed information offers advantages. The power of digital information is in personalization – the volume, and flexible format of information ensures that most patients can find a resource that is well matched to their level of literacy, interpretive skills, online expertise, cultural sensitivities, and time constraints – these factors, together with the accessibility and exclusivity of the resource, will determine an individual’s resource of choice.

However, there is some evidence of the emergence of a “digital divide” – differing levels of patient empowerment between those who have technology and internet fluency, and those without, who may be from certain socio-economic backgrounds [7]. Digital information is powerful and vast, but not available to everyone. To truly empower patients, accessibility is required.

Traditional, Online Resources

In the digital age, patients are now able to access a vast wealth of information online. This includes online versions of traditional patient information “leaflets” (for example, NHS Choices, <http://www.nhs.uk>).

Innovative Online Resources

The Life and IBD Web site from EFCCA (<http://www.lifeandibd.org>) takes better advantage of the digital platform. It offers more structured, modular information on IBD, which can be presented

according to a person's specific disease, age, and topic. Structured navigation helps readers to more quickly locate information of relevance. The ability to manipulate and personalize resources, in rich interfaces may even make these resources more effective in practice [8].

Rather than static information content, many resources now encourage users to store personal information for later retrieval, or to submit data for processing. For example, the Six Parts Water Web site offers tools to help people with IBD gather, manage and control personal health information (<http://www.sixpartswater.org>). This innovation is not limited to web browsing – the emergence of mobile devices such as “smart phones,” and the famous iPhone, means that users can carry resources with them which help track or manage diseases, or offer a specific service. For example, the “Crohn's Diary” and “GI Monitor” iPod applications allow users to record daily symptoms for discussion in the consultation. Crohn's and Colitis New Zealand has recently developed an iPhone application to help people with IBD locate and access toilet facilities quickly (<http://www.toiletmap.co.nz>). EFCCA's national IBD associations in Denmark (<http://www.flush-it.dk>) and Belgium (<http://www.controlezcrohn.be>) have also developed Web site versions, and EFCCA is developing a pan-European tool.

Users of services providing collection of personal or medical data need of course to be aware of security and confidentiality issues, which may not always be assured or apparent, given the variance in the reputability of different publishers. In the UK, the Information Commissioner's Office provides practical advice about the privacy and security implications of a modern web, at <http://www.ico.gov.uk>.

Peer-to-Peer Resources: Patient Experiences as a Success Factor

Rather than resources constructed by authorities to “educate” patients, some resources encourage a peer-to-peer exchange of experiences. DIPEX (<http://www.healthtalkonline.org>), for example, carefully sources and quality assures patient “stories.” The realization that more effective patient information includes the patient's perspective, directly through quotes, stories, and case-studies, has now spread to widespread acceptance. Education and medical knowledge may be the primary goal of resources, but this learning process may be more effective, and people more empowered, through inclusion of qualitative experience [9]. People with IBD often report that healthcare professionals and even family members cannot truly understand the perspective of battling with this chronic disease, so in many countries, discussion forums have been developed to enable people with IBD to share thoughts and reflections, ask questions and find answers, and perhaps above all, to find a sense of community and solidarity with those who share first-hand experience of IBD. EFCCA's member associations have developed forums in Finland (<http://www.crohnjacolitis.fi>), the United Kingdom (specifically for young people, <http://www.ibdandme.org.uk>), the Netherlands (<http://www.crohn-colitis.nl>), and many other countries. Issues such as moderation need careful consideration, but must not pose a barrier to the huge potential positive impact of these resources, recognizing that uncensored and free discussion can be of good value. Static information resources which seek to educate often have a limited lifespan, but dynamic information resources which offer a first-person perspective inspire users to return, and to contribute. The sharing of the patient experience is paramount if a resource for people with IBD is to be successful, and peer-to-peer information is the cornerstone of this approach.

Real stories may not be the best medicine, but they are nutritious and sustaining. They feed the mind with information and the heart with hope and strength.

Phillip Pullman

New Media

The easy online sharing of video and audio has made it possible for people with IBD to directly reach out to one another, without editorial constraint or regulation. The volume of material available has greatly increased, and some viewers find candid information of great value. However, views are often subjective, not quality assured or evidence-based, and can occasionally even be distressing, despite community-based editorial controls. However, patient associations in Canada and the Netherlands have demonstrated that editorial review processes, and careful management, can ensure that first-hand, experience-based video resources are robust, without censoring, and ensuring free contribution. CCFC in Canada has made a collection of experiences of young people with IBD available in video, at http://www.ucandcrohns.org/my_story.php. In the Netherlands, CCUVN have a similar resource for young people (<http://www.crohnjuwelen.nl>).

Web2.0 and The “Participatory Web”

The emergence of “Web2.0,” the “participatory web,” means that patients need no longer passively consume information – we can now author and share information for others to respond to, and actively connect and network with communities through social networking. EFCCA has developed several Facebook sites (<http://www.facebook.com>), since it offers an easy mechanism to raise awareness, create a sense of community, and allow the sharing of information across and between communities. The notion of “critical mass” is important here – social network and community initiatives can only succeed if they capture the imagination of large numbers of users, to make the online community viable [10]. This is perhaps why Facebook itself continues to dominate, while attempts at bespoke medical or patient group social networking remain small-scale to date.

Resources for Patients and Resources for Healthcare Professionals Need Not Be Separate

A fundamental dichotomy between resources for healthcare professionals, and resources for patients, is a mis-conception. In some situations, information specifically for patients is of clear benefit; however, the objectives and content are often identical for both audiences. Furthermore, expert patients are often familiar with the “hierarchy of evidence” proposed by Haynes, and engage with it at every level [11].

The National Library for Health project in the UK (<http://www.library.nhs.uk>) has granted equal access to resource listings for healthcare professionals and patients alike, including:

- Guidelines, for example, those from NICE (<http://www.nice.org.uk>) or NHS Clinical Knowledge Summaries (CKS, <http://www.cks.nhs.uk>) in the UK. Patient associations are also motivated and skilled to produce guidelines – EFCCA’s member, Crohn’s and Colitis UK has devised well-researched IBD health service guidelines – <http://www.ibdstandards.org.uk>. When access to treatment, and varying treatment quality, are common issues throughout many European countries, people with IBD are found to be highly motivated to interpret treatment standards, and examine guidelines.
- Systematic reviews (for example, those from the Cochrane collaboration – <http://www.thecochranelibrary.com>). People with IBD understand that one trial may contradict another, and are

increasingly turning to high-quality secondary evidence such as systematic reviews to provide a better answer to an information need.

- High-quality randomized controlled trials – our members are committed to supporting research, our members request us to review and publish trial-based news in the EFCCA newsletter (<http://www.efcca.org>).

Once a patient becomes fluent in the language of their condition, we may become “expert patients” discerningly seeking high-quality, reputable publications that have high status in the hierarchy of evidence. Gray states that equity of access to resources is important because 5% of the public are as well educated as clinicians, most healthcare information is relatively simple, and a patient can spend a large amount of time learning about their condition, whereas a general practitioner has hundreds of conditions to cover [2]. Gray concludes that “it makes no sense to separate knowledge resources for patients and clinicians” [2].

The Information Revolution and the Need to Appraise Resources

In the twenty-first century, there is a vast ocean of information at a patient’s fingertips. However, this ocean is turbulent – Taylor concisely states that “all evidence is not equal” – the majority of the information available electronically is not evidence-based [12]. It may be tainted by subjective opinion, lack of evidence, and even outright bias – making it misleading, wrong, or even harmful. Furthermore, information must be correctly applied – Gray states that “Knowledge alone will not create the resourceful patient [2]. Patients also need skills.”

Patients can be forced to make a trade-off between accessibility and quality – to find a specific answer that fulfills their needs, quality must be sacrificed, and in the process of learning to live with a chronic condition like IBD, finding this specific answer might be vital to a patient’s quality of life, and perhaps even the efficacy of their care.

The development of quality standards such as the UK Department of Health Information Standard, which can be used to flag high-quality publishers, is a useful step forward. In addition, the application of appraisal tools such as DISCERN (<http://www.discrim.org.uk>), or the OHRI decision aid evaluation methods (<http://decisionaid.ohri.ca>) to specific publications, is also helpful. Patients should be assisted in developing critical appraisal and interpretive skills.

In the same way that people need clean, clear water, they have a right to clean, clear knowledge.

Sir Muir Gray

Communities for People with IBD: EFCCAs Core Resource

The most crucial and valuable resource is each other. IBD can be an isolating condition, and where people with IBD can come together in a free environment, we can be greatly empowered by sharing experience and knowledge, in a community of mutual support. Our members tell us that the first experience of freely socializing with others who also have IBD can be life changing – this positive impact is one of the most rewarding aspects of the work of our 25 national IBD associations, and EFCCA itself. The benefits of community-based mobilization are well documented, and part of EFCCA’s core philosophy – the whole is greater than the sum of its parts. The community groups of EFCCA and its members are flourishing, using web technology to bridge distances, cross borders, and demonstrate a universal commonality in overcoming IBD. In the UK, the Department of Health Expert Patients’ Program (<http://www.expertpatients.co.uk>) offers a formal approach to

patient communities, providing structured, skills-based learning meetings, to assist those with certain chronic conditions in acquiring coping strategies and skills.

Wherever possible, people with IBD should be supported in accessing communities and groups, and this can be facilitated at the level of specific clinics, or even individual clinicians. Community-based support is not only immensely rewarding for those who take part, but it also offers a wealth of benefits for family members and friends, who may not be present at medical consultations. EFCCA offers national IBD patients' associations with resources and knowledge to establish these communities – projects such as EFCCA's summer camps for young people and children with IBD (<http://www.catchyourdream.org>) have been proven in efficacy – research conducted in the US in association with the Crohn's and Colitis Foundation of America (CCFA), clear benefits are demonstrated, which EFCCA aims to replicate in Europe (<http://www.efcca.org>) [13].

Resources to Enable Choice: Healthcare and Treatment Quality

Increasingly, patient choice of health service, healthcare provider, and treatment, is being advocated [4]. In the UK, NHS Choices (<http://www.nhs.uk>) and the Care Quality Commission (<http://www.cqc.org.uk>) provide statistical information, and qualitative comments, to enable service users to make informed health service choices.

EFCCA supports informed, evidence-based patient choice, except where this fine notion may be used as an opportunity to transfer responsibility for health service quality from government, to the choice of individuals.

One's Own Resource: Owning the Medical Record

Many patients are not aware that European law gives them a legal right to access their own patient data. One's own medical records are also a valuable resource, and ownership of them can often be the beginning in a journey of understanding about IBD, and can also be vitally useful when transferring between clinicians (particularly internationally), when traveling, or in medical emergencies. Electronic medical record projects often drawn controversy, but also offer the potential of significant rewards.

Patients' Rights as the Foundation Resource

Upholding patients' rights is one of the key challenges that any legislation has to face. Although recognizing the importance of the notion of the "patient as a consumer," the priorities for people with IBD are, at the moment, different.

It is not only difficult to diagnose and differentiate ulcerative colitis and Crohn's disease, but even more difficult to stipulate when a person's IBD meets the requirements of a formal definition of a "disability." This is not helped by the fact that IBD is often classified as a "rare disease," even though its incidence and prevalence is greater than many more well-known conditions. These circumstances have a clear cascade-effect on people with IBD – they sustain the isolating nature of IBD on patients, and distance patients from their right to health.

A united, focused voice, amongst healthcare professionals, together with patients, can help to overcome this situation. People with IBD must also join with people who have diseases similar

in terms of characteristics, or social consequences. This approach can offer a realistic chance of success, and the importance of patient-centric organizations is vast – at the national level in EFCCA's 25 associations, at the international level with EFCCA itself, and reaching on into umbrella organizations of which EFCCA is part, such as the European Patients' Forum, the European Disability Forum and the International Alliance of Patients' Organizations.

Conclusions: Patients' Group as "Trait D'Union" for Describing a New Conception of Resource

This article demonstrates the difficulty of defining "resources for patients," but also how many and varied they are, especially in terms of quality and access.

Organizations such as EFCCA are a source of resources, a source of assistance in patients who seek help in accessing or interpreting resources, and also a beacon of equilibrium and solidarity amongst the difficult conditions in which people with IBD live each day.

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