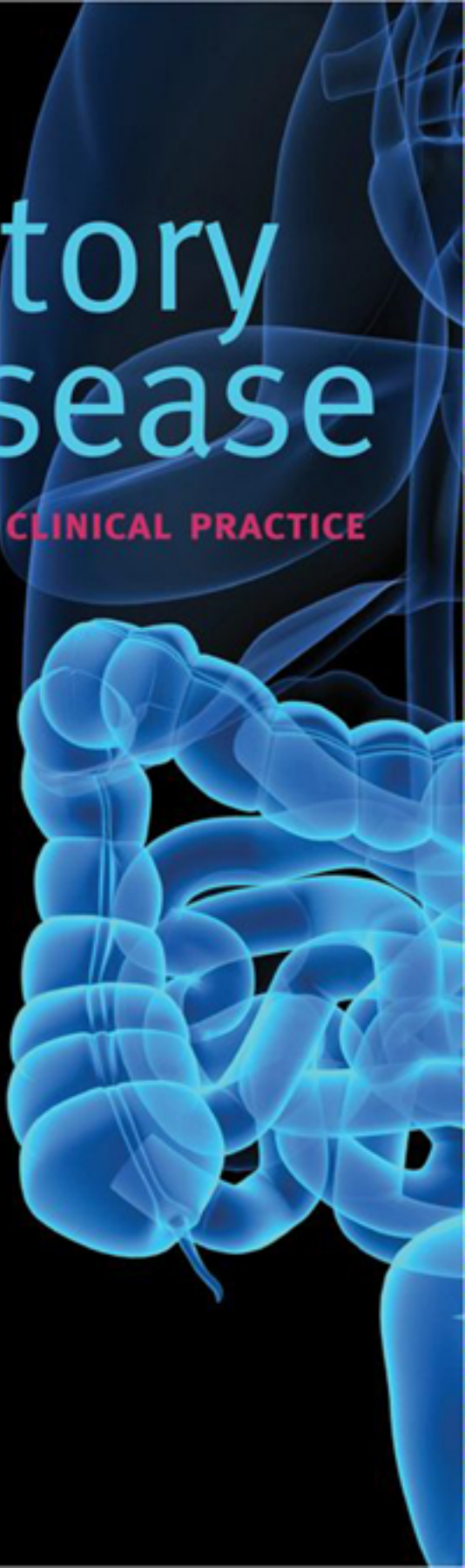
Inflammatory Bowel Disease

TRANSLATING BASIC SCIENCE INTO CLINICAL PRACTICE

Edited by
Stephan R. Targan
Fergus Shanahan
Loren C. Karp



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Preface

Inflammatory bowel disease research is changing. Progress in defining and treating these diseases is advancing in lock step with the furious pace of technological advances that continue to refine the tools of discovery. With sequencing of the entire genome completed, genetics research is providing direction for molecular and immunological *in vivo* and *in vitro* investigation, which in turn directs the development of targeted therapeutics. As translational investigation evolves, what is learned in clinical research is combined with what is learned in basic science research and is leading to a "personalized medicine" approach for managing inflammatory bowel diseases and is bringing the potential of prevention into view.

As Editors, our intention is that this book will provide insight along the entire continuum from basic science to clinical practice. The basic science chapters present findings in the context of what has already been established about the clinicopathological nature of the diseases. The clinical chapters describe the most effective applications of all available diagnostic and therapeutic approaches. This book reflects today's trends toward globalism and is a truly international effort. We encouraged our contributors to editorialize and provide thought-provoking, progress-stimulating content in their manuscripts. Now, more than ever, is the combination of all disciplines working in concert with the pharmaceutical industry key to the development of better treatments, with fewer side effects, and for predicting patient responses. As drugs become more specialized, it is vitally important to describe carefully patient populations both for study and for treatment. With ever increasing evidence that the inflammatory bowel diseases are heterogeneous disorders, drugs will likely only be effective in certain subpopulations of patients.

Above all, we hope that this book will stimulate future research to the point that achieving a diagnosis and development of a treatment plan will be directed by genetic, immunological and clinical markers of phenotypic distinctions.

We would like to express our sincere gratitude to each of the authors, our colleagues and partners, for nearly three decades of commitment to inflammatory bowel disease, and for their insightful, field-leading contributions. We would also like to acknowledge the commitment, patience and support of our publishers, Wiley-Blackwell, particularly Alison Brown, Adam Gilbert, Gill Whitley, Elisabeth Dodds and Oliver Walter.

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Chapter 1
Introduction: the Science and the Art of Inflammatory Bowel Disease

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This book is about the science *and* the art and the science *of* the art of gastroenterology as it pertains to inflammatory bowel disease. Once described as disabling and under-researched diseases, the inflammatory bowel diseases now attract intense interest from clinical and basic investigators, but remain an important cause of suffering and a major burden on healthcare resources.

Why another textbook, in this era of rapid information access? The answer is simple – there is a continuing need for informed opinion and perspective on the deluge of data generated in recent years spanning a diversity of aspects of inflammatory bowel disease. Many wish for a single repository of information from authoritative sources. With this in mind, the authors for this textbook were selected because they are expert and currently active contributors to their respective areas of the field. Each was charged with delivering a crisp, timely and opinionated account of their area with a futuristic perspective.

A recurring theme within modern biology in general and inflammatory bowel disease, in particular, is the need to think across traditional boundaries of intellectual pursuit and to be aware of research at the interface of disparate disciplines. The convergence of different research avenues in inflammatory bowel disease is represented by the host–microbe interface; other pertinent examples have been variably expressed as the brain–gut axis, immunoepithelial dialogue and neuroimmunology. Each is embraced in this textbook in various chapters dealing with disease mechanisms.

One of the great lessons of the recent past in gastroenterology was the failure of traditional epidemiologic and biologic approaches to identify a transmissible agent as the cause of peptic ulcer disease. A more important lesson was that the solution to some complex diseases may never be found by research focused exclusively on the host, without due regard for host–environment interactions, particularly host–microbe interactions. In the future, investigators involved in epidemiologic, genetic or other areas of research in inflammatory bowel disease will have to approach their challenge with some form of rapprochement with disease mechanisms. It is noteworthy, for example, that the genetic risk factors for inflammatory bowel disease are responsible for sensing and interpreting the microenvironment (e.g. NOD2/CARD15) or are involved in the regulation of the host immune response to that microenvironment (e.g. autophagy, IL23R). The complexity and clinical implications of these interactions are discussed by several authors in this volume.

Advances in technology have greatly facilitated research in inflammatory bowel disease. These include automated approaches to gene sequencing and genotyping large numbers of study subjects and molecular strategies for studying the intestinal microbiota, most of which is still unculturable and, therefore, neglected or considered until recently to be obscure. The human organism is now viewed as a composite of the human genome and its commensal microbial genome (microbiome), both of which interact with environmental and lifestyle modifying factors. As the human microbiome project and other similar metagenomic collaborations around the world deliver new information on the diversity and individual variations in the intestinal microbiota, it is anticipated that some of the heterogeneity of inflammatory bowel disease may be resolved. Thus, genetic risk factors will have to be reconciled with variations in microbial composition and with patterns of immunologic responsiveness to the microbiota. The challenge for epidemiologists and biologists will be to relate the aspects of a modern lifestyle with changes in the microbiota and thence with immunologic behavior and susceptibility to disease. Thus, the elucidation of the "IBD genome" provides the foundation for micro- and macro-environmental epidemiologic investigation. The contributing authors to this text have provided the background to this futuristic scenario.

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Has the relentless march of the biotech and genotech era of research delivered for the patient? Unquestionably patients are better off today than they were only a generation ago. A more coherent understanding of fundamental disease mechanisms is being translated into improved patient management with a progressive shift toward evidence-based approaches and away from therapeutic empiricism. This is reflected throughout those chapters of this book dedicated to patient care.

Although not quite at the stage of personalized healthcare, the splitters are in the ascendancy over the lumpers in today's approach to the patient with inflammatory bowel disease. Refinement of clinical phenotypes by fusing genetic variation and the functional consequences thereof will lead to the reclassification of standard clinical phenotypes into physiologically determined subgroups and ultimately to individualized therapeutic targeting. These critical steps will continue to inform the interpretation of data on the genotype. This represents just one of many opportunities for clinicians and basic scientists to engage in a mutually beneficial manner in translating bench-to-bedside research to improved management of inflammatory bowel disease.

But some things never change. Clinical care of chronic disease will always require attention to detail, compassion and a commitment to long-term follow-up. In the face of the extraordinary advances in therapeutics, which continue apace, there is substantial patient dissatisfaction with modern medicine, either because of increasing expectations or reduced tolerance of illness. Most patients place greatest emphasis on the doctor–patient relationship. In this relationship, the attitude and level of interest of the former will always be a major determinant of the outcome of the latter.

Textbooks like this cannot confer attitude, energy or enthusiasm on the reader, but they can sensitize and equip the reader with the necessary background information, opinion and perspective. Therein lies the essence of what is intended with this book – to provide stimulus and steerage for the interested clinician, scientist and clinician–scientist in what is already an intriguing and rewarding field of endeavor.

Chapter 2
Heterogeneity of Inflammatory Bowel Diseases

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Summary
- Heterogeneity in the inflammatory bowel diseases exists at the genetic, immunologic, subclinical and clinical levels.
- The mucosal inflammation that characterizes inflammatory bowel diseases is underpinned by multiple combinations of genes and innate and/or adaptive immune responses that determine disease expression and behavior.
- Serum immune responses are markers of underlying disease activity.
- Multiple genetic variants have been associated with inflammatory bowel diseases.
- Combinatorial genomics, studying the genetic variants and associated immune pathways in combination with disease markers, is leading to the development of distinct phenotypic subgroups and is identifying targets for the development of personalized therapeutic approaches.

Introduction

The chapters in this book describe the foundation of our premise about the heterogeneous nature of the inflammatory bowel diseases (IBDs). In the basic science chapters, we learn that mechanisms underlying disease expression vary genetically and immunologically and that potentially, the possibilities are as many as can be made with the known genes and variants, cells and microorganisms. In the translational and clinical chapters, we read evidence that distinct genetic and immunologic underpinnings differentiate groups of patients, setting the stage for a personalized medicine approach to treating these disorders.

Heterogeneity of inflammatory bowel diseases has been documented in the medical literature for more than a century. In 1905, Dr J.E. Summers Jr wrote, "Colitis of its different types is not uncommon; clinically, they are at some stages so much alike that a proper classification has not been made" [1]. In one simple sentence, we learn that early in the 20th century it was acknowledged by the medical field that there are many types of colitis, but defining them is confounded by their similarities and differences. Clinical heterogeneity of Crohn's disease is mentioned in the literature as early as 1932, when Dr Burrill Crohn published the first report of what he called "regional enteritis" in *JAMA* [2]. Dr Crohn described four "various types of clinical course under which most of the cases may be grouped: (1) acute intra-abdominal disease with peritoneal irritation, (2) symptoms of ulcerative enteritis, (3) symptoms of chronic obstruction of the small intestine and (4) persistent and intractable fistulas in the right lower quadrant following previous drainage for ulcer or abdominal abscess." Similarly, in 1953, Dr Bryan Brooke, writing about ulcerative colitis in reference to the likelihood that no single pathogen can be identified as causal, stated, "It is suggested that ulcerative colitis is not a specific disease, but a pathological state . . ." [3]. Dr J.B. Kirsner, in noting that ulcerative colitis has symptoms similar to other diseases, said, "Ulcerative colitis is merely a name for a class of disease which hitherto had been included under the name dysentery" [4]. From this era, when original observation and description were the hallmarks of excellence in medical research, decades of scholarly activity ensued, with an emphasis on trying to categorize the vast variability in clinical expression of inflammatory bowel diseases into descriptive categories for the purpose of diagnosis and treatment.

Attempts by physicians and scientists to harness IBD heterogeneous expression into the foundation of a framework by which to study these disorders has evolved into the modern hypothesis of disease pathogenesis. Early theories were based on the expectation that a single pathogen was to blame, although in the 1970s and 1980s this notion was abandoned by many and the immune response became the focus. By 1989, many of the elements of the contemporary hypothesis were in place. At that time, it was hypothesized that "tissue damage might be due to a

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direct attack by the mucosal immune system on a specific target, such as the surface, or glandular epithelial cell" [5]. The possibility of "a non-specific outcome of disordered mucosal immune regulation" was suggested, "with uncontrolled over-reactivity to environmental antigens based on a defective downregulation of this response" [5]. It was further postulated that "genetic predisposing factors and exogenous triggers might operate at the level of the 'target' cell or at the level of the mucosal immune system" [5]. In 1990, Dr Stephan Targan, leading an effort by a panel of experts to set a scientific agenda for inflammatory bowel disease research, advanced the concept of "reagent grade populations" [5]. Available treatments at the time were not aimed at any particular cause of disease. In the resulting "white paper", he described the need for defined populations of subgroups of patients with varying clinical and subclinical markers should be assembled. He further stated that:

> Such "reagent-grade" populations will be invaluable in reducing the time and improving the accuracy of all studies using tissues or dependent upon clinical signals from patients. These patients would be a source of materials for the tissue banks and would serve as an extant "pure" population for clinical trials of new therapeutic agents.

Over the last 20 years, three working parties have attempted to formalize an inflammatory bowel disease classification system. In 1991, an international working party assembled in Rome devised a classification for Crohn's disease based on anatomical distribution, surgical history and disease behavior. Seven years later, the "Rome Classification" was re-evaluated by a group attending the World Congress of Gastroenterology in Vienna. The resulting "Vienna Classification" of Crohn's disease proposed the parameters of age of onset, disease location and disease behavior. Most recently, a group meeting in Montreal expanded upon the three phenotypic parameters and modified the criteria. The "Montreal Classification" added distinctions made by serum immune markers and genetic markers and also proposed a classification for ulcerative colitis. The changes were "supported by an evolving body of evidence demonstrating that site of disease, behavior and disease progression are all variables that are likely to be identified by genetic and serological markers" [6].

It was not until the study of serological markers and their use for identifying pathophysiologically distinct subgroups that science yielded to the biologic reality that although it may be of clinical benefit and of benefit to researchers to define subgroups, numerous types of disease expression, with unique biologic processes and distinctive genetic, immunologic and clinical manifestation, exist. Nevertheless, to rein in the possibilities, focus investigation and to test treatments, groups of patients must be identified based on common, known variables. In the current hypothesis, that IBD results in a genetically susceptible individual via a dysregulated immune response to commensal flora, it has been established that there are multiple gene variants that are conferring susceptibility and that IBD patients mount immune responses to numerous microbes.

These authors long ago proposed that the classifications of Crohn's disease, ulcerative colitis and indeterminate colitis are somewhat false. This assertion was based on our emerging understanding of the underlying pathogenesis. Somewhat homogeneous groups of patients can be determined by similar genetic and immunologic and clinical data. Already a case is being made for determining whether to start biologic therapy early in the disease course for certain patients whose profiles suggest the likelihood of more severe disease. In the coming year, the first clinical trials of patients selected not by diagnosis of ulcerative colitis and Crohn's disease, but by a range of genetic and immunophenotypic characteristics, will begin.

Classical clinical heterogeneity

Classically, three major entities of IBD have been defined based on symptoms of disease and standard clinical laboratory, radiologic and histologic parameters: Crohn's disease, ulcerative colitis and indeterminate colitis. Abdominal pain, weight loss, diarrhea, urgency bloody stools and fever may be seen in all three. Crohn's disease is characterized by transmural inflammation with the potential to affect the entire gastrointestinal tract from mouth to anus. In ulcerative colitis, inflammation is superficial and localized to the large intestine and rectum. Indeterminate colitis is the term applied to 10–15% of IBD patients for whom the distinction cannot be made.

Disease behavior is also variable across subtypes of patients with Crohn's disease and ulcerative colitis. Although both disorders are considered to be relapsing and remitting diseases, some patients experience one flare and others experience constant symptoms. Some patients will have a mild course of disease, treatable with 5-ASA products, and others will have very severe disease that is refractory to all modalities attempted. Of course, presentations by individual patients will vary, with some at every point along the continuum. A somewhat arbitrary distinction has been made between Crohn's disease that is "inflammatory" or stricturing and penetrating. The presentation of extra-intestinal manifestations of inflammatory bowel diseases can often be heterogeneous. Some patients may develop rheumatologic, hepatic, ophthalmic and dermatologic effects secondary to their intestinal inflammation and others may not. Any potential combination of these is also possible.

Pouchitis, an inflammatory disease of the reservoir surgically constructed in ileal pouch–anal anastomosis, is

generally thought to occur in patients with underlying ulcerative colitis. The pathogenesis of pouchitis is not firmly established; however, consistent with the hypothesis described above, it is likely the result of an immune response to microbes in the pouch. As described in Chapter 30 by Shen, specific genetic variants have been associated with pouchitis, including IL-1 receptor antagonist [7,8] and NOD2/CARD15 [9]. Expression of a serum immune marker profile including perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), anti-*Saccharomyces cerevesiae* antibodies (ASCA), antibodies to *Pseudomonas fluorescens* (anti-I2) and antibodies to the *Escherichia coli* outer membrane porin-C (anti-OmpC) is associated with chronic pouchitis [10–12].

Classical diagnostic aids are used to differentiate from among many disorders with overlapping symptoms. In Chapter 21 by Schlenker, Eng and Surawicz, we learn that infectious colitides can be confused with IBD, as can other colitides, including diverticular disease and ischemia and colitis caused by therapeutics and radiation treatment for cancer. In the chapter on pathology by Royston and Warren (Chapter 17), we likewise learn that there are multiple potential pitfalls to histopathologic differentiation of these disorders.

One diagnostic tool, capsule endoscopy, has been useful in differentiating Crohn's disease in a specific subset of patients. In Chapter 18 by Dai and Lo, we learn that capsule endoscopy may discover Crohn's-like lesions in 16% of symptomatic patients with a prior diagnosis of indeterminate or ulcerative colitis [13].

Laboratory heterogeneity

C-reactive protein (CRP) is an important acute phase protein. In the acute phase of inflammation, CRP production is increased resulting from influence of interleukin (IL)-6, tumor necrosis factor α (TNF-α) and IL-1β. CRP is generally highest at the onset of a flare of inflammation and decreases in association with treatment. Patients with Crohn's disease tend to have elevated CRP responses, whereas patients with ulcerative colitis tend to have low or no CRP response. Ulcerative colitis and Crohn's disease have heterogeneous CRP responses [14]. Whereas Crohn's disease is associated with a strong CRP response, ulcerative colitis has only a modest to absent CRP response. Simple biologic explanations have failed to understand the reason for this difference; however, recently it has been reported that polymorphisms in the CRP gene may explain the differences in CRP production in humans [15–17]. In another study, however, no association was found [18]. A recent study demonstrated that the CRP 717 mutant homozygote and heterozygote status is associated with lower levels of CRP and that CRP levels are influenced by specific genetic polymorphisms [19].

Genetic heterogeneity

The symptomatic and clinical and immunologic heterogeneity of IBD summarized above is underpinned by multiple genetic variations. To date, 33 variants have been defined and many more are expected. These genetic associations can roughly be considered to contribute to either innate or adaptive immune responses. In Chapter 4 by Vermeire, McGovern, Van Assche and Rutgeerts, the genetic underpinnings of IBD heterogeneity are explored. Variants of the CARD15 gene have received by far the most attention and account for only about 20% of susceptibility in Crohn's disease, highlighting the certainty that many variants are at play in producing IBDs. Studies of the functional effects of the relevant genes in unaffected individuals and IBD have demonstrated the importance of immune pathways in the disease pathogenesis. This chapter also introduces the emerging role of autophagy in pathogenesis. The autophagy-related 16-like 1 gene (*ATG16L1*) and the IRGM gene [20,21] are both involved in autophagy, a process involved in the elimination of intracellular bacteria, and suggest that autophagy may play a protective role.

With genetic research ever more rapidly producing data, efforts to associate disease behaviors are making rapid progress. Specific gene variations have been associated with particular disease phenotypes (reviewed in [22]). For example, *NOD2/CARD15* variants are associated with onset at a young age and with complicated ileal disease (reviewed in [22]). Further studies of IBD subgroups with homogeneous clinical phenotypes may increase the likelihood of finding new susceptibility genes that are specific to those phenotypes.

Since the advent of techniques such as genome-wide association studies (GWAS), the rate of discovery has skyrocketed. Using findings from GWAS as a starting point, new pathways associated with disease pathogenesis are being discovered, as has been mentioned above with autophagy. This pathway was discovered only after the two related genes had been found. Also described in Chapter 4 is the developing information regarding *TNFSF15*. TL1A, the product associated with this gene is considered to be a master regulator of mucosal inflammation and among other functions, induces NFkB. In a sub-population of patients with IBD, TL1A levels are elevated in the mucosa. It has been shown recently that that *TNFSF15* haplotypes are associated with TL1A expression that is further delineated when considered with serologic responses and ethnic background [23]. Genetic information has also helped to elaborate understanding of other IBD processes. For example, the innate immune and the IL23/IL17 pathways, both of which contribute to an increased risk of developing IBD.

Multiple combinations of genetic variants and immunologic pathways lead to IBD. Therefore, it is likely that

progress in understanding susceptibility, improving tools for diagnostic accuracy and developing new treatment targets will depend on parallel investigations that pursue both the genetic underpinnings and the resultant pathway abnormalities. Dubinsky and Denson, in Chapter 20, suggest that the future application of candidate genes is that they may ultimately be used as predictors of immune responses to drugs designed to intercede at the relevant immunologic pathway, in keeping with trends toward personalized medicine.

Biomarkers of disease

Much progress has been made in identifying biomarkers, discovering the underlying inflammatory processes and sub-stratifying disease groups based on these markers and certain genetic variants.

Chapter 20 by Dubinsky and Denson delineates the currently known array of serologic markers associated with IBD. ANCA, ASCA, anti-OmpC, anti-I2 and antibodies to the CBir1 flagellin (anti-CBir1) have been associated with IBD. The presence of one or more antibody and the level of expression have been linked to different disease phenotypes. Levels and combinations of antibody expression have been linked to inflammatory bowel disease phenotypes.

pANCA is associated with ulcerative colitis and with an ulcerative colitis-like presentation of Crohn's disease. Some 60–80% of ulcerative colitis patients express pANCA, as do approximately 20% of patients with Crohn's disease. The pANCA associated with ulcerative colitis is distinguished by perinuclear highlighting upon immunofluorescence staining and by DNAse sensitivity. The ulcerative colitis-related pANCA differs from those associated with vasculitides. Anti-*Saccharomyces cerevisiae* antibody (ASCA) is a marker that is present in approximately 60% of Crohn's disease patients and 10% of ulcerative colitis patients. Antibodies to the *E. coli* outer-membrane porin C (OmpC), the *Pseudomonas fluorescens* Crohn's disease-related protein (I2) and the CBir1 flagellin have also been associated with IBD, predominantly Crohn's disease. Antibodies to OmpC are found in 30–60% of patients with Crohn's disease, sero-reactivity to I2 has been demonstrated in 55% of Crohn's disease patients and an immune response to CBir is detected in 50% of patients with Crohn's disease.

As mentioned, IBD has a vast spectrum of clinical presentations that range from purely inflammatory disease to that which progresses to severe, as defined by fibrostenotic/obstructive or penetrating features, usually associated with fistulization and/or abscess formation. Much progress has been made in the effort to define the nature of the relationship of immune responses to the different phenotypic expressions.

It has been established that subgroups of patients can be stratified based on antibody expression: (1) patients who respond to only one microbial antigen such as either oligomannan ANCA, ASCA, OmpC, CBir or I2, (2) patients who respond to two or three antigens, (3) patients who respond to all known antigens and, finally, (4) patients with no reactivity to any of the confirmed antigens. Patients with the highest complication rate (stricturing, fibrostenosis, etc.) are those who react to most or all of the microbial antigens and those who had the lowest complication rate or progression were in the group without antibody expression. When factoring in amplitude of antibody response, the patients with the highest level antibody expression had the highest complication rate and those in the low level or no response group were least likely to develop complications. Virtually all patients with the highest level response to all antigens experience at least one of these complications, compared with less than a 5% chance among patients with low level antibody expression.

Associations have been found between variants in NOD2/CARD15 and disease phenotypes [24,25], leading to the supposition that the severe innate immune responses lead to higher adaptive immune responses, and thus a more severe disease phenotype. In this model, more genetic defects in innate immunity (NOD2−/NOD2− vs NOD2+/NOD2+) result in a more aggressive adaptive immune response as expressed by higher serum immune markers, and thus a more severe dusease course [26]. See Figure 20.3 in Chapter 20 by Dubinsky and Denson.

Heterogeneity of treatment responses

Why do some patients respond to some therapies and others do not? Why does the effectiveness of a certain therapy wane over time? These are ongoing questions with better and better answers. For example, in Crohn's disease, lack of anti-TNF effectiveness in some patients could be because the immune process may be TNF-α independent. Decreasing response could be because the global suppression of TNF may result in activation of a different immune pathway (see Chapter 7 by Abreu, Fukata and Breglio, and Chapter 8 by McDonald and Monteleone.

In the chapters on cytokines and chemokines by Maillard and Snapper (Chapter 10) and healing/repair by Playford and Podolsky (Chapter 12), we learn about their multiple effects and the potential presented by many as targets for therapeutic development. Because of the complex interrelationships among growth factors/cytokines/chemokines, targeting one specific cytokine might have considerable effects on a large number of others. There is an ever-growing number of these targets, but even those seeming to be the most central to inflammation do not necessarily render a therapeutic that will work in more than a subset of patients, as demonstrated by the

experience with antibodies to TNF, antibodies to IFN and others.

Evidence of IBD heterogeneity from animal models

Over the last two decades, the technology for development of animal models has become increasingly exact. In Chapter 5 by Elson and Weaver, we learn that many combinations of gene protein insertions and deletions result in colitis. The numerous animal models that emerged over the last two decades show that the final common pathway of many alterations is mucosal inflammation. Animal model investigation has highlighted the roles of both innate and adaptive immunity in IBD. This process is revealing the genes, proteins and pathways that are likely to produce dysregulated inflammation and also the key elements of gut homeostasis. The work is becoming increasingly translational, with findings from animal models quickly tested *in vitro* in humans and findings from human research to be researched in animals. As genetic research identifies the relevant immunologic disease pathways, this information will result in improved animal models, an example of which is described below in the case of TL1A.

Harnessing heterogeneity – the future of IBD research

An excellent example in which utilizing concepts of heterogeneity translates to clinical care is found in a review of the recent work on *TNFSF15* and TL1A. This work has taken a linear path of investigation and demonstrates the foundation of a basic, translational and potentially clinical opportunity. The initial discovery of TL1A has given way to subsequent genetic, human and animal investigation at the bench and will reach the bedside in the form of a clinical trial in 2009–10. Furthermore, *TNFSF15* and TL1A fit superbly into the personalized medicine paradigm, in which the combination of genetic, biologic and microenvironmental information may well combine to inform the design of a therapeutic for the subgroup of CD patients that will be uniquely likely to benefit.

TL1A protein was first cloned in 2002 at Human Genome Sciences [26]. TL1A is a very potent enhancer of IFN-γ production. Microbial activation of TL1A plays an important role in modulating the adaptive immune response. TL1A levels are elevated in the mucosa of patients with Crohn's disease. Work in animal models has shown that neutralizing TL1A antibodies attenuates colitis. In genetic research, GWAS have established that the *TNFSF15* gene is a Crohn's disease susceptibility gene [27]. Variants of the *TNFSF15* gene have been found in all ethnic groups studied. Interestingly, however, the associations vary among the cohorts in terms of diagnosis and conferred risk. A recent GWAS revealed a significant association of genetic variants of the *TNFSF15* gene with Crohn's disease in a large cohort of Japanese patients, in several European cohorts [27,28], in US Jewish patients [29] and combined data from the NIDDK IBD Genetics Consortium, Belgian–French IBD Consortium and the WTCC [30]. Haplotypes A and B are associated with susceptibility in non-Jewish Caucasian Crohn's disease and ulcerative colitis. In addition, *TNFSF15* haplotype B is associated not only with risk, but also with severity in Jewish Crohn's disease [23,29,31]. We recently discovered that in addition to Crohn's disease, variants in the *TNFSF15* gene are also associated with both Jewish and non-Jewish severe ulcerative colitis needing surgery. Moreover, monocytes from Jewish patients carrying the risk haplotype B express higher levels of TL1A in response to FcγR stimulation [23]. These results show that Crohn's disease-associated *TNFSF15* genetic variations contribute to enhanced induction of TL1A that may lead to an exaggerated Th1 and/or Th17 immune response, resulting in severe, chronic mucosal inflammation. TL1A is an ideal molecule to link genetic variation and functional protein expression to *severity* and, ultimately, to targeted therapy in the appropriate subset of CD patients. If the results of animal model, genetic and immunologic investigation are combined to select the population of patients most likely to respond to TL1A blockade, it is expected that increased efficacy will be shown in that population. Such investigations are already producing results consistent with this expectation. Current research efforts are aimed at defining mechanisms of TL1A expression and function in inducing a more severe Crohn's disease mucosal inflammation and at defining the population of patients who will respond best to therapeutic blockade of TL1A function.

Conclusion

With more complete understanding of the "IBD genome", genomic-based epidemiology can guide our efforts to determine the process by which disease is initiated and perpetuated in groups of patients with specific profiles. As technology improves, further definition of the microbiome may prove that in different populations, different types of bacteria may be most relevant. These micro-epidemiologic findings can be linked with macro-epidemiologic information to reveal these precise relationships.

As biomedical progress moves more closely to the personal medicine paradigm, the understanding of the heterogeneous nature of IBDs will highlight potential targets for therapeutic development at the genetic and immunologic levels. The most productive avenues of investigation will select populations of patients for study, based on specific phenotypic criteria. The ultimate goal of harnessing

heterogeneity of IBD is an integration of scientific discovery that impacts on patient care. In this scenario, a patient presenting with symptoms would receive a panel of laboratory tests to establish their serotype, genotype and phenotype. The specific IBD phenotype will indicate the likely prognosis of the patient's disease and will further indicate a patient-specific treatment plan using newly discovered, integrated, target-specific therapeutics.

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Chapter 3
Epidemiology of Inflammatory Bowel Disease: the Shifting Landscape

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Summary

- One theory proposed to explain the emergence of IBD as a disease of western or developed nations through the middle of the 20th century has been the "hygiene hypothesis". This hypothesis posits that IBD (and other chronic immune diseases) emerged in the developed world, concurrent with a marked enhancement of personal and societal hygiene and decrement in infant mortality. This meant that there emerged a loss of tolerance to organisms that might otherwise be encountered in childhood, in a dirtier environment, which leads to aberrant immune responses when those organisms or mimicking antigens are presented at an older age.
- The hygiene hypothesis can apply to the emergence of late of IBD in the developing world where the developing world is now encountering more and more IBD, as it becomes "cleaner". Other environmental and societal factors in the developed world include westernization of diets and the broader introduction of western medicines including antibiotics and vaccines.
- Data from the past decade from developed countries have revealed that the incidence rate of Crohn's disease has overtaken that of ulcerative colitis. In areas where the incidence rate of ulcerative colitis is still higher, the trends are suggesting increasing rates of Crohn's disease. In developing countries ulcerative colitis is the predominant form of IBD.
- There seems to be a rising incidence of isolated colonic disease among Crohn's disease phenotypes, begging the question as to whether the emergence of a greater incidence of Crohn's disease over ulcerative colitis was real or whether much of the former high rates of ulcerative colitis encompassed misdiagnosed colonic Crohn's disease.
- Clues to disease etiology are more likely to arise from studies in pediatric and developing world populations where dietary and environmental impact may be more evident than in studies from developed nations with longstanding burdens of IBD.

Introduction

The epidemiology of inflammatory bowel disease (IBD) has been described for over 50 years with early population-based data being available from the Olmsted County, MN, USA and Northern Europe. In the past decade, there has been an onslaught of data from a variety of countries, including developing and Asian countries where IBD had rarely been seen. For the casual reader of the IBD epidemiology literature, it is easy to gloss over the study details and simply focus on the reported incidence and prevalence rates. However, epidemiology studies are conducted very differently in different jurisdictions. The study process is often dictated by what type of data collection or access is available. For example, in Scandinavia, the UK and Canada, administrative health data are collected comprehensively, inclusively and are accessible to researchers. In developing nations and countries of Eastern Europe, there are not only poor administrative data collection resources, but also variable access to care and in some instances various standards of care. This clouds the interpretation of clinic-based or single hospital-based studies and lessens the applicability of their findings to the broader population of the area under study. Some studies are presented as being population based where they are derived from a compilation of practices and not administrative data. Although the comprehensiveness of data collection from any one center is believable, the percentage of non-participation or a comparison between participating practices and those not participating are often missing from the methodology description. In the USA, arguably the country with access to the most advanced health technology for some sectors of its population, population-based studies are nearly impossible, because of the varied health insurance programs that exist. Multi-clinic and/or hospital studies in the USA are always subject to criticisms of bias.

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It is clear in western Europe, Canada and the USA that IBD has emerged as predominately an outpatient disease at diagnosis and for chronic management. Most diagnoses are made on outpatients and 50% of patients avoid hospitalization in the first 15 years from diagnosis [1]. However, it is possible that in eastern Europe and the developing world and in developed nations where IBD is uncommon (such as Japan and Korea), a sizeable number of IBD patients will be diagnosed in a hospital setting. Hence hospital-based or centralized specialty clinic-based studies may be more representative of the whole population than if such a study was conducted in the USA.

Pediatric studies, like adult studies, are more robust and representative when population based. However, pediatric studies conducted from hospital practices or centralized specialty clinics are more likely to be representative and less subject to bias than adult studies since it is more typical for children to be referred to centralized specialist care than adults. Pediatric gastroenterologists are fewer in number and more likely to congregate in group practices, particularly in pediatric hospitals. When assessing pediatric IBD studies, it is important to note the ages included. For many it is 15 years or younger. However, for some it is less than 18 years and those extra 2–3 years can markedly affect the final incidence data. On the one hand, children ages 16 and 17 years may be evaluated by adult gastroenterologists and not captured in pure pediatric settings. On the other hand, the incidence rates typically rise through later teenage years into the third decade and the inclusion of older children will increase incidence rates.

Why do we care so much about IBD epidemiology data and why does this topic still warrant a chapter in a state-of-the-art textbook on IBD where new gene discoveries and biological therapies are reviewed? First, it is important to appreciate the burden of disease with regard to sheer numbers. It is important to consider in the allocation of research resources as to whether the disease is rare, common or increasingly emerging. Second, patterns of disease can give clues to disease etiology. Just because researchers have yet to assemble conclusively the epidemiological clues into a defining etiologic paradigm in IBD does not mean that the clues are not emerging accurately. The failure to have clinched an etiologic cause(s) does not negate the potential that epidemiologic observations provide. The hygiene hypothesis [2] is one hypothesis to emerge from epidemiologic studies. In brief, with the epidemiology showing an emergence of IBD in the developed world, concurrent with a marked enhancement of personal and societal hygiene and decrement in infant mortality, this hypothesis suggests that it is in fact the loss of tolerance to organisms that might otherwise be encountered in childhood, in a dirtier environment, that leads to aberrant immune responses when those organisms or mimicking antigens are presented at an older age. This hypothesis can be applied to the evolving epidemiology where the developing world is now encountering more and more IBD, as the developing world becomes "cleaner". The emergence of IBD in the developing world in the past decade may also be a side effect of globalization. People in Asia, Africa and the former Soviet Union are now doing what people in the developed world have been doing for decades, including diets higher in fat and refined sugars, fast foods, reduced in fiber and reduced physical activity and increased refrigeration of foods. There is also increasing ingestion of pharmaceuticals even in the developing world, including therapeutics, additives and vaccines. Hence the clearer the picture of IBD presentation can be made based on a global view, the more clues will emerge as to what may be causing it. In parallel with genetics research, it is fascinating to observe the increasing incidence rates in various countries that are far outpacing what genetic evolution could instigate.

In this chapter, the recent epidemiology of IBD will be reviewed. The chapter is focused on reports published since 2000 and, where possible, on data from the mid-1990s into the 2000s.

The emergence of Crohn's disease as the most common form of IBD

Almost uniformly, the data from the past decade from developed countries have revealed that the incidence rate of Crohn's disease has overtaken that of ulcerative colitis. In areas where the incidence rate of ulcerative colitis is still higher, the trends are suggesting increasing rates of Crohn's disease. Not unexpectedly, where available, prevalence data have lagged behind and in many jurisdictions the prevalence of ulcerative colitis remains higher than that of Crohn's disease. The incidence data for Crohn's disease have been remarkably consistent in Northern Denmark (9.3/100,000) [3], Copenhagen County, Denmark (8.6/100,000) [4], Northern France (8.2/100,000) [5] and Olmsted County, MN, USA (8.8/100,000) [6]. The incidence rates of ulcerative colitis are more varied, including Northern Denmark (17/100,000), Copenhagen County, Denmark (13.4/100,000), Northern France (7.2/100,000) and Olmsted County, MN, USA (7.9/100,000) [3–6]. A very interesting contrast is posed by data from Canada and New Zealand [7,8].

Using the administrative definition of IBD validated in Manitoba in 1995 [9], investigators applied the definition to similar administrative health databases in British Columbia, Alberta, Saskatchewan, Manitoba and Nova Scotia for the years 1998–2000 [7]. The mean incidence rate for Crohn's disease was 13.4/100,000. An interesting finding was that the incidence rate in British Columbia was 8.8/100,000, which was significantly lower than that in the other provinces. It is interesting to speculate whether

Table 3.1 A summary of European IBD epidemiology data from 1991 to 1993.

	Males	Females
Crohn's disease		
North	6.2/100,000	7.9/100,000
South	3.8/100,000	4.0/100,000
Ulcerative colitis		
North	12.5/100,000	11.1/100,000
South	10.3/100,000	6.9/100,000

Based on data from Shivananda S, Lennard-Jones J, Logan R *et al.* Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* 1996; **39**:690–7.

the lower rates in British Columbia relate to its environment (i.e. Pacific coast, Rocky Mountain range) or to the fact that nearly one-quarter of its population are visible minorities, many of whom were recent immigrants within the past two decades. Nonetheless, the incidence rates for Crohn's disease in Canada are among the highest in the world. Considering its northern location, it reminds us of the original hypothesis proposed from Europe in the 1980s that the high rates in the UK and Scandinavia versus the low rates in Mediterranean countries reflected a north–south gradient. Might this gradient be explained by sunlight exposure or climate differences? Incidence data from 2004–05 from New Zealand were 16.3/100,000, comparable to Canadian data [8], diminishing the likelihood that lack of sunlight or temperate versus tropical climate is an important disease trigger.

A re-evaluation of European data in 1996 suggested that perhaps the earlier proposed north–south gradient was overstated based on primitive southern European data, since the updated data revealed a lessening of the gap between northern and southern European data [10]. Table 3.1 reveals rates reported from Europe up to the 1990s adapted from the European Collaborative Study on IBD [10]. These data show not only differences between northern and southern European rates of Crohn's disease and ulcerative colitis but also summarize an era when ulcerative colitis was more common than Crohn's disease. A recent study from northern Spain suggested incidence rates of 9.5/100,000 for Crohn's disease and 7.5/100,000 for ulcerative colitis (ever closer to rates from northern Europe) [11], while a study from northwest Greece reported low rates for both diseases (rates could not be calculated) [12]. In Greece, like other emerging countries, however, the incidence of ulcerative colitis far exceeded that of Crohn's disease [12].

There have been population-based data from France and Scotland that suggested within each country that the northern areas have higher rates than southern areas [13,14]. However, the fact that the northern areas may have higher rates than southern areas may be more coincidental and less informative than more global north versus south patterns. Within each country there may be ecological, topographical, socioeconomic or genetic factors that drive higher rates in some areas versus others. Perhaps etiological clues can emerge from these differences within any one country, and these differences should be sought. However, the likelihood that these differences reflect something specific about a northern location within any one country is low. In Manitoba there is wide variation between areas in terms of incidences of ulcerative colitis and Crohn's disease, but these did not follow a north–south pattern [15]. In fact Canada's north has very low rates of IBD compared with Canada's south, owing in part to the genetic makeup of the majority of northern dwellers in Canada (mostly Aboriginals). However, the sparseness of the population, the topography or the different dietary and childhood patterns of disease and infection may in fact provide important clues. However, this pattern of higher disease density in the south of Canada does not refute an overall north–south gradient of disease, much as the higher density of IBD in France's north does not prove that on a global basis northern countries have higher incidence.

It is unknown whether the high Canadian rates reflect a north–south gradient within North America. The only population-based data from the USA are from Olmsted County, MN [6], which is only 400 miles south of the Canadian border, and rates there have been reported to be just over half the rates reported from Manitoba. However, an unpublished update of the Olmsted County data to 2004 suggest rates much closer to those of Manitoba (Crohn's disease 12.9/100,000 and ulcerative colitis 12.5/100,000) [16]. Unfortunately, data from the southern USA where there is greater ethnic diversity are unavailable. Previously, using Veterans Administration data and also Medicare data, a north–south gradient within the USA was reported [17,18]. Hence it may be premature to dispense with the possibility that a north–south gradient exists. Even if the gap narrows in incidence rates between north and south (such as the high rates reported in New Zealand), it does not negate the potential clues to etiology that might exist by having seen high rates in northern countries initially and the recent emergence of the disease in the south. If the incidence rates in southern Europe are rising, then what is driving this progression?

It was recently suggested that much can be learned by studying the western Europe–eastern Europe dichotomy in IBD incidence [19]. Recently, the incidence rates of Crohn's disease and ulcerative colitis, respectively, in Hungary were 2.2/100,000 and 5.9/100,000 [20], in the Czech Republic were 3.1/100,000 for ulcerative colitis (rates for Crohn's disease in the non-pediatric population are lacking) [21], in Romania were 0.5/100,000

Table 3.2 Studies of adults with IBD.

Jurisdiction	Years	Study design*	Incidence rate per 100,000	
			Crohn's disease	Ulcerative colitis
North Denmark [3]	1998–2002	Population based	9.3	17
Copenhagen County, Denmark [4]	2003–2005	Population based	8.6	13.4
North France [5]	2000–2002	Population based	8.2	7.2
Olmsted County, MN, USA [6]	1990–2000	Population based	8.8	7.9
Canada [7]	1998–2000	Population based	13.4	11.8
New Zealand [8]	2004–2005	Population based	16.3	7.5
Northern Spain [11]	2000–2002	Clinic based	7.5	9.1
Hungary [20]	1997–2001	Clinic based	2.2	5.9
Czech Republic [21]	1999	Clinic based	1.5	1.5
Romania [22]	2002–2003	Clinic based	0.5	0.97
Croatia [23]	2000–2004	Clinic based	7	4.3
French West Indies [33]	1997–1999	Clinic based	1.9	2.4

*Clinic based refers to hospital- and/or outpatient clinic-derived data. In some instances these sources may facilitate a population-based study, but where there was any uncertainty the references were identified as being clinic based.

and 0.9/100,000 [22], in Croatia were 7/100,000 and 4.3/100,000 [23] and in Poland ulcerative colitis was considerably more common than Crohn's disease (rates were not calculated) [24]. These studies are mostly specialty clinic or hospital derived, although in Hungary an extensive effort has been made to recruit gastroenterologists across the country. What can be learned from these data is that in general the rates of Crohn's disease and ulcerative colitis were lower than elsewhere in Europe and that mostly ulcerative colitis is more common than Crohn's disease. This is typical of the emergence of IBD in developed nations and hence we can expect to see rates of Crohn's disease overtaking those of ulcerative colitis over the next decade.

Data from emerging nations such as South Korea [25], China [26,27], India [28,29], Iran [30], Lebanon [31], Thailand [32] and the French West Indies [33] reveal a clear pattern of greater rates of ulcerative colitis over Crohn's disease. Although these rates are lower than in the developed world, there are indications that they are greater than what might have been seen two decades ago. Of course, in many of these countries there remain issues of the comprehensiveness of data collection, and also access to care of the populations. It is noteworthy that amidst Manitoba's high rates of IBD exists the First Nations Aboriginal community (comprising 10% of the entire population). Much of the First Nations communities are located in the more sparse north of Manitoba and have living conditions that in some communities are more akin to the developing world, without flush toileting and in crowded conditions. Another sizeable First Nations community exists in the core of the city of Winnipeg. All of these communities, both rural and urban, have been shown to have significantly lower rates of Crohn's disease and ulcerative colitis than the non-First Nations Manitoba population [15]. However, the rate of ulcerative colitis is approximately four times that of Crohn's disease. This mirrors the rates of IBD from the mid-20th century in developed countries and from the developing world at present. Table 3.2 lists recent era studies of incidence data among adults with IBD.

Pediatric IBD

With peak incidence rates typically in the third decade of life, it is possible, if not probable, that events in childhood are shaping the ultimate development of IBD. The other aspect of assessing pediatric data is that children's lives are sufficiently short that clues to etiology might be more easily discerned. Dietary intake, living conditions and demographics can be more easily and accurately recorded in children than for adults. Almost uniformly, the modern pediatric data are from northern Europe and much of it is population based. Nearly all of the data show higher incidence rates for Crohn's disease than ulcerative colitis. These rates for Crohn's disease ranged from 2.3/100,000 to 4.9/100,000 and for ulcerative colitis from 0.8/100,000 to 2.4/100,000 (Table 3.3) [4,33–39]. Data from Finland in 2003 were contrary to this trend, with incidence rates in Crohn's disease of 2.6/100,000 and in ulcerative colitis of 3.2/100,000 [40]. In Copenhagen County, Denmark, the incidence rates in 2003–05 for Crohn's disease were 2.7/100,000 for those aged 15 years and under and 4.4/100,000 including all those younger than 18 years [4]. In fact, for ulcerative colitis the incidence rate for those younger than 18 years was 5.0/100,000, greater than the rate for Crohn's disease. Even in southern and eastern European countries such as Northern Spain [11] and the Czech Republic [41,42], Crohn's disease is outpacing ulcerative colitis among the pediatric population. In Saudi Arabia, in a single hospital in Riyadh, the rates of

Table 3.3 Studies of children with IBD.

Jurisdiction	Years	Age (years)	Study design*	Incidence rate per 100,000	
				Crohn's disease	Ulcerative colitis
Copenhagen County, Denmark [4]	2003–2005	<15	Population based	2.7	2.4
Copenhagen County, Denmark [4]	2003–2005	<18	Population based	4.4	5.0
Scotland [14]	1981–1995	<16	Population based	2.3	1.2
France [34]	1988–1999	<17	Population based	2.3	0.8
Sweden [35]	1990–2001	<16	Population based	4.9	2.2
Norway [36]	1999–2004	<16	Clinic based	3.6	2.1
The Netherlands [37]	1999–2001	<18	Population based	2.1	1.6
South Wales [38]	1996–2003	<16	Population based	3.6	1.5
Wisconsin, USA [39]	2000–2001	<18	Population based	4.6	2.1
Canada [7]	1998–2000	<20	Population based	8.4	3.9
Finland [40]	2003	<18	Clinic based	2.6	3.2
Northern Spain [11]	2000–2002	<15	Clinic based	5.7	1.4
Eastern Czech Republic [41]	1998–2001	<16	Clinic based	2.7	1.8
Czech Republic [42]	1998–2001	<16	Clinic based	1.3	Not stated

*Clinic based refers to hospital- and/or outpatient clinic-derived data. In some instances these sources may facilitate a population-based study, but where there was any uncertainty the references were identified as being clinic based.

ulcerative colitis were similar to those of Crohn's disease [43]. For those studies reporting trends, all reported an increase of Crohn's disease with either stagnation or a decrease in rates of ulcerative colitis (Copenhagen, France, Sweden, Norway, Czech Republic) [4,34–36,41,42]. Hence it is clear that Crohn's disease is emerging as the predominant form of IBD. In adults, rates of ulcerative colitis are not diminishing but in some areas they are in children. Hence the environmental factors contributing to Crohn's disease persist and may even be more easily identified. Children have led shorter lives than adults with likely more routine eating and lifestyle habits and with little movement between jurisdictions. Their habits are also often carefully tracked by parents and caregivers, making survey data potentially more reliable than in adults, who present at various ages and are asked retrospectively to consider events of the distant past.

The demographics of IBD

While Crohn's disease has emerged as the more predominant form of IBD, there has also been a swing towards more males with disease than females. In the past, the sex ratio has been mostly equal for ulcerative colitis, with a 30% predominance of females in Crohn's disease. While a female predominance of Crohn's disease has remained in northern Denmark, France, Canada, New Zealand, Northern Spain and French West Indies [3,5,7,8,11,33], rates are similar between males and females in Copenhagen County, Denmark, Olmsted County, MN, USA and Hungary [4,6,20]. Further, there was a male predominance in emerging IBD areas such as Greece, China, Lebanon, Romania and Croatia [12,22,23,26,27,31]. In the pediatric literature, a male predominance was evident in all studies in which it was reported (Scotland, Sweden, The Netherlands, Wisconsin, Czech Republic [14,35,37,39,42]), except for France and Finland, where the sex distribution was equal [34,40]. Hence for Crohn's disease the trends amongst children and emerging nations and in many of the adult studies are of an increase in males presenting with Crohn's disease. In ulcerative colitis, the equality of the incidence by sex was evident in studies from northern Denmark and Copenhagen County, Denmark, Canada, Northern Spain, Hungary and Croatia [3,4,7,11,20,23], with a male predominance in studies from France, Olmsted County, MN, USA, Romania, China and Lebanon [5,6,22,26,27,31]. In ulcerative colitis, the equality of the incidence by sex was evident in pediatric studies from Scotland, Sweden, Finland and the Czech Republic [14,35,40,42], with a male predominance in studies from The Netherlands and Wisconsin [37,39]. Only in the French West Indies was there a female predominance [33]. So what is it about males that has led to their emergence as the increasingly more affected sex by IBD, all over the world?

Another uniform finding in areas where IBD has long been established, such as northern Europe and North America, and also in emerging areas such as southern Europe, eastern Europe and Asia, is that the peak age of onset for Crohn's disease is typically in the third decade (northern Denmark, Copenhagen County, Denmark, France, Canada, Olmsted County, MN, USA, New Zealand, Hungary, Croatia, Korea, China, Lebanon) [3–8,20,23,25–27,31]. For ulcerative colitis, the peak age is typically in either the third (northern Denmark, Copenhagen County, Denmark, Olmsted County, MN, USA,

Korea, Lebanon) [3,4,6,25,31] or the fourth decade (France, Hungary, Croatia, China) [5,20,23,26,27]. Furthermore, recent large population-based studies show no second peak of either Crohn's disease (France, Olmsted County, MN, USA, Canada) [5–7], although this is not uniform (Copenhagen County, Denmark, New Zealand) [4,8].

As the incidence of Crohn's disease has overtaken the incidence of ulcerative colitis, it is of interest to consider whether the pattern of presentation has changed over time. Isolated colonic disease has been estimated formerly to be primary locus of disease in approximately 20%. Recent data show isolated colonic disease in approximately 30% in such disparate jurisdictions as Olmsted County, MN, USA, Croatia and China [6,23,26,27] and 50% of cases in northern Denmark [3]. In pediatric studies, the prevalence of isolated colonic disease ranges from 10% in France [34], to 17% in Copenhagen County, Denmark [4], to 25% in Norway [36], to 32% in Wisconsin [39], to 50% in Finland [40] and to 55% in Sweden [35]. In a study from six major pediatric referral centers in the USA, not only was Crohn's disease more commonly seen than ulcerative colitis, but of all cases of Crohn's disease 30% were isolated colonic disease [44]. Is it real that an emergence of colonic Crohn's disease, particularly in adults, but also in some pediatric studies, contributed to more Crohn's disease overall than ulcerative colitis, or was much of the former high rates of ulcerative colitis encompassing misdiagnosed colonic Crohn's disease?

Conclusion

The recent trends in the epidemiology of IBD show that there are higher incidence rates of Crohn's disease than ulcerative colitis in northern European and North American studies. While incidence rates of Crohn's disease in Manitoba have been high for several years, they appear to be rising in most other countries. This trend has emerged both in hospital- and clinic-based studies and in population-based studies. There remain higher rates of ulcerative colitis in the developing nations of eastern Europe and Asia, mimicking what was originally evident in the developed western world decades ago. The peak age of onset has been constant for years, with most cases of Crohn's disease presenting in the 20s and of ulcerative colitis presenting in the 20s to 30s. However, there has been an emergence of Crohn's disease among males and more IBD cases overall are males than females. What clues can we draw from this in terms of seeking etiologies? Pediatric environmental studies should be pursued. Dietary changes can likely be more easily tracked in children and over shorter lifetimes. It is important to explore differences between males and females, for instance vaccine patterns or hormones. In particular, environmental studies in the developing world are critical. Changes in dietary and lifestyle patterns of communities may be more evident over the past decade in Asia or eastern Europe, where an introduction to western lifestyles has been very recent. The introduction of cleaner water sources, diets higher in fats and refined sugars, electronic technology, novel food additives, broader access to antibiotics and other medications, lower infant mortality rates secondary to lesser critical pediatric infections and vaccine programs may all in some way contribute to the emergence of IBD in the developing world. The etiologic clues may be hidden amongst these observations.

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Chapter 4
Genetics of Inflammatory Bowel Disease: How Modern Genomics Informs Basic, Clinical and Translational Science

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Summary

- **Lessons which can be learned**: It is clear that the research on IBD genetics has proven to be one of the most successful of all complex polygenic traits. Both linkage and association scans have identified many genes and the first conclusion is that these genes can be grouped into biological pathways (see Figure 4.4). New pathways (for instance autophagy) have been identified, which need to be explored, and other pathways (role of barrier integrity and of bacterial recognition) were emphasized.
- **Remaining challenges**: Despite the success, many more significant signals were found and it will be a difficult task to separate noise from true associations. Furthermore, fine mapping and other approaches will need to be adopted to identify the true genetic associations at a number of the loci.
- So far, ***NOD2* is the most important gene** and explains around 20% of the overall genetic risk. All other identified genes carry effect sizes which are much more modest.
- It still remains an enigma why in Asian countries, despite a similar clinical phenotype of IBD, no *NOD2*, *ATG16L1*, *PTGER4* or *IL23R* variants can be found.
- **The clinical translation of the study of IBD genetics** is still limited and studies now need to be performed looking at risk prediction and integrating molecular tools in the diagnostic and prognostic work-up of our patients.

Introduction

The inflammatory bowel diseases (IBDs) are chronic relapsing inflammatory diseases of the gut. The exact causes of IBD are unknown but are accepted to be multifactorial. An interplay of environmental risk factors and immunologic changes will trigger onset of the disease in a genetically susceptible host. The progress, in recent years, in identifying susceptibility genes for IBD has been amazing. Crohn's disease (CD) and ulcerative colitis (UC) are the two major phenotypes of IBD, although the disease carries a very heterogenic presentation with respect to disease location, behavior and severity.

CD and UC are both complex polygenic disorders. A number of genetic variants, in the face of environmental stimuli, all contribute to the final clinical phenotype. This in part may explain the wide variety and heterogeneous nature of phenotypes seen by clinicians. It is not yet known how many susceptibility genes underlie the IBDs and it is also unclear how these susceptibility variants interact both with each other and with environmental factors. Epidemiologic evidence suggests that CD and UC are likely to share some susceptibility genes; however, disease-specific genes will also exist since CD and UC are very distinct in clinical features.

Methods used in the study of complex genetics

Until very recently, two main approaches could be undertaken to identify genes in complex diseases: the positional cloning approach, based on linkage analysis, and the candidate-gene approach, based on association analysis. Linkage analysis studies the co-segregation of the disease with a marker within families. The first genome-wide linkage scans were published just over 10 years ago and 11 of these scans have been undertaken in IBD identifying susceptibility regions on chromosomes 1, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19 and X (Table 4.1 and Figure 4.1) [1–11].

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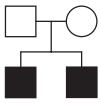
Table 4.1 Genome-wide linkage studies in IBD using the affected sibling pair method (pedigree).



Authors	Year	*N* siblings	Major region identified
Hugot *et al.*	1996	112	IBD1
Satsangi *et al.*	1996	186	IBD2
Cho *et al.*	1998	151	IBD1–7
Hampe *et al.*	1999	353	IBD1–2–3
Ma *et al.*	1999	65	IBD4–5
Duerr *et al.*	2000	94	IBD4
Rioux *et al.*	2000	183	IBD3–5–6–9
Paavola-Sakki	2003	138	IBD2
Vermeire *et al.*	2004	125	IBD4
Barmada *et al.*	2004	260	IBD2–3

Follow-up studies of fine mapping of these regions were performed. Alternatively, a candidate gene approach was adopted where a specific gene of potential interest was studied. The first gene identified for CD, *NOD2/CARD15*, was identified by two groups simultaneously using these methods.

More recently, with the completion of the human genome sequence, the development of the HapMap and the significant reduction in genotyping costs, whole genome association studies (WGAS) have now become possible. A number of WGAS have now been published in both UC and CD and more than 30 susceptibility loci have been identified for both CD and UC, including ATG16L1, IL23R, PTGER4, IRGM, IL10 and NELL1 [12–19] (Table 4.2 and Figure 4.2).

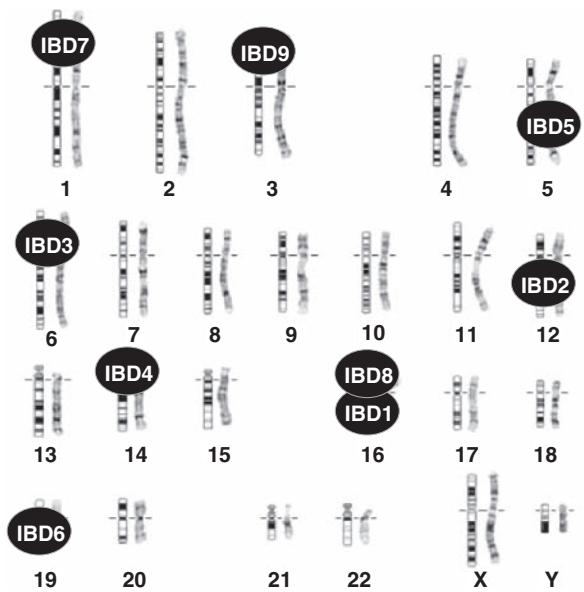


Figure 4.1 Replicated linkage regions for IBD (IBD1 to IBD9).

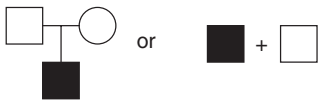
One of the largest WGAS was undertaken by the Wellcome Trust Case Control Consortium (WTCCC) [20]. This involved a joint GWA study in the British population which examined seven inflammatory conditions (hypertension, coronary heart disease, bipolar disorder, type 1 and 2 diabetes, Crohn's disease, rheumatoid arthritis). Crohn's disease was the most successful disease with nine independent association signals identified at the level of $p < 5 \times 10^{-7}$. Many of the identified loci only had modest effect sizes, hence stressing the importance of large sample sizes and independent confirmatory cohorts.

Identification of susceptibility genes in IBD

NOD2/CARD15

Hugot *et al.* were the first to report linkage to 16q in 1996 and, 5 years later, identified the underlying gene through a fine mapping and positional cloning approach as the *CARD15* (originally reported *NOD2*) gene [21]. Simultaneously, Ogura *et al.* also identified *CARD15* but by means of the candidate gene approach [22,23]. Thirty non-conservative polymorphisms have been identified within the gene and all seem associated with CD, but only three are common (Arg702Trp, Gly908Arg and Leu1007insC) (Figure 4.3). The three common variants account for approximately 82% of the mutated alleles [24]. *CARD15* variants are only associated with CD and not with UC. *CARD15* codes for the NOD2 protein expressed in monocytes, macrophages, dendritic cells, epithelial cells and Paneth cells [25]. NOD2 is a pattern recognition receptor (PRR) and senses bacterial peptidoglycan-derived muramyl dipeptide (MDP) through its leucine-rich-repeat (LRR) domain [26]. In its turn, but through yet unknown mechanisms, sensing of MDP stimulates secretion of antimicrobial peptides including α-defensins (also called cryptdins), and will in this way protect the host from invasion [27]. In CD, a reduced expression of α-defensins has been demonstrated and is even more reduced in patients carrying *CARD15* mutations [28]. The frameshift mutation 1007fsinsC leads to a truncated protein lacking the 33 distal amino acids and *in vitro* data showed impaired activation of NF-kappa B (NF-κB) after stimulation [22]. However, more than 8 years after the original publications of the association between CARD15 and CD the functional consequences of these genetic variants that lead to an increased risk of developing CD remain controversial. Debate continues as to whether these mutations are "gain" or "loss" of function mutations and controversy continues as to which of the *CARD15*-associated pathways is the most important in CD pathogenesis.

Table 4.2 Genome-wide association studies in IBD using case–control or parent–child trios (pedigrees).



Authors	Year	*N*	SNPs	Major findings
Yamazaki *et al.*	2005	484 CD Japanese + 363 CD UK + 347 IBD UK	80,000	***TNFSF15***
Duerr	2006	567 ilCD + 401 ilCD+ 883 IBD	300,000	***IL23R***, *NOD2*, *ATG16L1*, *PHOX2B*, *NCF4*, *PTPN2*
Rioux	2007		300,000	
Hampe	2007	735CD + 498CD + 509CD + 788UC	20,000	***ATG16L1***, *NOD2*, *5q31*
Libioulle	2007	547 CD Belgium + 1266 CD Belgium	300,000	***PTGER4***, *NOD2*, *IL23R*, *ATG16L1*
Parkes	2007	1748 CD UK + 1182 CD UK	500,000	***IRGM***, *NKX2–3*, *PTPN2*, *IL23R*, *PTGER4*
Franke	2007	393 German + 942 CD + 454 trios Quebec + 1059 UC + 453 CD UK	116,000	***NELL1***, *NOD2*, *PTGER4*, *5q31*
Raelson	2007	382 Quebec CD trios +521 German trios +750 German cases	164,000	*NOD2*, *IL23R*, *5q31*, *ATG16L1*, *IRGM*, *PTGER4*

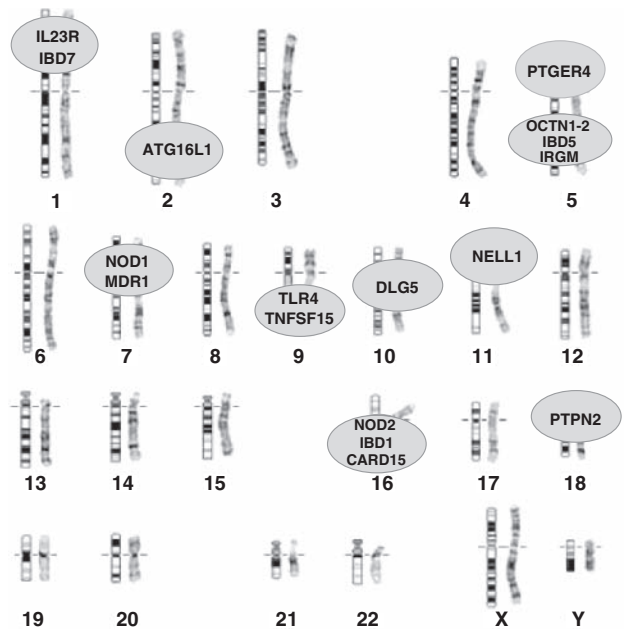


Figure 4.2 Identified genes for IBD, 2008.

DLG5

The linkage region reported on chromosome 10 by Hampe *et al.* [4] was refined by positional cloning and identified as containing *DLG5* (for its homology with Drosophila Discs Large Homolog 5) as the causal gene for IBD [29]. One haplotype in this gene, characterized by the haplotype-tagging SNP *G113A* [leading to a change from arginine to glutamine at amino acid position 30 (R30Q)], was over-transmitted to affected offspring with CD and UC. In an independent case–control sample, 25% of IBD patients carried at least one 113A risk allele, compared with 17% of healthy controls ($p = 0.001$). The overall risk for IBD associated with the 113A variant in their original study was, however, moderate [odds ratio (OR) = 1.6]. DLG5 is a widely expressed protein found in the placenta, small bowel, colon, heart, skeletal muscle, liver and pancreas. It is a member of the membrane-associated guanylate kinase (MAGUK) family of scaffolding proteins, which are important in signal transduction and epithelial cell integrity. Meanwhile, replication studies have emerged, but results are conflicting and pointing towards an even lower RR of approximately 1.25 [30–32].

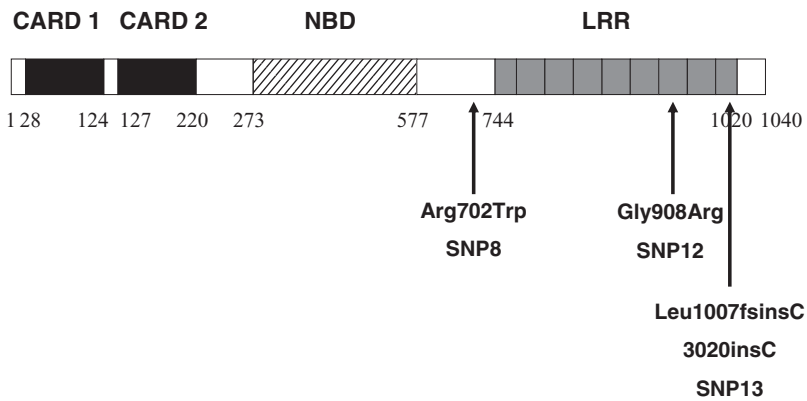


Figure 4.3 Structure of the *CARD15* gene indicating the three main variants associated with Crohn's disease.

IBD5 and *OCTN 1–2*

A 2004 study by Peltekova *et al.* suggested that the genes underlying the *IBD5* locus were the *SCL22A4* and *SLC22A5* genes, coding for the *OCTN1* and *2* (novel organic cation transporter) proteins, respectively [33]. Rioux *et al.* first reported linkage for CD on *5q31* in the Canadian population [7]. *IBD5* is a very attractive candidate region for IBD, since it harbors a cytokine gene cluster. Fine mapping of this locus refined the region to a 250 kb risk haplotype (surrounding the OCTNs) but precise identification of the underlying causal genetic variants was impossible due to strong linkage disequilibrium (LD) across the region [34]. By re-sequencing the known genes in the IBD5 region, 10 new single nucleotide polymorphisms (SNPs) were identified. Two of these were predicted to have functional effects: a missense substitution in *OCTN1 (L503F)* and a G → C transversion in the promoter of *OCTN2*. In the study by Peltekova *et al.*, these SNPs were associated with susceptibility to CD. The OCTNs are a family of transporter proteins for organic cations and carnitine, an essential co-factor of the metabolism of lipids [35,36]. Carnitine is involved in the transport of long-chain fatty acids into mitochondria where fatty acids will undergo β-oxidation. There is evidence that inhibition of fatty acid oxidation in the epithelium of the colonic mucosa is associated with the development of UC. Inhibition of β-oxidation by rectal administration of sodium 2-bromooctanoate induces weight loss and bloody diarrhea in rats with histological signs of ulcers, mucus cell depletion, vessel dilatation and an increase in acute inflammatory cells [37].

NOD1/CARD4

Another region of linkage which was further pursued using a candidate gene approach was *7p14*, identified in the original genome scan from Oxford, UK [2]. An association between a complex functional *NOD1 (CARD4)* insertion/deletion polymorphism [ND(1) + 32656*1] and IBD was found by the same investigators [38]. *NOD1* shows homology with *CARD15* [2]. There is, again, lack of wide confirmation.

MHC region

The MHC region is the region of most interest probably from a candidate gene approach. HLA class II molecules present partially digested antigen to the T-cell receptor and play a central role in the immune response. In contrast with other immune-mediated complex diseases such as rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes, studies on the role of the MHC complex in IBD have yielded inconsistent, heterogeneous and often very weak results [39–41]. *HLA DR2 (DRB1*1502)* has been implicated in Japanese patients with UC, whereas *HLA DR3 (HLA DRB1*0103)* has been implicated in European studies. HLA associations have been less convincing, although there has been some association with *HLA DR1*.

Toll-like receptor genes

Following the identification of the role of *CARD15* in CD, there has been major interest in other pattern recognition receptors (PRRs) like the membrane-expressed toll-like receptors (TLRs). A Belgian collaborative study described an association between the *TLR4 Asp299Gly* polymorphism and IBD in two independent cohorts of patients [42]. This polymorphism is associated with impaired LPS signaling and increased susceptibility to Gram-negative infections. The allele frequency of the *TLR4 Asp299Gly* polymorphism was significantly higher in CD [11% vs 5%; OR = 2.31; 95% confidence interval (CI) = 1.28–4.17; $p = 0.004$] and UC patients (10% vs 5%; OR = 2.05; 95% CI = 1.07–3.93; $p = 0.027$) compared with the control population. A transmission disequilibrium test on 318 IBD trios demonstrated preferential transmission of the *TLR4 Asp299Gly* polymorphism from heterozygous parents to affected children (T/U 68/34; $p = 0.01$). These associations have been replicated in a number of studies [43–46]. Toll-like receptor 5 (TLR5) has also been studied in detail partly because in animal models of colitis, flagellin acts as a dominant antigen, capable of activating the innate immune system and this via the TLR5. Flagellin-specific CD4(+) T cells, when transferred into naïve SCID mice, developed severe colitis, as shown by the study of Lodes *et al.* [47]. Furthermore, from a clinical perspective, antibodies directed against cBir1 flagellin are found in increased amounts in CD patients [48]. A genetic association has been described between Jewish CD patients and a TLR5-stop variant [49].

IL23R

The seven GWAS most recently published studied between 20,000 and 500,000 SNPs. These huge efforts were technically not feasible before completion of the Human Genome Project and HapMap projects or before the development of much cheaper genotyping capabilities. The results of these scans have identified additional genes, which were previously not picked up through linkage analysis. One of the first GWA studies performed by the North American NIDDK Consortium focused on ileal CD only and found a highly significant association with the interleukin 23 receptor gene (*IL23R*) on chromosome 1p31 [13]. An uncommon coding variant (rs11209026, Arg381Gln) confers strong protection against CD with an odds ratio of approximately 0.35. Replication studies confirmed *IL23R* associations in independent cohorts of patients with CD and UC [50–53]. Further studies looking at *IL23R* have demonstrated a much larger disease effect in CD than that

seen with the Arg381Gln SNP alone [52]. Furthermore investigation of the IL23/IL17 pathway demonstrated a number of other genes associated with CD, including *IL12RB1*, *IL12RB2*, *IL17A*, *IL17RA* and *IL17RD* [52]. This study also suggested that the association with *IL23R* and CD is conditional on the presence of other genetic variations within this pathway, although these findings will need to be confirmed.

The role of autophagy: *ATG16L1* and *IRGM*

Among the most intriguing novel genetic variants identified are the autophagy-related 16-like 1 gene (*ATG16L1*) and the *IRGM* gene [15–17]. Both genes are involved in autophagy. This is a fundamental biological process, also called "self-eating", which was originally described as an adaptation of the cell to starvation. During the process of autophagy, cytoplasmic components become sequestered by the membrane to form an autophagosome, which is then delivered to the lysosomes to form an autolysosome. Autophagy is also involved in the elimination of intracellular bacteria and may therefore play a protective role in infectious diseases. Gutierrez *et al.* showed that autophagy inhibits the survival of *Mycobacterium tuberculosis* in infected macrophages [54]. Furthermore, knockdown of *ATG16L1* in HeLa cells following *Salmonella typhimurium* infection is associated with fewer intracellular bacteria targeted to autophagic vacuoles. From the GWA studies, it seems that the *ATG16L1 Ala197Thr*, located in exon 8, carries all the risk. The minor allele is exerting a protective effect and the effect size of the risk variant is modest (OR 1.45 for heterozygotes and 1.77 for homozygotes). The protein is widely expressed in ileum, colon, intestinal epithelial cells and T cells and splice variants have been described.

IRGM or immunity-related guanosine triphosphatase family M on *5q33* is a GTP-binding protein, expressed in small bowel, colon and leucocytes. A 313 T → A silent variant is associated with CD and this gene is also known to induce autophagy [55]. The *IRGM* mouse homolog LRG-47 controls intracellular pathogens by autophagy and *IRGM* −/− mice have increased susceptibility to *Toxoplasma gondii* and *Listeria monocytogenes* [56]. One of the benefits of the hypothesis free approach in WGAS is the identification of new pathways that are associated with disease pathogenesis. This is the case with autophagy as its involvement in CD pathogenesis was unknown prior to the identification of these two genes.

TNFSF15

The first published WGA study in CD was performed by a Japanese group who identified variation in a *TNFSF15* with Japanese CD [12]. This association was confirmed in a British cohort with susceptibility to both CD and UC. The association with *TNFSF15* and CD has been widely replicated in a number of different ethnic groups and remains the only consistent genetic association in Asian CD [57–61]. *TNFSF15* encodes the protein TL1A which is upregulated in biopsy specimens from patients with both CD and UC and has a number of diverse functions including induction of NFκB. More recently, association between *TNFSF15* haplotypes and expression of *TL1A* has been established. These expression profiles can be further delineated by serological profile and ethnicity [62]. *TNFSF15* remains an excellent target for therapeutic intervention in IBD.

PTGER4

A GWA study from Belgium identified a novel region on *5p13.1* that was associated with CD. This region is a 1.25 Mb gene desert [16]. In this study, it was suggested that the underlying disease-associated alleles correlate with quantitative expression levels of the prostaglandin receptor EP4, *PTGER4*, located 270 kb proximal of the gene desert. *PTGER4* plays a role in the regulation of the epithelial barrier and thus fits very well in the model of IBD. It is hypothesized that regulatory elements in this gene desert control the expression of the gene. The gene is further implicated in IBD pathogenesis as the *PTGER4* knockout mouse develops severe colitis when exposed to dextran sulfate sodium in drinking water [63].

To date, more than 30 genetic loci have been identified for CD [64], although these regions only explain approximately 20% of the genetic variance in Caucasian CD. One thing that has become clear as a result of these gigantic steps in the understanding of genetic underpinning of CD is just how heterogeneous IBD is. This is true both from a clinical/phenotype perspective and from a genetic angle. Genetic alteration within a wide variety of processes including autophagy, the innate immune pathway and the IL23/IL17 pathway can all lead to an increased risk of IBD (Figure 4.4). This genetic heterogeneity may, in part, explain the broad clinical presentations and varying natural history of IBD.

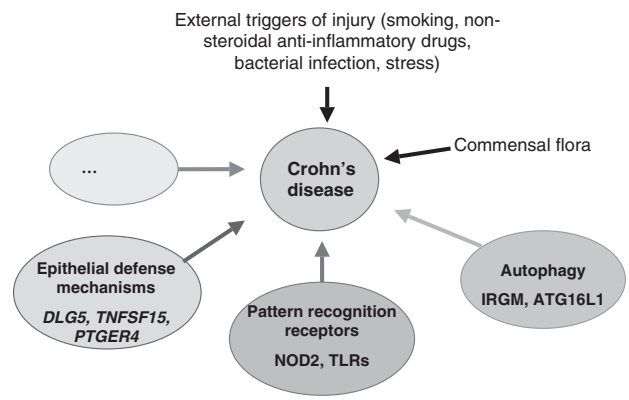


Figure 4.4 The study of IBD genetics has identified biological pathways.

Recent advances in UC genetics

From both a therapeutic and a genetic perspective, advances in UC have lagged behind those in CD. Genetically, this is due in part to a lower genetic influence in UC rather than CD. However, more recently significant advances have been made in UC genetics. Using the WGA approach, a number of loci, in addition to the HLA (see above), have been identified that increase the risk of developing UC, including ECM1 (extracellular matrix protein 1) [65], IL10, ARCP2 [66] and a number of signals on chromosome 1p36 including variation near *OTUD3/PLA2G2E (1p36)* and a couple of signals near *IFNg/IL26/IL22* on chromosome *12q15*. More recently, genetic variation at IL2/IL21 has also been associated with UC [67]. Furthermore, evidence is increasingly suggesting that there is shared genetic association between IBD and a number of the other autoimmune conditions including shared loci between UC susceptibility and susceptibility to celiac disease [67–69].

Translation of IBD genetic research into the clinic

Despite the significant advances in our understanding of genetic variation and its effect on susceptibility to IBD, there has been little translation of the use of this information through into clinical practice. A genetic profile of an individual is unlikely to allow the development of a diagnostic test given the low prevalence of the disease within the general population. Despite this, a number of companies now sell a "test" to indicate whether a diagnosis of CD is "higher or lower than average" based on genotypes extracted from a saliva sample. This sort of testing has not been validated in any form of trial and cannot be recommended.

An increased prevalence of *CARD15* variants is found in most Caucasian patients with CD. Although prevalence varies from study to study, around 35–45% of CD patients will carry at least one *CARD15* variant compared with 15–20% in healthy controls [70–76]. A much lower prevalence of *CARD15* variants is observed in Scandinavian [77], Irish [78] and Scottish [79] CD patients and in the Japanese [80], Chinese [81] and African-American population these variants are absent [82]. The relative risk of developing CD in the presence of one mutation is 2–4, but increases to 20–40 in the case of two mutations (compound heterozygous or homozygous).

The phenotypic expression of *CARD15* variants is widely replicated and has shown consistent associations with small bowel disease and less importantly with a stricturing behavior (53% vs 28%; OR = 2.92; $p = 0.00003$).

Several authors have also shown that patients carrying *NOD2/CARD15* variants need surgery earlier in the disease course and also are at higher risk for surgical recurrence [83,84] . However, it appears that *CARD15* status alone will not be robust enough to influence clinical practice.

CARD15 seems also implicated in graft-versus-host disease (GvHD) and complications following allogeneic stem cell transplantation [85]. In patients receiving stem cell transplantation, the transplant-related mortality in donor–recipient pairs with mutations was much higher (49%) than the mortality in donor–recipient pairs without mutated *CARD15* (20%). The mortality was even higher (83%) in pairs with mutated alleles in both donors and recipients ($p < 0.001$).

For the other reported genes *DLG5*, *OTCN*, *NOD1*, *TLR4*, *IL23R*, *ATG16L1*, *IRGM* and *PTGER4*, phenotypic associations have been less consistent. For *IBD5* and *OCTN1* and *-2*, associations with perianal disease [28,86,87] and with ileal disease [88] have been reported. The association with perianal fistulizing disease is probably the most replicated. The reason for the discrepancy in phenotypic associations for these genes is not clear, but the modest relative risk associated with each of them probably needs large sample sizes. Furthermore, definitions of phenotype need to be consistent across studies and patients probably need time to "declare" their phenotype before inclusion in such a study.

Combinations of genetic variants may provide sufficient utility for translation into the clinical setting. If the known genetic susceptibility variants together with novel variants that affect natural history (discovered in large well-characterized cohorts) can build on the *CARD15* association with the need for earlier intervention/surgery in CD, then this may allow risk stratification of CD patients. Furthermore, a similar approach incorporating clinical factors, serology and genetics to predict responses to therapies will be extremely useful and the pharmaceutical industry needs to be persuaded of the need for this type of translational surgery in their trials of novel therapies in IBD.

Genetic research may also direct investigators to novel therapeutic targets in IBD and an understanding of which pathway is important in which individual's disease may allow a much more targeted or individualized approach to therapy. For example, the understanding that a number of genetic variants within the IL23/IL17 pathway increase susceptibility to CD may allow individuals who carry that specific genetic profile to be targeted for therapies that interfere with that pathway. In the future, at diagnosis, patients may have a genetic profile to go with their clinical assessment, which may allow a better prediction of their natural history and response to therapy.

Conclusion

The recent advances in the genetics of IBD have been tremendous. Whole genome linkage and association scans

have already led to the identification of a number of susceptibility genes (*CARD15*, *DLG5*, *OCTN1* and *-2*, *NOD1*, *IL23R*, *PTGER4*, *ATG16L1* and *IRGM*), of which the *CARD15* gene is undoubtedly most understood at present. However, even for the *CARD15* gene, a number of questions remain, especially concerning the mechanisms of signaling. Answers to these questions will further improve our knowledge on the pathogenesis of the disease in the coming years. Genetic research in IBD has advanced our understanding of the different pathways involved in the disease and have underlined the heterogeneity of the disease. It is anticipated that in the future, these discoveries will be translated back into clinical practice, where genetic markers will find their place in an integrated molecular diagnostic and prognostic approach to our patients.

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Chapter 5
In Vivo Models of Inflammatory Bowel Disease

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Summary
- Experimental model data strongly support the immunologic hypothesis of IBD pathogenesis.
- Critical genes, molecules and pathways maintaining intestinal homeostasis with the microbiota are being defined.
- Epithelial cell mutations can result in pathogenic adaptive immune responses to the microbiota and IBD.
- Innate, adaptive and regulatory immune defects can result in IBD.
- Multiple different mutations and mechanisms can result in experimental colitis with a similar histopathology.

Introduction

The inflammatory bowel diseases (IBDs) appear to involve complex interactions among immunologic, environmental and genetic components. Experimental models allow such interactions to be dissected because each of these components can be controlled or defined. Although no animal model exactly reproduces human IBD, the experimental models allow us to approach the complex, multifaceted processes and mechanisms that can result in chronic intestinal inflammation. There are now many dozens of experimental models of IBD available and these have generated a burgeoning literature that is daunting. This chapter will organize the various models into categories, which admittedly are somewhat arbitrary, and summarize some of the new understanding about the pathogenesis of IBD that has been generated to date.

Most of the new models of IBD involve some form of genetic manipulation, either insertion (transgenic) or selective deletion (knockout) of a gene. Mice resulting from such genetic manipulation are now collectively referred to as "induced mutants" to distinguish them from mice with spontaneously occurring mutations. The induced mutants that develop IBD, usually in the absence of any further manipulation, represent a small subset of the total number of genes that have been mutated. This argues that the mutations that have resulted in disease must represent genes involved in pathways critical to the maintenance of mucosal homeostasis. The results that have been obtained from these models to date provide strong support for the *immunologic hypothesis* that a dysregulated mucosal immune response, particularly a $CD4^+$ T cell response, to antigens of the enteric bacteria in a genetically susceptible host, can result in chronic intestinal inflammation. Given the large number and variety of microbes resident in the intestine, which outnumber the number of cells in the body by 10 to 1, the mystery has been why all of us do not have IBD. The answer to that mystery is now emerging through the study of these new models. These studies have shown that the host interaction with the flora is complex, but that there are a select number of cells and molecules that are critical to this effort. When these key pathways are impaired, the host response to the bacterial flora results in IBD.

In multiple models, animals rendered germ-free do not get IBD unless they are reconstituted with an enteric bacterial flora. This should not be surprising because even in normal hosts, acquiring a microbiota has profound effects on the host, including development or maturation of the epithelium, the enteric nervous system, the intestinal vasculature and the mucosal immune system. With regard to the immune system, it is clear that the host devotes enormous resources in responding to the microbiota, including the majority of T cells and B cells in the body. The innate and adaptive immune response to the microbiota begins at birth and continues throughout the lifetime of the host. Many "background" genes can influence the host-microbiota relationship, as evidenced by the varying susceptibility of certain inbred mouse strains to IBD, even when they are carrying the same induced mutation such as IL-10 deficiency [1]. The interaction between the host and its microbiota is complex but is the nexus of the puzzle of IBD.

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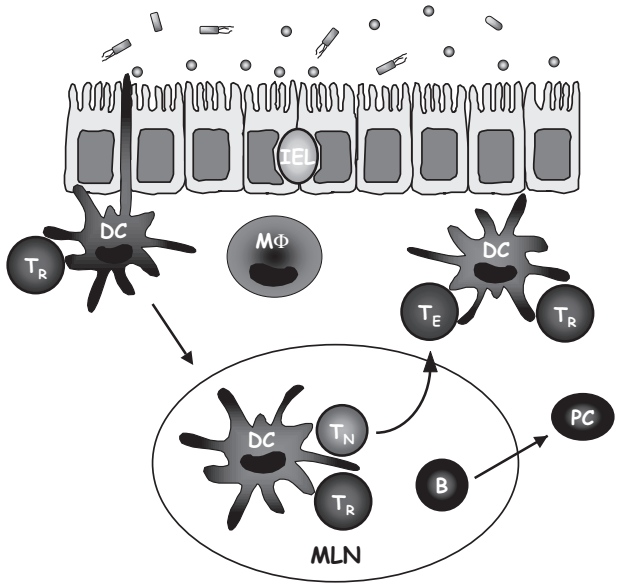

Figure 5.1 Epithelial cells are in close contact with the commensal microbiota and form a barrier to them. Nevertheless, both microbes and their products do translocate across the epithelium, where they encounter macrophages that will destroy them and dendritic cells that will phagocytose them. Bacteria loaded dendritic cells migrate to the mesenteric lymph node (MLN) and activate IgA-producing B cells. Such IgA decreases the translocation of commensal bacteria [198]. Dendritic cells loaded with commensal antigens can also activate either regulatory cells or potential effector T cells. In the normal host, regulatory T cells are dominant over effector T cells. Defects in these innate, adaptive, effector or regulatory mechanisms can result in IBD. T_E, T effector cells; T_R, T-regulatory cells; T_N, naïve T cells. Reproduced with permission from Elson CO, Cong Y, McCracken VJ *et al.* Experimental models of inflammatory bowel disease reveal innate, adaptive and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005; **206**:260–76.

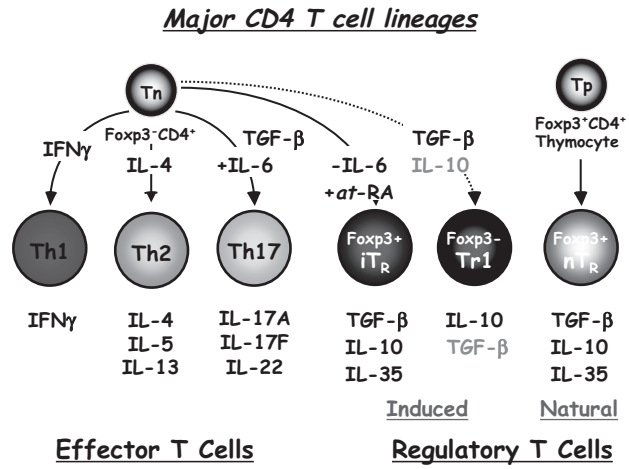

Figure 5.2 Diversity of CD4 T cells. Recent progress in defining additional developmental programs of CD4 T cells with distinct functional roles in adaptive immunity has expanded the original Th1–Th2 paradigm to at least six major arms: three effector lineages and three regulatory lineages. The most recent addition to the effector T cell family is Th17, so-named for the two IL-17 family cytokines (IL-17A and IL-17F) that are characteristic of mature Th17 cells. This effector cell subset has been shown to play a central role in host protection against pathogens at mucosal interfaces, especially the intestines. When uncontrolled, Th17 cells appear to be the dominant autoinflammatory T cell responsible for at least some types of inflammatory bowel disease. Induction of Th17 cells, like that of Th1 and Th2 cells, is strongly influenced by cytokines derived from innate immune cells. The cytokines TGFβ and IL-6 cooperate to induce Th17 development from antigen-activated naïve CD4 precursors. (The cytokines/factors associated with arrows indicate dominant cytokines involved in specification of the indicated lineages; cytokines listed below each cell type indicate key effector or regulatory cytokines produced by differentiated cells of that lineage.) One class of regulatory T cells, which develops in the thymus during selection for self reactivity and is referred to as "natural" regulatory T cells or nT_R, is characterized by expression of the transcription factor Foxp3. The other two classes of regulatory T cells develop from naïve CD4 T cells in the extrathymic environment, so-called "induced" regulatory T cells, and are subdivided into $Foxp3^+$ "induced" T_R or iT_R and $Foxp3^-$ Tr1 cells. In contrast to Th17 cells, TGFβ acts with all-*trans*-retinoic acid (*at*-RA) produced from vitamin A by dendritic cells – in the absence of IL-6 – to drive iT_R development. Thus, there is a tight developmental coupling of Th17 and iT_R, contingent upon the relative balance of IL-6 and *at*-RA. Tn, naïve, post-thymic CD4 T cell precursors; Tp, thymic precursors. Dotted lines represent less well-defined lineage relationships. Adapted from Weaver CT, Harrington LE, Mangan PR *et al.* Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006; **24**:677–88.

The first responder to the microbiota is the innate immune system, which includes intestinal epithelial cells (Figure 5.1). Mutations in multiple genes encoding molecules of innate immunity have been implicated in both mouse (see below) and human IBD [2,3]. Mice that lack adaptive immunity are able to live with their microbiota, although they are highly susceptible to pathogens. Thus, the innate immune system has evolved effective mechanisms of interaction with the microbiota. Defects in these mechanisms result in activation of adaptive immunity towards the microbiota and thus in IBD. Experimental models with innate immune defects will be the first category to be considered.

The critical component of the pathogenic adaptive immune response to the microbiota is the $CD4^+$ T cell. CD4 T cells can be placed into several functional subsets based on the types of cytokines that they produce (Figure 5.2). Th1 cells produce IFN-γ, IL-2 and TNFβ, cytokines that are important in delayed hypersensitivity and resistance to intracellular pathogens. Th2 cells produce instead IL-4, IL-5, IL-6, IL-10 and IL-13, cytokines important in humoral immunity and host resistance to parasites. Both subsets have been found to mediate colitis in various mouse models. A third effector CD4 T cell lineage has recently been identified, Th17 cells, which produce IL-17, IL-21 and TNFα, and are important for colitis progression in multiple models. At present the data are compatible with the concept that

excessive responses of either the Th1, Th2 or Th17 effector subsets are detrimental and can result in IBD. The tissue damage resulting from the $CD4^+$ T cell in each case is likely to be indirectly mediated through cytokines rather than by direct cytotoxicity and a critical molecule in the Th1 and the Th17 pathways is likely to be TNFα [4]. Experimental models involving excessive adaptive T cell responses will be the second category to be considered.

Both innate and adaptive effector immune responses in the intestine are inhibited by cells whose major function is to dampen or limit immune reactions (Figure 5.2). Many different lymphoid cells can have regulatory function but the dominant players are regulatory T cells (Tregs). A large fraction of the T cells in the lamina propria are Tregs, which play an important role in intestinal homeostasis. Although the experimental data indicate that $CD4^+$ T cells are the key regulatory cells in the intestine, other cell types also very likely contribute to maintaining mucosal homeostasis. Data supporting such a role for $CD8^+$ T cells [5,6], NK cells [7], NK T cells [8] and B cells/antibody [9] have been generated in certain experimental model systems. In some models, impairment of immune regulation appears to be the major mechanism leading to IBD and therefore will comprise the third category of models to be considered.

Innate immune models – epithelial

The intestinal epithelium is recognized as an active partner in the mucosal immune system. Intestinal epithelial cells (IECs) produce and respond to a wide variety of cytokines and express molecules able to interact with lymphoid cells. Epithelial cells signal the innate immune system by rapid release of chemokines upon bacterial invasion [10,11]. The epithelial layer is exposed to and interacts also with lumenal bacteria. IECs express a number of toll-like receptors (TLRs), proteins that bind to and thus recognize certain classes of microbial products based on molecular patterns. Eleven TLR genes have been identified to date and each recognizes a different assortment of microbial molecules, e.g. TLR2 binds CpG nucleotides, TLR4 binds lipopolysaccharides and TLR5 binds flagellins. IECs alter the expression of some genes based on the composition of the bacterial flora and so are able to detect changes in the flora via TLRs or other pattern recognition receptors [12]. Hence one could more accurately view the dynamic interactions at the mucosal surface as a bacterial–epithelial–lymphoid circuit with each component communicating with the others. It is of some interest that NOD2, the first gene identified as conferring susceptibility to Crohn's disease, appears to be a pattern recognition receptor [13] which is expressed in IEC.

The epithelial hypothesis of the pathogenesis of IBD proposes that abnormal epithelial cell function can result in chronic intestinal inflammation even in a host with a normal immune system. The inflammation is viewed as secondary to the epithelial abnormality. Support for this hypothesis is the observation that some patients with Crohn's disease have increased intestinal permeability for small molecules [14] and that enterocytes from patients with IBD demonstrate abnormal stimulation of allogenic T cells due to deficient expression of a glycoprotein (gp180) on their surface [15]. Further support for this hypothesis comes from experimental animal models in which the major abnormality appears to be epithelial. The best example of this is the mdr1α-deficient mouse, as discussed below. However, this hypothesis is not incompatible with immune-mediated mechanisms of intestinal injury, particularly when one views the epithelial layer as an active player in a dynamic bacterial–epithelial–lymphoid circuit at the mucosal surface (Figure 5.1).

Considering that the epithelium is a "first responder" to the microbiota, it is not surprising that mutations that affect epithelial cells can result in perturbation of the mucosal immune system and thus eventuate in chronic intestinal inflammation (Table 5.1). Providing an impermeable barrier to small molecules is one important function of the epithelium, but only one of them [16]. Although increased permeability is generally assumed to equate to increased mucosal immune reactivity and thus chronic intestinal inflammation, direct data supporting this point are lacking. The chronicity of the altered barrier function is likely an important variable. For example, instillation of acetic acid into the colon of mice, which destroys the mucosal barrier for days, results in inflammation and increased mRNA for IL-1, TNF and IL-6 but no upregulation of T cell cytokine mRNA despite the presence of memory T cells in the lamina propria [17]. The best support for a role of increased permeability leading to IBD comes from the N-cadherin-dominant negative mutant chimera, discussed below, and in this model the inflammation takes months to develop and remains histologically mild [18]. Given the advances in the understanding of the many roles that epithelial cells play in mucosal immune homeostasis, concepts of their potential role in intestinal inflammation need to be expanded beyond alterations in permeability.

Multi-drug resistance gene 1a (*mdr1a*)-deficient mice

The murine multiple drug resistance gene, *mdr1a*, encodes a 170 kDa transmembrane transporter protein that is expressed by intestinal epithelial cells, and also by subsets of lymphoid cells and hematopoietic cells. The *mdr1a* gene is one of a family of transporters known as ATP-binding transporters that are characterized by their ability to transport small amphiphilic and hydrophobic molecules across cell membranes in an ATP-dependent manner. Three *mdr* genes have been identified in rodents, each with a restricted pattern of tissue expression and presumably distinct functions; two *mdr* genes have been identified in

Table 5.1 Innate immune models – epithelial.

Model	Area involved	Effector cell	Altered immune component(s)*	Bacterial flora driven	Strain variation/ genetic modifiers	Ref.
Multi-drug resistance gene 1α deficient	Colon	$CD4^+$ Th1	I	Probable: antibiotics prevent *and* treat	FVB	20
Epithelial NEMO deficient	Colon	Unknown	I	Yes	Unknown	31
Muc2 deficient	Cecum, colon	Unknown	I	Unknown	Unknown	32
Epithelial XBP1 deficient	Small bowel, colon	Unknown	I	Probable (Paneth cell defect)	Unknown	33
NCADΔ chimera	Small bowel, cecum	Unknown	I	Unknown	Unknown	18
Gai2 deficient	Entire colon	$CD4^+$ Th1	I, R	Unknown	129, C3H > (129 × B6)F1 > B6	22,25
Keratin 8 deficient	Cecum, colon	Unknown	I	Unknown	FVB/N strain. Embryonic lethal in 129, B6	29

*I, innate; A, adaptive; R, regulatory.

humans. Although originally defined on the basis of their ability to confer resistance to chemotherapeutic agents in neoplasia, the physiologic function of these transporters is not known. Polymorphisms in the *mdr1* gene are associated with human IBD [19].

Mice with a targeted deletion of the *mdr1a* gene on an FVB background developed colitis [20]. The majority of infiltrating cells are $CD4^+$ $TCR\alpha\beta^+$ cells, plus granulocytes and clusters of B cells. There is increased expression of IFN-γ, TNFα, IL-12, IL-6 and IL-1 in the colon lesions compared with controls. There was also increased expression of the chemokine receptors CCR2 and CCR5 and the chemokines MCP-1, MIP1a and RANTES.

Treatment of $mdr1a^{-/-}$ mice with broad-spectrum antibiotics both prevented disease development and treated active disease [20]. In mice with active disease that showed clinical improvement following 10 weeks of treatment with antibiotics, there was no evidence of persistent granulocytic infiltrates or B cell follicles, although increased numbers of $CD3^+$ T cells remained in the colon lamina propria. Characterization of the intestinal flora showed only commensal organisms, although there were differences in the relative frequency of species between experimental groups.

Interestingly, T cells isolated from the mesenteric lymph node of *mdr1a*-deficient mice with clinical evidence of IBD demonstrated proliferative responses to bacterial antigens, whereas cells from non-colitic $mdr1a^{-/-}$ and control FVB mice did not. The absence of T cell or antibody reactivity to the intestinal flora in non-colitic *mdr1a*-deficient mice suggests that antigenic reactivity to the intestinal flora followed rather than preceded disease development.

Because the *mdr1a* transporter is expressed in both intestinal epithelium and mucosal lymphocytes, irradiation bone marrow chimeras were generated to determine which cell populations might be responsible for disease. Bone marrow chimeras in which irradiated FVB animals were reconstituted with bone marrow from $mdr1a^{-/-}$ or FVB donors and irradiated $mdr1a^{-/-}$ recipients reconstituted with FVB or $mdr1a^{-/-}$ bone marrow were generated. Only $mdr1a^{-/-}$ recipients developed disease, whereas FVB recipients failed to develop disease irrespective of the origin of the bone marrow donor. Increased bacterial translocation has been found in $mdr1a^{-/-}$ mice prior to onset of colitis, which provides additional support for a primary defect in the epithelial barrier [21]. Thus, defects in the *mdr1a* expression by the gut epithelium appear to be the primary abnormality associated with the development of colitis in these mice. These data are compatible with the idea that spontaneous colitis can develop from an epithelial abnormality in animals with a normal immune system.

N-cadherin-dominant negative mutant chimeric mice

This model provides support also for the concept that primary abnormalities of epithelial barrier function can cause chronic intestinal inflammation. A dominant negative N-cadherin mutant (NCADΔ) gene lacking an extracellular domain was transfected into 129/Sv embryonic stem cells, which were then introduced into normal C57BL/6 blastocysts [18]. The chimeric mice that resulted had patches of mutant 129/Sv-derived epithelium dispersed in normal C57BL/6-derived intestinal epithelium. Cells derived from the two sources could be distinguished by differences in lectin binding. The 129/Sv epithelium, but not the C57Bl/6 epithelium, had a defect in epithelial adhesion due to disruption of E-cadherin expression, a molecule

critical to cell–cell and cell–matrix adhesion. Two types of chimeras were generated using different promoters, one that caused expression of the NCADΔ in both the small intestinal crypt and villus cells and another that caused expression only in villus cells. Focal inflammation and adenomas occurred in the chimeras that expressed the epithelial defect in both crypt and villus cells, but neither occurred in mice expressing NCADΔ only in villus cells. By age 3 months, the inflammation in the 129/Sv mucosal patches became transmural and was associated with numerous lymphoid aggregates, lymphangiectasia, cryptitis and some small ulcerations. This model gives support to the idea that primary abnormalities of the epithelial barrier could result in significant secondary inflammation because there should be no direct effect of NCADΔ on the immune system.

Gαi2-deficient mice

G proteins are important signal transducers that couple cell surface receptors to various effector pathways inside the cell. G proteins are composed of alpha, beta and gamma chains. The alpha subunit of Gi2 is part of the heterotrimeric complex that regulates signal transduction through adenylate cyclase. Gi2 is widely distributed in most cell types, including gut epithelial cells and lymphocytes. In lymphocytes, Gi2 is known to regulate certain events in thymogenesis, T cell recirculation, T cell activation and production of certain cytokines. Gαi2-deficient mice are normal at birth, but exhibit slow growth and increased mortality due to a pancolitis that begins at 8–12 weeks of age. The colitis is gradually progressive and more severe in the distal than the proximal colon [22], with rectal prolapse being common. Other organs are not involved. The colon mucosa has foci of intense regenerative proliferation bordering on dysplasia. Indeed, up to one-third of animals develop adenocarcinoma of the colon between 15 and 33 weeks of age [23]. Thymic abnormalities have also been noted in Gαi2 mice, but the role that this plays in their colitis is unknown [24].

Gαi2-deficient mice manifest a variety of immune abnormalities, particularly in lymphocytes isolated from the colon, with immunologic changes consistent with an unrestrained Th1 response in the colon [25]. Activation of the mucosal immune system precedes development of the colitis by several weeks [26] and defects in certain B cell populations have been found [27], but these would not exclude a major role for an abnormal epithelium in the process. Mice housed under specific pathogen-free conditions continue to develop the disease at a similar frequency, including the development of adenocarcinoma. Genetic background has an important influence (Table 5.1): the Gαi2 knockout mutation on the 129/Sv background results in severe disease, but the same mutation on a mixed 129/Sv × C57BL/6 background has a much lower severity and incidence of colitis [25].

Intestinal trefoil factor-deficient mice

Intestinal trefoil factor (ITF) is a member of the trefoil family of proteins, which are expressed by goblet cells in the intestinal mucosa. Members of this family share a distinctive three-loop secondary structure that confers upon them resistance to acid and proteases. ITF has been shown to enhance epithelial restitution *in vitro* in a wounded monolayer system. Its role *in vivo* was explored by the generation of an ITF-deficient mouse [28]. These mice developed normally, but ITF-deficient mice given DSS in their drinking water developed more severe colitis than wild-type controls and half of them died. Histologic sections revealed a marked defect in epithelial repair. ITF-deficient mice given acetic acid enemas also developed colitis with markedly impaired healing, but this was corrected by delivery of exogenous recombinant ITF to the colon mucosa. Although these studies involved acute injury to the colon mucosa , they do provide a proof of principal that impaired healing can contribute to the severity and progression of colitis. These data also provide support for the notion that agents that enhance healing of the inflamed mucosa might be useful in the therapy of IBD.

Keratin 8-deficient mice

Keratin filaments are present in all epithelial cells and are thought to provide strength to the cell. The keratin 8 gene encodes a type II keratin filament which pairs with type I filaments to form extended keratins within single-layered epithelia such as that in the intestine. Disruption of the keratin 8 gene is embryonic lethal in mice of a mixed B6, 129 genetic background. However, a proportion of keratin 8-deficient FVB/N strain mice do not die *in utero* but are born and develop [29]. These mice develop colitis between 2 and 12 months of age while housed under SPF conditions. The colitis is heralded by rectal prolapse and involves the whole colon and the cecum, but not the small intestine. There is epithelial hyperplasia and inflammation of the colon lamina propria and submucosa, but no ulcerations, goblet cell depletion or crypt distortion. Curiously, deletion of keratin 18, the filament that pairs with keratin 8 in enterocytes, does not result in IBD [30].

Epithelial NEMO-deficient mice

NF-κB is an important signaling pathway for inflammatory responses and has also been implicated as important in maintaining normal epithelial integrity. Mice deficient in intestinal epithelial cell IKKγ (NEMO) were generated by crossing mice carrying LoxP-flanked NEMO alleles with villin Cre transgenics [31]. These mice develop colonic inflammation at 1 week of age while they would still be suckling and just beginning to acquire an enteric flora. There was early infiltration with innate cells such as neutrophils and dendritic cells with T cell infiltration being a later event. These mice demonstrate an increased epithelial apoptosis and bacterial translocation with uptake

of the bacteria by dendritic cells and polymorphonuclear leukocytes. The development of colitis required MyD88 and TNFR1 expression. Interestingly, the overall cryptdin expression of the epithelium was only mildly impaired. These studies demonstrate that epithelial NF-κB signaling is required for maintenance of the epithelial barrier.

Muc2-deficient mice

Goblet cells play an important role in innate immunity in the intestine by secreting various mucins and trefoil factors. Among the mucins, Muc2 is produced by goblet cells in the greatest amount. To determine the role that this mucin plays in host protection, Muc2 129SV-deficient mice were generated. The Muc2-deficient mice developed spontaneous colitis starting at 5 weeks of age, which progressively worsened with age [32]. Their colons showed mucosal thickening, increased epithelial proliferation and superficial ulcerations. Goblet cells were small and condensed but were shown to contain trefoil factor 3. Although goblet cells were distributed throughout the intestine and the Muc2-deficient mice were runted, the small intestine did not show any significant changes. Homozygote muc2-deficient mice and heterozygote littermates were both more susceptible to DSS-induced colitis. There was a mild CD3 T cell infiltration in the inflamed mucosa and mild increases of TNFα and IL-1β, but not IL-6. These studies indicate that mucin is an important factor in intestinal homeostasis and its deficiency, even partial, could contribute to the onset or perpetuation of IBD.

Epithelial X-box-binding protein 1 (XBP1)-deficient mice

XBP1 is a transcription factor that is involved in the endoplasmic reticulum stress response. XBP1 directs transcription of a set of genes involved in maintenance of endoplasmic reticulum function. This molecule was hypothesized to be crucial in epithelial cells that have high secretory activity. A conditional deletion of XBP1 in epithelial cells resulted in spontaneous enteritis in the small intestine and an increased sensitivity to DSS-induced colitis [33]. Paneth cells were dramatically reduced, which was shown in ancillary studies to be due to apoptosis. Reduced expression of XBP1 was associated with heightened JNK signaling in epithelial cells in response to inflammatory stimuli such as flagellin or TNFα. These results prompted analysis of human IBD mucosa, which also exhibited signs of ER stress. This, in turn, led to a search for genetic variants of XBP1 that might be linked to IBD susceptibility. A number of SNPs in the XBP1 gene were identified and then replicated in an independent series of patients. Two of the variants that were identified result in diminished XBP1 activity, thus linking the results in human IBD to the XBP1-deficient mouse. These data implicate XBP1 as playing a role in human inflammatory bowel disease and represent another mechanism by which primary epithelial cell abnormalities can lead to intestinal inflammation.

Innate immune models – myeloid and other

A20-deficient mice

A20 is a cytoplasmic deubiquinylation enzyme that inhibits the TLR and TNFα-stimulated activation of NF-κB signaling at multiple points [34]. Mice deficient in the A20 protein were generated by gene targeting [35]. Mice heterozygous for the A20 deficiency ($A20^{+/-}$) develop normally with no evidence of pathology. In contrast, homozygous A20-deficient mice ($A20^{-/-}$) are runted and within a few weeks die of severe intestinal inflammation. The inflammatory lesions have increased numbers of activated T cells, granulocytes and macrophages. Interestingly, $A20^{-/-}$ mice bred into a RAG-1-deficient background demonstrated similar multi-organ inflammation, indicating that the innate immune response is sufficient for disease to develop and adaptive immune cells are not required. Macrophages from $A20^{-/-}$ mice produce large amounts of TNFα when stimulated via TLR4 [36]. Similar multi-organ inflammation has been described in IkBα-deficient mice, indicating that attenuation of TNFα-mediated inflammatory effects by regulators of NFκB are essential for maintenance of gut homeostasis.

STAT $3^{-/-}$-deficient mice

STAT3 (signal transducer and activator of transcription-3) mediates the cell signaling pathway of IL-10, IL-6 and other cytokines. Conditional deletion of STAT3 in a mouse line was accomplished by using CreLox technology using the lysozyme M gene regulatory region to target STAT3 deletion to macrophages and neutrophils [37]. Macrophage–neutrophil STAT3-deficient mice produce high amounts of inflammatory cytokines when stimulated with LPS which was resistant to inhibition by IL-10. These mice develop an enterocolitis that requires IL-12p40 and an adaptive immune system in that it does not occur in IL-12p40/STAT3 and RAG2/$STAT^{-/-}$ double knockout mice. The intestinal inflammation is also ameliorated in TLR4/$STAT3^{-/-}$ double knockouts [38]. These data are consistent with a role for STAT3 in regulating the innate and adaptive immune response to the microbial flora.

A second conditional STAT3-deficient mouse line has been generated in a similar manner but using TIE2 gene promoter to target the deletion to all bone marrow-derived cells. These mice develop severe enterocolitis and wasting and die by 2 months of age [39]. There is extensive granulocyte infiltration in the ileum, cecum and colon. These lesions also occur in RAG/$STAT3^{-/-}$ double knockouts, indicating that the adaptive immune system is not required for the colitis to occur. Consistent with that, innate

immune function is impaired at multiple levels. This STAT3-deficient mouse line is one of the few models of IBD in which the adaptive immune system is not required. The other is A20-deficient mice, discussed above.

NOD2-deficient mice

NOD2 (nucleotide-binding oligomerization domain 2) is an intracellular pattern recognition receptor whose ligand is muramyl dipeptide. Mutations in the gene encoding NOD2 result in a higher risk of getting Crohn's disease, particularly Crohn's disease of the small intestine [40–42]. NOD2 is expressed in monocytes, intraepithelial cells and Paneth cells in the terminal ileum. In order to elucidate the role of NOD2 mutations in IBD, three different groups have generated mice with a deletion or knock-in mutation in the NOD2 gene [43–45]. None of these mouse strains have developed spontaneous intestinal inflammation. However, similarly to what has been found in patients with Crohn's disease, NOD2 knockout mice had a decrease in Paneth cell cryptdins (analogous to human a defensins), similar to what has been noted in patients with Crohn's disease. The mechanism by which NOD2 increases susceptibility from Crohn's disease remains unclear. The results coming from these three different mouse lines are somewhat conflicting, suggesting that there may be multiple mechanisms by which NOD2 contributes to IBD susceptibility.

Anti-CD40 agonist-induced colitis

As noted elsewhere in this chapter, interactions between CD40 and CD40L (CD154) are important in the initiation and maintenance of T cell-mediated intestinal inflammation. CD40 stimulation of myeloid cells is important in IL-12 production, among other things. In order to assess the effects of CD40 stimulation on innate immune cells *in vivo*, an agonist CD40 monoclonal antibody was injected into immunodeficient RAG1-deficient mice [46]. The mice developed diarrhea and anal inflammation and rapidly lost up to 20% of their body weight within 4 days. In addition, there was marked splenomegaly and enlargement of the mesenteric lymph node. At 7 days after the injection the mice had histologic colitis with epithelial hyperplasia and a marked leukocyte infiltration of the lamina propria, goblet cell depletion and epithelial cell damage. All of these changes reversed such that by 3 weeks after the injection all histologic changes had resolved. In this system, local intestinal inflammation was dependent on the presence of IL-23 and was independent of IL-12, whereas the splenomegaly and wasting disease were dependent on IL-12, but not IL-23. Interestingly, the anti-CD40 treatment induced comparable changes in germ-free mice as compared with conventional SPF mice, which is surprising given the large number of ligands that would come across the mucosa in the latter but not the former. This is an interesting innate immune model that may be valuable in dissecting how defects in myeloid cells, particularly dendritic cells that express CD40, lead to susceptibility to IBD.

Dextran sulfate sodium (DSS)-induced colitis

Addition of 30–5 kDa DSS to the drinking water at 3–10% will induce colitis in hamsters, rats and mice [47], which is manifested by bloody diarrhea, weight loss, shortening of the colon, mucosal ulceration and neutrophilic infiltration. In some strains of mice, a chronic colitis can be induced by feeding multiple cycles of DSS [48]. Prolonged low-dose feeding of DSS has resulted in colitis, dysplasia and colon cancer in hamsters [49] and rats [50].

The earliest change of acute DSS-induced colitis is a progressive non-inflammatory dropout of crypts [48], indicating a primary effect on epithelial cells. Indeed, DSS inhibits proliferation of mouse epithelial cells *in vitro* [51]. Early lesions occur mainly in the left colon and over lymphoid aggregates. Colitis occurs in SCID mice fed DSS, indicating that the T cells and B cells are not required for acute colitis to develop [51]. Lumenal bacteria may play a role in the pathogenesis of these lesions in that concomitant metronidazole therapy prevents DSS colitis [49].

The DSS model has some advantages and some limitations. It is a fairly simple method of inducing damage in the colon of most strains of mice. The lesions are reproducible and the clinical and histologic severity can be quantitated. Because of these features, DSS colitis is popular for screening of potential therapeutic agents and a large number of agents have shown benefit in this model, indicating that it is a sensitive screening system. There are also limitations of this model, mainly that it represents a non-specific injury model that does not require either T cells or B cells, hence it is not well suited to address immunologic or therapeutic issues involving the adaptive immune system.

There is a genetic variation in DSS-induced colitis among inbred strains of mice. The location of a number of DSS modifier genes has been identified through quantitative trait locus mapping [52]. Interestingly, the same pattern of susceptibility and resistance among strains as found with DSS is seen in a number of induced mutant mouse models (Tables 5.1–5.6), suggesting either that the same set of genes or different genes affecting similar pathways may be involved.

Trinitrobenzenesulfonic acid (TNBS)–ethanol enema-induced colitis

This model involves both chemical damage and T cell immune reactivity. The administration of an enema containing the contact sensitizing agent TNBS in 50% ethanol induces colitis in rodents. The ethanol breaks the mucosal barrier and is a crucial component; no colitis ensues if TNBS is given alone [53,54]. Because TNBS is a covalently reactive compound, its administration results

Table 5.2 Innate immune models – myeloid and other.

Model	Area involved	Effector cell	Altered immune component(s)*	Bacterial flora driven	Strain variation/ genetic modifiers	Ref.
Dextran sulfate sodium	Colon	Macrophage	I	Probable	C3H>BALB/c>C57B1/6	(169)
STAT3$^{-/-}$	Colon	Macrophage	I	Probable	Unknown	(37–39)
A20$^{-/-}$	Colon	T & B cell independent; macrophage?	I	Unknown	Unknown	(34–36)
TNBS–ethanol	Colon	CD4^{+}, Th1	I, A	Probable	SJL, C3H, BALB > B6 > DBA/2	56,202
Oxazalone	Colon	CD4^{+}, Th2	I, A	Unknown	SJL	61
Acetic acid	Colon	Innate cells	I	No	BALB/c	17

*I, innate; A, adaptive; R, regulatory.

in acute necrosis of the wall of the distal colon due to oxidative damage. The occurrence of such necrosis appears to be an important factor for the development of the colitis, which may explain why the effective dose of TNBS in mice is close to the lethal dose. In a few strains of rats and mice, a single enema can result in a prolonged chronic colitis [55]. However, in most strains of mice, multiple enemas are required to generate chronicity and the duration of colitis following administration is a matter of days.

In mice, TNBS colitis appears to be a classic delayed-type hypersensitivity response mediated by T cells responding to "hapten-modified self antigen". The latter is formed by the covalent attachment of the hapten, trinitrophenyl (TNP), to self peptides. Such reactions in mice are mediated by CD4^{+} T cells and are under complex regulation by T cell and B cells. The colitic mucosa and submucosa are infiltrated with CD4^{+} T cells [55] and increased IgG- and IgA-producing B cells [56], the latter being reminiscent of the changes in plasma cells that occur in the mucosa in human IBD. There is a marked increase in B cells producing IgG anti-TNP in the inflamed colon, including the IgG1, IgG2a and IgG2b subclasses.

Mucosal CD4^{+} T cells produce increased amounts of IFN-γ and IL-17, but not IL-4 [55], consistent with a Th1 and Th17 effector response. Administration of monoclonal anti-IL-12p40 to mice with TNBS–ethanol colitis significantly prevents or treats the colitis [55], indicating that the interaction between antigen presenting cells producing IL-12/IL-23 and T cells contributes to both the induction and progression of colitis. Antibody blockade of CD40L (CD154) prevents induction of TNBS colitis by reducing IL-12 production [57]. Furthermore, other agents that can inhibit IL-12 production such as IL-10 [58] or an anti-sense oligonucleotide to the transcription factor NF-κB p65 [59] have similar beneficial effects.

A significant finding originating from the TNBS model is that regulatory cells able to inhibit colitis can be manipulated. Contact allergens are classic oral tolerogens and thus TNBS itself [56] or TNP-conjugated tissue homogenates [60] have been fed to mice to induce oral tolerance prior to the induction of colitis. The resulting colonic inflammation was less severe, the mucosal IgG anti-TNP cells were markedly reduced and mucosal CD4^{+} T cells produced less IFN-γ and more TGFβ1, IL-10 and IL-4. Mucosal production of IL-12 was also reduced. Administration of antibodies to TGFβ reversed the protective effects of TNP-colon homogenate feeding in one study [60], suggesting that the regulatory cells induced by feeding were producing TGFβ.

Oxazolone–ethanol-induced colitis

Similarly to the induction of colitis initiated by TNBS administration, administration of the contact sensitizing agent oxazolone in 50% ethanol as an enema also induces distal colitis in mice. In contrast to the colitis initiated by TNBS administration, however, colitis induced by high doses of oxazolone given in an ethanol enema to SJL mice has distinct clinical, histopathologic and immunologic features [61]. Colonic instillation of 6 mg of oxazolone in 50% ethanol as an enema results in rapid onset of distal colitis, diarrhea and weight loss in SJL mice which peak on day 2. Approximately 50% of the animals given this dose died by day 4. Surviving animals showed progressive clinical improvement with apparent complete resolution by 10–12 days.

Examination of the cytokine profiles of T cells isolated from the lamina propria of distal colon at day 2 demonstrated elevations of both IL-4 and IL-5 in CD3/CD28-stimulated T cell cultures, with no change in IFN-γ production. Mice that received a single neutralizing dose of IL-4 at the time of oxazolone–ethanol administration displayed an abbreviated and attenuated transient weight loss and only minimal inflammation. In contrast, treatment of mice with neutralizing antibodies to TGFβ or IL-12 resulted in more severe disease and weight loss.

Although these studies implicate IL-4 as playing a role, the time course of these responses is too rapid for Th2 cells to develop. Subsequent studies identified NK-T cells

Table 5.3 Adaptive immune models with excessive effector cell function.

Model	Area involved†	Effector cell	Altered immune component(s)†	Bacterial flora driven	Strain variation/ genetic modifiers	Ref.
STAT-4 transgenic	Colon, ileum	$CD4^+$ T cell	A	Probable	FVN/NHSD	67
IL-7 transgenic	Colon	$CD4^+$ T cell	A	Unknown	B6	71
$TNF\alpha^{\Delta ARE}$*	Terminal ileum > proximal colon	T cell	A	Unknown	B6 × 129	73
CD40L transgenic*	Colon, SB, other organs	T cell	A	Unknown	B6	77

*Multi-organ inflammation not limited to intestine.
SB, small bowel.
†I, innate; A, adaptive; R, regulatory.

rather than $CD4^+$ T cells as the effector cell mediating oxazalone colitis [62]. Moreover, these NK-T cells exert their negative effects by producing IL-13, which is known to have negative effects on the intestinal epithelium. IL-13 is involved in induction of fibrosis in this and other models of inflammation by inducing the IL-13 receptor $a_{(2)}$ in macrophages, then signaling via this receptor to activate the TGFβ promoter and subsequently, fibrosis [63]. Lamina propria cells from patients with ulcerative colitis produce high amounts of IL-13, a cytokine that impairs epithelial barrier function [64], consistent with the results in the oxazalone colitis model. These data support a trial of anti-IL-13 in patients with ulcerative colitis, which will be the ultimate test of its pathogenic role in human disease.

Adaptive immune models – effector cell function

If normal mucosal homeostasis is maintained by a balance between regulatory and effector adaptive lymphocytes, then disease could result either from excessive effector cell function that overcomes a normal regulatory tone or from impaired regulation that allow unrestrained effector cell activity. The net effect of either is the same. In this section, we will consider some models where the former appears to be occurring (Table 5.3).

CD4 effector T cells can be divided into several phenotypic and functional subsets based on the pattern of cytokines that they produce after antigen-driven clonal expression [65]. These subsets include the well-known Th1 and Th2 CD4 cell subsets, plus a recently discovered third subset, Th17. CD4 Th1 cells develop under the influence of IL-12p70, express the transcription factor T-bet and produce IFN-γ; Th1 cells are important in host defense against intracellular pathogens and can be involved in various immune pathologies, including IBD. Th2 cells develop under the influence of IL-4, express the transcription factor GATA3 and produce IL-4, IL-5, IL-13 and IL-25; Th2 cells are important in host defense against parasites and are involved in allergy and asthma. Th17 cells develop under the influence of TGFβ and IL-6 in mice, express the transcription factor RORγt and produce IL-17A, IL-17F, IL-6, G-CSF and TNFα. Th17 cells protect the host against certain extracellular bacteria and fungi and are major contributors to immune pathology in multiple inflammatory diseases, including IBD [65]. IL-23 is required for survival of Th17 cells [66] and, interestingly, variants of the IL-23R gene confers susceptibility to human IBD [2], indicating that the Th17 subset may play a particularly important role in these diseases.

STAT-4 transgenic mice

The clearest example of experimental models that involve excessive effector cell function leading to colitis is the STAT-4 transgenic mouse. STAT-4 is a transcription factor that is phosphorylated (along with STAT-3), following IL-12 binding to its receptor on the surface of $CD4^+$ T cells. The phosphorylated STAT-3–STAT-4 complex translocates into the nucleus of the cell and activates the expression of Th1 pathway genes such as IFN-γ. Mice transgenic for STAT-4 under control of the CMV promoter were generated in the FVB/NHSD strain [67]. Interestingly STAT-4 mRNA was not increased in cells from unperturbed transgenic mice. However, when STAT4 Tg mice were challenged by immunization with dinitrophenylated keyhole limpet hemocyanin (DNP-KLH) in CFA, transgene expression was increased in both spleen and colon and 7–14 days later these mice developed an unremitting colitis manifested by diarrhea, weight loss and severe transmural inflammation of ileum and colon and dense infiltrates of $CD4^+$ T cells expressing nuclear STAT-4 and producing IFN-γ and TNFα. Lymphocytes isolated from colitic STAT-4 transgenic mice proliferated and produced large amounts of IFN-γ when stimulated with lysates of intestinal bacteria. Transfer of bacterial antigen-activated transgenic $CD4^+$ T cells from spleen or lymph nodes of colitic STAT-4 transgenic mice into SCID mice transferred colitis to the recipients. Transfer of similarly treated wild-type, non-transgenic $CD4^+$ T cells did not.

STAT-4 transgenic mice would be expected to have a normal regulatory cell activity, hence this model is the

clearest example of excessive effector cell activity overwhelming endogenous regulatory mechanisms to cause disease. An interesting feature in this model was the requirement of antigen-specific activation for colitis to occur. Colitis did not occur in STAT-4 transgenic mice given complete Freund's adjuvant i.p. without the DNP-KLH incorporated into it. Thus, these genetically susceptible mice did not develop excessive $CD4^+$ T cell reactivity to the bacterial flora and subsequent colitis unless they sustained a *triggering event* that activated the pathogenic process. The exact relationship of DNP-KLH to the flora is unclear and other types of antigens were not tested. However, complete Freund's adjuvant contains mycobacterial antigens and these mycobacterial antigens were not sufficient to trigger disease on their own, suggesting that some specificity was required for triggering of disease. Little is known about how colitis is triggered in genetically susceptible hosts and this seems to be an excellent model to explore such questions further.

IL-7 transgenic mice

Interleukin-7 (IL-7) is a pleiotrophic cytokine with growth-promoting activity for both immature and mature lymphocytes. IL-7 is produced by stromal cells in the bone marrow and thymus, in addition to stroma in other organs. IL-7 mRNA and protein expression have been demonstrated in both mouse and human intestinal epithelium and the IL-7 receptor (IL-7R) is expressed by intestinal lymphocytes in both the IEL and lamina propria (LP) compartments [68,69]. Indeed, γδ IEL T cells are deficient in IL-7- and IL-7R-deficient mice [70] and IL-7 has been implicated as a growth factor for local, extrathymic T cell development in the intestine.

To examine the role of IL-7 in mucosal immune homeostasis, transgenic mice were generated that expressed IL-7 under control of the SV40/HTLV1 LTR viral promoter [71]. In at least one of the SRa/IL-7 transgenic founder lines, clinical evidence of colitis (diarrhea, weight loss, rectal prolapse and perianal bleeding) was observed at 6–10 weeks of age, although significant variability in the penetrance and age of onset of colitis was found. Intestinal inflammation was most severe in the rectum, although involvement of the ileum and colon was also found.

The composition of the infiltrating lymphocyte population was dominated by $CD4^+$ αβ T cells. *Ex vivo* stimulation of $CD4^+$ T cells isolated from colitic lesions demonstrated increased production of IFN-γ and IL-2 but decreased production of IL-4 compared with controls [71]. Curiously, increased IL-7 production in the inflammatory lesions of transgenic mice was due to infiltrating lymphocytes, not epithelial cells, and IL-7R expression was increased only on colonic lymphocytes. IL-17 may be essential for survival and/or expansion of pathogenic T cells in general in that transfer of pathogenic $CD4^+$ T cells into IL-7-deficient $RAG^{-/-}$ mice does not result in colitis [72].

TNFα "knock-in" ($TNF^{\Delta ARE}$) mice

Tumor necrosis factor alpha (TNFα) is a key cytokine in the pathogenesis of chronic intestinal inflammation in both experimental models and in patients with Crohn's disease and ulcerative colitis. A mouse model of excessive production of TNF develops chronic, unremitting inflammation in the intestines and joints [73]. In this model, deletion of a repeated octanucleotide AU-rich motif in the 3′-untranslated region of the TNFα gene (ARE) results in enhanced mRNA stability and increased TNF production by macrophages and other hematopoietically derived cells. Mutant mice homozygous ($TNF^{\Delta ARE/\Delta ARE}$) or heterozygous ($TNF^{\Delta ARE/+}$) for the ARE deletion have elevated circulating levels of TNFα. Homozygous animals fail to thrive and die between 5 and 12 weeks of age.

Both homozygous and heterozygous TNF ΔARE mice develop intestinal inflammation, although the tempo of disease development and progression is accelerated in homozygous mice. Disease is localized primarily to the terminal ileum and, less frequently, the proximal colon. Initial lesions consist of villus blunting and broadening that are associated with mucosal and submucosal infiltration of chronic and acute inflammatory cells, including mononuclear leukocytes, plasma cells and scattered neutrophils. Severe intestinal inflammation is usually observed by 4 weeks of age in homozygous mice and 8 weeks of age in heterozygous mice, with progression to transmural inflammation as heterozygous mice age. Focal skin lesions and severe symmetrical joint disease develop concomitantly with the intestinal lesions.

This model supports a central role for TNF in the pathogenesis of chronic intestinal inflammation. Interestingly, there is clearly a gene dosage effect of the mutated TNF allele because homozygous mice develop the clinical phenotype much more rapidly than heterozygotes. Dissection of the cellular source of TNFα using Cre/loxP-mediated recombination indicates that either myeloid cell or T cell-derived TNFα can mediate disease [74]. Moreover, mice expressing TNF receptor I only on mesenchymal cells develop both arthritis and enteritis, indicating that TNFα stimulation of non-immune mesenchymal cells in the joint and the gut is sufficient for disease development [75], possibly explaining the frequent association between gut and joint inflammation in humans.

CD40 ligand transgenic mice

CD40 ligand (CD40L) is a member of the TNF family that is expressed primarily by activated T lymphocytes. Engagement of CD40L on activated T cells by its receptor, CD40, provides an important signal for naïve T cell activation and differentiation. Conversely, binding of CD40L

expressed on the T cell surface by CD40 expressed on B cells during cognate B–T interactions provides a critical costimulatory signal for B cell proliferation, differentiation and Ig class switching [76]. CD40 is also expressed on other cell lineages, including monocytes and dendritic cells, where it plays an important role in proinflammatory signaling. Engagement of CD40 on monocytes and dendritic cells during interactions with activated T cells induces expression of proinflammatory cytokines including TNFα, IL-1, IL-8 and IL-12, and also various cell surface molecules. CD40L–CD40 interactions are required for sustained release of IL-12, which is needed to initiate and sustain Th1 responses.

CD40- and CD40L-deficient mice generated by gene targeting demonstrate profound defects in both cellular and humoral immunity [76]. In contrast, transgenic mice that overexpress CD40L under control of the LCK proximal promoter, which directs transgene expression to T cells and some B cells, demonstrate multi-organ inflammation and morbidity associated with inflammatory bowel disease [77]. Ectopic expression of CD40L on B cells as a transgene results in ileitis and colitis with massive infiltration of IgM^+ B cells in the lesions [78]. Transfer of $CD4^+$ T cells from colitic mice induced colitis, indicating that $CD40L^+$ B cells were causing the activation of pathogenic $CD4^+$ T cells *in vivo*. These studies reflect the potency and importance of the CD40L–CD40 molecular interaction in immune homeostasis.

Adaptive immune models – aberrant T cell development or activation

There are a number of models where the major abnormality appears to be defective T cell development or TCR activation (Table 5.4).

T cell receptor (TCR) α-chain-deficient mice

Mice with targeted mutations of each of the four T cell receptor chains have now been produced (α, β, γ and δ). Of these, spontaneous development of colitis has been observed in both TCRα- and β-deficient mice, although only TCRα-deficient mice have consistently developed spontaneous colitis [79,80]. TCRα-deficient mice develop normally for the first 3–4 months of life, but then develop unremitting chronic diarrhea, rectal prolapse and wasting [80]. Histologically, the colitis is characterized by marked epithelial hyperplasia and elongation of crypts with acute and chronic inflammation in the colonic lamina propria, mucin depletion and occasional crypt abscesses. Mucosal ulceration is unusual. No inflammation is found in the small intestine or in extraintestinal tissues.

TCRα-deficient mice demonstrate a number of immunologic abnormalities such as a poor immune response to protein antigens and deficient rejection of skin grafts [81]. There is polyclonal expansion and activation of B cells with the production of multiple autoantibodies [80], including anti-colon and anti-tropomyosin, which have been reported previously in patients with ulcerative colitis [82]. Mice also develop antibodies to multiple enteric bacterial antigens. The immunologic defect in TCRα-deficient mice results from aberrant thymic selection and deficiency of circulating αβ T cells. In developing thymocytes, gene rearrangement of the T cell receptor β-chain locus precedes that of the TCR α locus, resulting in "β-selection" by association of TCRβ chains with the pre-TCRα (pTα) molecule. In TCRα-deficient mice, functional TCRβ rearrangement and pairing with the pTα chain occurs; however, the absence of subsequent α chain rearrangement results in defective TCR repertoire selection in the thymus. Nevertheless, these mice populate peripheral immune tissues with a unique population of T cells that express the TCRβ chain without TCRα chain [81,83,84]. This novel $TCR\beta^+$ T cell

Table 5.4 Models with aberrant T cell development or activation.

Model	Area involved	Effector cell	Altered immune component(s)*	Bacterial flora driven	Strain variation/ genetic modifiers	Ref.
TCRα deficient	Cecum, colon	$CD4^+$, Th1	R	Yes	C3H, 129 > (129 × B6) F1 > B6	79
HLA-B27 transgenic rat	Entire intestine, other organs	T cell $CD4^+$ > $CD8^+$	A	Yes	Undefined	94–96
Wiskott–Aldrich syndrome protein deficient	Colon	?T cell	A, R	Unknown	Undefined	99
Fucosyltransferase transgenic	Cecum, colon	Unknown	A	Unknown	BALB/c	104
Pigeon cytochrome T cell receptor transgenic	Cecum, colon	$CD4^+$	A	Probable	B10, B6	102

*I, innate; A, adaptive; R, regulatory.

subset responds to polyclonal activators, is enriched in Peyer's patches and in colon lymphoid follicles early in life and is abundant in the colon lamina propria and draining mesenteric lymph nodes of mice with colitis. TCRβ$^+$ cells produce IL-4 and mediate disease, in that treatment of mice with antibodies to the TCRβ chain abrogates colitis and polyclonal B cell activation [85]. Analysis of the TCR repertoire indicates that the pathogenic T cell utilizes a restricted Vβ8.2$^+$ chain with a conserved motif in the CDR3 region and that this TCR might cross-react with both epithelial and bacterial antigens [86].

Similarly to several other colitis models, development of disease in TCRα-deficient mice requires the bacterial flora [84,87]. Removal of the distal cecum ("appendectomy") at 1 month of life reduces colitis incidence later in life from 80% to 3%, suggesting that the dysregulated immune response may develop in the cecum [88]. A transition from a polyclonal to oligoclonal antibody response to enteric bacterial antigens parallels the development of colitis [89].

In contrast to most other models, TCRα-deficient mice produce IL-4 predominantly [83] and the colitis appears to be Th2-mediated, i.e. IL-4-deficient TCRα knockout mice show markedly attenuated colitis development [84,90] and TCRα-deficient mice that are both IL-4 and IL-13 deficient show complete absence of colitis development. Hence it appears that both of these cytokines contribute to pathogenesis.

An interesting facet of the TCRα model concerns the role of the B cell response in modifying disease. Although B cells are not required for development of colitis [91], B cell-deficient, TCRα-deficient mice develop colitis at an earlier age and of greater severity than B cell-competent TCRα-deficient mice, indicating that B cells have a regulatory role in the colitis of TCRα-deficient mice. Indeed, B cells from TCRα-deficient mice inhibit colitis induction in RAG-1$^{-/-}$ recipients given TCRα-deficient mesenteric lymph node cells and administration of TCRα-deficient serum immunoglobulin or of a mixture of monoclonal anti-colon autoantibodies to mice deficient in both TCRα and B cells (Igμ$^{-/-}$) ameliorates their colitis [91]. A regulatory CD1d$^+$ B cell subset appears to regulate disease by production of IL-10 [92], and this IL-10-producing subset in turn stimulates IL-12 production by other B cells that inhibits also the CD4$^+$ Th2 response [93]. Thus, a novel B cell regulatory circuit has been revealed in this model.

HLA-B27/β2M transgenic rat

Because the human HLA-B27 class I MHC molecule is strongly associated with ankylosing spondylitis and the spondyloarthropathies, transgenic rats expressing the human HLA-B27 and β2 microglobulin genes were generated. Certain of these transgenic rat lines develop a multiorgan disease manifested by colitis, arthritis, orchitis and psoriasiform changes of the skin and nails in the absence of any further manipulation [94]. These features resemble the human spondyloarthropathies. Rats transgenic for other class I molecules such as HLA-A2 or HLA-B7 have not developed inflammatory disease. In the most susceptible 21-4H line, disease occurs in all animals surviving past 10 weeks of age and is manifest primarily by watery diarrhea (Table 5.4). A diffuse enteritis with mononuclear cell infiltrate is present that is variable in the stomach and small intestine, but prominent in the colon where it is associated with hyperplasia of crypts and mucin depletion. Crypt abscesses or transmural inflammation are uncommon.

The mechanisms by which the human B27 molecule induces this disease are unclear [94]. The HLA B27 transgene is not expressed on gut epithelial cells but is present on antigen-presenting cells. CD4$^+$ T cells are abundant in the intestinal lesions. Disease can be transferred with transgenic bone marrow and a critical role for T cells in disease is demonstrated by the observation that athymic (nude) rats bearing the transgene fail to develop disease, although bone marrow cells from these nude rats can transfer disease [95]. Interestingly, colitis and arthritis do not occur when B27 transgenic rats are raised under germ-free conditions [96], but both occur when the flora, particularly Bacteroides species, are restored [97]. Cecal bacterial antigen-pulsed APCs stimulate IFN-γ production by B27 transgenic CD4$^+$ T cells, and this response is blocked by antibody to class II MHC [98]. Thus, the commensal bacterial antigens appear to be driving the pathogenic CD4$^+$ T cell response.

Wiskott–Aldrich syndrome protein (WASP)-deficient mice

The Wiskott–Aldrich syndrome is an immunodeficiency disease due to mutations in a gene on the X chromosome encoding a cytoplasmic protein, WASP, which is expressed in lymphocytes and megakaryocytes. WASP appears to function in cell signaling and cytoskeletal interactions. WASP-deficient mice develop acute and chronic colitis by 4 months of age [99]. Large numbers of CD4$^+$ T cells infiltrate the mucosa of colitic mice and adoptive transfer of CD4$^+$ T cells from WASP-deficient mice to immunodeficient recipients results in colitis. Treatment of WASP-deficient mice with anti-IL-4 abrogates colitis, implicating a Th2 effector cell in the pathogenesis [100]. WASP-deficient mice have reduced numbers of natural Treg cells (CD4$^+$CD25$^+$foxp3$^+$) and those that they have function poorly [101], hence these mice have defects in both adaptive and regulatory immune components.

Lymphopenic T cell receptor transgenic mice

A report described an unusual form of spontaneous colitis that arose in a subset of T cell receptor transgenic mouse lines [102]. In this study, two lines of mice with rearranged, transgenic α and β TCR chains specific for the antigen cytochrome *c* (5C.C7-D, AND TCR transgenic lines). When

these TCR transgenes were crossed on to a SCID or RAG background, colitis developed early in life and was characterized by marked mucosal hyperplasia, crypt elongation and mixed inflammatory cell infiltrates with a predominance of mononuclear cells. Focal mucosal ulceration and crypt abscesses were also identified.

Intestinal inflammation in each of these transgenic lines seemed to correlate with low numbers of circulating $CD4^+$ T cells, suggesting that lymphopenia played a role. The dominant T cell population found in colon lesions expressed the transgenic β chain, but not the transgenic α chain ($T\gamma\alpha^-$), suggesting the pairing with non-transgenic, endogenous α chains, even in SCID and $RAG^{-/-}$ mice. It is postulated that this pathogenic $T\gamma\alpha^-$ cell with endogenous α chains was cross-reactive to intestinal antigens as the mechanism of colitis.

Fucosyltransferase transgenic mice

A primary abnormality in the glycosylation of colonic mucins resulting in a defective mucosal barrier function is hypothesized as a mechanism by which genetic factors may be involved in the etiology of ulcerative colitis. In a recent study, mice transgenic for human α-1,2-fucosyltransferase (hFUT1) were observed spontaneously to develop colitis with a mixed acute and chronic inflammatory infiltrate and crypt abscesses, similar to human ulcerative colitis. The inflammation was limited to the colon and cecum. The mucosa had altered glycosylation but barrier function was not altered. hFUT1 mice were lymphopenic and had thymic medullary hypoplasia. Thymocytes demonstrated increased TCR signaling and apoptosis of both double positive and single positive thymocytes [103]. The cellular mechanism responsible for the colitis is not known, but reconstitution of hFUT1 mice with normal bone marrow restored normal thymic morphology and prevented colitis [104].

Models of impaired regulation

Although multiple cell types can have immunoregulatory effects (e.g. CD8 T cells, B cells, NK cells), lineages of $CD4^+$ T cells appear particularly important for immune homeostasis. At least two major subsets of regulatory T cells (Tregs) have been identified: natural Tregs (nTreg) and adaptive or induced Tregs (aTregs). nTregs express the transcription factor Foxp3 and are selected in the thymus by high-affinity interactions with self-MHC II [105,106]. Foxp3 induces a variety of genes that are necessary for the development and maintenance of nTregs. nTregs constitutively express CD25, CTLA4 and GITR on the cell surface, all of which seem necessary for the survival or function of this subset [107–109]. Adaptive Tregs include $Foxp3^+$ T cells that are induced extrathymically and have the same surface markers and functional features as nTregs, as well as $Foxp3^-$ aTregs that produce high levels of IL-10 (Tr1 cells) or TGFβ (Th3 cells). nTregs and both types of aTregs are well represented in the intestine [110].

Local mucosal regulatory mechanisms limit the immune response to antigens and mitogens of the bacterial flora. Multiple experimental models demonstrate that disruption of such regulation results in excessive responses to intestinal bacteria and thus chronic intestinal inflammation (Table 5.5). The occurrence of IBD in mice deficient of certain immune molecules is helping to identify the critical, non-redundant pathways of mucosal immune regulation.

$CD4^+$, $CD45RB^{hi}$ transfer model

The adoptive transfer of naive $CD4^+$ T cells that express high levels of the surface molecule CD45RB ($CD4^+$, $CD45RB^{hi}$) to immunodeficient SCID or RAG deficient mice results in colitis and wasting over the following weeks to months. Transfer of the whole $CD4^+$ T cell subset does not result in disease in this time frame, nor does

Table 5.5 Models with deficient immune regulation.

Model	Area involved	Effector cell	Altered immune component(s)†	Bacterial driven	Strain variation/ genetic modifiers	Ref.
CD4+, $CD45RB^{hi}$ transfer	Cecum, colon	$CD4^+$Th1	R	Yes	BALB > B6	112
IL-10 deficient	Cecum, colon	$CD4^+$Th1	I, R	Yes	C3H, BALB, 129 > (129 × B6)F1 > B6	128
CRF 2–4 (IL-10R2) deficient	Cecum, colon	$CD4^+$Th1	I, R	Probable	B6	140
IL-2 deficient	Cecum, colon	$CD4^+$Th1	R	Yes	C3H, BALB > B6	142
IL-2 receptor alpha deficient	Cecum, colon	$CD4^+$Th1	R	Probable	Undefined	203
Bone marrow transfer to Tgε26	Cecum, colon	$CD4^+$Th1	R	Yes	AKR	150
TGFβ1 deficient*	Multiple organs	$CD4^+$	I, R	No	Undefined	150,204
TGFβRII deficient*	Multiple organs	Unknown	R	Unknown	Undefined	160
Smad3 deficient*	Multiple organs	Unknown	I, R	Unknown	Undefined	162

*Multi-organ inflammation not limited to intestine.

†I, innate; A, adaptive; R, regulatory.

transfer of the reciprocal CD4 subset expressing low levels of the CD45RB molecule ($CD4^+$, $CD45RB^{lo}$) [111,112].

SCID or $RAG^{-/-}$ mice receiving $CD4^+$, CD45RBhi T cells develop weight loss and diarrhea within weeks of the transfer secondary to a colitis that is unremitting and eventuates in the death of the animal. The colon is markedly thickened due to both hyperplasia of the epithelium and infiltration of the lamina propria and the submucosa by lymphocytes and macrophages. The small intestine is usually unaffected. Disease can be prevented by treatment with anti-IFN-γ, anti-TNFα or murine IL-10 but not by administration of IL-4 [113], indicating that the colitis is mediated by Th1 effector cells.

One of the most important findings in the CD45RB transfer model is that colitis is abrogated by co-transfer of the $CD4^+$, $CD45RB^{lo}$ T cell subset or of whole CD4 T cells along with the pathogenic $CD4^+$ $CD45RB^{hi}$ T cell subset [112]. Prevention of colitis by the $CD4^+$, $CD45RB^{lo}$ subset can be abrogated by administration of either anti-TGFβ [114] or anti-IL10R1 [115]. These results are consistent with the presence in this subset of regulatory population(s) producing IL-10 and TGFβ1. Indeed, both $CD4^+CD25^+foxp3^+$ and $CD4^+foxp3^-IL\text{-}10^+$ regulatory T cells have been identified in the normal lamina propria [110]. The former may be derived from the $CD4^+$, $CD25^+foxp3^+$ subset that is generated in the thymus and acts to maintain peripheral tolerance for autoantigens or may be generated in the intestine by local TGFβ [106]. $CD4^+$, $CD25^+$ cells constitutively express CTLA-4 and this molecule may play a role in their regulatory activity [108,116].

Exogenously generated Tr1 cells producing high levels of IL-10 have been shown capable also of inhibiting the induction of colitis in this model [117]. Somewhat similar results were obtained with T cells from a mouse transgenic for IL-10 under the regulation of the IL-2 promoter [118]. Because this promoter is restricted to the T cell lineage, IL-10 is overproduced only when such transgenic T cells are activated. $CD4^+$, $CD45RB^{hi}$hi T cells from these IL-10 transgenic mice did not induce disease in SCID recipients and moreover they prevented colitis when co-transferred with control, non-transgenic pathogenic $CD4^+$, $CD45RB^{hi}$ T cells. Thus, T cell production of interleukin-10 either by the Tr1 subset or by a transgenic, IL-10-producing T cell subset can prevent the induction of colitis in the $CD45RB^{hi}$ transfer model. In addition, both Tr1 cells and nTregs can treat established colitis in this model [119,120].

After transfer to SCID mice, both $CD4^+$, $CD45RB^{hi}$ and $CD4^+$, $CD45RB^{lo}$ T cells traffic to the intestine and reconstitute both lamina propria and intraepithelial compartments [121]. The cell surface markers that they express are typical of mucosal lymphocytes, i.e. $\alpha E\beta 7^{hi}$, $CD69^{hi}$, L-selectin^{lo} and $CD45RB^{lo}$. Inhibition of cell trafficking to the intestine with anti-β7 integrin or anti-MAdCAM-1 attenuates disease [122], as does disruption of secondary lymphoid tissue organization by administration of lymphotoxin β-immunoglobulin fusion protein [123]. Although lymphocytes migrate to both colon and small intestine, lesions occur only in the colon. IL-12 is required for disease initiation and perpetuation and antibody blockade of CD40L, which is required for sustained IL-12 production, prevents colitis and ameliorates established disease [124]. When $CD4^+$, $CD45RB^{hi}$ T cells are transferred to SCID recipients with a reduced flora [121] or to recipients that are treated with antibiotics [125], the colitis is ameliorated. These results strongly implicate the bacterial flora as driving the colitis and indeed the T cells became oligoclonal after transfer [126] and demonstrate reactivity to antigens of the bacterial flora [127]. This model does illustrate two important concepts, namely that normal T cells can cause intestinal inflammation and, second, that such inflammation is prevented in normal mice by the effects of regulatory cells.

Interleukin-10-deficient mice

Interleukin-10 (IL-10) is an important cytokine produced by T cells, certain B cells, macrophages, thymocytes and keratinocytes. IL-10 is a potent direct inhibitor of macrophage function and an indirect inhibitor of Th1 and NK cells. IL-10-deficient mice have normal lymphocyte development and antibody responses initially; however, with age the animals develop anemia, growth retardation, and chronic inflammatory bowel disease [128]. The bowel lesions consist of focal ulcerations and focal epithelial hyperplasia. The lamina propria and submucosa of affected areas are heavily infiltrated with T cells, macrophages, neutrophils, B cells, plasma cells and occasional multinucleated giant cells. Some animals develop perforating ulceration. There is an increased and aberrant expression of MHC class II on the epithelium of both small intestine and colon. More than 60% of mice surviving 6 months or more develop colon adenocarcinomas. The disease is progressive and does not remit.

The effector cell mediating colitis in the IL-10-deficient mouse is the $CD4^+$ T cell. Thus, transfer of $CD4^+$ or $CD4^+CD8^+$ T cells isolated from the lamina propria (LP) of IL-10 mice into syngeneic $RAG\text{-}2^{-/-}$ recipients results in colitis, whereas the transfer of $CD8^+$ LP T cells does not. Although the $CD4^+$Th1 subset may contribute to the pathology, colitis does not occur in mice deficient also for IL-23, indicating that the Th17 effector subset is necessary and sufficient for colitis to occur [129]. A role for the CD4 Th1 subset is suggested by experiments showing that anti-IFN-γ therapy given to of young IL-10-deficient mice attenuates their colitis [130]. Anti-IL-12p40, which inhibits both IL-12 and IL-23, can prevent disease and treat established disease in adult IL-10-deficient mice [131]. These data indicate that the pathogenic mechanism is an enhanced Th17 and possibly a Th1 response in the mucosa due to a lack of inhibition by IL-10. This leads to

macrophage activation and overproduction of inflammatory cytokines such as IL-1, IL-6, TNFα and IL-17, all of which have been demonstrated in the lesions. There is also an underlying innate immune defect in that TLR-driven cytokine production is excessive in the absence of feedback inhibition by IL-10 in macrophages [132] and in epithelial cells [133].

Although IL-10 deficiency in these mice is global, the lesions are confined to the colon and are focal. The presumption is that the disease is localized in the colon because of the large quantities of bacteria there. Certainly the environmental conditions in which the mice are housed can have a major effect on disease expression, i.e. mice held in SPF conditions do not have small bowel lesions [128] and mice raised under germ-free conditions do not develop colitis at all [134]. The bacterial species and antigens that are involved remain unclear [134,135]. IL-10-deficient mice have serum IgG antibodies to a highly selective number of antigens of the enteric bacteria including commensal flagellins, a selective reactivity also identified in a C3H/HeJBir mouse [136]. At least one of the putative autoantibodies that develop in IL-10 knockout mice, anti-pANCA, is likely to be due to a cross-reactivity to conventional enteric bacterial antigens in that this reactivity can be removed by absorption with homogenates of normal enteric bacteria [137].

In addition to environmental influences, the background genes of the inbred strains carrying the IL-10 null mutation strongly influence the age of onset and severity of disease. Thus, 129SvEv, BALB/c and C3H/HeJBir strains have severe disease early in life, 129SvEv × C57BL/6 mice have intermediate disease and C57BL/6 mice have disease onset >3 months of age [130]. This pattern of susceptibility and resistance among inbred strains is similar to that identified in other models. The difference in susceptibility for colitis in IL-10-deficient C3H/HeJBir versus C57Bl/6 strains has been shown to be a genetic trait [1]. Mapping studies have identified multiple different genes with a major locus on Chr3, termed cytokine-dependent colitis susceptibility gene 1 (Cdcs1). The Cdcs1 locus regulates innate immune responses to TLR and NOD2 ligands, and also adaptive T cell responses to enteric bacterial flagellins [138]. Interestingly, a syntenic locus on human Chr.4 appears to regulate susceptibility to Crohn's disease [139].

An IL-10 pathway to colitis

IL-10 mediates its effects by binding to a specific IL-10 receptor on target cells such as macrophages and neutrophils. After binding its ligand, the IL-10R activates STAT-3 (a member of a family of cytoplasmic proteins named Signal Transducers and Activators of Transcription) by tyrosine phosphorylation via receptor-associated Jak kinases. The phosphorylated STAT proteins translocate to the nucleus and induce gene expression. Two genes encoding proteins along this IL-10 pathway have been mutated and in both instances a disease similar to that seen in IL-10-deficient mice has resulted. Deletion of the gene encoding the IL-10Rβ chain (CRF2-4) results in a lack of response to IL-10 and the mice develop colitis by 12 weeks of age [140]. Deletion of STAT-3 in macrophages and neutrophils renders these cells unresponsive to the suppressive effects of IL-10 on the production of inflammatory cytokines such as TNFα, IL-1, IL-6 and IFN-γ. Macrophage–neutrophil STAT-3-deficient mice develop a chronic enterocolitis similar to IL-10-deficient mice as discussed above. These results indicate that STAT-3 is critical for transduction of IL-10–IL-10R signaling in macrophages and neutrophils. Taken together, these experiments define an IL-10 pathway in which deficiency of any one of a number of molecules can result in disease. This is important conceptually in thinking about genetic susceptibility to disease among different human populations or among different species. Apparently discordant results may be due to involvement of different genes along the same pathway.

Interleukin-2-deficient mice

Interleukin-2 (IL-2) is an important cytokine with multiple effects, including the growth, expansion and eventual activation-induced cell death of T cells, differentiation of B cells and activation of macrophages and NK cells. Mice homozygous for a disrupted IL-2 gene develop normally for the first 4 weeks of life [141]. However, approximately half of the animals die between the fifth and ninth week of age with splenomegaly, lymphadenopathy and a severe autoimmune hemolytic anemia. Mice surviving past 10 weeks of age uniformly develop a pancolitis with diarrhea, intermittent bleeding and frequent rectal prolapse [141]. There is a pronounced thickening of the colon due to hyperplasia of the epithelial layer, an extensive infiltration of the lamina propria with acute and chronic inflammatory cells and ulceration. The small intestine is not affected. The colitis is unremitting and results in progressive weight loss and death. Immune abnormalities include a high number of activated T cells and B cells, elevated IgG1 and IgE levels, anti-colon antibodies and increased expression of MHC class II antigens on colon epithelial cells.

As IL-2 deficient mice age, a polyclonal B cell and T cell activation occurs with lymphocytic infiltration of the gut and other organs and production of multiple autoantibodies. Several studies have shown that the crucial effector cells in the colitis of IL-2-deficient mice are $CD4^+$ T cells [142]. Both IFN-γ and IL-12 are increased in the colon mucosa [143], suggesting that the pathogenic $CD4^+$ T cells are of the Th1 subset and, consistent with this, administration of antibodies to IL-12p40 abrogates colitis. Antibody blockade of the α4β7 gut homing receptor on lymphocytes prevents and partially treats colitis in B6.IL-2-deficient mice [144]. Lastly, IL-2 is an important permissive cytokine for activation-induced programmed cell

death and, not surprisingly, IL-2-deficient mice have defective T cell apoptosis [145]. However, the role that this defect plays in disease is unclear.

Why do $CD4^+$ Th1 cells develop sustained activation in IL-2-deficient mice? Several studies point to a defect of regulatory T cells. IL-2-deficient mice fail to generate the $CD4^+$, $CD25^+$ regulatory T cell subset which has been found to be important in maintaining peripheral tolerance [146]. IL-2 is known to be required for this natural Treg population to expand. Thus, IL-2 receptor α chain (CD25)-deficient mice also develop a disease very similar to that of IL-$2^{-/-}$ mice and they also lack this subset.

The environment strongly influences whether and how the disease is expressed in IL-2-deficient mice. IL-2-deficient mice derived germ free do not develop colitis, although they still develop anemia, extraintestinal lymphoid hyperplasia and autoimmunity [147]. Indeed, C56Bl/6.IL-$2^{-/-}$ housed under certain specific pathogen-free (SPF) conditions did not develop colitis [143] unless challenged with parenteral immunization with KLH. The exact mechanism by which the enteric flora induces colitis in conventional IL-2-deficient mice remains unknown.

The disease course and its mortality have been found to be strongly influenced by the genetic background of the mice. BALB/c.IL-2^{-1-} mice develop a severe hemolytic anemia and die by 5 weeks of age [142]. C3H/HeJ.IL-$2^{-/-}$ mice also develop early and severe anemia whereas C57BL/6J.IL-$2^{-/-}$ mice develop anemia at 3–6 months of age. Although the time course to development of lesions differs among these strains, the histopathology is similar, namely mononuclear cell infiltration of most organs, with the intestine most severely affected.

Transgenic epsilon 26 bone marrow transfer model

Mice transgenic for the human CD3ε chain gene (Tgε26) have a block at a very early stage of thymic T cell development, resulting in complete T cell and NK cell deficiency [148]. The thymus develops abnormally and becomes involuted in these mice unless they receive normal bone marrow cells early in life, which corrects the thymic abnormality, a result demonstrating that T cells participate in normal thymic development [149]. The transfer of normal T cell-depleted bone marrow into adult Tgε26 mice whose thymus is already abnormal does not restore a normal thymus, but instead results in marked weight loss and diarrhea, lymphadenopathy, especially in the mesenteric lymph node, and a severe pancolitis [150]. This disease does not occur in Tgε26 mice if a normal fetal thymus is transplanted into them at the same time that they receive the transfer of T cell-depleted normal bone marrow. These data are consistent with disease being due to either abnormal thymic selection of pathogenic T cells or alternatively the lack of development of a critical regulatory T cell population that does develop in the normal thymus, such as the $CD4^+$, $CD25^+$ natural Treg lineage.

The effector cell mediating disease is an αβ TCR cell, as shown by the transfer of colitis into C57BL/6.RAG–$2^{-/-}$ mice by transfer of peripheral (PLN or MLN) αβ T cells from bone marrow-transplanted, colitic Tgε26 donors [151]. Transfer of similar cells from control animals does not cause colitis. There is increased production of IFN-γ and TNFα by both $CD4^+$ and $CD8^+$ T cells in the lesions. Production of IL-12 is required for colitis to occur, in that antibody to IL-12 or antibody blockade of CD40L, which is required for sustained production of IL-12, prevents disease [124]. Response of effector cells to IL-12 is also required, in that transfer of STAT-4-deficient, IL-12-unresponsive bone marrow results in only mild disease. $CD4^+$CD25 T cells are reduced in number in bone marrow-transplanted Tgε26 mice due to thymic epithelial abnormalities [152] and adoptive transfer of Tregs can prevent colitis [153].

Colitis does not occur in the absence of the bacterial flora [154]. Interestingly, transfers of fully pathogenic T cells from colitic mice to germ-free recipients did not result in colitis, even though these T cells migrated to the intestine and maintained their activation and memory markers. This result demonstrates that perpetuation of colitis in this model was completely dependent on the continual presence of enteric bacteria and moreover that the T cell response to bacteria did not cross-react with any host autoantigens.

Transforming growth factor β1 (TGFβ1)-deficient mice

TGFβ is a member of a family of cytokines involved in growth and differentiation. TGFβ has pleiotropic effects, including inflammation, fibrosis and immunosuppression. It is produced by most cells, including intestinal epithelial cells, as an inactive precursor and must be enzymatically activated to exert its effects. Mice deficient in TGFβ1 develop a multi-organ inflammatory disease involving the heart, lungs, diaphragm, salivary glands and pancreas and die by 5 weeks of age [155,156]. Gastritis, enteritis and colitis can occur on certain genetic backgrounds but is mild. Germ-free TGFβ-deficient mice develop the same lesions as conventionally reared mice [157]. TGFβ1 mice crossed on to a SCID or MHC class II deficient background do not develop inflammation [158,159], nor do TGFβ1-deficient mice treated with monoclonal anti-CD4, indicating that CD4 T cells mediate the inflammation.

TGFβ1 exerts its effects on target cells by binding to its specific receptor. The TGFβ receptor is a heterodimer consisting of an RI chain that binds TGFβ on the cell surface and then associates with the RII chain, which has an intracellular kinase domain that transmits the signal

Table 5.6 Models with spontaneous intestinal inflammation.

Model	Area involved	Effector cell	Immune component(s)*	Bacterial flora driven	Strain variation/ genetic modifiers	Ref.
C3H/HeJBir	Cecum > colon	$CD4^+$ Th1	I, A	Yes	Yes. Multiple loci Chr 3 > 8, 1	165,196
SAMP1/Yit	Ileum > cecum, perianal	$CD4^+$ Th1	I, A	Yes	AKR	173–175

*I, innate; A, adaptive; R, regulatory.

by causing the phosphorylation of SMAD2 and SMAD3. These SMADs translocate to the nucleus and mediate activation of target genes. Similarly to the IL-10 pathway, this TGFβ signaling pathway can be interrupted at multiple points. Two groups have blocked TGFβ signaling in T cells by expressing a dominant negative form of the TGFβR II as a transgene under the control of a T lineage-specific promoter. One line of such mice, on a mixed genetic background and using a CD4 promoter, developed wasting and diarrhea and were found to have severe colitis [160]. There was also mononuclear infiltration of multiple other organs. Interestingly, another mouse line expressing this transgene on the C57Bl/6 background using a CD2 promoter did not develop any inflammation but did demonstrate a marked expansion of $CD8^+$ T cells [161], for reasons that are not clear. Targeted disruption of the SMAD3 gene blocks T cell and neutrophil responses to TGFβ. SMAD3-deficient mice develop runting and wasting and most die between 1 and 3 months of age with multifocal pyogenic abscesses in the wall of the intestine and at other mucosal surfaces. Cultures of these abscesses grew commensal bacteria [162]. The small fraction of mice which lived more than 6 months had chronic inflammatory bowel disease. The effector cell mediating this disease has not been identified nor have the cytokines expressed in the lesions.

The phenotypes expressed due to deficiency of TGFβ1, TGFβRII or SMAD3 are remarkably variable, although multiorgan inflammation is a common feature. Colitis occurred in some but not others, which is likely due to differences in the genetic backgrounds and possibly in the enteric bacterial flora. This variability illustrates the difficulty in studying and understanding this complex cytokine. The pathogenesis of the inflammation likely involves deficiency of TGFβ1-producing regulatory cells, which have been implicated in a number of models including TNBS colitis [60] and IL-2-deficient mice [163]. Yet TGFβ1 deficiency itself has resulted in only mild enteritis and colitis. Moreover, the TGFβ-producing regulatory cell does not protect IL-10-deficient mice from developing colitis. Clearly, many questions remain to be answered about this cytokine and its role in IBD.

Models of spontaneous IBD

IBD has been reported to occur in spontaneously in animals, but this has been sporadic and uncommon. There are now several instances in which IBD has occurred in high frequency in a strain of animals: C3H/HeJBir mice, SAMP1 Yit mice and the Cotton-top Tamarin (Table 5.6). The Cotton-top Tamarin is an interesting model but little is known about the pathogenesis. The interested reader is referred to an earlier review [164].

C3H/HeJBir mice

C3H/HeJBir is a substrain of C3H/HeJ mice, which was generated by a program of selective breeding at the Jackson Laboratory. These mice are highly susceptible to the development of colitis and under certain housing conditions can develop colitis spontaneously. In the original studies, the spontaneous colitis was localized mainly to the cecum and right colon of young mice, with onset in the third to fourth week of life. Colitis was usually mild, resolving by 10–12 weeks of age. The lesions were focal, mainly in the cecum, but sometimes extending into the right colon. There was acute and chronic inflammation, ulceration, crypt abscesses and epithelial regeneration, but no thickening of the mucosal layer or granulomas [165].

C3H/HeJBir mice have no significant B cell or T cell reactivity to food or intestinal epithelial cell antigens, but do have strong B cell and T cell responses to cecal bacterial antigens. Serum IgG antibodies, particularly IgG2a, bind to a set of antigens on Western blots of electrophoresed homogenates of cecal bacteria. This antibody reactivity is highly selective, recognizing a limited but reproducible subset of antigens from the many thousands of proteins present [166]. These antibodies probably do not play a major role in disease pathogenesis but do provide insight into what antigens are stimulating pathogenic T cells in that the predominant IgG2a isotype requires T cell help.

C3H/HeJBir $CD4^+$ T cells demonstrate strong proliferative and cytokine responses to cecal bacterial antigens that are detectable as early as 4 weeks of age. This T cell response is directed at protein antigens and is MHC class II restricted. Adoptive transfer of bacterial

antigen-activated $CD4^+$ T cells from C3H/HeJBir but not from control C3H/HeJ mice into histocompatible C3H/HeSnJ *SCID/SCID* recipients induces colitis [167]. This formally demonstrates that $CD4^+$ T cells reactive with conventional antigens of the enteric bacterial flora can mediate chronic inflammatory bowel disease. A number of $CD4^+$ T cell lines reactive with enteric bacterial antigens have been generated *in vitro*. These lines are predominately Th17 and Th1 phenotypes and cause colitis in all recipients after adoptive transfer into C3H SCID recipients. High levels of IL-17, IFN-γ and IL-12 are found in colon explant cultures of colitic mice. The sustained IL-12p40 production in the mucosa that is required for disease to occur depends on CD40L–CD40 interactions in that antibodies to CD40L block IL-12p40 production *in vitro* and prevent colitis *in vivo* [168]. IL-12p40 is present in two cytokines, IL-12 and IL-23. An anti-IL-23p19 monoclonal antibody was able to prevent and treat colitis in this model, an effect accompanied by apoptosis of $CD4^+$ Th17 cells, indicating that Th17 cells are the major pathogenic phenotype in this model [66].

Spontaneous colitis is reduced or eliminated when the mice are housed in SPF conditions. C3H/HeJBir (and C3H/HeJ) remain very susceptible to several forms of colitis, including both TNBS-induced and DSS-induced colitis [169]. C3H/HeJBir.IL-10-deficient mice get severe disease in the first weeks of life [1], whereas C57Bl/6.IL-10-deficient mice develop a mild colitis only after 3 months of age. This marked difference in colitis susceptibility in these two strains mapped to five chromosomal loci [170] of which a locus on Chr.3 had the greatest contribution. This locus was designated cytokine deficiency-induced colitis susceptibility (Cdcs1) [138]. The C3H/HeJBir Cdcs1 allele reduced innate cell responses to bacterial TLR ligands but increased $CD4^+$ T cell responses to commensal bacterial antigens compared with C57Bl/6J.

The selective serum IgG antibody reactivity of colitic C3H/HeJBir mice [166] led to an effort to identify the cecal bacterial antigens involved. Serologic expression cloning, using colitis sera to probe a DNA library derived from cecal bacteria, identified some 60 bacterial antigens, among which one-quarter were bacterial flagellins [136]. One of these flagellins, CBir1, was recognized by serum IgG from multiple other mouse models and strains and, importantly, by half of sera from Crohn's disease but not ulcerative colitis or controls [136] (Figure 5.3). Subsequent studies have found that anti-flagellin seroreactivity is associated independently with complicated Crohn's disease [171] and may have prognostic import. Interestingly, anti-flagellin responses in Crohn's disease are genetically regulated by a locus on Chr.4, in a region syntenic to mouse Cdcs1 on Chr.3 [139]. This locus contains an NF-κB1 haplotype associated with reduced NF-κB activation in patients with Crohn's disease [146]. In addition, patients with Crohn's disease demonstrate increased innate and adaptive T cell responses to CBir1 flagellin [172]. Thus, the response to enteric flagellins appears to be highly conserved between human and mouse, probably reflecting the potent immunostimulatory properties of these molecules.

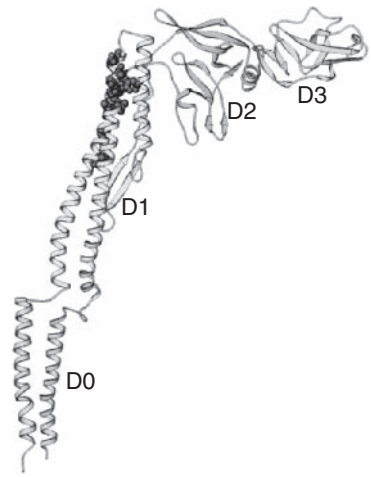

Figure 5.3 Molecular model of flagellin. This molecular model of CBir1 flagellin is based on the molecular structure of *Salmonella* flagellin. CBir and related commensal flagellins are immunodominant antigens that stimulate pathogenic T cells in mice and in half of patients with Crohn's disease (but not patients with ulcerative colitis). The immune responses to flagellins are highly conserved across species. It seems likely that there are other types of immunodominant antigens of the microbiota that will also cross species and provide opportunities for antigen-directed therapy in the future This is an example of translation of discoveries in mouse models to human IBD. Reproduced with permission from Duck LW, Walter MR, Novak J *et al.* Isolation of flagellated bacteria implicated in Crohn's disease. *Inflamm Bowel Dis* 2007; **13**:1191–201.

SAMP1/Yit mice

Another model of spontaneous intestinal inflammation is the recently described SAMP1/Yit mouse [173]. The SAMP1/Yit strain is a sub-line of the SAM (Senescence Accelerated Mice) P1 strain, which was derived by extended sibling mating of AKR/J mice. SAMP1 mice are so named because of their shortened life span (approximately 9 months) and propensity to develop early senescence associated with spontaneous amyloidosis, alopecia and osteoporosis. The SAMP1/Yit sub-line of the SAMP1 strain was derived by selective sibling breeding of SAMP1 mice with spontaneous skin ulcerations [173]. Unlike the parent SAMP1 strain, the SAMP1/Yit strain shows no shortened life span or features of early senescence, but does develop a spontaneous enteritis and cecitis under SPF conditions.

Unlike most of the IBD models described in this chapter, the intestinal inflammation in SAMP1/Yit mice is unusual in its preferential localization to the small intestine;

the absence of colonic involvement is characteristic. Animals maintained under SPF conditions develop discontinuous inflammatory lesions of the distal small intestine as early as 10 weeks of age and show 100% penetrance by 30 weeks of age [174]. Histologically, the disease is most severe in the terminal ileum. Early lesions are characterized by neutrophilic infiltrates over Peyer's patches or pre-existing lymphoid follicles, resembling the aphthous ulcers in human IBD. Mature lesions are characterized by villus atrophy and crypt hyperplasia with transmural inflammatory cell infiltrates. Of note, the SAMP1/YitFc substrain bred at the University of Virginia develops perianal fistulous disease resembling that seen in Crohn's disease [175].

The inflamed ileum contains increased numbers of activated T cells [174] and ileitis can be transferred into AKR SCID mice by unfractionated or $CD4^+$ T cell-enriched mesenteric lymph node (MLN) cells from 30-week-old SAMP1/Yit mice in a dose- and time-dependent manner. Both IFN-γ- and IL-4-producing T cells are able to transfer ileitis, so both Th1 and Th2 cells are postulated to play a role. The Th1 response is more prominent early in the course and the increase in Th2 cytokines later, during chronic ileitis [176]. Anti-TNFα therapy is beneficial, with a marked decrease in intestinal inflammation and epithelial cell damage, the latter associated with reduced epithelial cell apoptosis [177]. Administration of antibodies to adhesion molecules that have been implicated in T cell homing and neutrophil trafficking (E- and P-selectin and α4-integrin) also attenuates disease development in SAMP1/Yit mice [178].

Development of chronic ileitis in SAMP1/Yit mice occurs in the absence of the commensal flora, but is mild and delayed compared with SAMP1/Yit mice held under SPF conditions [179]. In the latter, commensal bacterial lysates stimulate T cell production of IL-4, compatible with the idea that T cell reactivity to bacterial antigens localized in the terminal ileum exacerbates the inflammation.

As in the C3H/HeJBir model, quantitative trait locus mapping has been used to identify genes involved in SAMP1/Yit ileitis. A locus on Chr.9 that promotes inflammation-induced epithelial damage [180] and another on Chr.6 in the region of PPARγ [181] have been identified. The genetic basis of this ileitis may be primarily epithelial, in that abnormalities of the ileal epithelium have been noted as early as 4 weeks of age and prior to the onset of inflammation [182]. In addition, bone marrow chimera experiments found that ileitis occurred after AKR bone marrow was transferred to SAMP1/Yit recipients, but not vice versa, compatible with a defect in non-hematopoietic cells. In the AKR → SAMP1/Yit chimeras, increased permeability and reduced barrier function in the ileum were found prior to disease onset and this was associated with reduced expression of tight junction proteins elandin-2 and occludin [183].

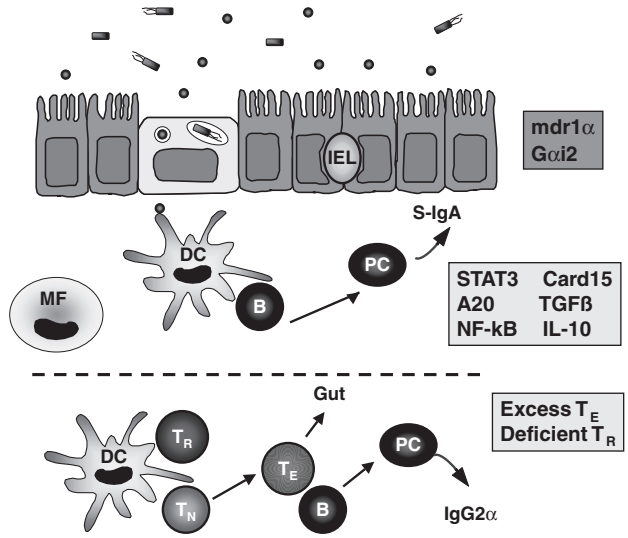

Figure 5.4 Multiple defects in homeostatic mechanisms are likely required for IBD to occur. The normal mucosal innate immune response to the microbiota is depicted above the line. When these normal innate mechanisms are impaired, there can be a systemic $CD4^+$ T cell priming. Either excess T-effector function or deficient T-regulatory function can result in inflammatory bowel disease. In the boxes are denoted molecules or models in which defects result in IBD. In spontaneously occurring disease it is likely that defects in multiple components are involved. Reproduced with permission from Elson CO, Cong Y, McCracken VJ *et al.* Experimental models of inflammatory bowel disease reveal innate, adaptive and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005; **206**:260–76.

Discussion

The inflammatory bowel diseases represent a complex interplay among environmental, genetic and immunologic components (Figure 5.4). New insights into each of these factors have been generated from experimental models.

Environmental factors

The major environmental factor revealed in the different experimental models is the enteric bacterial flora. On the lumenal side of the epithelial layer, there are a large number of bacterial species, estimated at 100 trillion organisms in the human intestine. On the other side of the epithelium are a large number of immune cells and molecules. The concept of a microbial–epithelial–lymphoid circuit with each component in communication with one another has already been presented, as have examples where perturbations of either epithelial or myeloid innate cells can lead to chronic intestinal inflammation (Tables 5.1 and 5.2). It is unknown whether abnormalities of the flora alone ("dysbiosis") can lead to disease in a similar manner, but this is possible. An altered or abnormal flora has been raised as a possible explanation for the rapid increase in immune-mediated diseases, including IBD, in the western

world. Sometimes called "the hygiene hypothesis", this theory postulates that early childhood exposure to microbes primes the immune system to respond in certain ways later in life. In the western world, such priming either does not occur or is abnormal and results in a variety of immune diseases occurring later in life, including autoimmunity, atopy, asthma and IBD. One factor that has changed substantially in the western world is the bacteria flora and a leading hypothesis is that this altered flora is responsible for abnormal priming of the immune system early in life and thus later immune-mediated diseases. This theory is being tested by interventions to alter the bacterial flora early in life and initial results indicate a 50% reduction in atopy in young children from atopic families who were given a probiotic flora as newborns [184].

The microbial flora is very complex and comprises thousands of different organisms [185]. Many have never been cultured but are being identified by new molecular methods that can identify novel organisms without having to culture them. The bacterial flora is the major stimulus to the development of the intestinal immune system. For example, germ-free animals have little or no intestinal lymphoid tissue or IgA despite exposure to a variety of food antigens. When germ-free animals are reconstituted with an intestinal flora, they develop abundant mucosal lymphoid cells along the length of the intestine and secretory IgA is produced in large amounts [186]. However, not all organisms among the flora are equally able to stimulate the mucosal immune system [187–190]. For example, *Helicobacter* are a species that appear to be immunostimulatory in the intestine and some *Helicobacter* strains can contribute to colitis in selected immunodeficient strains, whereas in most mice with an intact immune system *Helicobacter* species are commensals and do not cause disease. At the other end of the spectrum there may be organisms that are delivering inhibitory signals to the mucosal immune system [191–193] and thus some commensal bacteria may actively inhibit mucosal immune responses. Certain bacterial strains have been classified as "probiotics", i.e. bacteria that promote health. Probiotic bacteria are being tested in both experimental models and in humans with IBD with some promising early results [194]. In the future, the probiotic approach may be taken one step further by genetically altering commensal bacteria to express cytokines. This has been done in a recent report in which *Lactococcus* were genetically altered to express IL-10 [195]. As we learn more about the bacteria flora, its manipulation may represent an effective treatment modality particularly for maintaining remission of disease.

The variation in immune stimulating capability among organisms extends to their antigens. For example, in the colitic C3H/HeJBir mouse only a small fraction of the total enteric bacterial proteins present in the cecum are recognized by serum antibody and this set of antigens is highly reproducible among individual members of this strain [136]. The implication is that there are not only immunodominant organisms, but also a limited set of immunodominant antigens recognized by the host immune system which may vary from one strain or individual to another. Such immunodominant antigens need to be defined further in both mouse and humans.

Genetic factors

Disease expression can vary greatly when the same mutant gene is bred on to different inbred mouse strains. Each inbred strain carries a set of genes that are identical within that inbred strain but differs from other inbred strains. These "background genes" can greatly influence or modify the expression of a disease resulting from a defined mutation. With regard to experimental IBD, many of the same strains appear to be susceptible or resistant regardless of how the disease was induced (Tables 5.1–5.6). This variation has been examined in detail in DSS-induced colitis where reproducible quantitative differences were found among the inbred strains. These differences were shown to be heritable and due to the effects of multiple genes [169]. Using quantitative trait locus mapping, the locations of a number of genes responsible for this variation have been identified [52]. A similar effort using IL-10 deficiency as the stimulus for colitis has also revealed the influence of multiple genes determining susceptibility [1,170,196]. These results in mice mirror the results of similar efforts in patients with IBD in whom a multiple genetic loci contributing to susceptibility have been identified. Once these genes are identified, the creation of mouse mutants will be needed to clarify how these susceptibility genes work. Thus, genetic studies in experimental models and in human patients complement one another.

Immunologic factors

The immunologic pathogenesis has been discussed in the introduction and sections on each experimental model. However, there are some overall themes that recur through multiple models that are worth considering. These include triggering factors, progression or perpetuation of disease and immune regulation, i.e. the immunologic stages of IBD.

A number of models demonstrate that an animal that is genetically susceptible to IBD may not become ill unless the process is triggered by some event that perturbs the system. Initiating or "triggering" factors seem to operate also in humans with IBD whose disease may initiate from a viral or bacterial infection, emotional shock, etc. Very little is known about these initiating factors but several examples of their requirement for disease to occur are demonstrated in the experimental models. One example is the specific pathogen-free C57Bl/6.IL-2-deficient mouse, which under strict SPF housing conditions can remain healthy. However, immunization with DNP-KLH in complete Freund's adjuvant triggers the typical disease

associated with IL-2 deficiency, including a severe colitis [143]. Another is the STAT-4 transgenic mouse, which remains healthy unless immunized with the same material [67]. The ITF deficient mouse, which has a genetic defect in the epithelial healing, remains well as long as epithelium is not perturbed. Feeding of DSS to ITF-deficient mice injures the epithelium and thus results in a severe colitis [28]. Non-steroidal anti-inflammatory drugs (NSAIDs) have long been thought to activate human IBD but data supporting this idea are lacking. Hence it is interesting that an NSAID, piroxicam, can accelerate the onset of colitis in C57Bl/IL-10-deficient mice [130]. Even from this short list, it is clear that the triggering factors can be diverse and can involve either systemic or mucosal perturbations. Experimental models should allow further dissection and understanding of this aspect of IBD.

Initiating factors and those responsible for disease perpetuation or progression are likely to be different. With regard to Th1-mediated disease, a key factor in disease perpetuation is sustained IL-12 production. The latter in turn requires interactions of CD40L on T cells and CD40 on antigen-presenting cells. Antibodies to IL-12 and to CD40L have been shown to be effective in multiple models and each of these agents is currently being tested in patients. Another factor required for perpetuation of the disease is the recruitment of new effector cells into the mucosa. Effector cells have fairly short life spans, including dendritic cells, macrophages, neutrophils and activated lymphocytes. Hence all these different cell types need to be renewed for chronic inflammation to persist and this involves recruitment of such cells from the circulation. Supporting this idea are the findings that agents which block trafficking of cells into the intestine are able to prevent and treat colitis in a number of experimental models.

A third immunologic aspect important to pathogenesis of IBD is immune regulation. Deficiency of regulatory cells has been implicated in a significant number of models (Table 5.5). One of the most exciting developments in modern immunology is the identification of these regulatory cells. Their effects have long been seen, but identification of the cells responsible for those effects have been elusive. Many different types of regulatory cells are being defined and a great deal of information is being generated. Much of the basic immunology will come from experimental systems in the mouse and will need to be translated to humans. This area has great promise for novel therapeutic approaches to IBD, therapies that could potentially alter the natural history of these diseases. Based on what has been learned from the models to date, the most rational therapy for IBD is to enhance regulatory cell function, to inhibit effector cell function or to accomplish both. Such an approach is within sight at present but will need to be validated in experimental models before it is translated to the treatment of human IBD.

Conclusion

There are now more than 30 distinct genes or gene loci that contribute to susceptibility for human Crohn's disease [2]. Some of these same genes and a variety of others contribute to susceptibility for ulcerative colitis [3]. Many of these gene loci involve innate immunity. Some would be predicted to be expressed by epithelial cells and others by the adaptive immune system. The first susceptibility gene identified, NOD2, is a pattern recognition receptor for bacterial component MDP and is expressed by a variety of cells, but is particularly important in Paneth cell function. There has been a remarkable coherence between the genes identified in genome-wide association studies and the results of the experimental mouse models of IBD, although the genes identified in humans versus mice are not the same. Nevertheless, the gene defects in humans seem to involve the same compartments as do the gene mutations in mice. Although the findings in NOD2 mutant mice have failed to identify a specific mechanism, the mouse and human data for another gene, ATG16L1, have been fairly coherent, implicating Paneth cells as a major target [197].

Defects in innate immunity induced by these mutations seem to result in increased activation of pathogenic $CD4^+$ T cells that, in turn, are the mediators of the inflammation. How these abnormalities in innate immunity result in pathogenic T cell activation has yet to be defined, but it is possible that either deficient or excessive innate responses might result in pathogenic T cell activation depending on the genetic background of the host and on the microenvironment where the interactions occur. Identifying how defects in innate immunity in both epithelial or myeloid cells leads to excessive T cell responses that result in IBD remains an important but challenging question. The intestine is enriched in regulatory T cells and likely other types of regulatory cells, which are clearly important in maintaining homeostasis. Where and how these regulatory cell effects are exerted and exactly how they influence and the dialogue of the host with its microbiota remain unclear.

The different models discussed in this chapter have been divided fairly arbitrarily into innate, adaptive and regulatory components. This classification parallels the involvement of innate, adaptive and regulatory cells in the complex interaction of the host with its microbiota. However, most of the models involve defects in more than one of these components, as noted in the tables. Given the multilevel and redundant nature of the host immune response to the microbiota, we propose that a combination of two or more immune defects is probably necessary for IBD to occur. This is particularly likely in humans where the gene defects likely lead to a relative reduction in gene function, rather than an absence that is seen in mouse knockout mutations. In this view, a "multiple hit" is required for susceptibility to IBD to be expressed as overt disease.

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Chapter 6
Factors Affecting Mucosal Homeostasis: a Fine Balance

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Summary

- In most cases, the intestinal antigen presentation leads to local tolerance or a contained, non-systemic, immune response that can be characterized as a low-grade or "physiological" inflammation.
- The intestinal immune system must clearly discriminate between pathogenic and harmless antigens, to determine which antigens will be tolerated and which will elicit an immune response.
- The state of non-responsiveness that often characterizes the mucosal immune system is not a passive process but rather involves the active suppression of a potential exuberant immune response mediated by a network of sophisticated mechanisms of immunoregulation.
- The gut mucosal immune system is structurally and functionally divided into inductive and effector sites.
- Specialized cell types have been shown to contribute to immune regulation in the mucosal immune system. Among these are specific dendritic cells, immunoglobulin A (IgA) producing plasma cells and $CD4^+$ regulatory T cells (Tregs).

Introduction

The entirety of the mucosal surface and their associated lymphoid structures are subsumed under the term mucosa-associated lymphoid tissue (MALT), among which the gut-associated lymphoid tissue (GALT) with its enormous surface area is the largest lymphoid formation. The GALT mainly consists of Peyer's patches, lymphoid follicles and lymph nodes, representing the cardinal sites for the initiation of lymphoid responses against antigens. The normal intestinal mucosa contains an highly intricate and specialized immune system that has unique features compared with other compartments of the immune system, as it is constantly exposed to an enormous amount of luminal antigens, consisting of dietary agents and numerous commensal bacteria and viruses. Furthermore, it represents a cardinal entry point for potential pathogens to which the immune system must respond. Therefore, the intestinal immune system must clearly discriminate between pathogenic and harmless antigens, to determine which antigens will be tolerated and which will elicit an immune response. This is ensured by a network of complex interactions between the host's innate immune system, which represent the first line of defense against pathogens and the adaptive immune system for an antigen-specific humoral and cellular immune response. In most cases, the intestinal antigen presentation leads to local tolerance or a contained, non-systemic, immune response that can be characterized as a low-grade or "physiological" inflammation. A malfunction of these immune-regulatory mechanisms, which physiologically prevent an active immune response towards dietary antigens or commensal bacteria, leads to an ongoing intestinal inflammatory reaction, causing gluten-sensitive enteropathy or inflammatory bowel disease. The pathogenesis of these diseases provide a striking example of an errant local immune response that has serious systemic consequences. Therefore, one major requirement towards the mucosal immune system is the maintenance of immunoregulatory mechanisms that downregulate responses to luminal antigens, while still retaining its ability to respond strongly to potential pathogens. To meet this task, the intestinal mucosal immune system maintains a delicate balance between responsiveness and non-responsiveness (tolerance). There are specific features that enable the mucosal tissue to convert the principle of tolerance induction. Among these is a unique anatomical setup with an array of specialized cells that maintain a state of physiological inflammation, which is tightly controlled to prevent an aberrant immune reaction. The gut mucosal immune system is therefore structurally and functionally divided into inductive and effector sites. Inductive sites comprise

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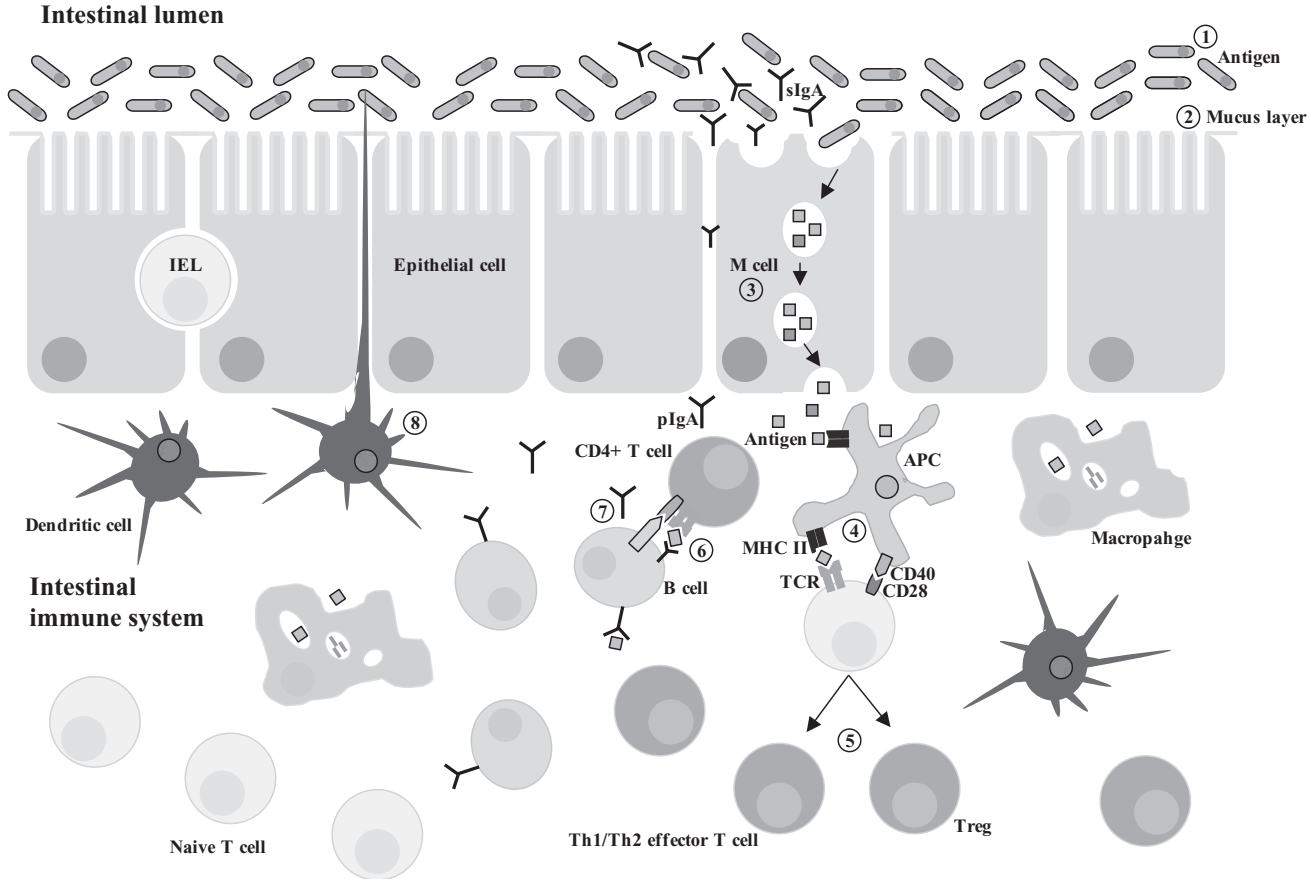

Figure 6.1 Schematic representation of antigen processing and induction of the immune response in the intestinal immune system. (1) Luminal antigens consisting of dietary agents and numerous commensal bacteria and viruses. (2) Mucus layer secreted by goblet cells and an overlying coating, consisting of a glycocalyx formed by mucins anchored to the apical surface of the epithelial cell membrane. (3) M cells transport and deliver antigens to APC. (4) APC process antigens and present them to naïve T cells. (5) Naïve T cells differentiate into TH1/Th2 effector cells or Tregs. (6) Interaction between T and B cells. (7) pIgA produced by plasma cells, which is latter released as sIgA. (8) Dendritic cells sampling antigens by penetrating the epithelium with their dendrites

organized lymphoid tissues in the intestinal mucosa and the mesenteric lymph nodes for the uptake and processing of antigens, whereas effector sites consist of a network of immune cells scattered throughout the intestinal epithelium and lamina propria. Many cell types have been shown to contribute to immune regulation in the mucosal immune system. Among these are specialized dendritic cells, immunoglobulin A (IgA)-producing plasma cells and $CD4^+$ regulatory T cells (Tregs). The function of these Tregs exemplifies the general demand towards the intestinal immune system, as they inhibit the development of pathologic immune responses to self commensal antigens without compromising the immune response to potential pathogens. The heightened understanding of the gut mucosal immune mechanisms that mediate the balance between immunity and tolerance have special implications regarding diseases that are associated with a loss of tolerance, such as celiac disease and inflammatory bowel disease, as it may allow the development of more specific therapies in these intestinal maladies. Furthermore, the insights that unravel could also lead to more efficient therapeutic applications in the fields of organ transplantation and organ-specific autoimmune diseases.

The gastrointestinal immune response – the inductive site

The epithelial layer as the first line of defense

The structure of the GALT can be structurally and functionally divided into inductive sites for the uptake and processing of antigens, which takes place in organized secondary lymphoid tissue and effector sites. The inductive sites include the Peyer's patches, mesenteric lymph nodes and isolated lymphoid follicles that are scattered throughout the intestine. The anatomical structure of this organized tissues is consistent, with the outer layer

consisting of epithelial cells. These epithelial cells are closely joined together by tight junctions that are composed of proteins such as ocludin and claudin, forming a physical barrier that prevents pathogenic organisms from invading the underlying lymphoid tissue. The intraepithelial cells feature extensions of their apical surface called microvilli, which in their totality form the epithelial brush border. This epithelial barrier is supported by an overlying coating, consisting of a glycocalyx formed by mucins anchored to the apical surface of the epithelial cell membrane and a mucus layer secreted by goblet cells. The subsequent epithelial layer is made up of epithelial cells that are derived from basal crypts and differentiate into villus or surface epithelium and Paneth or goblet cells [1]. Paneth cells are secretory epithelial cells which are predominantly found in the small-intestinal crypts of Lieberkuhn and are able to release secretory granules containing peptides with microbicidal properties, like lysozymes, RegIIIγ or α-defensins, into the lumen. Another subgroup of these microbicidal peptides, that are constitutively expressed in the epithelial compartment upon inflammatory conditions, are β-defensins. Apart from their role in innate immune defense, defensins also have chemotactic properties for dendritic cells and lymphocytes, indicating a participation in regulating adaptive immunity. The group of defensins therefore provide a first physical barrier that is able to prevent or at least regulate the entry of potential pathogens into the body and, moreover, regulate the composition of the bacterial flora [2]. Apart from dendritic and B cells, enterocytes are also able to express major histocompatibility complex (MHC) class I and II molecules, in addition to MHC I-like molecules, leading to the ability to take up and process antigens, which enables them to function as antigen-presenting cells (APCs). In order to activate T cells, the antigen must be presented by the APCs to the T cell receptor (TCR) in the context of an MHC molecule and, furthermore, it requires the interaction of a co-stimulatory molecule on the APC with its counter ligand on the T cell. This interaction is mediated by members of the B7 family of proteins or other molecules such as CD40 or CD54, which are expressed on APCs, with their corresponding receptor CD28 and CTLA-4 on T cells. Recent data indicate that although intestinal epithelial cells express MHC molecules, they are not able to induce full T cell activation, as they do not seem to express the required co-stimulatory molecules such as CD54 [3]. Consistent with these findings, other data suggest that gut epithelial cells are unable to promote antigen-driven activation of $CD4^+$ T cells, even in the presence of exogenous co-stimulatory signals, and moreover are even able to prevent T cell activation by APCs. Apart from the barrier function of the epithelial layer, this might explain the non-responsiveness of mucosal T cells to the numerous enteric antigens sampled by epithelial cells, contributing to the maintenance of mucosal homeostasis through T cell tolerance [4]. Recent data also demonstrated that epithelial cells themselves are able to internalize enteric antigens in a process resembling phagocytosis, mediating their translocation across the epithelium. It could be shown that intestinal epithelial cells that expressed the Toll-like receptor (TLR) 4 were capable of this form of phagocytosis, contributing to the maintenance of mucosal homeostasis [5].

The mucosal immune system is furthermore marked by intraepithelial lymphocytes (IELs), which predominantly develop independently of the Peyer's patches and reside above the basement membrane within the mucosal epithelium. These lymphocytes are a heterogeneous group with many subtypes that differ substantially with regard to their phenotype and function. The intestinal intraepithelial lymphocytes can be divided into two major subpopulations based on the TCR and co-receptors that they express. These are the predominant TCRαβ-expressing T cells that also possess CD4 or CD8αβ co-receptors and TCRαβ- or TCRγδ-expressing T cells without conventional co-receptor expression. Although the exact mechanisms that lead to this differentiation of IELs is unknown, the variety of the luminal flora along the gut may have a major influence. Whereas TCRαβ T cells are mostly located in the large intestine, the other type is enriched in the small intestine. Most of the resident IELs are $CD8^+$ and express the TCRαβ and some of them are cytotoxic T cells that participate in the lysis of virus-infected cells. IELs also contain $CD4^+$ T cells, which can be differentiated into typical $CD4^+CD8^-$ and atypical $CD4^+CD8^+$ cells, which both endorse B cell activation [6]. TCRγδ-expressing lymphocytes can directly recognize antigens in the form of intact proteins or non-peptide compounds, unlike TCRαβ, which recognize antigens bound to major MHC molecules [7]. Due to their distinctive antigen-recognition properties, γδ- and αβT cells have different antigen-recognition requirements and almost certainly recognize different set of antigens [8]. Another important function of γδT cells in the mucosal immune system is the homeostatic regulation of the epithelial tissue through the production of insulin-like growth factor-1 and keratinocyte growth factor, which modulate the growth and differentiation of epithelial cells [9].

In addition to intraepithelial lymphocytes, the epithelial layer is further characterized by specialized epithelial cells which are known as M cells. These are localized in the follicle associated epithelium of the Peyer's patch overlying the dome of Peyer's patch follicles. Unlike other enterocytes, they do not contain a thick mucus layer or microvilli on their apical surface, but broader microfolds that give the cells their name. M cells differentiate from enterocytes under the influence of membrane-bound lymphotoxin $\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) that is present on local lymphocytes, mainly B cells [3]. M cells contain endocytic vesicles to uptake sampled antigens from the intestinal lumen and transport them across the epithelial layer. The transcytosed antigens are delivered to lymphocytes and macrophages located in

an intraepithelial pocket-like structure of the M cells and then carried to innate and adaptive immune cells in the underlying subepithelial dome region of the Peyer's patch, where an active immune response evolves. The transport of antigens by M cells therefore represents a first important step in the mucosal immune response. Furthermore, M cells are also believed to provide co-stimulatory signals leading to the initiation of lymphocytes located in the Peyer's patch. On the other hand. M cells are not believed to process antigens themselves, as they do not express MHC class II molecules, and instead they probably solely transport the intact antigens to professional APCs in the epithelium or subepithelial dome of Peyer's patches [1].

Lymphocyte response in Peyer's patches

Peyer's patches are the primary inductive site for the mucosal immune response and consist of multiple lymphoid follicles that are located in the submucosa of the intestine. The unique structure of Peyer's patches is emphasized by the fact that they do not contain any afferent lymphoid vessels, as lymphocytes migrate from the bloodstream across the high endothelial venules into the Peyer's patches. The lymphoid areas of the Peyer's patches are separated from the intestinal lumen by a single layer of columnar epithelial cells, classified as the follicle associated epithelium (FAE), and an area underlying the epithelium known as the subepithelial dome (SED). The FAE differs from the surrounding villous epithelium because of the high density of lymphocytes, macrophages, dendritic cells and the aforementioned M cells. B lymphocytes are localized in the central region of Peyer's patches beneath the SED where the B cell follicles form around follicular dendritic cells. Peyer's patches are also the source of intestinal $CD4^+$ and cytotoxic T cells, which are enriched in the periphery of the B cell follicles from where they subsequently can migrate out of the GALT and reach mucosal and also peripheral non-mucosal tissue [1,10,11]. The immune response in Peyer's patches requires the recognition of the antigen by T helper cells and the subsequent cooperation between antigen specific B and T lymphocytes.

T-helper cells occupy a pivotal position in the humoral and cell-mediated mucosal immune response. $CD4^+$ T lymphocytes can differentiate into functionally distinct subpopulations, T-helper 1 (Th1) and T-helper 2 (Th2) cells. The activation and differentiation of naïve T precursor cells is partly mediated by signals received from the T cell receptor (TCR)/CD3 complex after its interaction with antigens presented by APCs. The second signal is produced through the interaction of co-stimulatory or accessory molecules on the APCs with corresponding ligands on T cells. The major factor in T-helper polarization is the impact of cytokines that most probably are produced by dendritic cells. Recent data suggest that specific subsets of $CD11c^+$ dendritic cells induce interleukin-12 (IL-12)-dependent Th1 differentiation, whereas other dendritic cells may favor a Th2 response [12]. Th1 cells are characterized by the production of interferon-γ (IFN-γ) and tumor necrosis factor α (TNFα) and mainly affect cell-mediated immunity. Th2 cells secrete IL-4, IL-5 and IL-13 and are predominantly involved in humoral immunity. Furthermore, Th3 and regulatory $CD25^+CD4^+$ T cells (Trs) produce transforming growth factor β (TGFβ) and IL-10, respectively.

Mucosal B cells are stimulated in a T cell-dependent manner and under the influence of secreted TGF-β, a μ to α class switch recombination occurs. Moreover, interaction between B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) on antigen-presenting cells and the BAFF receptor on B cells further enhance activation [13]. The activation of the B cells leads to IgA isotype switching and a subsequent emigration via the lymphatic drainage to mesenteric lymph nodes, where they proliferate further and differentiate into B blasts. These cells migrate through the thoracic duct and the bloodstream to the final effector site (e.g. the gut lamina propria). This migration is facilitated by changes in the expression of adhesion molecules and chemokine receptors, as B cells express α4β7 integrin and later CCR9, which interacts with MAdCAM-1 and TECK expressed by blood vessels in the lamina propria and the intestinal epithelium, respectively. Under the influence of IgA-enhancing cytokines such as IL-5 and IL-6 produced by Th2 cells, they enter the terminal differentiation process, converting to polymeric IgA (pIgA)-producing plasma cells. After the transport across the epithelium through a poly Ig receptor expressed on the basal membrane of epithelial cells and subsequent cleavage at luminal surfaces, the polymeric IgA is released as surface IgA (sIgA) together with a bound secretory compound. This secretory component is the cleaved extracellular portion of the pIgR which protects the sIgA against degradation by intraluminal enzymes, enabling sIgA to exert its role in mucosal defense.

IgA has multiple important functions considering the mucosal epithelium, as IgA prevents adhesion of antigens to the epithelium. During the transport across the epithelium, IgA can inhibit virus production and neutralize antigens inside infected epithelial cells and in addition IgA in the lamina propria binds and excretes antigen in the lumen. Moreover, as IgA does not activate complement or bind to Fc receptors, an uncontrolled inflammatory reaction is averted. In addition, the intestinal immune system exhibits a Peyer's patch-mediated re-entry mechanism of sIgA across the intestinal mucosa, as binding of IgA to luminal antigens facilitates adherence to the apical membrane of M cells and the transport of IgA-bound antigens across the epithelial barrier. The presentation of these antigens to immature Peyer's patch dendritic cells that are less activated than dendritic cells that have migrated to the mesenteric lymph nodes induces a less pronounced antigen-specific response, favorable to preserve gut mucosal homeostasis. In accordance with this mechanism, sIgA also forms luminal immune complexes

with commensal bacteria, which are thereafter presented in the Peyer's patch and induce diminished immune response [14,15]. This is consistent with observations that dendritic cell bound commensal bacteria in the SED of Peyer's patches do not advance further than the mesenteric lymph nodes. The ensuing commensal-specific IgA response is independent of T helper cell activity and facilitates the destruction of commensal bacteria by mucosal phagocytes, leading to local confinement of the immune response and avoiding systemic reactions [16]. The generation of IgA by plasma cells is therefore one of the hallmarks of the mucosal immune response as it combines the properties of a neutralizing antibody and of an immune-regulator that induces the effector side of the immune response in a restrictive way, preserving gut homeostasis.

Immunological properties of mucosal dendritic cells and macrophages

The ensuing activation of the naïve T cell response is mainly caused by dendritic cells that migrate or reside in the interfollicular regions of the Peyer's patch where T cells and high endothelial venules are located. Several different subsets of dendritic cells have been identified, exhibiting unique functional and phenotypic characteristics. Most of the immature dendritic cells within the lymphoid organs are classified as blood-derived dendritic cells. This subgroup includes $CD8\alpha^+$ dendritic cells that are capable of presenting viral pathogens to T cells but can also induce tolerance upon contact with commensal antigens. The other main population are $CD8\alpha$–$CD11\beta^+$ dendritic cells, which in the absence of antigen exposure may be involved in the generation of Th2 or Th3 response, which is characteristically mediated by TGF-β. The dendritic cells residing in the lamina propria are directly exposed to the antigen overload in the gut and have the crucial task of eliciting an immune response against pathogens while maintaining tolerance to commensal microflora. It has been shown that in the absence of pathogenic antigens these dendritic cells are able to prime a Th3 response with ensuing TGF-β production in the mesenteric lymph nodes.

Immature dendritic cells originally reside in peripheral tissues with an emphasis on its phagocytic abilities and diminished expression of MHC class II or co-stimulatory molecules. Dendritic cells are directly activated following engagement with bacterial antigens through TLRs or indirectly through the influence of inflammatory cytokines such as TNFα or IL-1 produced by activated macrophages. These matured dendritic cells now are potent APCs, that are capable of presenting bacterial antigens to naïve and primed T cells, processing the antigens that were afore transported by M cells. Moreover, lamina propria dendritic cells express tight junction-associated proteins (e.g. occluding, claudin 1, zonula occludens 1) that enables them to extend their dendrites to penetrate the epithelial layer without disrupting its integrity and sample luminal antigens directly from the intestinal epithelial surface. The now matured dendritic cells exhibit a reduced phagocytic activity, while migrating from the periphery to the draining lymph nodes to present the processed pathogenic peptides to naïve T lymphocytes through the now expressed MHC class II molecules. This migration is mediated by TLR-induced downregulation of inflammatory chemokine receptors and upregulation of the receptors for lymphoid chemokines. The myeloid $CD11\beta^+CD8\alpha^-$ dendritic cell subset has been shown to migrate to the subepithelial dome of Peyer's patches and the intestinal epithelium through engagement of its chemokine receptor CCR6 with the chemokine macrophage inflammatory protein 3α (MIP 3α). The activated dendritic cells do not migrate to other lymphoid compartments as they contain the immune response to a local level. Inside the lymph node the dendritic cells induce the activation and differentiation of antigen-specific T cells into effector cells. This activation is mediated by an antigen-specific signal that is induced upon binding of the TCR to the pathogenic peptides presented by MHC molecules and by a second signal provided by co-stimulatory molecules (e.g. CD80 and CD86) which are expressed by dendritic cells and trigger CD28 expression on naïve T cells. The differentiation of activated $CD4^+$ T cells is in part influenced by TLR-mediated production of IL-12 by dendritic cells, which lead to the induction of a Th1 response. In addition, recent reports indicate that dendritic cells might also be able to polarize antigen-specific T cells into producing Th2 cytokines [3,17,18]. Intestinal lymphoid dendritic cells are also capable of modulating naïve T cells to differentiate into Treg subsets and moreover to support them through increased production of the immunregulatory cytokine IL-10. Recent studies have indicated that the IL-10 production of these dendritic cells is induced by cyclooxygenase 2 (COX2)-dependent prostaglandin E_2 (PGE_2), which is constitutively produced by mucosal stromal cells upon exposure to physiological levels of the bacterial component LPS [19]. This mechanism reflects a basal state of the lamina propria in which commensal bacteria promote the downregulation of the intestinal immune response. In addition, dendritic cells can modulate class switching of B cells to secrete IgA through direct interaction with B cells via cell surface BAFF and APRIL or indirectly through increased IL-6 production [20]. These subsets of dendritic cells are conditioned by epithelial cells through the constitutive release of thymic stromal lymphopoietin (TSLP) and other mediators [21].

Located in the subepithelium is also a population of intestinal macrophages that do not express innate response receptors or produce proinflammatory cytokines in response to antigen uptake, but have retained their phagocytic and bactericidal activity. These findings indicate a mechanism of destroying luminal antigens that have

leaked across the epithelium without inducing an inflammatory reaction, thereby preserving gut homeostasis [22].

Migration of mucosal lymphocytes

Initially naïve T cells only migrate between secondary lymphoid organs and do not enter the peripheral tissue. After the aforementioned antigen exposure, the now primed T cells alter their migratory behavior and leave the Peyer's patches through the draining lymphatic vessels to the mesenteric lymph nodes and the thoracic duct to the systemic circulation. These T cells are still able to return to the lymphoid organs but are now also able to migrate into peripheral tissues. The activated effector and memory T cells migrate preferentially to tissues that are connected to the secondary lymphoid organs where the antigen was first encountered and which are most likely still to contain their cognate antigen. This gut-homing specificity on T cells is imprinted by dendritic cells from gut-associated lymphoid tissues. The mechanism for dendritic cell-dependent imprinting of gut specificity is still largely unknown, but recent results indicate that intestinal dendritic cell-produced retinoic acid is essential for the induction of gut-homing receptors. This selective expression of adhesion molecules recognizing tissue-specific vascular addressins on the endothelium is essential for intestinal homing. Two main gut homing molecules identified are the integrin α4bβ7 and the chemokine receptor CCR9. CCR9 is expressed in the majority of small bowel lamina propria mononuclear cells and essentially all $CD4^+$ and $CD8^+$ T lymphocytes of the small intestine. This enables the lymphocytes to migrate to the thymus-expressed chemokine (TECK or Ckβ-15/CCL25), which is constitutively expressed by intestinal epithelial cells and endothelial cells of the lamina propria in the small intestine but not in the colon. The other major adhesion molecule expressed on mucosal lymphocytes is the α4β7 integrin. This integrin is a heterodimer membrane glycoprotein that consists of two non-covalent linked α and β subunits. Several studies have shown the decisive role of α4β7 integrin in the migration of lymphocytes into the mucosal tissue. For example, it could be demonstrated that lamina propria lymphocytes express high levels of α4β4 on their surface, as do lymphocytes located near high endothelial venules of Peyer's patches. Other adhesion molecules that are also involved in the migration process include L-selectin, which participates in the homing of lymphocytes to mucosal sites, especially Peyer's patches, but does not contribute substantially to the migration of T cells into the intestinal wall. This finding supports a major, but not exclusive role for the α4β7 integrin in lymphocyte migration to mucosal sites. The main ligand for the α4β7 integrin is the mucosal addressin cell adhesion molecule 1 (MAdCAM-1), which is selectively expressed on high endothelial venules of Peyer's patches and mesenteric lymph nodes, and also on vascular epithelium of the lamina propria, but not on high endothelial venules of non-mucosal lymph nodes. Circulating lymphocytes recognize MAdCAM-1, which promotes their rolling and adhesion to the vascular wall and subsequent extravasation into the lymphoid tissue.

These findings implicate a distinctive mechanism of lymphocyte recruitment that permits functional specialization of immune responses in specific segments of the gut. Correspondingly T cells that are primed in peripheral lymphoid organs acquire the α4β1 integrin VLA4 and the chemokine receptor CCR4, which hinders them from migrating into mucosal surfaces. This is consistent with the model of a compartmentalized mucosal immune system where stimulated mucosal lymphocytes preferentially migrate into the mucosal tissue where the immunological response was induced.

The gastrointestinal immune response – the effector site

Introduction to the lamina propria lymphocytes

The effector site of the intestinal immune response is the lamina propria, where the mature lymphocytes migrate into following their induction in the Peyer's patches and differentiation in mesenteric lymph nodes. Upon entering the mucosa, they are allocated into different compartments. The B cells that undergo terminal differentiation into IgA-producing plasma cells, as well as $CD4^+$, and a large number of $CD8^+$ T lymphocytes remain in the lamina propria, while another fraction of $CD8^+$ T cells migrates to the epithelium. Apart from these effector cells, the lamina propria also contains a regulatory subset of T lymphocytes. Among these are Treg cells, which are characterized by a low proliferative capacity, expression of the cell-surface CTLA-4 receptor and augmented production of immunosuppressive cytokines such as TGFβ and IL-10. Both TGFβ-producing Th3 cells and $CD4^+CD25^+$ Tregs have been implicated to be generated in the induction of oral tolerance. Apart from the large amount of lymphocytes, the lamina propria also harbors macrophages, dendritic cells, mast cells and neutrophils.

Lamina propria T cells

The intestinal lamina propria and the epithelium is the largest single T cell site in the human organism and these lymphocytes display unique characteristics that reflect their specific roles in the mucosal immune system. The majority of these lymphocytes that migrate to the mucosa and therefore extravasate into the lamina propria or the epithelium are memory cells that have already been in contact with antigens in the organized lymphoid tissue. These lymphocytes are a specialized subset of conventional memory cells that predominantly are $CD45R0^+$ and

express the TCRαβ. These $CD4^+$ and $CD8^+$ T cells distribute in different sections of the intestinal tissue, as $CD4^+$ T cells are largely located in the lamina propria and $CD8^+$ lymphocytes reside along the epithelium. Recent studies have elucidated that the dendritic cells of the inductive sites determine the gut tropism of lamina propria lymphocytes by the induction of specific adhesion molecules [23].

The mucosal immune response is therefore normally characterized by the major expansion of antigen-specific T cells, in order to elicit a strong immune response against potential pathogens, but at the same time also harbors the risk of substantial autoreactivity or the induction of mucosal inflammation. Therefore, the intestinal immune system has evolved several effector mechanisms that contain excessive immune responses.

Due to the continuous exposure to antigens from the gut lumen, the T lymphocytes reside in an increased state of activation exemplified by increased IL-2Rα, HLA-DR and transferrin receptor expression. Intestinal lamina propria T cells were found to produce spontaneously the classical Th1 cytokine IFN-γ, and also to a lesser extent IL-4. Stimulation of these lymphocytes via the alternative pathway of activation (CD2 and CD28) induces a strong cytokine response, again dominated by IFN-γ. This cytokine response upon stimulation is due to the differentiation of naïve T cells in Peyer's patches to Th1 cells after antigen exposure. Correspondingly, compared with peripheral blood lymphocytes the proliferation of lamina propria T cells to TCR/CD3 engagement is markedly reduced, but relatively unchanged when stimulated via the CD2 accessory signaling pathway [24]. In addition, despite the aforementioned fact that lamina propria T cells have increased expression of IL-2R, TCR/CD3 stimulated T cells do not proliferate when cultured in IL-2. This indicates that lamina propria T lymphocytes have been rendered partially anergic upon TCR/CD3 stimulation. This anergic state of the T cells is principally reversible, when stimulated and cultured with IL-2, but this cytokine makes them also more susceptible to apoptotic cell death. It has been proposed that the effect of IL-2 on apoptosis depends on the pre-existing state of T cell activation. Therefore, in non-anergic T cells, reactivation in the presence of IL-2 may lead to apoptosis, whereas relative unresponsive cells exhibit reduced anergy and thus reduced apoptosis in the presence of IL-2.

The programmed cell death (apoptosis) can occur via an active mechanism following TCR stimulation or via a passive mechanism following cytokine (e.g. IL-2) withdrawal. This leads to the elimination of lamina propria T cells before they can mediate an exaggerated immune response [25]. Unstimulated lamina propria T cells exhibit increased susceptibility to Fas-mediated apoptosis compared with peripheral blood lymphocytes. In addition, these lymphocytes also exhibit augmented spontaneous apoptosis, which is probably associated with IL-2 withdrawal. Consistent with these findings, stimulation of lamina propria T cells via CD2 leads to further immunologically induced apoptosis through the Fas/FasL pathway [26]. This pathway is in part regulated by cellular expression of Fas and FasL, as Fas is solely activated by the membrane bound form of its ligand FasL. The cellular mechanisms that control the expression of both molecules seem to be independent of each other. Recent data suggest that strong upregulation of FasL occurs at an early stage of T cell activation whereas Fas is expressed later in the course of activation. Therefore, the expression of Fas on T cells could have the effect of an inhibited immune response through the mediation of apoptosis upon ligation with FasL [27]. The mechanism of activation-induced apoptosis among activated lamina propria T cells strictly regulates the expansion of antigen-stimulated T cells and thereby promotes intestinal homeostasis. Consequently, various data indicate that augmented T cell resistance against apoptosis contributes to inappropriate T cell accumulation and the perpetuation of chronic mucosal inflammation in inflammatory bowel disease [25].

Consistent with the expression of CD8αβ T cells, the α chain of the CD8 lymphocytes conveys cytotoxic activities against MHC I class restricted antigens, whereas the β chain acts as a TCR co-receptor for the recognition of cytotoxic T cell epitope antigens. These CD8αβ T cells therefore express the pore-forming protein perforin and cytolytic granules containing granzyme proteases, exhibiting cytotoxic activity against pathogenic antigens.

The role of regulatory T cells in maintaining homeostasis in gut mucosal immunity

Among the many cell types that have been shown to contribute to immune regulation in the intestine, $CD4^+$ Tregs are one of the most important as they exert various suppressive functions through a number of independent pathways. Different types of Tregs have been described based on distinct expression patterns of surface markers and cytokines.

Recent studies underlined the central role of naturally occurring $CD4^+CD25^+$ cells in maintaining self-tolerance and a possible role in oral tolerance. The cells that mediate this effect have been identified as nTregs that make up 90% of the $CD4^+CD25^+$ cell subset. nTregs can develop in the thymus and have the ability to recognize commensal antigens and foreign antigens. The most specific marker for nTregs is the transcription factor Foxp3, a member of the forkhead box family that is crucial for nTreg generation and activity. Antigen-specific Tregs can be also be generated through induction from Foxp3 precursors under specific conditions that seem to be TGFβ dependent. These cells are phenotypically and functionally similar to thymic-derived $Foxp3^+$ cells as they up regulate CD25 and cytotoxic T lymphocyte antigen-4 (CTLA-4) and produce TGFβ. CTLA-4 is a member of the CD28

costimulatory/inhibitory family of cell surface receptors that is upregulated upon T cell activation. CTLA-4 has an important function in controlling the immune response, as it inhibits T cell activation and therefore plays a decisive role in Treg activity. Another class of induced Tregs is Th3 cells that were first identified following oral tolerance induction. Their suppressor activity is characteristically mediated by TGFβ, which also promotes Th3 generation. Currently, it is not definitely clarified whether Th3 and $Foxp3^+$ Tregs are independent populations. Another well-described induced Treg population is IL-10-secreting Tr1 cells. These are induced following mucosal administration of antigen and their development and function are IL-10 dependent, inhibiting pathological response to normal flora.

The production of the immunosuppressive cytokine TGFβ through which numerous immune-regulative effects are steered is one of the most outstanding characteristics of these cells. TGFβ is a potent regulatory cytokine produced by various cell including Tregs. The pivotal function of TGFβ in the immune system is to maintain tolerance via the regulation of antigen-induced proliferation and differentiation of T lymphocytes. TGFβ also exerts its function on various other cell types of the immune system as it inhibits dendritic cell maturation, leading to tolerogenic antigen presentation, and furthermore controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation and survival of natural killer cells, macrophages and granulocytes. Apart from suppressive effects TGFβ is also involved in inducing $FoxP3^+$ Tregs and is involved in the survival and proliferation of nTregs. The regulatory activity of TGFβ in turn is also tightly controlled, as it is normally secreted in a latent form that can only bind to its receptor upon activation, which depends on the cell differentiation state and the presence of inflammatory cytokines and co-stimulatory molecules [17,28].

Another mechanism through which Tregs exert their suppressive functions is the production of the anti-inflammatory cytokine IL-10, which is also produced by many other cell types, such as dendritic cells or monocytes. IL-10 is a multifunctional cytokine which exerts its diverse effects on various intestinal cells, as it can inhibit their activation, effector function, growth and differentiation. In activated macrophages and monocytes, IL-10 potently inhibits the production of the pro-inflammatory cytokines IL-1 and TNFα, which often have synergistic activities on inflammatory pathways and processes, and in addition also enhance the production of the anti-inflammatory IL-1 receptor antagonist. Moreover IL-10 also inhibits the production of distinct chemokines that are implicated in the recruitment of different cell types to the site of inflammation, by activated monocytes. Furthermore, it inhibits the expression of MHC class II antigens on monocytes and dendritic cells, which significantly diminishes their antigen-presenting capacity towards T cells, leading to inhibited cytokine production and proliferation of $CD4^+$ T cells. IL-10 also directly affects the function of T cells as it inhibits IL-2 and TNFα production, but on the contrary also has stimulatory effects on $CD8^+$ T cells, as it induces their recruitment and cytotoxic activity. The pivotal role of IL-10 in the survival, proliferation and differentiation of B cells has been extensively studied and in the gut, IL-10 contributes to isotype switching of B cells to IgA-producing plasma cells. The distinct effects of IL-10 in comparison with TGFβ in the intestinal immune system is best visible in knockout mouse models for these cytokines. Whereas TGFβ-deficient mice develop a fatal multiorgan inflammatory disease, IL-10-deficient mice exhibit an inflammatory reaction that is limited to the intestines. These experiments elucidate two separate regulatory mechanisms, suggesting a TGFβ-mediated suppressive effect for the generalized immune system and a more mucosa-specific prevention of inflammatory reactions for IL-10 [28,29].

Tregs have an important function in the intestinal immune system as they exert their various regulative effects on both the adaptive and innate immune system. On the one hand they are able to prevent T cell activation and proliferation in lymphoid organs to prevent an inflammatory response and on the other they can also accumulate in the inflamed gut to suppress the aberrant immunological response in an ongoing mucosal inflammation.

General mechanisms of oral tolerance

Oral tolerance is defined as a physiological mechanism that downmodulates systemic immune response to a soluble antigen following previous introduction of the antigen orally. This mechanism basically prevents the development of inappropriate immune responses against food antigens or commensal flora which could otherwise lead to a chronic inflammatory state or delayed-type hypersensitivity reactions. In general, the type of immune response tolerated depends on the amount of antigen applied and the frequency of oral administration. Tolerance of cell-mediated immunity and IgE responses requires lower doses of antigen than tolerance of IgG response and similarly tolerance of Th1 cells needs fewer antigens than tolerance of Th2 cells. In humans, oral feeding of antigen results in systemic T cell tolerance visible in diminished T-cell proliferation, whereas B cell response in the form of IgM and IgA production is unaffected. The major mechanisms of tolerance induction are clonal deletion or clonal anergy of antigen-specific $CD4^+$ T cells and inhibition of the immune response through the induction of regulatory T cells. In general, low-dose uptake of oral antigen favors active suppression, whereas high-dose feeding protocols favor clonal anergy or clonal deletion. Anergy describes the inactivation of $CD4^+$ T cells leading to an absent

immunological reaction upon antigen contact. T-cell anergy is partly induced by T cell recognition of antigens displayed by APCs in the absence of inflammatory stimuli. The APCs express low levels of co-stimulatory molecules that particularly engage the inhibitory T cell counter-receptor cytotoxic T lymphocyte antigen-4 (CTLA-4) [30]. Recent data elucidated the dependence of antigen-specific T cells towards the expression of this regulatory molecule for oral tolerance [31]. The induction of Tregs that mediate their suppressive effects through production of TGFβ and IL-10 or through modulation of cell–cell contact involving cell surface TGFβ and CTLA-4 is also a crucial step in oral tolerance. The widely accepted concept that intestinal immune responses depend exclusively on antigen uptake by M cells in Peyer's patches has been challenged by recent reports that observed induction of oral tolerance in Peyer's patch-deficient intestines [32]. Mesenteric lymph nodes, on the other hand, are believed to be the pivotal site for induction of oral tolerance. Recent experiments indicated that T cells do not necessarily only migrate to the mesenteric lymph nodes after activation in Peyer's patches, but that they are instead primed in the mesenteric lymph nodes after CCR-7-dependent migration of antigen-loaded dendritic cells from the lamina propria or Peyer's patches [33]. The influence of the commensal flora on the induction of oral tolerance is not yet fully understood, but it could be found that its presence was critical for the suppression of systemic antigen-specific IgE responses and Th2 cytokine response after oral intake of the antigen. Indirect evidence of the importance of the intestinal bacterial flora for oral tolerance induction is also derived from studies in patients with inflammatory bowel disease, where an aberrant immune response is elicited against the endogenous flora. Correspondingly, oral antigen administration did not result in oral tolerance in inflammatory bowel disease patients, but actually led to active immunity [34].

The relationship between commensal bacteria and the mucosal immune system

The human organism is constantly exposed to and interacts with resident bacteria and transiently present microbes, as the number of the autochthonous microbes living on mucosal surfaces (10^{14}) exceeds the number of cells forming the human body (10^{13}). The largest amounts of bacteria are colonized in the gastrointestinal tract and mainly reside in the lumen, outside the mucus layer [35]. The fetal gut is initially sterile, but colonization begins immediately after birth and is influenced by the mode of delivery, the infant diet, hygiene levels and medication [36]. The bacterial density gradually increases from the proximal to the distal regions of the intestine, as the duodenum and jejunum harbor only a few bacteria, whereas the ileum and especially the colon contain a large and diverse microbial population. More than 90% of the diverse microbial population are obligate anaerobes which consist predominantly of Bacteroides, Eubacterium, Bifidobacterium, Fusobacterium and many others [35].

The role of the commensal flora in several important intestinal functions has been intensively studied in animal models reared under germ-free conditions. The reconstitution of these gnotobiological models with defined commensal microbes permits the differentiation between genetically determined developments in the intestine from those induced by commensal bacteria. These studies revealed that commensal bacteria are able to modulate the expression of genes involved in several important physiological intestinal functions. It could be shown that commensal bacteria lead to the fortification of the intestinal epithelial barrier, which is critical in constraining intestinal microbes [35]. Furthermore, microbials improve nutrient absorption and processing and raise gut motility and angiogenesis. Furthermore, they enhance the hosts capacity to metabolize xenobiotics and endogenous toxins [37]. These findings underline the enormous metabolic activity of the intestinal microbial flora that can therefore be classified as a virtual organ within an organ. Apart from these metabolic properties, the commensal microflora also has a profound impact on various immunologic mechanisms of the host, as it exerts important regulatory mechanisms that contribute to the delicate balance of both mucosal and systemic immunity. While the components of the microflora play a crucial role in stimulating postnatal development of the local and systemic immune system, they later have a fairly important role in inhibiting a strong mucosal immune response. In animals reared under germ-free conditions, major alterations of the GALT structure and function were described, as these animals have fewer intraepithelial lymphocytes and reduced serum immunoglobulin levels and are more susceptible to infections. The reconstitution of these germ-free mice with the intestinal microflora, however, is sufficient to restore the mucosal immune system [35]. The analysis of these data elucidates the pivotal role of commensal bacteria in modulating fundamental intestinal functions in the host and in return is tolerated by the host providing a niche for colonization and growth, resulting in a mutually beneficial relationship. Therefore, the host defense requires the ability to distinguish accurately between commensal organisms and pathogens. The ability of immune cells to discriminate reliably pathogenic from commensal bacteria is mediated by a host pattern recognition system that detects bacterial pathogens and triggers anti-microbial host defense responses. This strategy is based on the detection of a limited set of conserved molecular patterns that are invariant among microbial pathogens, the so-called pathogen-associated molecular patterns (PAMPs). These PAMPs are

the target of pattern recognition receptors (PPRs) that signal the host the presence of infectious pathogens and trigger a multitude of anti-microbial and inflammatory responses. PPRs consist of the family of TLRs and the nucleotide-binding oligomerization domain/caspase recruitment domain isoforms (NOD/CARD) receptors and are expressed on a variety of cell types, including epithelial cells, dendritic cells and even lymphocytes. The TLR family is the best characterized class of PPRs and is expressed, among others, on enterocytes and dendritic cells. The pathogen-responsive TLRs detect multiple microbial-associated molecular patterns, including lipopolysaccharides (detected by TLR4), bacterial lipoproteins and lipoteichoic acids (detected by TLR2), flagellin (detected by TLR5), unmethylated bacterial and viral CpG DNA motifs (detected by TLR9), double-stranded RNA (detected by TLR3) and single-stranded viral RNA (detected by TLR7). The recognition of these PAMPs through TLRs initiates the migration of cells to the site of the infection through expression of chemokines and cell surface adhesion molecules [12]. The activation of TLRs leads to the expression of selectins on the endothelial cells, such as the intercellular cell adhesion molecule-1 (ICAM-1), which leads to the adhesion of leukocytes to the endothelium. The secretion of chemokines, like IL-8 and MCP-1, also leads to the activation of monocytes, neutrophils and NK cells and to their subsequent migration into the mucosa, in addition to the recruitment of various other tissue-resident innate cells, such as dendritic cells that are crucial for naïve T cell activation and differentiation [12].

When a microbial component engages a TLR, a Toll/IL-1R homology (TIR) cytoplasmatic signaling domain dimerizes and recruits TIR domain-containing adapter molecules to its domain. The ensuing effector functions downstream of TLR signaling are dependent on the differential recruitment of these adapter molecules. Moreover, some intestinal enterocytes constitutively or inducibly express high levels of the Toll-interacting protein (Tollip) that down regulates TLR surface expression upon repeated contact with bacterial antigens [38]. This mechanism could be a direct result of the continuous presence of specific bacterial components leading to a status of hyporesponsiveness in otherwise reactive IECs and thereby contributing to mucosal homeostasis. On the other hand, stimulation of dendritic cells with a corresponding TLR ligand can also lead to an enhanced immune response as this activation of dendritic cells leads to enhanced IL-6 secretion, which in turn reduces Treg activity. However, reversal of Treg anergy is also dependent on TLR activation of dendritic cells and subsequent IL-6 production, as soon as the pro-inflammatory signal is absent, leading to Treg proliferation. In addition to these indirect effects on Tregs mediated by dendritic cells, PPRs can also act directly on TLR-expressing Tregs, leading to enhancement or suppression of the immune response. In addition, TLR activation modulates migration of intestinal dendritic cells and generation of T cell-dependent antigen-specific antibody response in B cells. Moreover, TLRs are also involved in the presentation of the complex of phagocytosed antigen and MHC class II molecules by dendritic cells. Recent evidence suggests that NOD2, which is expressed by monocytes, dendritic cells and some epithelial cells, also has the property to mediate pro- and anti-inflammatory signals. These findings show that commensal bacteria are recognized by PPRs under normal steady-state conditions and that this interaction plays a crucial role in the maintenance of intestinal immune homeostasis [12]. On the other hand, PPRs are also vital for the host's immune reaction against potential microbial pathogens and are critically involved in the initiation of adaptive immune responses. PPRs therefore mediate a delicate balance between effector and regulatory T cells and an abrogation of this equilibrium can lead to augmented immune reactions, as in inflammatory bowel disease where mutations in the NOD2 gene possibly contribute to disease pathogenesis. Another mechanism that mediates mucosal tolerance is the inhibition of the transcription factor nuclear factor (NF)-κB by commensal bacteria. It is well known that that control of the inflammatory response to microbial and viral infections is closely related to the activation of NF-κB signaling pathways through degradation of the NF-κB counter-regulatory factor IκBα. Several distinct mechanisms by which commensal bacteria inhibit NF-κB signaling have been elucidated. These include inhibition of IκBα degradation and nuclear export of the activated NF-κB subunit p65, through a peroxisome proliferator-activated receptor (PPAR)-γ-dependent pathway, thereby terminating promoter activation [36].

Nevertheless, the exact mechanisms by which immune cells discriminate commensal from pathogenic organisms on the basis of TLR signals are only vaguely understood.

Conclusion

This overview has illustrated that the intestinal immune system has a remarkable capacity to maintain a state of equilibrium in spite of its constant encounter with commensal and pathogenic antigens. It has been shown that the gut mucosal surface is an active site for immune suppression of unnecessary harmful reactions (oral tolerance) and for the generation of protective responses (controlled inflammation) that maintain mucosal homeostasis. The state of non-responsiveness that often characterizes the mucosal immune system is not a passive process but rather involves the active suppression of a potential exuberant immune response mediated by a network of sophisticated mechanisms of immunoregulation. This network is formed by an array of constitutive, non-specific defense mechanisms acting together with an inducible, highly

specialized local immune system. These findings underscore the unique nature of immunoregulation at the intestinal mucosal site. The prevention of a systemic immune response upon antigen encounter in the gut is based on the restriction of the local immune response against antigens to its compartment, thereby making the suppression of a systemic immune response unnecessary. The aforementioned mechanisms underline that the barrier function of the mesenteric lymph node is critical to contain the gut mucosal response, as dendritic cells loaded with antigens cannot penetrate beyond the mesenteric lymph node and are thus hindered to reach systemic secondary lymphoid structures. This barrier function avoids unnecessary systemic exposure of antigens with accompanying inflammatory reactions. Overall, further insights into the mechanisms that maintain mucosal homeostasis could lead to better strategies for suppressing harmful immune responses and for augmenting and sustaining beneficial immune responses to microbial vaccines and tumors.

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Chapter 7
Innate Immunity and its Implications on Pathogenesis of Inflammatory Bowel Disease

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Summary
- The intestinal epithelium forms a barrier against luminal microorganisms. Innate immunity plays a crucial role in maintenance of that barrier, including induction of antimicrobial peptides and wound healing.
- In the normal mucosa, downregulation of innate immunity contributes to intestinal homeostasis.
- Recent genetic, serological and animal studies support dysfunction of the mucosal innate immune response to commensal flora as a central mechanism in the pathogenesis of IBD.
- Toll-like receptors (TLRs) are the principal component of mucosal innate immunity. In general, the pathogenesis of ulcerative colitis and Crohn's disease may be due to increased or decreased TLR signaling, respectively.
- Our recent research demonstrates the possible role of TLR signaling in the development of colitis-associated neoplasia. Better understanding of the innate immune response to commensal bacteria will lead to the development of targeted and more effective therapy for patients with IBD.

Introduction

A great deal of research into the pathogenesis of inflammatory bowel disease (IBD) has painted a picture of a dysregulated adaptive immune response to the presence of luminal bacteria. No specific pathogen has thus far been identified as the cause of ulcerative colitis or Crohn's disease. The differences in the clinical manifestations of these two disorders in their extreme forms points to different pathogenetic mechanisms. The focus of this chapter is innate immunity as it relates to IBD.

The innate immune system provides immediate protection from microbes [1,2] (Figure 7.1). The initial role of the innate immune system is to identify the presence of an infection, orchestrate an inflammatory response to clear the pathogen and prime the adaptive immune response to provide an anamnestic response to the pathogen in the future [3]. Deviations from this tight distinction between a pathogen and commensal bacterium may be crucial to the pathogenesis of IBD. The cells of the innate immune system as relates to the intestine include dendritic cells, macrophages, neutrophils and epithelial cells. The vascular endothelium may also be called upon to recruit inflammatory cells in response to perceived or real pathogens. In this chapter, we describe what is known about innate immune responses in human and murine models of IBD. Innate immune defects are at the center stage of the identified genetic polymorphisms present in individuals with IBD. Aberrations in innate immunity may underlie IBD susceptibility (especially Crohn's disease), complications of IBD such as cancer and response (or lack of response) to medical or biological therapy in IBD.

In normal intestinal mucosa, the presence of bacterial invasion provokes an activation of rapid innate immune response that induces inflammatory cytokine release followed by phagocytic macrophage and neutrophil influx, resulting in clearance of intruders. Crohn's disease may result from defective function of this intestinal innate immune defense against the persistent hazard of bacterial invasion into the mucosa. For example, Marks *et al.* [4] reported a defective acute inflammatory response in the intestinal mucosa in patients with Crohn's disease. They assessed the acute inflammatory response provoked by a biopsy in normal-appearing ileal and rectal mucosa in healthy individuals and patients with Crohn's disease. After a repeat biopsy from the same site in 6 h, they found decreased IL-8 expression and accumulation of

Inflammatory Bowel Disease. Edited by S. R. Targan, F. Shanahan and L. C. Karp.

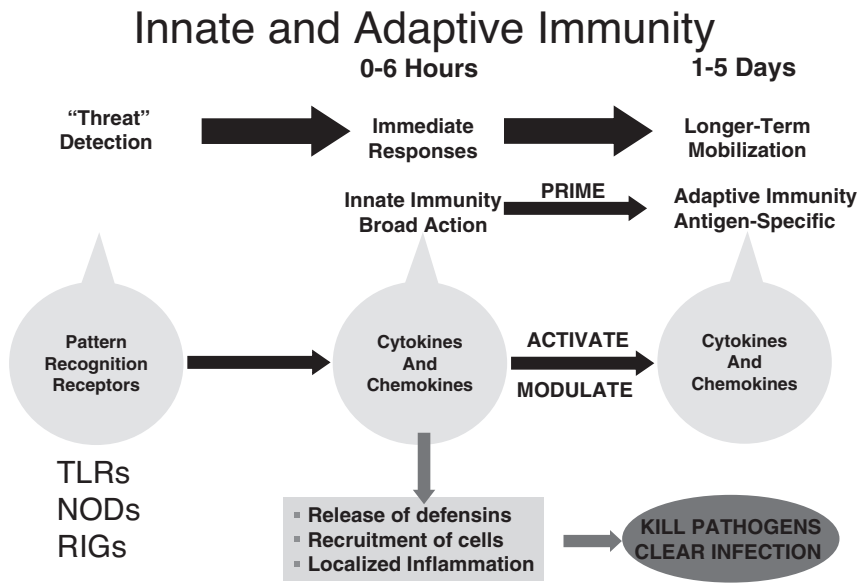

Figure 7.1 Innate and adaptive immunity. The innate immune response is characterized by rapid recognition of a pathogen through gene-encoded pattern recognition receptors. This early recognition results in the production of chemokines and cytokines that aim to recruit inflammatory cells that clear the invading pathogen or in some cases clear commensal bacteria that have gained access to the lamina propria through some break in the mucosal barrier. The innate immune response then is critical for priming an adaptive immune response through the generation of activated, mature macrophages and dendritic cells which present antigen and determine the type of T cell response most appropriate for the pathogen.

neutrophils in patients with Crohn's disease compared with healthy controls. These results suggest that stimulation or restoration of innate immunity may be effective therapy of Crohn's disease by addressing a primary defect. In this regard, GM-CSF may bypass an unknown mucosal innate immune defect underlying the pathogenesis of Crohn's disease. Of course, in treating human disease one must always grapple with attempting to stop a process that is already well entrenched. Therefore, restoring innate immunity may work better to prevent than to treat IBD.

Overview of the innate immune system

The intestinal innate immune system is composed of several components that contribute to the barrier against luminal pathogens. The intestinal mucosa must avoid an excessive immune response to commensal bacteria in order to coexist with the extremely high concentration of microbes and their pathogen-associated molecular patterns (PAMPs). First, the surface epithelium serves as an initial defense in the mucosal innate immune system. The recognition of pathogens is an important function of innate immune cells as they express selective microbial sensing receptors, such as TLRs and Nods [5,6]. In their normal state, intestinal epithelial cells do not react to luminal commensals, yet can control against microbial invasion [7–9]. Under a single layer of intestinal epithelial cells, many other innate immune cells, including macrophages, dendritic cells (DCs) and B cells, can also express microbial sensing receptors [10]. Once stimulated, these antigen-presenting cells (APCs) immediately progress to perform their respective effector functions. Acute inflammatory cells, including neutrophils, will be triggered by secreted chemokines. Innate immune signaling leads to DC maturation, which is crucial for appropriate activation of adaptive immunity (Figure 7.2). This comprehensive system of innate immunity finally induces regulatory T cells (Tregs), which act to suppress excess inflammation. Damage of the epithelial defense can allow increased contact of luminal agents with the inflammatory and immune cells in the lamina propria. Thus, a primary epithelial defect can trigger the activation of the sequential innate immune responses resulting in mucosal inflammation.

The innate immune system defends the host from infection by potential pathogens in a generic fashion and does so within minutes to hours of an infection. Unlike the adaptive immune system, which can take days to cultivate a specific T cell or B cell response through gene rearrangements of their respective receptors, the innate immune system uses germline-encoded receptors called pattern-recognition receptors (PRRs), that recognize classes of PAMPs. These PRRs include Toll-like receptors (TLRs), nucleotide oligomerization domain proteins (Nods), scavenger receptors and the cytosolic RNA helicase family (RIG-1, MDA5). Certain PRRs are expressed as transmembrane proteins (most TLRs), whereas others are only intracellular sensors (Nods and RIG-1, MDA5). Nods (mainly NOD1 and NOD2) are involved in intracellular antibacterial response that have caspase recruitment domains (CARDs) as PRRs and activate NF-κB as a result of its activation. However, little is currently understood about how bacterial products obtain intracellular access to PRRs. RIG-1 and MDA5 are RNA helicases. These also have CARDs and are involved in sensing of intracellular dsRNA and induction of type I IFN in response to RNA virus replication [11]. Cytoplasmic RIG-1 and MDA5 activate NF-κB and IRF3 via the adaptor molecule IPS-1 located on mitochondria. These cytosolic RNA helicases

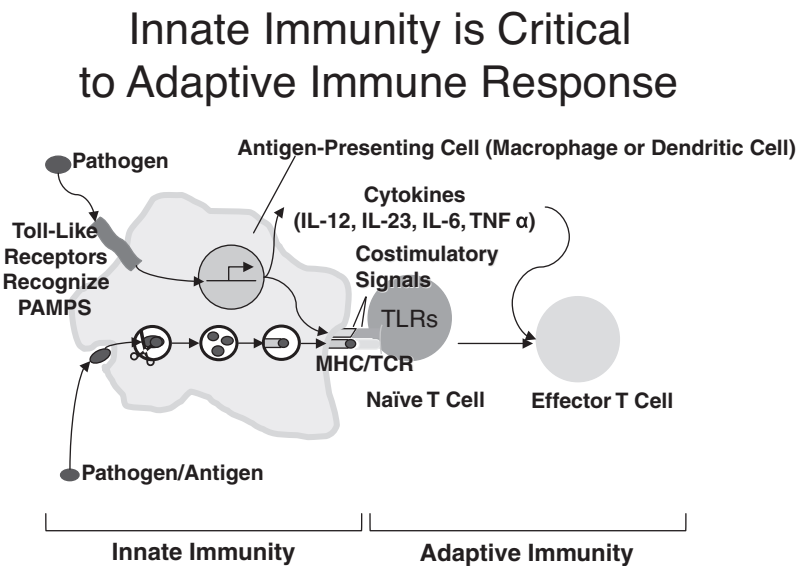

Figure 7.2 Innate immunity is required for T cell activation. Not only does exposure of pathogens or PAMPs result in maturation of macrophages and dendritic cells resulting in T cell activation, but TLRs are also present on naïve T cells and serve as co-stimulatory signals.

have been demonstrated to be expressed in human IECs and suppressed viral replication, suggesting an important role of these molecules in mucosal innate immune defense in the gut [12]. Each of these families of PRRs recognizes a different type of PAMP. PAMPs are characterized by common structural motifs and are often an essential part of the organisms such as the cell wall for bacteria and fungi or double-stranded RNA for viruses.

Innate and adaptive immunity exist to protect the host from danger, in particular microbial pathogens. Both innate and adaptive immunity, however, can serve as a double-edged sword, i.e. exuberant protection against pathogens may result in tissue damage to the host; insufficient responses to even healthy bacteria may result in bacterial invasion and secondary adaptive immune activation. As research into innate immunity continues to unfold, it will be nearly impossible to tease apart innate versus adaptive immunity since the generation of adaptive immunity is dependent on innate immune responses.

TLRs are members of a conserved interleukin-1 (IL-1) superfamily of transmembrane receptors that recognize PAMPs. In some cases, TLRs also recognize "damage-associated molecular patterns" such as hyaluronic acid, raising the intriguing possibility that TLRs can recognize both self and non-self danger signals. With respect to IBD, expression and function of TLRs and Nods have been most extensively studied. Traditionally, innate immune cells have been thought primarily to be antigen-presenting cells, i.e. macrophages or dendritic cells or neutrophils. More recently, however, intestinal epithelial cells and T cells have been found to express functional TLRs. The type of cell expressing the receptor, for example an intestinal epithelial cell versus a macrophage, provides another level of specificity for an innate response.

At present, a total of 13 TLRs have been identified in mammals (for a good review, see [13]). The first TLR described in mammals was TLR4, which is required for recognition of lipopolysaccharide (LPS) [14,15]. Other TLRs such as TLR2 in combination with TLR6 or TLR1 are required for recognition of peptidoglycan, lipoarabinomannan (TLR2), triacyl lipopeptides (TLR1/2), diacyl lipopeptides and lipotechoic acid (TLR2/6). Given the diversity of flora present in the intestine, it becomes readily apparent that TLRs must be carefully regulated in the intestine to avoid a needless inflammatory response to commensal organisms. Conversely, genetic data suggest that defective innate immunity may play a role in a subset of patients with Crohn's disease. In the following sections, we describe what is known about TLR regulation in the intestine in health and IBD and how the seeming paradox of innate immunity in IBD can be reconciled. Where possible, we identify specific cell types that express TLRs in the intestine.

Expression of TLRs in health and disease

On the basis of PAMPs, there is nothing that distinguishes commensal bacteria from pathogenic bacteria. Given the complexity of the bacterial flora in the human intestine, careful regulation of TLR signaling must take place in order to avoid an inappropriate inflammatory response. One of the emerging themes of research in innate immunity in the gut is the important role of TLR signaling in maintenance of intestinal homeostasis. This will be discussed in greater detail below. Since IBD is associated with inappropriate inflammation in the presence of seemingly normal commensal bacteria, several groups have studied expression of TLRs and Nods in human and murine IBD in the hope of identifying how bacterial sensing contributes to IBD pathogenesis. Like all research involving human IBD

Table 7.1 The expression profile of TLRs and their respective ligands in the gut.

TLR	Species	PAMPs*	Large intestine expression	Small intestine expression
TLR1	Human/mouse	PAM3CSK4 [199]	(+) RNA [17]	(+) RNA [18]
TLR2	Human/mouse	PAM2CSK4, MALP2, LTA, ZYM [200]	(+) RNA [17]	(+) Ileum protein [19], RNA functional [8]
TLR3	Human/mouse	dsRNA, poly-IC, viral RNA [54]	(+) Protein [19] (+) RNA [17]	(+) Protein [19]
TLR4	Human/mouse	LPS, MMTV, VSV-G, taxol, F protein, fibronectin, HSP60, HSP70, hyaluronan [201]	(+) RNA [7], (+) protein [19], (+) RNA [17]	(+) Protein [19]
TLR5	Human/mouse	Flagellins [202]	(+) Protein [19], (+) RNA [17]	(+) Ileum protein [19]
TLR6	Human/mouse	MALP2, LTA, ZYM [203,204]	(+) RNA [16,17]	Unknown
TLR7	Human/mouse	ssRNA, siRNA, IAQ (R848) [205–214]	(+) RNA [17]	(+) RNA [20]
TLR8	Human/mouse	ssRNA, siRNA, IAQ (R848) [205–209,211–214]	(+) RNA [17]	(–) RNA [20]
TLR9	Human/mouse	CpG-ODN [211]	(+) RNA [17]	(+) Ileum protein [61]
TLR10	Mouse	Unknown [215]	Not expressed [215]	Unknown
TLR11	Mouse	Profilin, uropathogenic bacteria [216,217]	Unknown	Unknown
TLR12	Mouse	Unknown [218]	Unknown	Unknown
TLR13	Mouse	Unknown [218]	Unknown	Unknown

*CpG-ODN, synthetic oligodeoxyribonucleotides containing CpG motifs; IAQ, imidazoquinolines, including resiquimod and imiquimod; LTA, lipoteichoic acid; MAL, MyD88 adapter-like; MALP2, macrophage-activating lipopeptide 2; MMTV, mouse mammary tumor virus; PAM3CSK4, synthetic triacylated lipopeptide Pam3Cys-SKKKK × 3 HCl; PAM2CSK4, synthetic diacylated lipopeptide Pam2Cys-SKKKK × 3 TFA; poly-IC, polyinosinic-polycytidylic acid; VSV-G, vesicular somatitis virus G protein; ZYM, zymosan.

samples, several caveats should be made. First, human tissue is generally taken from adult patients with established IBD wherein the changes in gene or protein expression may represent a late phase of the disease. In addition, immunohistochemical detection of TLRs or Nods has been limited by the difficulties in staining human intestine with its high endogenous peroxidase activity and the relatively poor quality of commercially available antibodies. Nevertheless, it is tempting to speculate that inappropriate TLR signaling may contribute to the loss of tolerance to the normal flora seen in IBD, thereby initiating and perpetuating intestinal inflammation.

The expression and function of several TLRs have been examined in the gut (Table 7.1). Almost all of the TLRs, TLR1–TLR9, are expressed in intestinal epithelial cells and also in other types of cells in the intestine [16–20]. Specialized intestinal epithelial enteroendocrine cells in the intestine express a variety of TLRs including TLR1, -2 and -4 [21]. Given that enteroendocrine cells secrete neuropeptides such as serotonin, somatostatin, motilin and cholecystokinin, it suggests that stimulation of these cells by pathogens or PAMPs can induce a secretory diarrhea and/or increased intestinal motility directed at flushing away luminal pathogens. In general, there is cross-talk between activation of TLRs and the up- or downregulation of signaling by other TLRs. For example, stimulation of TLR5 in intestinal epithelial cells appears to upregulate expression of TLR2 and TLR4 [22]. However, activation of TLR2 or TLR4 results in damped signals via the other TLR [17]. At least in part, this is due to increased expression of inhibitors of TLR signaling such as Tollip.

In the following sections, we break down the contribution of individual TLRs to gastrointestinal health and disease. By way of an oversimplified model, one can imagine that ulcerative colitis is a mucosal disease characterized by increased bacterial responsiveness in a relatively non-specific way. This hyper-responsiveness may be mediated by increased TLR signaling or failure to downregulate TLR signaling. Conversely, Crohn's disease, especially transmural small bowel disease, may represent a defect in innate immune signaling (TLRs and Nods), the consequence of which is bacterial translocation and a secondary adaptive immune response to luminal bacteria.

TLR4: the receptor for LPS and its role in intestinal homeostasis and IBD

As mentioned at the beginning of this chapter, innate immunity is a double-edged sword. TLR4 was the first-described TLR and therefore has received the most attention in gastrointestinal health and IBD. In the absence of TLR4 (C3H/HeJ mice or TLR4 knockout mice), animals are unable to recognize LPS. Protection from LPS-induced septic shock is unfortunately followed by death from overwhelming Gram-negative sepsis. As far as we know, humans lacking TLR4 function either do not exist or are extremely rare. Mutations in the signaling molecules

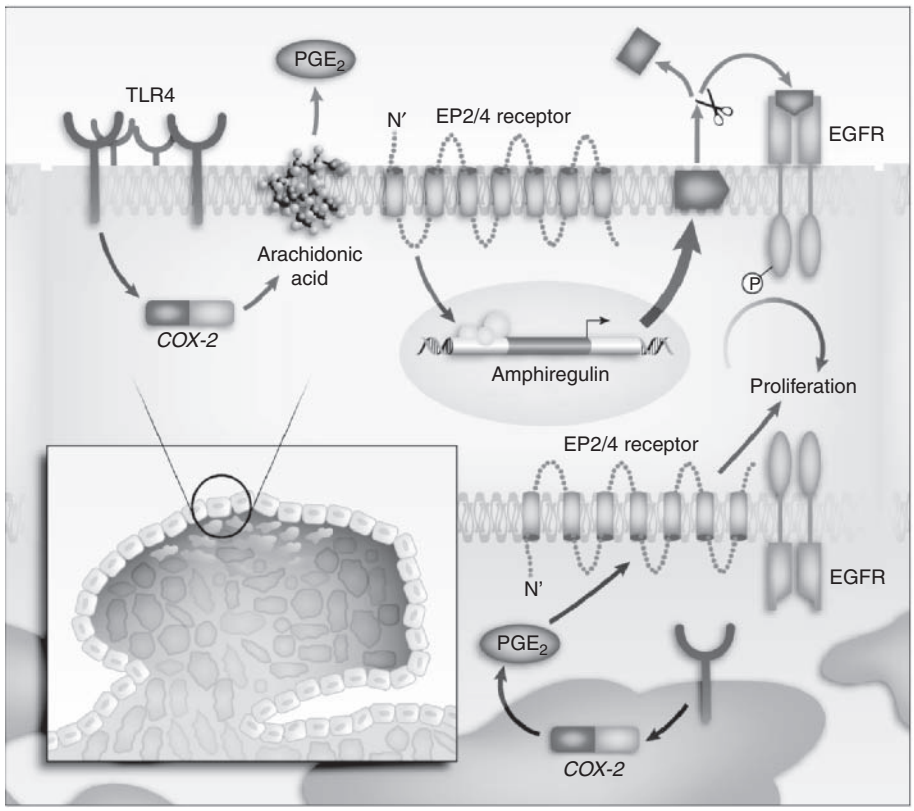

Figure 7.3 Increased TLR4 signaling may increase susceptibility to colitis-associated cancer. We have shown that TLR4 expression is increased in human colitis-associated cancer. TLR4 may increase susceptibility to cancer by activating potent growth pathways including the Cox-2–PGE_2 axis and EGFR signaling.

downstream of TLRs such as IRAK4 are associated with recurrent and often fatal bacterial infections in children [23]. There are, however, functional polymorphisms in TLR4, which are associated with protection from cardiovascular disease but susceptibility to sepsis [24]. A recent meta-analysis of the two common polymorphisms in TLR4, Asp299Gly and Thr399Ile, found an association between carriage of Asp299Gly and Crohn's disease with an odds ratio of 1.45 [25]. These data give support for studying TLR4 signaling in IBD.

Using PCR or immunohistochemical approaches, human colonic epithelial cells have been shown to express relatively little TLR4, especially when compared with peripheral blood mononuclear cells [7,16,17,19,26]. Functional TLR4 has been described in small intestinal epithelial cells [27–30], suggesting that expression of TLRs may be different between the small bowel and colon. Immunohistochemical examination has revealed that TLR4 is upregulated in both CD and UC [19]. Lamina propria macrophages isolated from small intestinal surgical specimens are poorly responsive to LPS, implying functional downregulation of TLR4 signaling [31]. Expression of TLR4 and TLR2 is increased in lamina propria macrophages and in dendritic cells in IBD [9,32]. MD-2 is a secreted molecule necessary for TLR4-mediated recognition of LPS. IFN-γ regulates expression of MD-2 in intestinal epithelial cells [33]. Given that CD lamina propria lymphocytes express IFN-γ, MD-2 expression may also be increased. It is possible that increased expression of TLR4 and MD-2 could result in LPS hyper-responsiveness, leading to proinflammatory cytokine secretion [34]. In animal models of IBD, such as dextran sodium sulfate (DSS)-induced colitis, TLR4 and MD-2 expression is increased [29,35].

Human studies of TLR4 function are limited to studies in cell lines, which are derived from colon cancer cell lines. A larger body of work is derived from studying animal models of colitis. Animals deficient in TLR4 have decreased ability to repair epithelial damage created by DSS-induced injury because they have decreased intestinal epithelial cell proliferation [36,37]. We have shown that underlying this defect in repair of the epithelial barrier is blunted expression of Cox-2 and PGE_2 [38]. Given the critical role of TLR4 in intestinal epithelial cell proliferation, we asked whether TLR4 played a role in colitis-associated cancer. We found that most human colitis-associated cancers have dramatically increased expression of TLR4 even compared with inflamed ulcerative colitis–mucosa [35] (Figure 7.3). Animals deficient in TLR4 are likewise protected from murine colitis-associated neoplasia. These

data demonstrate that TLR4 signaling in the intestine is necessary, especially in the setting of mucosal damage, for signaling proliferation and repair. Unfortunately, in chronic idiopathic colitis, whether Crohn's disease or ulcerative colitis, the sustained activation of TLR4 may culminate in colon cancer. Although we have focused primarily on TLR4, it is likely that other TLRs also contribute to proliferation, repair and cancer. Intestinal epithelial cells from animals with a much broader TLR defect, MyD88 knockout mice, have a greatly decreased ability to proliferate and are very susceptible to death following DSS [36,37]. The phenotype of MyD88$^{-/-}$ mice with respect to colitis-associated neoplasia cannot be properly assessed because they succumb to DSS injury. Recent data have shown that MyD88$^{-/-}$ mice crossed to Apc/min mice, an animal model of familial adenomatous polyposis, have decreased intestinal polyps compared with the background Apc/min mice [39].

Given that defective innate immune signaling protects against colitis-associated neoplasia, the prediction would be that increased TLR signaling should be pro-inflammatory and pro-neoplastic. The single immunoglobulin IL-1 receptor-related molecule (SIGIRR) acts as a negative regulator of TLR signaling [40]. SIGIRR$^{-/-}$ animals demonstrate increased intestinal inflammation and increased tumorigenesis following treatment with AOM–DSS. Restitution of SIGIRR expression in the epithelium reduces inflammation and tumors, suggesting a role for epithelial TLR signaling in tumor development.

The pro-inflammatory and repair-related functions of TLR4 clearly exist in a balance in the intestine. Mice orally infected with *Toxoplasma gondii* develop ileal inflammation [41]. Studies have shown that animals deficient in TLR4 develop less ileal inflammation than wild-type animals. Although in this artificial animal model the absence of TLR4 results in decreased inflammation, one can imagine that during infection with a natural pathogen, TLR4 is very important for its clearance.

TLR2 signaling in the intestine

TLR2 in combination with TLR1 and TLR6 is primarily involved in the recognition of Gram-positive bacteria and fungal species. It may also recognize fimbrial structures on pathogenic *Salmonella* [42]. Relevant to Crohn's disease, mycobacterial antigens are recognized by the TLR2 complex. Relatively little TLR2 is expressed by the intestine compared with peripheral blood mononuclear cells [7,16,17,19]. A recent study found that TLR2 was expressed in the ileum of ulcerative colitis patients [30]. Expression of TLR2 is increased in lamina propria macrophages and dendritic cells from patients with active IBD [9,32]. Isolation of dendritic cells from Crohn's disease patients stimulated with TLR2 ligands resulted in increased expression of IL-12 and IL-6 [32]. At least *in vitro*, expression of TLR2 is regulated by the availability of polyamines [43].

As stated earlier, it is more difficult to examine the function of TLRs. Using TLR2$^{-/-}$ mice, investigators have found that mice given DSS colitis have increased bleeding compared with wild-type mice, suggesting a role for TLR2 in epithelial repair [36]. We discuss the role of TLR2 in epithelial barrier function below.

TLR5: the receptor for flagellin and its role in IBD

TLR5 is the receptor for monomeric flagellin, a component of bacterial flagella and a virulence factor for both Gram-negative and Gram-positive bacteria. Although many organisms are flagellated, only pathogenic organisms release monomeric flagellin capable of activating TLR5 [44]. Investigators have described that *Salmonella* senses lysophospholipids from host intestinal epithelial cells and produces monomeric flagellin rather than the polymeric flagellin associated with its bacterial cell wall [45]. One can see how both the host and luminal pathogens have evolved to maximize recognition of pathogens and minimize inflammation in response to commensals.

TLR5 is expressed on the basolateral surface of polarized intestinal epithelial cells [46,47]. *In vivo*, activation of TLR5 by flagellin in the setting of a disrupted epithelial surface results in increased histological and biochemical inflammation [48]. Immunohistochemical examination has shown that the expression of TLR5 remains relatively unchanged in IBD [19]. This expression pattern of TLR5 may protect against dysregulated inflammation, since flagellin would normally be found on the apical surface in the lumen. Patients with CD express serum antibodies reactive against a specific flagellin derived from commensal bacteria termed CBir [49]. Recent work using 16S ribosomal DNA has identified that the CBir flagellin is likely derived from the family Lachnospiraceae of the phylum Firmicutes [50]. Why there should be a predilection for recognizing this particular flagellin in Crohn's disease is unclear.

Gewirtz *et al.* found that patients with CD express more generalized anti-flagellin antibodies than just anti-CBir and that truncating mutations in TLR5 prevent the development of these antibodies [51,52]. They also reported that about one-quarter of mice null for TLR5 develop spontaneous IBD [53]. Since only a subset develop disease, this points to a defect in clearing bacteria which is exemplified by their high intestinal bacterial load. Therefore, TLR5-dependent recognition of flagellin may play a role in the pathogenesis of IBD. TLR5 may be necessary to clear or contain a subset of luminal bacteria, which, if left unchecked, may be pathogenic.

TLR7 and TLR8

TLR7 and -8 recognize single-stranded RNA (depending on the species). These TLRs can sense synthetic RNA

homologs such as imidazoquinolines: imiquimod (R-837) and resiquimod (R-848). TLR7 and TLR8 are close phylogenetic relatives and TLR8 is believed to be inactive in mice. Their function or expression in the intestine has not been characterized. Oral administration of TLR7 or -8 ligands to mice results in recruitment of myeloid dendritic cells to the lamina propria. These data suggest that signaling by TLRs can result in distinct intestinal events presumably best suited to fight off a pathogen.

TLR3: the unique TLR that senses dsRNA and its possible role in IBD

TLR3 is a receptor for dsRNA, presumably to recognize viral PAMPs. In the laboratory, synthetic poly(I:C) is used as an analog to dsRNA. Stimulation of myeloid DCs with these ligands results in type I IFN expression [54]. Among TLRs, TLR3 uses a unique downstream pathway. In contrast to the other TLRs which use MyD88 to initiate the downstream signaling, the TLR3 pathway only signals through TRIF, resulting in IRF3 activation [55]. Although part of the TRIF signaling pathway is shared by TLR4 signaling, TLR3 signaling may have a distinct role in mucosal innate immune functions. In addition, the TLR3 gene is located on chromosome 4 in a region where several IBD-susceptible loci have been identified [56]. The expression of TLR3 in intestinal epithelial cells is downregulated in active CD, but not in UC [19]. This decreased expression of TLR3 has not been found in the colonic mucosa of children with Crohn's disease [57]. Decreased expression of TLR3 has also been demonstrated in pouchitis compared with normal ileal mucosa [58]. Interestingly, pretreatment with poly(I:C) protected against induction of acute colitis in the DSS-induced colitis model [59]. These results suggest that activation of mucosal TLR3 using synthetic dsRNA may be a potential therapeutic or preventive strategy for IBD.

TLR9 recognizes bacterial DNA and may downregulate inflammatory signals

TLR9 recognizes bacterial methylated (CpG) DNA or oligodeoxynucleotides (ODNs), which are used experimentally to recapitulate the effects of CpG DNA [60]. TLR9 signaling in the intestine has been examined in a variety of contexts. TLR9 is expressed by Paneth cells and exposure of Paneth cells to ODNs causes the release of defensins from their granules [61], suggesting that defensin release is another way in which TLR signaling protects the gut from pathogens. Probiotics can be protective in experimental colitis and investigators have suggested that bacterial DNA, rather than live bacteria, can be protective [62]. The role of TLR9 signaling in the intestinal mucosa during mucosal inflammation is complicated. For example, mice given pretreatment with CpG–ODN to mimic bacterial DNA were resistant to acute DSS-induced mucosal inflammation [63,64]. In contrast, treatment with CpG–ODN after the induction of colitis exacerbated mucosal inflammation in the acute DSS model [64,65]. TLR9-deficient mice showed increased susceptibility to acute DSS-induced colitis but were protected from chronic colitis induced by four cycles of DSS treatment [66,67]. These results suggest that the role of TLR9 is different between induction (prevention) and perpetuation (suppression) of the mucosal inflammation.

Investigators have addressed whether manipulation of TLR9 signaling can be used to ameliorate established murine colitis [66]. AV-ODN (sequence motifs in adenoviral DNA) antagonizes the effect of bacterial DNA. Mice with established colitis (chronic DSS, IL-$10^{-/-}$ or T cell adoptive transfer colitis) were given oral AV-ODN. Administration of AV-ODN was associated with decreased pro-inflammatory cytokine expression and improved histology scores. This study offers indirect evidence that TLR9 signaling can be exploited for the treatment of IBD. The caution is that individual TLRs may play distinct roles at different phases of the IBD timeline and may have different effects on different cell types.

General role of TLRs and Nods: maintenance of barrier function, induction of IgA and expression of antimicrobial peptides

Unlike other epithelial surfaces, which are normally sterile, the intestinal epithelium must maintain a physical and immunologic barrier against the presence of luminal bacteria. The interaction between the commensal flora and the mucosal barrier plays a crucial role in IBD. Increased intestinal permeability may predispose to the development of CD [68] and increased permeability may precede the onset of the symptomatic disease [69]. Increased intestinal permeability is also observed in asymptomatic first-degree relatives of CD patients [70].

TLRs may play an important role in enhancing the barrier function in the gut. In intestinal epithelial cells, TLR2 signaling via protein kinase C and PI3-kinase is associated with enhanced transepithelial resistance through apical tightening and sealing of the tight junctional protein ZO-1 [71,72]. TLR2 appears to be very important in protection from early disruption of tight junction structure during colitis [72]. We have shown that healing of injured intestinal epithelium and clearance of intra-mucosal bacteria require the presence of intact TLR signaling [37]. Furthermore, LPS can induce cytoprotective heat shock protein expression by intestinal epithelial cells and protects against radiation-induced injury [73]. Therefore, innate immune dysfunction may lead to defective mucosal barrier function that can predispose to the initiation of intestinal inflammation in patients with IBD.

Intestinal epithelial cells have been shown to participate in the generation of IgA-secreting lamina propria B cells

through expression of chemokines and cytokines responsible for local class switching [74]. In addition to classical T cell-dependent B cell class switching, recent data demonstrate that lamina propria B cells can secrete non-specific IgA, of the IgA2 sub-type, to protect against commensal bacteria in a T cell-independent fashion. IgA2 predominates in the distal human colon compared with IgA1 found in the systemic immune system. The expression of the cytokines A proliferation-inducing ligand (APRIL), the B cell-activation factor of the tumor necrosis factor family (BAFF) and thymic stroma lymphopoietin (TSLP) appears to be very important for this process. At least *in vitro*, stimulation of intestinal epithelial cells with TLR ligands or commensal bacteria can induce expression of APRIL, suggesting that TLR signaling by intestinal epithelial cells may culminate in increased IgA production in the mucosa.

Antimicrobial peptide expression is one of the mechanisms by which bacterial concentrations in the small and large intestine are decreased. The intestinal epithelium produces defensins to limit bacterial growth in the intestinal crypts [75]. Defensins are antimicrobial peptides that are expressed by Paneth cells (cryptdins) or intestinal epithelial cells. Paneth cells located at the base of small intestinal crypts express a wide range of TLRs [76]. We have demonstrated that TLR4- and TLR2-dependent pathways can stimulate β-defensin-2 expression in human intestinal epithelial cells [77]. Therefore, TLRs may also be involved in controlling the local bacterial population in the gut through the expression of defensins. Defects in TLRs or Nods may result in a decreased ability to clear bacteria from the apical surface of the epithelium. In fact, IBD patients have a dramatic increase in the number of bacteria adhering to the intestinal mucosa, even in mucosa that is not inflamed [78]. It is also possible that expression or lack of expression, of defensins may characterize CD versus UC in addition to the location of the disease [79]. HD-5 and HD-6, human intestinal α-defensins, are diminished in CD patients with ileal disease [80,81]. In addition, patients with mutations in the NOD2 gene show decreased expression of Paneth cell α-defensins [80–82]. β-Defensins are localized in the colon and therefore defective β-defensin expression may contribute to the colonic involvement in IBD [83]. At least one way in which the level of β-defensin-2 expression is regulated is gene copy number. Investigators have demonstrated that patients with colonic Crohn's disease have a smaller number of gene copies than controls, suggesting that this locus could modify disease expression [84].

Investigators have addressed whether TLR expression affects the bacterial populations in the intestine. TLR5$^{-/-}$ mice do demonstrate an increase in bacterial colonization compared with wild-type littermates [53]. By contrast, the absence of TLR2 or TLR4 did not have an impact on the bacterial flora based on denaturing gradient gel electrophoresis and fluorescent *in situ* hybridization (FISH) [85].

Finally, investigators have examined the role of TLRs in the development of gut-associated lymphoid tissue (GALT) [86]. Although animals deficient in MyD88 or TLR4 have smaller Peyer's patches than normal mice or TLR2$^{-/-}$ mice, these defects seem to be overcome by adulthood, probably because of redundancy in innate immune function and microbial diversity.

Expression of Nods in health and disease

In 2001, two groups simultaneously reported that there is an association between genetic polymorphisms in NOD2 and susceptibility to CD [87,88]. NOD2's leucine-rich repeat (LLR) domain implicates its role as a PRR. NOD2 is expressed in monocytes [89], macrophages, T and B cells, DCs [90], Paneth cells [91] and intestinal epithelial cells [92,93]. Muramyl dipeptide (MDP), a specific motif derived from bacterial wall peptidoglycan, is the ligand for NOD2 [94,95]. In the presence of MDP, NOD2 induces NF-κB activation and the production of pro-inflammatory cytokines. NOD2 protein expression by intestinal epithelial cells may serve a protective, antibacterial function in the gut, based on the observation that the forced overexpression of NOD2 in intestinal epithelial cell lines protects against *Salmonella* infection [95]. However, this protective effect is lost in cells transfected with mutant gene constructs of NOD2. The fact that NOD2 is preferentially expressed in Paneth cells in the ileum may help to explain the strong association between these mutations and disease localization [96].

Hedl *et al.* examined NOD2 signaling in primary human monocyte-derived macrophages in a cohort of patients with and without Crohn's disease [97]. They found that activation of NOD2 with muramyl dipeptide resulted in subsequent decreased responses to TLR2 and TLR4 ligands. This effect was lost in patients carrying homozygous mutations in NOD2. Downregulation of TLR activation was likely due to activation of IRAK-1. These results indicate the cross-regulation of innate immune pathways to fight pathogens but to dampen over-exuberant inflammatory responses.

The role of NOD2 in innate and adaptive immunity was further elucidated by the use of genetically engineered mice. NOD2$^{-/-}$ mice had been previously described to show no spontaneous phenotype [98]. Further characterization has demonstrated that NOD2$^{-/-}$ mice have low levels of cryptdin expression by Paneth cells [99]. When these mice are orally infected with *Listeria monocytogenes*, the bacterial species is disseminated in a way not observed in wild-type mice. Mice carrying the CD-associated NOD2 mutation 3020insC exhibit elevated NF-κB activation in

response to MDP and more efficient IL-1β processing and secretion [100]. Also, these mice are more susceptible to DSS-induced colitis and show a greater expression of proinflammatory cytokines [100]. Indeed, several lines of evidence support a role for NOD2 in the regulation of IL-1β processing [101,102]. In contrast to the animal model, however, macrophages from Crohn's disease patients carrying NOD2 mutations have decreased IL-1β production in response to MDP consistent with a loss of function mutation [103,104].

Another function of Nods is to cooperate with TLRs for recognition of potential pathogens. For example, peripheral blood mononuclear cells stimulated with the NOD2 ligand muramyl dipeptide and the TLR9 ligand CpG DNA demonstrate synergistic activation of cytokine expression [105]. This synergism is lost in patients carrying NOD2 mutations. NOD2 in combination with TLR signaling results in activation of IL-23 production by dendritic cells and Th17 differentiation of T cells [106]. Although most studies have found a positive interaction between TLR signaling and NOD2, others have demonstrated that NOD2 damps TLR2 signaling and that the absence of NOD2 is associated with increased TLR2 signaling [107,108].

Link between innate immunity and adaptive immunity in the pathogenesis of IBD

We have described so far how innate immunity plays an important role in the pathogenesis of IBD. Dendritic cells, macrophages and epithelial cells may serve as the primary sites of innate immune defects, whereas aberrant T and B cell activation may be a secondary effect that tries to compensate for the defective innate immune response. One clinical manifestation of the defective innate immune response culminating in increased adaptive immune responses is the observed serum antimicrobial antibodies against commensal organisms seen in patients with IBD [109]. Patients with CD and UC show antimicrobial responses to various commensal flora including *Saccharomyces cerevisiae* (ASCA), *Escherichia coli* (Omp-C), *Pseudomonas fluorescens* (I2) and flagellin from *Clostridium* (CBir) [110,111]. Anti-CBir1 (anti-flagellin) expression has been independently associated with small-bowel, internal-penetrating and fibrostenosing disease features in CD [112]. Recently, the presence of antibodies to carbohydrate epitopes in patients with CD has also been reported [113]. These patients express antibodies against laminaribioside [anti-laminaribioside carbohydrate (ALCA)] and chitobioside [anti-chitobioside carbohydrate (ACCA)]. Devlin *et al.* have shown that mutations in NOD2 result in increased expression of antimicrobial Abs, demonstrating that defective innate immunity may culminate in aberrant responses to luminal bacteria [109].

Mucosal control of bacterial clearance is intimately involved in the ability to develop tolerance versus an adaptive immune response. We have examined the role of TLR signaling in bacterial clearance from the mucosa. We found that mice deficient in TLR4 or its adaptor molecule, MyD88, show decreased ability to clear intramucosal bacterial and experience as bacterial translocation to the mesenteric lymph node after DSS-induced mucosal injury [37]. Patients with CD also show an increased bacterial translocation into deeper layers of the mucosa [114,115]. The increased bacterial invasion of the mucosa may be caused by ineffective innate immune responses such as a mutated and defective NOD2 function and may be one factor culminating in an adaptive immune response to the commensal flora.

Abnormal T cell responses to luminal commensal bacteria remain crucial to the pathogenesis of IBD [116–119]. Mucosal immune homeostasis ultimately relies on the interplay between effector T cells and regulatory T cells (Tregs). The effector cells differentiated from naïve T cells can be divided into three distinctive types, Th1, Th2, Th17 cells, depending on the type of cytokines secreted. The fate of T cell differentiation into Th1, Th2 or Th17 type T cells or Tregs is largely regulated by DCs through engagement of TLRs [120,121]. The recognition of PAMPs by TLRs on DCs promotes antigen presentation, upregulation of costimulatory molecules and secretion of cytokines, which in turn leads to the induction of T cell differentiation, proliferation and survival of antigen specific $CD4^+$ T cells [122]. The lamina propria has unique DCs that extend their projections between intestinal epithelial cells in the small intestine [123,124] (Figure 7.4). More projections are seen in the proximal jejunum, but only a few were present in the terminal ileum [125]. It appears that pathogens increase the presence of projections. TLR signaling by intestinal epithelial cells also appears important in this process.

It has long been suggested that IL-12 is involved in Crohn's disease pathogenesis by triggering Th1 T cell responses [126–128]. More recently, IL-23 has been identified as a crucial innate immune effector cytokine in mouse models of IBD [129–131]. IL-23, a newly discovered member of the IL-12-related cytokine family, participates in the maintenance of IL-17-producing cells (Th17). Upregulation of IL-17 has been demonstrated in human IBD mucosa [127,132]. IL-23 is heterodimeric protein composed of p19 and p40 subunits. IL-23 shares its p40 subunit with IL-12. IL-12p35/p40 heterodimer and IL-23p19/p40 are potent regulators of adaptive immune responses. Animal studies have revealed that constitutive p40 promoter activity is found mainly in the terminal ileum and is associated with high expression of IL-23 p19/p40 from DCs [133]. Germ-free conditions prevent p40 promoter activity in DCs in the terminal ileum, indicating a key role of the intestinal flora to activate IL-23 expression. Therefore, IL-23 may be an important link between the recognition

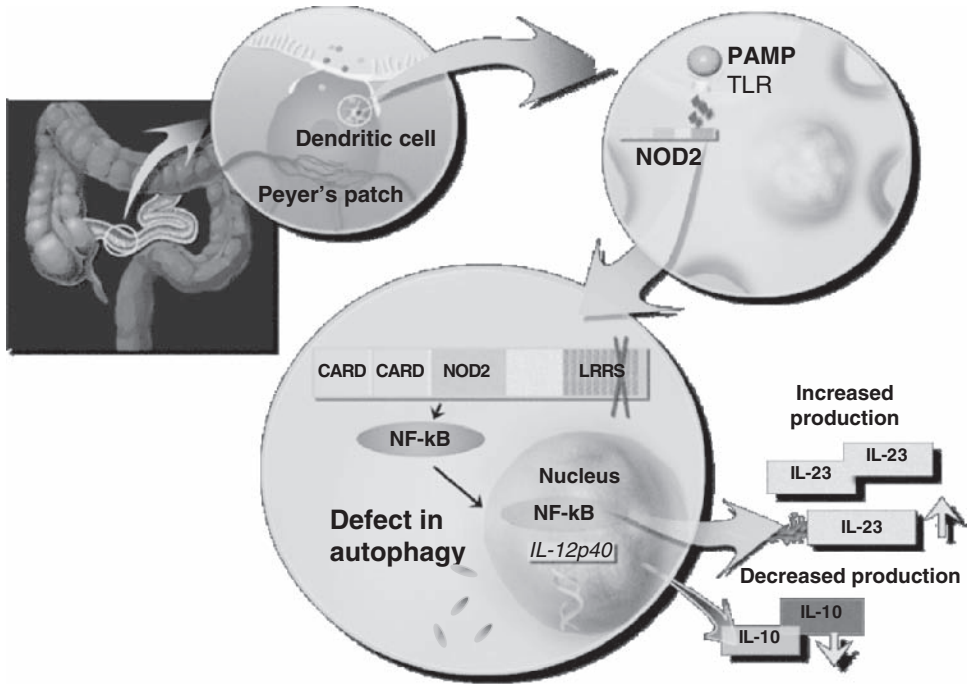

Figure 7.4 Model for defective innate immunity in Crohn's disease. Dendritic cells in the ileum sample luminal bacteria. In the presence of NOD2 mutations or mutations in autophagy genes, there is likely to be defective clearance of bacteria and a secondary maladaptive T cell response.

of the intestinal bacteria and T cell activation in the ileum. IL-23 has also been proposed to be more important than IL-12 for the expression of pro-inflammatory cytokines in murine models of colitis [130].

Overproduction of p40 is also essential for the development of chronic enterocolitis in myeloid cell-specific Stat3 mutant mice [134]. In this model, TLR4-mediated recognition of microbial components triggers aberrant IL-12p40 production by myeloid cells, leading to the development of enterocolitis. These data indicate that innate immune recognition of pathogens leads to adaptive immune responses. Abnormal T cell responses in IBD may therefore be secondary to the primary innate immune dysfunction caused by a combination of genetic and environmental factors.

Other data implicate a link between innate immune defects and abnormal activation of adaptive immunity. C3H/HeJBir mice, which carry a null mutation in TLR4, develop spontaneous colitis that is dependent on luminal bacteria [135]. T cells isolated from C3H/HeJBir mice are reactive to cecal bacterial antigens and transfer colitis to immunodeficient mice [135]. In spite of the fact that these animals have defective TLR4 signaling, the TLR4 gene is not implicated in pathogenesis in this model. Genetic mapping studies, however, have found decreased innate immune signaling as a cause of colitis in this model [136]. These results indicate that defects in innate immunity can activate pathogenic T cells that mediate intestinal inflammation.

Interestingly, most TLRs are expressed on $CD4^+$ T cell themselves, suggesting that TLRs may directly regulate the functional responses of $CD4^+$ T cells in a DC-independent manner [137–142]. For example, TLR2 is a potent costimulatory receptor found on $CD4^+$ T cells, which may increase proliferation and IFN-γ secretion by TCR stimulation [143]. TLR3 signals may prolong $CD4^+$ T cell survival [139]. TLR5 and -7 have been shown to enhance TCR stimulation in memory $CD4^+$ T cells [137]. These TLRs also influence Tregs by modulating their proliferation and suppressive capacity [144–146]. We have shown murine $CD4^+CD45Rb^{high}$ naïve T cells express TLR2, -4, -9 and -3. T cells from $MyD88^{-/-}$ mice do not induce wasting disease in the T cell adoptive transfer colitis model, due to defective differentiation into Th17-type effector cells (M. Fukata, in press). Therefore, innate immune signals may act through cells involved in adaptive immunity.

Innate immunity links genetic susceptibility to IBD and commensal bacteria

IBD results from a triggering event in the genetically-susceptible host. The data to support a role for luminal bacteria or an initial infection with a pathogen include the observation that restitution of the fecal stream induces inflammation in Crohn's disease patient [147,148] and that a pathogenic bacterial infection is associated with an

increased risk of IBD, especially during the first year after infection [149]. Antibodies against commensal flora are also seen [150]. However, it has not yet been completely understood how microbes, in particular commensals, can induce chronic intestinal inflammation.

Certain populations of intestinal flora may affect the induction of IBD. Commensal bacteria within individuals in industrialized countries demonstrate an increase in certain bacterial species, such as Bacteroides, and a decrease in Bifidobacteria compared with areas where IBD is rare, such as rural Africa [151]. Also, certain bacteria may actively suppress inflammation. Therefore, the system responsible for the clearance or tolerance of luminal bacteria after mucosal damage may be a key in understanding the initial defect that occurs mucosal immunity.

Genetic factors play a more dominant role in CD than in UC based on accumulated data from identical twin studies and familial clustering [152]. Approximately one-third of patients with CD carry one of three allelic variants of NOD2/CARD15 compared with 10–15% of the normal population or UC patients [153–156]. Homozygosity increases the relative risk of developing CD by as much as 40-fold compared with simple heterozygosity [87,88,95]. Clinical phenotypic manifestations of NOD2/CARD15 mutations include a slightly younger age of onset, ileal involvement and fibrostenotic disease [157–160]. Although NOD2 mutations do not consistently increase the risk of surgery in adults [161], children with mutations in this gene have an accelerated course towards their first surgery [162]. However, studies examining NOD2 mutations in Japanese, Korean and African-American individuals with CD have not shown an association with disease [163–165]. Therefore, other genes may be involved in IBD susceptibility in bacterial recognition. Polymorphisms in this gene result in a decreased ability to bind to the bacterial ligand and altered activation of NF-κB, which leads to a reduced capacity to activate pro-inflammatory signals [166,167].

The first potentially functional polymorphism of NFKB1 identified was associated with UC [168]. The NFKB1 gene encodes the NF-κB p105/p50 isoforms. A polymorphism in a gene within the NF-κB family (NFKB1A) has been associated with an increased risk for CD independent of NOD2 [169]. Interestingly, similarly to the CARD15/NOD2 mutation, the disease-associated NFKB1 allele showed less promoter activity compared with the activity of the normal allele [168], indicating again that an impaired innate immune function is more likely to be involved in the pathogenesis of IBD than a primary hyper-reactivity. Mice deficient in NF-κB subunits are susceptible to colitis caused by pathogenic bacteria [170]. Therefore, these results imply a robust NF-κB response from innate immune activation inhibits colitis.

TLR genes may be candidate genes for IBD. Two common co-segregating missense mutations in the extracellular domain of TLR4, Asp299Gly and Thr399lle, result in a diminished response to inhaled LPS and protection against atherosclerosis [171–173]. The allele and carrier frequencies for the Thr399Ile mutation in the TLR4 gene show a positive association with UC in a German population [174]. In addition, the TLR4 Asp299Gly polymorphism was found in both CD and UC in a Belgian population [175]. However, TLR4 (A299G) and CD14 (T-159C) variants did not differ between CD and controls in Scottish and Irish populations [176]. Increased susceptibility to IBD has been associated with coexistence of TLR4 and/or CD14 and NOD2 mutated alleles in a Greek population [177]. Japanese patients with UC, however, failed to show any increase in TLR4 polymorphisms within IBD patients [178]. A recent meta-analysis of the two common polymorphisms in TLR4, Asp299Gly and Thr399Ile, found an association between carriage of Asp299Gly and Crohn's disease with an odds ratio of 1.45 [25].

Deficient innate immune responses to bacteria due to variants in TLR1, TLR2 and TLR6 result in more extensive disease localization in UC and in colonic CD [179]. The -1237C promoter polymorphism of TLR9 is linked with CD in a German population [180]. The synergy between TLR9 and NOD2 has also been reported to be lost in the CD patients carrying a NOD2 mutation, indicating combinations of various innate immune gene polymorphisms may be further impaired in disease susceptibility of IBD [105].

Additional genetic evidence points to the recognition of PAMPs in the pathogenesis of IBD. Polymorphisms in the IL-1 receptor antagonist gene may affect the severity and extent of disease in UC patients, particularly in patients positive for perinuclear antineutrophil cytoplasmic antibody (pANCA) [181]. In addition, a common functional promoter region polymorphism (T-159C) of the CD14 gene shows a weak association with both CD and UC in German and Japanese patients [182,183]. Lack of bacterial responsiveness in promoter activation was observed with polymorphisms in the IBD5-associated organic transporter gene (OCTN) [184]. The OCTN promoter G207C variants increase the risk for CD by 2–2.5-fold when present as a single copy and by 4-fold in homozygous carriers [184,185], although these findings have not been widely reproduced. Drosophilia Discs Large Homolog 5 (DLG5) encodes cell scaffolding proteins involved in the maintenance of epithelial integrity and regulation of cell growth [186]. Interestingly, DLG5 genes are involved in the transport of key molecules for the homeostasis or exclusion of toxins (SLC and MDR1) [187,188]. Polymorphisms in DLG5 were reported to be associated with CD [189]. The human multidrug resistance 1 (MDR1) gene product P-glycoprotein is highly expressed in intestinal epithelial cells. P-glycoprotein therefore may play a role in the defense against intestinal bacteria [190]. MDR1-deficient mice spontaneously develop colitis depending on enteric bacteria [191]. C3435T and Ala893 polymorphisms of

MDR1 gene, which cause lower protein expression, are associated with the risk of developing UC or IBD [192, 193].

Finally, polymorphisms in the IL-23 receptor and in autophagy genes have been demonstrated in patients with Crohn's disease. The polymorphism in the IL-23 receptor protects against the development of Crohn's disease, but the mechanism by which this protection is afforded is unclear [194]. Cooperation with other genes in the IL-23–IL-17 pathway may be responsible for this protective effect. Autophagy-related genes such as ATG16L1 and IRGM are important in clearance of intracellular bacteria, especially mycobacterial organisms [195–197]. Thus mutations in this pathway, like the NOD2 pathway, could result in defective innate immunity.

Initially, it was difficult to reconcile why these mutations would increase susceptibility to a disease characterized by exuberant inflammation in response to commensal bacteria. As described above, recent advances in genetics and discoveries in the molecular mechanisms of the proteins encoded by these genes give rise to a new vision in understanding the cause of IBD. However, there is no single etiological factor responsible for the onset of IBD. The challenge therefore is to clarify functional gene–gene interactions and gene–environment interactions.

Conclusion and implications for therapy of IBD

The fundamental purpose of the innate immune system is to protect a host against pathogens. In this context, the innate immune system in the gastrointestinal tract is unique because of the numerous foreign organisms living within it. Through a better understanding of the innate immune response to commensal bacteria, we can develop targeted therapy for patients with IBD. Animal studies have provided a wealth of information. A number of relevant models of IBD have been described over the last decade in which chronic intestinal inflammation occurs spontaneously in mice with a specific genetic background or in mice that have been genetically manipulated (transgenic, knockout) in response to luminal commensal bacteria.

Although the development of anti-TNF agents has improved the quality of life in IBD patients, there appears to be a loss of efficacy, not only due to antibodies, but also due to a change in the mechanism of inflammation. If one imagines that the underlying problem is an innate immune defect in certain patients with IBD, then tonically inhibiting TNF or other pro-inflammatory cytokines will have limited utility. A subset may in fact benefit from strategies that boost innate immunity. One such example is GM-CSF, although toxicities limit its utility [198]. There continues to be blurring of the lines between innate and adaptive immunity. Manipulating TLR signaling could improve inflammation in patients with IBD. It may also be important for preventing colitis-associated cancer. Because of the broad implications of manipulating TLR signaling, it will be critical to select patients of the appropriate phenotype based on genetic, serologic and proteomic examinations.

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Chapter 8
Adaptive Immunity: Effector and Inhibitory Cytokine Pathways in Gut Inflammation

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Summary
- Therapy for mouse models of colitis is poorly predictive for human disease.
- TGFβ is the master negative regulator of inflammation in the gut.
- Overexpression of Smad7 in IBD prevents TGFβ from inhibiting inflammation.
- IL-21 appears to be playing a major role in human gut inflammation.
- Paradoxically, TGFβ and IL-21 synergize to produce Th17 cells in humans.

Introduction

The discovery of the cytokine/chemokine network is one of the major discoveries of modern biology. It is of particular relevance to gastroenterology because the gut is the main interface between the immune system and external antigens, is separated from the microflora only by a single layer of epithelium and one of the main effects of cytokines is on the epithelial barrier. The critical role of cytokines in controlling inflammation in the gut is also clearly demonstrated by the spontaneous colitis which develops in mice lacking cytokines such as TGFβ or IL-10. At the same time, as the role of different cytokines in the induction of inflammation in the gut has become clear, so the specific targeting of cytokines such as TNFα has been the great success of inflammatory bowel disease (IBD) research in the last 12 years.

There is continuing interest in this area because anti-TNFα antibodies have demonstrable risks, are only effective in 40–50% of patients and efficacy may wane. The reasons for this are not understood, but could be because, in a heterogeneous disease such as Crohn's disease (CD), in some patients the lesions may be TNFα independent. Likewise, loss of response could be because the anti-TNFα treatment itself has led to the immune system activating a different tissue-damaging pathway.

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For the non-cognoscenti of the arcane and complex world of cytokines in the gut, it is difficult to make relative judgments as to which anti-cytokine therapy would be the best to take into patients. Animal models are traditionally the way to obtain important clues as to the "best bet", but, as Table 8.1 shows, the list of treatments which prevent colitis in animal models is extensive and growing by the week. However, there is publication bias in that therapies which did not work may not make it into print and it may be reassuring to know that any treatment to be taken into humans does work in an animal model.

A more important question, however, is whether it is indeed true that, for example, in TNBS colitis in mice, IL-2, IL-6, IL-12, IL-16, IL-17, IL-21, interferon-γ, TNFα, MIF, adiponectin, leptin and various chemokines are all critical for development of disease. Is the immune system in the gut really so complex that all of these cytokines are needed or is there an invisible hierarchy of importance because "protection" from colitis may vary between the agents being studied and no-one ever does a comparative study to determine, for example, if blocking TNF is superior to blocking IL-12? In reality, we do not know the answers to these questions and we have to rely on supportive animal data, but critically, data from humans (*in vivo*, *in vitro* and *ex vivo*) to determine if the pathways investigated are relevant to human gut inflammation.

In this chapter, we will attempt to persuade the reader that continuing work on cytokine biology in the gut is relevant. We will focus particularly on work which shows

Table 8.1 A list of the cytokines/chemokines and other growth factors shown to have an effect in animal models of colitis.

Cytokine	Method	Model	Effect*
IL-1	IL-1 receptor antagonist	Rabbit immune complex colitis	Reduced inflammation[1]
IL-2	IL-2–IgG fusion protein	Mouse TNBS colitis	Reduced inflammation[2]
	IL-2 null mice	Spontaneous colitis	Develop inflammation[3]
IL-3	No data		
IL-4	Anti-IL-4 antibody	Mouse oxazolone colitis	Reduced inflammation[4]
IL-5	Anti-IL-5 antibody	SAMP1/Yit ileitis	Reduced inflammation[5]
IL-6	Anti-IL-6R, gp130-Fc	TNBS colitis IL-10 null mice Transfer colitis (SCID mice)	Reduced inflammation[6]
IL-7	IL-7-deficient mice	Transfer colitis	Reduced inflammation[7]
MIP-2†	MIP-2 overexpression	DSS colitis	Increased inflammation[8]
IL-9	No data		
IL-10	IL-10 null mice	Spontaneous colitis	Develop inflammation[9]
IL-11	Injection of IL-11	HLA-B27 transgenic rats	Reduced inflammation[10]
IL-12	Anti-IL-12 antibody	TNBS colitis in mice	Reduced inflammation[11]
IL-13	IL-13R–Ig fusion protein	Oxazolone colitis in mice	Reduced inflammation[12]
IL-14	No data		
IL-15	IL-15 null mice	DSS colitis	Resistant to disease[13]
IL-16	Anti-IL-16 antibody	TNBS colitis in mice	Reduced inflammation[14]
IL-17	Anti-IL-17 antibody IL-17R null mice IL-17R–Ig fusion protein Act-1 null mice (defective IL-17R signaling)	DSS colitis TNBS colits TNBS colitis DSS colitis	Enhances inflammation[15] Reduced inflammation[16] Reduced inflammation[16] Reduced inflammation[17]
IL-18	Anti-IL-18	DSS colitis	Reduced inflammation[18]
IL-19	No data		
IL-20	No data		
IL-21	IL-21 null mice	TNBS colitis	Reduced inflammation (Monteleone,-unpublished)
IL-22	No data		
IL-23	IL-10 null mice crossed with IL-23p19 null mice Anti-IL-23 p19 antibody	Spontaneous colitis Transfer colitis into SCID mice	No inflammation[19] Reduced inflammation[20]
IL-24	No data		
IL-25	No data		
IL-26	No data		
IL-27	IL-27 null mice	Oxazolone colits	Reduced inflammation[21]
IL-28	No data		
IL-29	No data		
IL-31	No data		
IL-32	No data		
IL-33	No data		
Interferon-γ	Anti-interferon-γ antibody IFNγR null mice	Tranfer colits into SCID mice TNBS colitis	Reduced inflammation[22] No change[23]
TNFα	anti-TNFα antibody	TNBS colitis	Reduced inflammation[24]
CSF-1	Anti-CSF-1 antibody	DSS colitis	Reduced inflammation[25]

(*Continued*)

Table 8.1 (*Continued*)

Cytokine	Method	Model	Effect*
MIF	MIF null mice	Transfer colitis into SCID mice	Reduced inflammation[26]
		TNBS colitis	Reduced inflammation
	Anti-MIF antibody	Transfer colitis	Reduced inflammation[26]
αMSH	MSH	DSS colitis in mice	Reduced inflammation[27]
Adiponectin	Adiponectin null mice	TNBS and DSS colitis in mice	Reduced inflammation[28]
Leptin	Leptin null mice	TNBS and DSS colitis in mice	Reduced inflammation[29]
Basic FGF	FGF per rectum	TNBS and DSS colitis in mice	Reduced inflammation[30]
MCP-1 (CCL2)	MCP-1 null mice	DNCB colitis	Reduced inflammation[31]
IP-10 (CXCL10)	Anti-CXCL10 antibody	IL-10 null mice	Reduced inflammation[32]
CXCR2‡	Anti-CXCR2 antibody	DSS colitis	Reduced inflammation[33]
MIP-3α (CCL20)	Anti-CCL20 antibody	TNBS colitis	Reduced inflammation[34]
MIP-1α (CCL3)	Recombinant MIP-1α	TNBS colitis	Exacerbates colitis[35]

*References: 1. *Gastroenterology* 1992; **103**:65–71. 2. *Gastroenterology* 1999; **117**:866–76. 3. *Cell* 1993; **75**:253–61. 4. *J Exp Med* 1998; **188**:1929–39. 5. *Eur J Immunol* 2004; **34**:1561–9. 6. *Nat Med* 2000; **6**:583–8. 7. *J Immunol* 2007; **178**:4737–48. 8. *Pediatr Res* 2003; **53**:143–7. 9. *Cell* 1993; **75**:263–74. 10. *Lab Invest* 1998; **78**:1503–12. 11. *J Exp Med* 1995; **182**:1281–90. 12. *Immunity* 2002; **17**:629–38. 13. *Gut* 2006; **55**:334–41. 14. *Gastroenterology* 2000; **119**:972–82. 15. *Clin Immunol* 2004; **110**:55–62. 16. *Inflamm Bowel Dis* 2006; **12**:382–8. 17. *Nat Immunol* 2007; **8**:247–56. 18. *Am J Physiol Regul Integr Comp Physiol* 2001; **281**:R1264–73. 19. *J Clin Invest* 2006; **116**:1310–6. 20. *Proc Natl Acad Sci USA* 2002; **99**: 16951–6. 21. *Immunity* 1994; **1**:553–62. 22. *Gastroenterology* 2007; **132**:2359–70. 23. *Eur J Immunol* 2000; **30**:1486–95. 24. *Eur J Immunol* 1997; **27**:1743–50. 25. *Inflamm Bowel Dis* 2007; **13**:219–24. 26. *Nat Immunol* 2001; **2**:1061–6. 27. *Peptides* 1997; **18**:381–5. 28. *Gastroenterology* 2007; **132**:601–14. 29. *Gastroenterology* 2005; **128**:975–86. 30. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**:G803–11. 31. *Inflamm Bowel Dis* 2005; **11**:799–805. 32. *Gastroenterology* 2002; **122**:2011–25. 33. *J Leukoc Biol* 2007; **82**:1239–46. 34. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**:G1263–71. 35. *Gut* 2005; **54**:1114–20.
†Mouse equivalent of human IL-8.
‡Receptor CXCR1-3, -5, -6, -8.

that manipulation of the TGFβ signaling pathway can be anti-inflammatory in the human gut and more recent data which suggest that the cytokine IL-21 is an important pro-inflammatory mediator in the gut and is critical for the development of the newest tissue-damaging cell type, namely Th17 cells.

CD4$^+$ T cells accumulate in IBD tissue

There is no doubt that CD4$^+$ T lymphocytes are major players in the immune–inflammatory response leading to IBD and that their survival in inflamed tissues is supported by cytokines which overcome programmed cell death and TGFβ-mediated immunosuppression. In both CD and ulcerative colitis (UC), the inflamed mucosa is heavily infiltrated with activated CD4$^+$ T lymphocytes. CD4$^+$ T cells accumulate in the inflamed gut as a result of enhanced production of chemoattractants within the inflammatory microenvironment. There is also evidence that, at least in CD, mucosal CD4$^+$ T lymphocytes exhibit enhanced cell cycling [1]. Compared with CD4 cells from the normal gut and those from patients with UC, CD CD4$^+$ cells show increased phosphorylated Rb, necessary to bring cells into S phase, and decreased phosphorylated p53, a negative regulator of S phase transit and excessive T cell replication. CD CD4$^+$ T lymphocytes also exhibit enhanced resistance to apoptosis, the programmed cell death that follows activation [2]. The molecular basis of this latter defect remains unknown, but activation via specific cytokine receptors would seem to contribute in prolonging T cell survival (Figure 8.1). In this context, studies in mouse models of IBD and humans have shown that blocking IL-6 activity enhances T cell apoptosis, with the downstream effect of inhibiting the mucosal inflammation [3]. Signals delivered through the common γ-chain receptor subunit, that is shared by IL-2, IL-4, IL-9, IL-13, IL-15 and IL-21 receptors, could also regulate mucosal T cell survival and may in part explain why blocking nearly all of these agents individually has some benefit in mouse colitis. However, regardless of the underlying molecular mechanism, the pathogenic relevance of the diminished susceptibility of T cells to undergo apoptosis in CD is substantiated by the demonstration that drugs that potentiate mucosal CD4$^+$ T cell death are useful for inducing clinical remission in CD patients [4,5].

TGFβ as the master regulator of inflammation in the gut in health and disease

Mice lacking TGFβ1 develop widespread inflammation including in the gut and die early in life [6], and animals

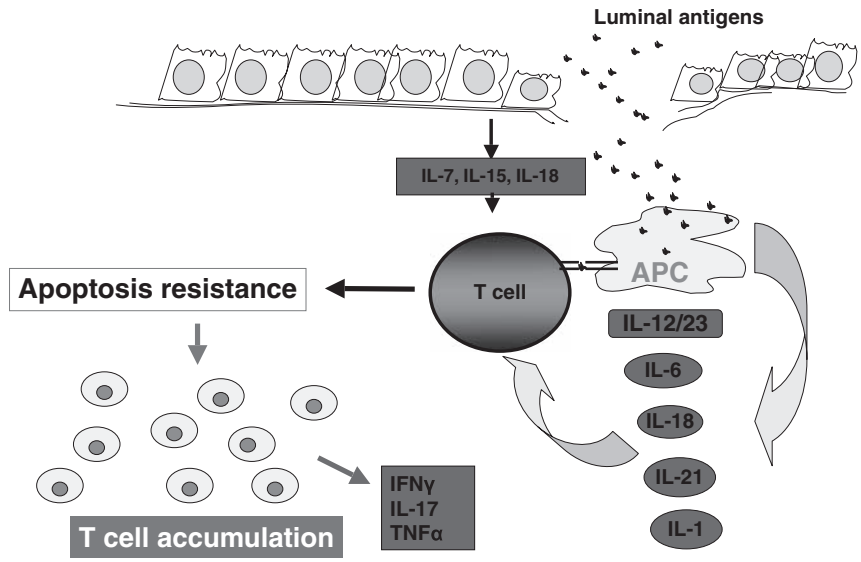

Figure 8.1 T cells in the healthy gut normally die by apoptosis. In IBD it is assumed that their survival is enhanced by presentation of bacterial antigens, but also through a large variety of cytokines which prevent apoptosis. T cells then accumulate in the tissues and secrete large amounts of tissue-damaging cytokines such as IFNγ, IL-17 or TNFα.

whose T cells cannot respond to TGFβ also die of wasting disease and gut inflammation [7]. It is therefore generally considered that TGFβ is a master negative regulator of intestinal inflammation. TGFβ is made by many cell types, but particular attention has been paid to its production by T cells, so-called regulatory cells. There is now evidence in a number of systems that TGFβ1 secreting T cells or membrane-bound TGFβ1 on regulatory cells can prevent inflammation, including experimental colitis [8–10].

This work, however, presents a problem when translated into clinical IBD, since in the inflamed gut TGFβ is made by many different cell types and is present in abundance in inflamed tissue. In this situation, it is very difficult to imagine how T cell-specific TGFβ1 could downregulate inflammation in the mucosa. There is the possibility that Th3 cells mediate their activity in gut-associated lymphoid tissues at the inductive phase of colitogenic T cells, but this is not amenable for study in humans. We have therefore been interested in the control of TGFβ1 signaling in normal and inflamed human intestinal mucosa.

Smad signaling

TGFβ1 initiates signaling through the ligand-dependent activation of a complex of heterodimeric transmembrane serine/threonine kinases, consisting of type I (TGFβ1 RI) and type II (TGFβ1 RII) receptors (Figure 8.2). Upon TGFβ1 binding there is phosphorylation and activation of TGFβ1 RI by the constitutively active and autophosphorylated TGFβ1 RII [11]. TGFβ1 signals from the receptor to the nucleus using a set of proteins, termed Smads, based on their high homology to the *Drosophila* Mad and the *Caenorhabditis elegans* Sma proteins. To date, nine different Smad genes which fall into three distinct functional sets have been identified: signal-transducing receptor-activated Smads, which include Smad1, 2, 3, 5, 8 and 9; a single common mediator, Smad4, and inhibitory Smad6 and 7 [12,13]. Activated TGFβ1 RI directly phosphorylates Smad2 and Smad3 at serine residues in the carboxy-terminal SSXS sequence [13,14]. Once activated, Smad2 and Smad3 associate with Smad4 and translocate to the nucleus where Smad protein complexes participate in transcriptional control of target genes [13–15]. Targeted disruption of Smad3 is associated with diminished cell responsiveness to TGFβ1 [16]. Mutant mice also exhibit

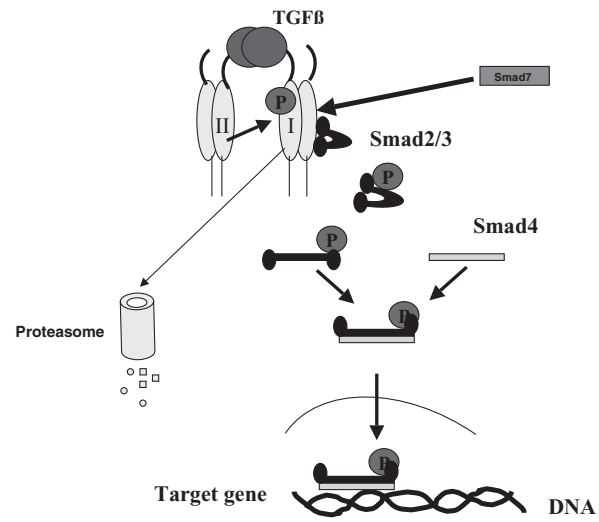

Figure 8.2 The canonical TGFβ signaling pathway involves activated TGFβ binding to its cell surface receptor. The type II receptor phosphorylates and activates the type I receptor, which then recruits Smad2/3, which is itself phosphorylated. This forms a heterodimeric complex with Smad4, which then moves to the nucleus and activates or suppresses gene expression depending on the cell type. The inhibitory Smad, Smad7, prevents Smad2/3 from binding to the type I receptor and, because it is complexed with a ubiquitin ligase, ubiquitinates the type I receptor for degradation in the proteasome.

massive infiltration of T cells and pyogenic abscess formation in the stomach and intestine, supporting the view that Smad3 is an essential mediator of the TGFβ1-induced anti-inflammatory and suppressive activities. The inhibitory Smad7 acts by occupying ligand-activated TGFβ1 RI and interfering with the phosphorylation of Smad2/Smad3 (Figure 8.2). Upregulation of Smad7 has been associated with an inhibition of TGFβ1-induced signaling [17,18].

Smad signaling in IBD

We have provided evidence for defective Smad signaling in patients with chronic gut inflammation in the colon in IBD [19] and the stomach in *Helicobacter pylori* gastritis [20]. Smad7 is overexpressed in IBD mucosa and purified mucosal T cells, and in both whole tissue and isolated cells there is defective TGFβ1 signaling as measured by reduced phospho-Smad3 immunoreactivity. Specific antisense oligonucleotides for Smad7 reduce Smad7 protein in cells isolated from IBD patients and the cells then become responsive to exogenous TGFβ1. TGFβ1 cannot inhibit pro-inflammatory cytokine production in isolated lamina propria mononuclear cells (LPMC) from CD patients, but inhibition of Smad7 with antisense oligonucleotides restores TGFβ1 signaling and allows TGFβ1 to inhibit cytokine production markedly. In inflamed mucosal tissue explants from CD patients, inhibition of Smad7 also restores p-Smad3 and decreases pro-inflammatory cytokine production, an effect which is partially blocked by anti-TGFβ1. We have extended these studies to examine the interactions between Smad signaling and NF-κB activation in the inflamed gut [21]. While TGFβ1 is a potent inhibitor of TNFα-induced NF-κB activation in the normal gut, it has no activity in the inflamed gut. This can be attributed to overexpression of Smad7 since treatment of cells from the inflamed gut with antisense to Smad7 allows TGFβ1 to downregulate NF-κB activation rapidly.

Our recent studies on *H. pylori* gastritis [19] have further emphasized the role of Smad7 in promoting gut inflammation. However, in this model we are able to show that blocking interferon-γ with antibody lowers Smad7 in gastric biopsies from *H. pylori*-infected patients. Furthermore, interferon-γ on its own induces Smad7 in normal gastric biopsies.

Regulation of Smad7 in IBD

Since Smad7 appears to be crucial in the regulation of TGFβ1 in the gut, understanding how this inhibitor is regulated in IBD could help in the design of new therapeutic interventions for patients. Experiments with cell lines have shown that activators of NF-κB (e.g. TNFα and IL-1β) and Stat1 (e.g. IFN-γ and IL-7) pathways can enhance Smad7 expression [22]. Therefore, upregulation of Smad7 was initially thought to be consequent to the sustained activity of both of these pathways in the gut of IBD patients. However, Smad7 protein expression remained unchanged after blocking IFN-γ/Stat1 or TNFα/NF-κB activities in IBD LPMC. Smad7 is also strongly and rapidly induced by TGFβ1 itself, thus representing an important effector in the feedback loop that controls TGFβ1/Smad signaling. However, it seems unlikely that this mechanism is responsible for increasing Smad7 expression in IBD, as p-Smad3 is reduced in samples exhibiting high Smad7 levels. These data suggest that the *in vivo* regulation of Smad7 is more complex than might be expected from *in vitro* studies. Moreover, a quantitative analysis of Smad7 RNA revealed no difference between IBD and normal intestinal samples, indicating that in IBD, Smad7 is mostly regulated at the post-transcriptional level [23].

One important mechanism of control of Smad7 protein expression relies on the dynamic post-translational modifications which make the protein resistant to proteasome-mediated degradation in the cytoplasm. The stability of Smad7 is controlled by competition between acetylation and ubiquitination for the same lysine residues [24]. Therefore, acetylation of lysine residues prevents ubiquitination and protects Smad7 protein against proteasomal degradation. Using *in vitro* and *ex vivo* assays, Smad7 is ubiquitinated and degraded in control but not IBD tissue. Moreover, inhibitors of the proteasome activity enhanced Smad7 protein in control LPMC, suggesting that Smad7 is ubiquitinated and targeted for proteasome degradation in normal but not inflamed gut. Smad7 was highly acetylated *in vivo* in IBD but not control samples. The transcriptional coactivator p300 is known to interact with Smad7 and promote its acetylation on lysine residues. Consistent with this, p300 protein is high in IBD compared with controls and Smad7 interacts *in vivo* with p300. Such an interaction seems to be functionally relevant, since siRNA-mediated inhibition of p300 in CD LPMC results in diminished acetylation and expression of Smad7. Overall, these results suggest that p300-mediated acetylation of Smad7 plays a key role in enhancing Smad7 protein expression in IBD and that manipulating p300 level in IBD tissue can be useful for controlling TGFβ1 activity and eventually limiting the local inflammation (Figure 8.3).

Smad7 protein turnover can be controlled by additional molecules that enhance its ubiquitination, such as Smurf1, Smurf2, Arkadia and Jun activation domain-binding protein 1 (Jab1), a component of the COP9 signalosome [25]. The exact contribution of each of these molecules in the regulation of Smad7 at the gut level remains, however, to be determined.

A further complication to the biology of Smad 7 is that it has recently been shown to inhibit NF-κB activation [26]. Further studies are needed to determine if this happens in the gut since high Smad7 and activated NF-κB are coexistent in the mucosal in IBD.

Additional insight into the factors involved in the genesis of high Smad7 levels can be inferred from the

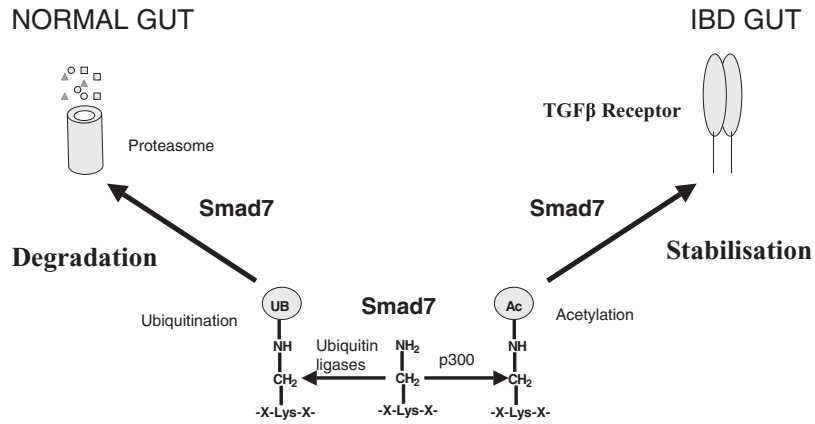

Figure 8.3 The biological activity of Smad7 in the gut is controlled by the balance between ubiquitination and acetylation by p300. Smad7 is produced all the time in the gut but is constantly ubiquitinated and degraded. When p300 levels are increased, the lysine residues targeted for ubiquitination are acetylated, making the protein resistant to degradation, allowing it to move to the membrane to interact with the type I TGFβ receptor.

pattern of Smad7 expression in various chronic gastrointestinal inflammatory processes. Upregulation of Smad7 occurs in *Helicobacter pylori* (Hp)-associated gastritis [20] but not in the duodenum of patients with active celiac disease (Monteleone G, MacDonald TT, unpublished work), clearly indicating that Smad7 upregulation is not a specific hallmark of IBD and that its induction is not simply an epiphenomenon of the ongoing mucosal inflammation.

A logical approach to treat IBD would therefore be to use p300 inhibitors, the notion being that inhibiting p300 would reduce Smad7 acetylation, leading to its degradation, then allowing endogenous TGFβ to inhibit ongoing inflammation. Synthetic p300 inhibitors have been described [27] but have not been formally tested in IBD. Diferuloylmethane, the major ingredient of curcumin, is a food additive, and pharmacologically inhibits lymphocyte proliferation, is an antioxidant and inhibits NF-κB activity [28], but is also a potent p300 inhibitor at low doses [29]. Based on its anti-inflammatory properties, previous studies have shown that it can inhibit TNBS colitis in mice by an unknown mechanism [30], and it has been demonstrated in a small open-label study that oral curcumin capsules show benefit in ulcerative proctitis and CD [31] and also help maintain remission in UC [32] . We suggest that this activity is mediated through the effects of curcumin on Smad7 acetylation.

A functional role of Smad7 in controlling gut inflammation in murine models of IBD

The above observations show that TGFβ1 signaling is inhibited by Smad7 in inflamed tissue of IBD patients, but little is known on the role of Smad7 in controlling *in vivo* the gut inflammation in mouse models. Levels of TGFβ1 are increased in the inflamed tissue of both TNBS- and oxazolone-mediated colitis, associated with low p-Smad3, due to high expression of Smad7 [33]. Oral administration of Smad7 antisense oligonucleotides reduces Smad7 and restores TGFβ1-associated p-Smad3 expression in the colon of mice with TNBS and oxazolone colitis. More importantly, treatment with antisense led to significant amelioration of both forms of colitis, as evidenced by a reduction in weight loss and of macroscopic and microscopic evidence of inflammation. The Smad7 antisense oligonucleotides also reduced the inflammation in established chronic colitis induced by repeated administrations of TNBS. Detailed analysis of cytokines produced in the colons of such animals revealed that restoration of TGFβ1 signaling by specific inhibition of Smad7 resulted in a significant downregulation of Th1-cytokines (IL-12 and IFN-γ) and reduced expression of Th1-associated transcription factors (i.e. T-bet and Stat1) in TNBS colitis. On the other hand, oral administration of Smad7 antisense oligonucleotides marginally reduced the production of IL-4 in mice with oxazolone-induced colitis, thus indicating that TGFβ1 exerts a negative effect on T cell function rather than on polarized T cell subsets. This study raises the possibility that resolution of gut inflammation may be accomplished by downregulating Smad7 and allowing endogenous TGFβ1 to inhibit inflammatory pathways which promote tissue injury. However, in this context, it is noteworthy that TGFβ1 has different effects on different cell types and inhibiting Smad7 in some cell types might enhance the detrimental effects of TGFβ1, such as production of collagen and fibrosis. Further studies are needed to elucidate this potential difficulty.

The emerging role of IL-21 in mediating the Th1 cell responses in Crohn's disease

In the preceding section, we attempted to demonstrate how the anti-inflammatory effects of TGFβ are nonfunctional in IBD. The opposite side of the coin, and the situation which prevails in active CD at least, is the continued survival and cytokine secretion of T cells in the gut wall which result in chronic inflammation. Generally, since the initial characterization of Th1 cells, it has become evident that, in addition to Th1-polarizing stimuli such as IL-12 or IL-23, various signals are required to drive and maintain ongoing Th1 cell responses. In addition to factors such as IL-2, IL-6, IL-15 and IL-18

(Figure 8.1), we have recently suggested that IL-21 may also play a major role in this phenomenon in patients with IBD [34].

IL-21 is made by activated $CD4^+$ T cells and natural killer T cells and mediates its function through a heterodimeric receptor, composed of a specific subunit, termed IL-21 receptor (IL-21R), and the common γ-chain [35]. IL-21 augments the proliferation of $CD4^+$ and $CD8^+$ T lymphocytes and regulates the profile of cytokines secreted by these cells [36,37]. It drives the differentiation of B cells into memory cells and terminally differentiated plasma cells and enhances the activity of natural killer cells [38,39]. Like other cytokines that signal through the common-γ chain subunit, IL-21 activates the JAK-family protein tyrosine kinases JAK1 and JAK3, leading to the activation of signal transducer and activator of transcription (Stat)1, Stat3 and, to a lesser degree, Stat4 and Stat5, depending on the cell type studied.

Analysis of IL-21 protein in biopsies of patients with IBD and controls showed that IL-21 is over-produced in the inflamed intestine of patients with CD in comparison with patients with UC and normal controls [34]. $CD4^+$ T cells infiltrating CD mucosa are the main cellular source of IL-21. Exogenous IL-12 further boosts IL-21 production. Antibody-blockade of IL-21 activity in cultures of CD LPMC reduces the expression of p-Stat4 and T-bet and the production of IFN-γ. Collectively, these results suggest that, in CD, IL-21 may be part of a positive feedback loop that expands and maintains the ongoing Th1 cell response [34].

Nonetheless, enhanced IL-21 production is also seen in mucosal samples taken from patients with UC, a disease that is not associated with a predominant Th1 cell response, which suggests that its role is more in the maintenance of T cell activation regardless of the polarized pathway used.

IL-21 controls the production of MIP-3α, a T cell chemoattractant, by human gut epithelial cells

Gut epithelial cells play an active role in the amplification and maintenance of chronic intestinal inflammation. Intestinal epithelial cells synthesize cytokines that control the survival and activity of mucosal lymphocytes. Epithelial cells also contribute to generate a chemoattractant milieu that sustains the recruitment of inflammatory cells from the circulation. Gut epithelial cells in turn are influenced by cytokines released by inflammatory cells.

Colonic epithelial cells constitutively express both IL-21R and the common γ-chain subunit and IL-21R is upregulated in the inflamed epithelium of both CD and UC patients [40]. IL-21R is also expressed by colonic epithelial cell lines and these cells respond to the *in vitro* stimulation with IL-21 by increasing the secretion of the chemokine, macrophage inflammatory protein (MIP)-3α. MIP-3α is upregulated on the gut epithelium in IBD and is thought to play a role in the mucosal recruitment of gut-homing α4β7 integrin-expressing T cells. *In vitro* studies aimed at investigating the IL-21 effects on T cell migration revealed that MIP-3α accounts for a significant component of IL-21-induced chemotactic activity, as a neutralizing anti-MIP-3α antibody significantly attenuates the chemotactic activity of conditioned media obtained from IL-21-stimulated colonic epithelial cells for blood $CD3^+$ T cells ([40], Figure 4). Blockade of endogenous IL-21 in mucosal explant cultures of IBD reduces MIP-3α and inhibited lymphocyte migration induced by supernatants of these explants. Analysis of intracellular pathways involved in the control of MIP-3α synthesis in colonic epithelial cells showed that IL-21 activates ERK1/2 MAP kinase and pharmacologic blockade of ERK1/2 kinases significantly inhibits MIP-3α secretion. Overall, these data suggest that IL-21 may be one important mediator by which immune cells interact with non-immune cells in IBD mucosa.

IL-21 enhances matrix metalloproteinase secretion by gut fibroblasts

CD and UC are characterized by the presence in the gut of extensive areas of erosions and ulcerations, which can lead to the development of complications, including bleeding, perforation and fistulae. In CD patients, prolonged mucosal injury leads also to dysregulation of repair mechanisms resulting in excessive collagen deposition and fibrotic strictures. Lamina propria myo-fibroblasts and fibroblasts are involved in these processes, since they make collagen, pro-fibrotic factors and, in the pro-inflammatory environment, large amounts of matrix metalloproteinases (MMPs). MMPs are a family of neutral endopeptidases that can cleave multiple components of the extracellular matrix. These enzymes are normally produced as inactive zymogens and are activated in the extracellular environment. Their activity is tightly controlled by tissue-specific inhibitors of metalloproteases (TIMPs). An altered balance between MMPs and TIMPs probably contribute to the tissue damage and mucosal remodeling seen in IBD [41]. Fibroblasts produce MMPs and/or collagen after stimulation with cytokines made by T cells and macrophages *in vitro*, implying that this is a typical response to inflammation.

We have recently shown that IL-21R is constitutively expressed by gut fibroblasts and that these cells secrete very large amounts of MMP-1, -2, -3 and -9 in their active forms but not TIMPs following exposure to IL-21 [42]. Additionally, neutralization of endogenous IL-21 markedly reduces the inducing effect of CD LPMC supernatants on fibroblast-derived MMPs. IL-21 does not enhance MMP production at the transcriptional or translational level, but rather promotes the secretion of MMPs from the intracellular stocks (Figure 8.4).

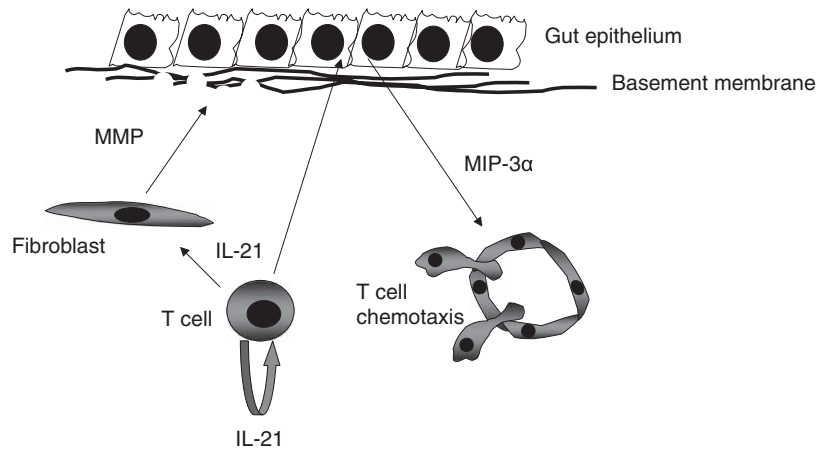

Figure 8.4 IL-21 has multiple effects in the gut. It helps T cell survival in an autocrine loop, induces epithelial cells to make the T cell chemoattractant MIP-3α and induces fibroblasts to make MMPs, which degrade the extracellular matrix and basement membrane.

Putative factors involved in the control of IL-21R on gut fibroblasts remain to be ascertained. However, TNFα and IL-1β can increase IL-21R expression in fibroblasts, suggesting that IL-21 signaling may be enhanced during inflammation. Indeed, IL-21 and TNFα cooperate in inducing MMP production by fibroblasts.

Although IL-21 is over-produced in fibrotic specimens of CD patients, its role in the pathogenesis of gut fibrosis remains unknown. Studies in other models of inflammation-driven fibrosis, however, suggest a potential involvement of IL-21 in this process. For example, mice with deletion of IL-21R gene display a greater than 50% reduction in hepatic fibrosis after infection with *Schistosoma mansoni* in comparison with wild-type mice [43]. Neutralization of IL-21R signaling by soluble IL-21R fusion protein also reduces the development of *Schistosoma mansoni*-induced fibrosis in infected wild-type mice.

Th17 cells, the link between TGFβ and IL-21

IL-17 is a cytokine which was described over 10 years ago. It is produced in multiple isoforms (IL-A–F). It also appears there is a distinct lineage of pro-inflammatory $CD4^+$ cells which preferentially make IL-17 and these cells have been implicated in a variety of autoimmune diseases. There is now some evidence they may also be involved in gut inflammation. For example, the numbers of T cells making IL-17 [44] and IL-17 protein are elevated in IBD [45], particularly in CD. IL-17R knockout mice are protected from TNBS colitis and an IL-17R fusion protein ameliorates TNBS colitis [46].

There has been considerable interest in the cytokines which induce Th17 cells. In mice, whereas TGFβ1 on its own induces virgin $CD4^+$ T cells to become $FoxP3^+$ Treg cells, the addition of IL-6 induces the cells to become pro-inflammatory Th17 cells [47]. IL-6, however, acts indirectly by inducing cells to make IL-21, which then functions in an autocrine loop to drive the generation of Th17 cells [47]. IL-21-deficient mice therefore cannot make Th17 cells [48]. Retinoic acid is also important in the development of mouse Th17 cells [49]. The situation, however, appears to be rather different in humans since TGFβ inhibits the generation of Th17 cells and their development is more dependent on IL-23 and IL-1β [50]. Further studies are needed to determine if IL-17 and Th17 cells are important mediators of inflammation in human IBD and represent a new therapeutic target. However, we have recently shown using human CD4 T cells that IL-21 strongly counteracts the TGFβ-mediated induction of Treg cells and, more importantly, that even in the presence of TGFβ, IL-21 directs the development of Th17 cells rather than Treg cells [51]. This work therefore defines another role for IL-21 in the gut and is also another example where the immunosuppressive effects of TGFβ (in this case the induction of Treg cells) is circumvented.

Conclusion

To put these observations into context, it is important to emphasize that gut inflammation evolved as a host response to eliminate infections and there is therefore a prerogative to maintain pro-inflammatory responses and damp anti-inflammatory responses until the pathogen has been eliminated – hence the need to damp TGFβ effects. The difficulty in CD is that the inflammatory response is directed against the normal microbial flora being seen by the immune system as a surrogate pathogen, but which is highly resilient and cannot be dislodged from its niche in the gut. Hence the most appropriate strategy is to identify the key molecules which maintain inflammation in different patients, disrupt their activity and restore mucosal homeostasis.

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Chapter 9
Host Response to Bacterial Homeostasis

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Summary
- The microbial ecosystem plays a role in both maintenance of homeostasis and the development of inflammation.
- Homeostasis is associated with "tolerance" to the enteric microbiota.
- Inflammation is due to improper innate (epithelial and non-epithelial) and adaptive immune sensing of the enteric microbiota.
- The enteric microbiota determines the immune structure and function of the intestines which in turn regulates the structure and function of the microbiota.
- The intestinal epithelial cell is a critical functional interface between the microbiota and gut associated immune cells.

Introduction

The precise molecular mechanisms by which the mucosal immune system regulates this intricate balance between tolerance and responsiveness is an area of great scientific interest [1,2]. Whereas most individuals maintain "homeostasis" and health in the face of this extreme immunologic challenge, others cannot. Indeed, the clinical entities collectively known as inflammatory bowel disease or IBD (i.e. Crohn's disease and ulcerative colitis) are conditions in which inflammation persists at various anatomic sites along the gastrointestinal tract [3]. IBD is the second most common immunologically mediated disease in the United States, affecting approximately 1 in 200 to 1 in 1000 individuals [4]. Although many details regarding the etiology of IBD remain unclear, a central hypothesis has emerged. Specifically, IBD can be said to represent a *dysregulated immune response to bacteria from the lumen of the intestinal tract in a subset of individuals with a genetic predisposition or propensity to develop chronic, mucosal inflammation.*

This is a broad hypothesis that needs to be viewed within the context of the current genetic data and clinical phenotypes of disease that are consistent with a marked heterogeneity of human IBD. Still, the microbial focus inherent to the hypothesis highlights a series of important cell types at the mucosal interface (T lymphocytes and antigen-presenting cells including intestinal epithelial cells) and their associated questions. For example, what is the nature of the bacterial species that are responsible? Do the predisposing genes relate to immunologic function within the gut-associated lymphoid tissue (GALT) or to intrinsic properties of the gastrointestinal tract (e.g. intestinal barrier function)? What are the immune effector cells that mediate IBD and how are they modulated by bacteria? In this chapter, we address these and other questions and early events relevant to the central hypothesis outlined; in particular, those involving interaction between bacteria and the lymphocytes of the GALT at the epithelial interface and their relationship with the intervening intestinal epithelial cell (IEC).

The microbial bionetwork

Humans exist in a completely open ecosystem – with the anatomy of the entire gastrointestinal tract from the mouth to the rectum in continuity with the external environment. In this context, one might consider the gastrointestinal tract as "outside-in" – with an extensive microbiota derived from the *outside* world resident *inside* the body, generally, with highly beneficial consequences. The human host lives in a complex microbial environment, with the diversity and number of prokaryotic cells far outnumbering the eukaryotic cells of their hosts. In no instance of human health or disease is this fact more important than in a discussion of immunity at mucosal surfaces and the pathogenesis of IBD.

Human and animal research has highlighted the critical nature of the intestinal microbiota in the establishment and maintenance of IBD. The data from human studies include the potential efficacy of antibiotics in treating patients with IBD [5], the resolution of inflammation distal to fecal diversion [6] and the data showing a beneficial

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effect of "probiotic" therapy [7]. The mouse data are even more compelling with observations from many laboratories demonstrating a lack of mucosal inflammation in established mouse models of IBD raised in either a "germ-free" (GF) or a clean "specific pathogen-free" (SPF) environment. These data have extended across the spectrum of mouse models described [3], including models dominated by "Th1" responses (e.g. IL-$10^{-/-}$, IL-$2^{-/-}$, $CD45RB^{hi}$ transfer model, SAMP1/YIT), models dominated by "Th2" responses (e.g. $TCR\alpha^{-/-}$, $WASP^{-/-}$) and models characterized by intrinsic "barrier" defects (e.g. $mdr1a^{-/-}$) (reviewed in [3,8] and Chapter 4). Recent data from a number of laboratories indicate that re-population of these mice with limited numbers of organisms (e.g. *Helicobacter hepaticus*, *Bacteroides vulgatus*, enteroadherent *Escherichia coli*, *Bifidobacterium animalis*, *Fusobacterium varium*, *Enterococcus faecalis*) in these 'clean' mice can trigger the inflammation previously seen when the mice were housed in conventional ('dirty') colonies [9,10]. Interestingly, the same bacterial challenge in normal mice has no untoward consequences and certain bacterial strains may be anti-inflammatory (e.g. *Bacteroides thetaiotaomicron*, *Lactobacillus* sp., *Bifidobacterium* sp., *E. coli* Nissle 1917). Although the molecular details as to how these bacteria induce mucosal inflammation in these genetically susceptible hosts remain to be determined, the mounting evidence favors the "bacterial hypothesis"; i.e. commensal bacteria are required for the development of colitis. Moreover, even in a genetically susceptible context (e.g. IL-$10^{-/-}$), pro-inflammatory bacteria only stimulate inflammation within certain genetic backgrounds, supporting the essential role of genetic composition in determining the inflammatory nature of bacteria [11,12]. Given the estimates of there being approximately 10^9 organisms per milliliter of fecal material (closely approximating the maximum packing density), it is perhaps surprising that not more individuals develop chronic mucosal inflammation.

The mucosal immune system has evolved to balance the need to respond to pathogens while maintaining active "tolerance" to commensal bacteria (and food antigens) [2]. Most individuals exist in a homeostatic balance with the complex microbiota in their intestinal tract [13], which is consistent with the data from mice and family studies in humans highlighting the genetic basis of susceptibility to IBD in certain individuals. Understanding why and how certain people exhibit a "dysregulated" (or pathogenic) response to bacteria that are not universally pathogenic remains central to our detailed understanding of IBD pathogenesis. Still, the microbiota itself can be considered a dynamic, living, organism – and is likely to shape the host mucosal immune response and balance [14]. Moreover, the immune state of the host is likely to shape the composition of the microbial milieu, which may lead in fact to a state that is pro-inflammatory with a propensity to promote IBD [15]. Hence a more detailed understanding of the bacterial populations of the GI tract is likely, as well, to offer important and critical clues to the cause(s) of IBD.

The microbiota of the gastrointestinal tract has been the topic of several recent thorough and insightful reviews from authors with extensive experience in this area [14,16–18]. In this part of the chapter, we focus on microbial aspects most relevant to IBD and not on providing the comprehensive microbiological overview seen in these recent summaries.

Although precise estimates are difficult, there appear to be >400 different species of bacteria present in the colon of the normal human. In actuality, the number is likely to be dramatically higher when viewed in the context that conventional culture techniques result in growth of less than 50% of the organisms present [19]. There are clear regional differences in bacterial populations identified along the cephalo-caudal axis of the gastrointestinal tract (see below), but the overall representation of bacterial genera is extremely broad. In the colon, where bacterial counts are four to five orders of magnitude higher than in intestinal regions proximal to the ileo-cecal valve, all major groups of bacterial species are present (Table 9.1). Specifically, one finds a diverse mixture of Gram-positive, Gram-negative and atypical bacteria (e.g. mycobacteria) that grow under varying conditions (e.g. aerobic, facultatively anaerobic and obligate anaerobic conditions) that achieves a total mass of $10^{13–14}$ bacterial cells. In comparison, the total number of cells in a human host is estimated to be $10^{12–13}$ [20].

A few important technical considerations are important in the attempt to interpret the literature in this complex area and its potential relationship to IBD. First, what culture conditions are used? For example, culture conditions for aerobes or facultative anaerobes are widely available, whereas anaerobic culture is still difficult and problematic. Second, what is the nature of the patient sampling? Certainly, the clinical and medication history (e.g. bowel preparation for surgery or endoscopy) will bias the results obtained from microbiological analysis. In addition, there are likely to be marked differences when biopsies and/or surgical specimens are used as a source for culture compared with stool samples. Finally, what is the cost of such detailed analysis? Given the biodiversity present, the answer is likely to be high. This final consideration has limited the detailed analysis of large numbers of individual patients and detailed "species" analysis. The increasing widespread use of 16S rRNA sequence analysis for microbial detection and analysis [21] is a promising approach now being extended to studies of the gastrointestinal tract [22]. However, analysis of 16S rRNA sequences is likely to be able to identify only a fraction of bacterial species within the gastrointestinal tract and will be unable to identify viruses, eukarya and archea [23]. Therefore, newer methods of analysis are required to characterize this

Table 9.1 Common bacteria in the human large intestine.

Organism	Description	Range
Bacteroides	Anaerobic Gram-negative rods; most common species include *B. thetaiotaomicron*, *B. vulgatus*, *B. ovatus and B. fragilis*	9–12
Fusobacterium	Anaerobic Gram-negative rods; most common species include *F. mortiferum* and *F. necrophorum*	7–12
Peptostreptococcus	Anaerobic Gram-positive cocci; most common species is *P. productus*	5–12
Bifidobacterium	Anaerobic Gram-positive, non-spore-forming rods; most common species include *B. adolescentis*, *B. infantis* and *B. longum*	6–12
Eubacterium	Anaerobic Gram-positive, non-spore-forming rods; most common species include *E. aerofaciens*, *E. lentum* and *E. contortum*	9–12
Lactobacillus	Facultative and obligately anaerobic Gram-positive rods; most common species include *L. acidophilus*, *L. fermentum* and *L. plantarum*	4–12
Clostridium	Anaerobic, spore-forming, Gram-positive rods; most common species include *C. perfringens*, *C. bifermentans* and *C. ramosum*	7–12
Enterococcus	Facultative Gram-positive cocci; *E. faecalis* and *E. faecium* are the most common species	5–10
Streptococcus	Facultative Gram-positive cocci; *S. lactis* and *S. intermedius* are the most common species	5–9
Staphylococcus	Facultative Gram-positive cocci	3–6
Escheria coli	Facultative Gram-negative cocci	3–9
Enterobacter	Facultative Gram-negative cocci	3–9
Klebsiella	Facultative Gram-negative cocci	3–9

important ecosystem and its function such as that provided by bacterial metagenomics [24].

In this context, it should not be surprising that there are few data on the "complete" analysis of the gastrointestinal tract mucosal microbiota – either in normal individuals or individuals with IBD. The "best" analyses are those completed by Gorbach *et al.* [25], Eckburg *et al.* [26] and Ley *et al.* [27]. The current consensus will be briefly summarized.

Stomach/small intestine

The stomach and small intestine, once thought to be "sterile", are sites of considerable microbiologic diversity. The proximal gastrointestinal tract contains abundant flora, derived predominantly from the mouth. These include, but are not limited to, α-hemolytic streptococcus, S*taphylococcus epidermidis*, and various yeast species. The hostile acidic environment of the stomach results in a rapid decline of culturable organisms. Still, the obvious presence and functional consequences of *Helicobacter pylori* infection in the stomach and proximal small intestine highlight the importance of bacterially driven mucosal inflammation even in the proximal gastrointestinal tract [28].

In the small intestine, there is a "transition" from oral to colonic flora. In this organ, one needs to consider ingested food as a microbiological reservoir and source for colonization. For example, dairy products, meats and fruits/vegetables are rich sources of bacteria. The substantial antimicrobial effect of low gastric pH is likely responsible for the fact that bacterial counts are not higher in the proximal gastrointestinal tract. Still, as one moves more distally, one sees increasing concentrations of bacterial counts and increasing numbers of Gram-negative species (e.g. Enterobacteriaceae, *Bacteroides*, *Enterococci* spp.) as one approaches the terminal ileum. The total counts in the ileum are 10^3–10^4 times higher than in the jejunum and one study has estimated the counts at the terminal ileum as approximating those seen in the proximal colon [29].

Colon

In both mass (estimated to be 10^{11} organisms per gram of dry weight of stool) and number (estimated to be >400 species) of bacterial organisms, tremendous biodiversity is present within the colonic microbiota. Consistent "themes" regarding the nature of colonic bacteria exist. First, obligate anaerobes are the predominant bacterial species in the colon, comprising >99% of the total population. Reflecting a delicate balance between diet, environmental influences and host genetic factors, considerable genus and species variation in this microbiological population exists between individuals. Still, species of the Gram-negative, obligate anaerobe *Bacteroides* spp. (e.g. *B. vulgatus*, *B. fragilis*, *B. thetaiotoamicron*) comprise the major subset of bacteria in humans and several other carnivores

and represent a large portion of the so-called "normal" flora which is always present in large numbers in the population. Facultative aerobes (e.g. *Escherichia coli*, *Enterococcus* spp., *Staphylococcus* spp.) are also common in the colon, but are present at concentrations typically two logs less than the obligate anaerobes. As mentioned, a large variety of other bacteria, including the Gram-positive rods of the genus *Lactobacillus* and *Bifidobacteria* (common bacteria used as "probiotics"), are also seen in the colon and are summarized in Table 9.1.

Two more recent analyses characterizing the intestinal microbial flora in humans and mice using 16S rDNA amplification confirmed the above-described data and revealed interesting new findings [26,27]. First, the studies demonstrated that both human and mouse intestine contains relatively few bacterial and archaeal divisions compared with other microbial habitats. The human intestinal samples contained members of nine divisions of bacteria (Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Spirochaeates, VadinBE97 [26,30]) and the mouse survey showed a very similar number and set of divisions [27]. At these broad phylogenetic scales, the intestinal communities of all human hosts appeared fairly similar [31], with the Firmicutes and the Bacteroidetes (two of at least 50 known bacterial phyla) accounting for more than 98% of all 16S rRNA sequences in each mammalian host. This lack of diversity among phyla in the gut, however, dramatically contrasted with a high diversity on the subspecies level. Over half of the 13,335 16S rRNA sequences in the human colonic data set were sampled only once and therefore can be considered to represent strains (subspecies) [26]. This high number of strains was derived from about 395 species according to 16S rRNA sequence identity [26]. At this phylogenetic fine scale level, with respect to strain identity and relative abundance, the microbiota of an individual appeared to be as personalized as a fingerprint [31]. However, bacterial communities sampled at different points along the colon were similar within each of the three human hosts and their composition overlapped with the fecal microbiota [26].

Two important points should be mentioned in the context of this microbial diversity. First, the mucosal microbiota is a dynamic "structure" – with changes capable of resulting in significant clinical impact. A notable example is the expansion and "overgrowth" of *Clostridium difficile* in a subset of patients treated with broad spectrum antibiotics [32]. The resulting diarrhea and pseudomembranous colitis that results from the elaboration of *C. difficile* toxin in these patients is a reminder of how a "dysregulated" microbiota may result in untoward mucosal inflammation. In contrast to the example of *C. difficile*, wherein a common commensal may overwhelm the microbial ecosystem, are the recent examples which suggest that overall changes of microbial ecology may be linked to disorders of metabolism (obesity) and inflammation (inflammatory bowel disease) [8,27]. On the other hand, the use and limited efficacy of "probiotic" therapy [7] highlights the possibility of manipulating the colonic flora to suppress mucosal inflammation. Second, one must realize that our current view of the colonic microbiota is truly an "overview". Subpopulations of microbes within specific microenvironments or "niches" are likely to exist and are overlooked by even sophisticated methods of analysis of the isolated samples currently obtained in clinical and/or experimental studies. Both the changing flora and local microenvironments are likely to have significant implications in the microbial pathogenesis of IBD.

Relationship of microbial populations to IBD

Comparisons of the microbial populations between "normal" controls and patients with IBD are critically important to understanding the pathogenesis of IBD; however, such analysis is replete with potentially misleading information and needs to be viewed with caution. For example, to date, the analysis of mucosal microbiota in IBD has been performed in patients with chronic disease who have been treated medically and/or surgically [33]. Although an important first step, it remains to be seen if these data reflect the early events associated with IBD pathogenesis. One notable exception is a study from Scandinavia looking at microbial differences in children with IBD [34]. In addition, one needs to consider phenotypic differences between the same genus/species of bacteria isolated from a patient with IBD compared with control patients. The report of increased adhesion of bacteria from patients with IBD is notable in this regard [35], wherein recent studies implicate enteroadherent *E. coli* species in disease, interestingly through their ability to bind members of the carcinoembryonic antigen cell adhesion molecule (CEACAM) family expressed on intestinal epithelial cells [36,37].

Mechanisms of bacterially driven IBD

A relative "consensus" is emerging regarding the fact that luminal bacteria are involved in the establishment and maintenance of IBD, but several hypotheses have been advanced to explain the mechanism(s) underlying this bacterial "trigger" [17,38] . In general, these can be broadly defined into those that are directly related to pathogenic factors of microbes, those that are related to alterations of luminal energy metabolism, those that are related to activation of innate immune factors through interaction between microbial products and "pattern recognition" receptors of the host and those that are related to specific nominal antigens (peptide, glycolipid) which stimulate adaptive (or acquired) immune responses associated with the interactions between antigen-presenting cells and lymphocyte populations (T and B cells).

To date, attempts to prove that IBD is the result of mucosal infection have consistently proven negative [17], yet

our current level of understanding of the pathogenesis of IBD does not completely rule out an infectious etiology or component of a pathogenic organism. A pathogen can be defined as a microorganism that employs a particular virulence determinant that allows the microorganism to bypass host protective mechanisms and directly elicit cellular and tissue injury, which is further promulgated by the effects of host immune (innate and adaptive) responses. It could be argued, for example, that in the genetically susceptible host, the normal (putatively nonpathogenic) microbiota of the intestine is perceived as if it were a pathogen [3]. Regardless, it is clear that so-called pathogenic microorganisms utilize a wide variety of mechanisms either to parasitize and/or to inflict injury upon the host [38]. These include the ability to adhere to IECs through a variety of mechanisms, to invade IECs directly, to spread between IECs and to secrete a variety of injurious toxins (Table 9.2).

It has been hypothesized that IBD is a disease caused by IEC starvation, hence an interesting aspect of the luminal microbiota that is important relative to IBD is the concept that bacteria may affect the energy metabolism of IECs [39]. Specifically, it has been proposed that subsets of anaerobic bacteria, through their production of hydrogen sulfide, inhibit the metabolism of short-chain fatty acids (SCFAs), an important energy source for IECs. Interestingly, there are some data supporting this notion in that a beneficial role for topical SCFA therapy has been observed in human IBD [40].

Pattern recognition receptors are components of the host immune system (either soluble or membrane associated) that recognize characteristic structural features of the microbial world [41]. As such, they are part of the innate or so-called natural immune, system, a "hard-wired" system that allows for rapid responses to microbes in contrast to the delayed responses associated with the adaptive immune system (see below). These receptors (summarized in Table 9.2) recognize specific structures, usually carbohydrates, associated with microbes that either indirectly through stimulation of other innate and adaptive components or directly through initiation of phagocytosis promote the removal of microbes and their products. Arguably, these host structures may also conceivably aid in the maintenance of immune tone such as that associated with the GALT. Indeed, in genetically susceptible hosts, bacterial products such as peptidoglycan-polysaccharides

Table 9.2 Mechanisms of bacteria-driven IBD.

Pathogenic category	Functional category	Organism, molecule or cellular subset	Mechanism of action
Microbial virulence factors	IEC adherence	ETEC	Specific colonization factors
		EPEC	Attachment and effacement via Bfp pili
		Yersinia enterocolitica	Intimin binds to $\alpha 4\beta 1$ integrin
	IEC invasion	*Shigella* spp.	Type III secretion system
		Salmonella spp.	Type III secretion system
	Cytotoxins	*Clostridium difficile*	*C. difficile* toxins A and B
		EHEC	Shiga-like toxins
Luminal dysbiosis	Abnormal metabolism	*Eubacteria* spp.	Hydrogen-sulfide inhibition of SCFA utilization
Pattern recognition molecules of innate immune system	Microbial polysaccharide recognition	C-reactive protein	Activates complement and phagocytosis
		Serum amyloid protein	Facilitates phagocytosis
		Mannose binding protein	Binds C19 receptor and promotes phagocytosis
	Microbial glycolipid recognition	DEC-205	Cell surface protein that targets MHC class II pathway
		Lipopolysaccharide-binding protein	Inactivates LPS
		Soluble CD14 and CD14	Binds and regulates pro-inflammatory response to LPS
		Surfactant protein A	Binds and aggregates lipids
Adaptive (specific) immune responses	Cellular	$CD4^+$ T cell	Recognition of bacterial peptide antigens on APC in context of MHC class II leading to excess pro-inflammatory (TNFα, IFN-γ) relative to anti-inflammatory (TGFβ, IL-10) cytokines
	Humoral	B cell	Production of autoantibodies that cross-react with microbial antigens (e.g. ANCA cross-reactivity with I2 sequence of *Pseudomonas* spp.)

ETEC, enterotoxygenic *E. coli*; EHEC, enterohemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; ANCA, anti-neutrophil cytoplasmic antibody.
Adapted from [8,17,38].

from Eubacteria can elicit IBD-like pathology, perhaps in part through innate immune mechanisms [17]. Alternatively, elimination of normal pattern recognition signaling such as through deletion of that which is mediated by Toll-related receptors (TLRs) through interruption of MyD88 disrupts mucosal homeostasis, creating a susceptibility to inflammation [42]. Thus pattern recognition receptors both promote and protect from mucosal inflammation.

Finally, specific immune responses, which are initiated by presentation of nominal antigens from microbiota to T and B cells, provide long-term protection to microbial pathogens through the generation of memory responses and maintenance of immune homeostasis (tolerance) to non-pathogens (normal microbiota) [1,43]. These include the classical antigen presentation pathways associated with the major histocompatibility complex (MHC) class I and II molecules for presentation of peptides and the non-classical MHC class I-like molecule, CD1, for presentation of glycolipid antigens (see below) [44,45]. Dysregulation of this aspect of the immune system, as clearly seen in a large variety of animal models and implicated in humans, leads to inappropriate tissue damage and/or autoimmunity through molecular mimicry between microbial and host antigens [46–50].

The intestinal epithelial barrier

In the context of the growing body of evidence linking luminal bacteria to the pathogenesis of IBD, one needs to consider the nature of the anatomical barrier that separates the overwhelming number of bacterial organisms from the underlying mucosal immune system. In addition to the marked differences between the microbiota of the small intestine and that of the colon, there are clear distinctions in the microarchitecture and organization of lymphoid tissue that are likely to affect the interaction between bacteria and immune cells at these sites. For example, in the small intestine, one sees classical lymphoid follicles [Peyer's patches (PPs)] in addition to villous structures composed of columnar epithelial cells. It appears that certain pathogenic bacteria selectively gain access to the mucosal immune system via the intestinal epithelial cells characterized by microfold villous architecture (M cells or follicle-associated epithelium, FAE [51]) overlying these follicles [52]. It remains to be determined whether "non-pathogenic" bacteria, which are likely to be responsible for stimulating IBD in genetically susceptible hosts, contact the underlying immune system via FAE or by interacting with the conventional epithelial surface. Given the fact that the absorptive columnar epithelial surface area is orders of magnitude greater than that of the FAE and that many bacteria adhere to and invade columnar epithelial cells, it is likely that bacterial entry is not restricted to lymphoid follicles. Indeed, in the colon, "classical" lymphoid follicles (PPs) are absent, although small lymphoid aggregates, presumably with modified FAE, can be seen (see below), yet bacteria are able to cross the mucosal barrier and trigger IBD. Moreover, it may be possible that bacterial translocation *per se* is not necessary for development of IBD but rather the movement of bacterial products (antigens) across the IEC via a process termed transcytosis. Such a possibility of apical to basal transport of bacterial antigens has now been described through the appreciation that the neonatal receptor for IgG transport functionally exists in adult human IECs and is able to transport IgG–antigen complexes across the IEC to professional antigen-presenting cells (APCs) within the lamina propria [53,54].

The barriers to bacterial entry across the intestinal mucosal surface are numerous and varied. From an anatomical standpoint, there is an extensive mucinous glycocalyx extending from the apical (i.e. luminal) surface of epithelial cells that can trap bacteria and prevent adherence [55]. In addition, epithelial cells are connected to one another by so-called tight junctions that effectively limit the paracellular transport of macromolecules across the epithelial barrier [56]. Furthermore, various populations of cells along the gastrointestinal tract (e.g. Paneth cells) are capable of secreting small peptides (e.g. defensins) that have potent, local, antimicrobial properties (see below).

How do bacteria breach this formidable barrier and stimulate mucosal inflammation? The answer is complex, but derives in part from the growing realization that alterations in intestinal barrier function are seen in IBD—and that this "dysregulation" of barrier function may contribute to the establishment of IBD. Indeed, the term "dysregulation" (and not simply disruption) underscores the fact that the barrier is a dynamic structure that is actively regulated and maintained and is composed of more than just the IEC itself but includes the products of the IEC in addition to locally associated lymphoid and myeloid cells and their products. Based on seminal studies using polarized monolayers of intestinal epithelial cells *in vitro*, it is clear that various cytokines present in the intestinal mucosa regulate intestinal barrier function. Notably, tumor necrosis factor, γ-interferon, IL-4 and IL-13 all attenuate barrier function [57–60], whereas IL-10 appears to "enhance" the barrier [61]. Although the precise mechanisms underlying this regulation are not known, recent data suggest that alterations in the apical actin cytoskeleton might disrupt the structural integrity of the tight junction [62].

Tight junctions consist of a complex and tissue-specific composition of a variety of proteins including tricellulin, occludin and the members of the claudin, zonula occludens and junctional adhesion molecule families [56]. The barrier function of tight junctions is primarily regulated by claudins, a family of at least 24 proteins which are

expressed in a tissue-specific pattern and which largely differ in their barrier characteristics, thereby exhibiting sealing (e.g. claudin-1, -4, -5) or pore-forming properties (claudin-2, -16) [56]. Alterations of the composition of tight junctions was reported for both Crohn's disease (CD) and ulcerative colitis (UC) and consisted in upregulated expression of the pore-forming tight junction protein claudin-2 (CD and UC) and also in decreased expression and intracellular redistribution of several sealing claudin family members (CD) [60,63,64]. In the case of UC, these changes were mainly the consequence of IL-13 signaling, a pro-inflammatory cytokine which is likely to be derived from intestinal CD1d-restricted natural killer T (NK-T) cells [60].

Finally, it is important to consider how alterations in intestinal barrier function (with the untoward effect of increased transit of bacteria and/or their antigenic components into mucosal tissues) may contribute to an underlying genetic predisposition to the development of IBD. As noted, decreased barrier function is a hallmark of established IBD [65]. Although controversial, some investigators have suggested that alterations in barrier function actually precede the appearance of IBD and are seen to a greater degree in healthy, first-degree relatives of patients with IBD [66]. These human data are supported by mouse data showing alterations in barrier function preceding histological inflammation in the IL-10 knockout mice [67] and spontaneous ilieitis in the SAMP1/yit mouse model [68] and the enhanced mucosal inflammation that could be induced in mice deficient for intestinal trefoil factor, a molecule important in intestinal epithelial repair and restitution [69]. Taken together, the information suggests that subtle alterations in barrier function (e.g. abnormal regulation of tight junctions, impaired epithelial restitution) may contribute to the underlying genetic susceptibility to IBD seen in humans and some experimental animals. One might predict that the function of one of more of the IBD susceptibility genes being mapped by various groups will relate to intestinal barrier maintenance. Indeed, taking a broad definition of barrier function as expounded above, this may indeed be the case with the identification of DLG5, an IEC scaffold protein encoded on chromosome 10 and an organic cationic transport encoded on chromosome 5 as genetic risk factors for IBD [70,71].

Immune cell types

Phenotype of T cells

Exemplary of the fact that the mucosal surfaces of the intestine must confront a large microbial antigenic burden, it is not surprising that it has been estimated that approximately 10% of the T lymphocytes in the normal host are associated with the GALT [72]. These GALT-associated T cells are organized into three compartments connected by distinctive and highly regulated trafficking pathways. These include the PPs and related isolated lymphoid follicles that are considered important sites of immune response induction and the loosely affiliated lamina propria and intraepithelial lymphocyte compartments, which are considered effector compartments [73]. Consistent with this, most T cells within the PPs are naïve, thymically derived T cells that are characterized as $CD3^+$ $TCR\alpha\beta^+$ $CD45RA^+$ $\alpha4\beta7^{hi}$ L-selectin $(CD62L)^+$ $CD44^{lo}$ $\alpha^E\beta_7^-$ with a CD4:CD8 ratio of 3.5:1. These naïve T cells, and also their naïve (IgM^+ IgD^+) B cell counterparts, are directed to the PPs via interactions between $\alpha^4\beta_7$ and L-selectin on the T cell and the protein and carbohydrate constituents of MadCAM1 on the high endothelial venule (HEV), respectively. In addition, contributions are provided from interactions between LFA1 ($\alpha^L\beta_2$, CD11a/CD18) and either ICAM-1 (CD54) or ICAM-2 (CD120) on the lymphocyte and HEV, respectively. Consistent with this naïveté, most of the T cells possess a diverse array of TCR-αβ chains [74]. Few TCR-$\gamma\delta^+$ T cells can be observed within the PPs, consistent with the notion that they have received their priming earlier in ontogeny within the thymus prior to the development of the PPs and/or are not directed to this location by lack of appropriate homing properties [75].

At any one time, a significant subset of these naïve T cells within the PPs are likely the recipients of their cognate antigen-derived signal from the luminal milieu as transported to the lymphoreticular structures of the PPs by specialized microfold villous (M) cells. This is based on the fact that the CD45RA:CD45RO ratio within the organized GALT is 1:1 and the majority of these $CD40RO^+$ (memory) cells are congregated within the M cell pocket immediately adjacent to the lumen in the context of professional APCs [76]. The range of luminal antigens, including antigens from commensal bacteria, that are specifically sampled by the M cell and/or breach the M cell barrier through pathologic mechanisms (e.g. pathogenic microbes) thus dictates the generation of antigen-specific B and T cell blasts which emigrate from the PPs via efferent lymphatics.

The antigen-specific $CD4^+$ and $CD8^+$ T cell blasts, including memory populations, which are largely $\alpha^4\beta_7^{hi}$ L-$selectin^{lo}$ $CD45RO^+$, presumably migrate from the PPs and disseminate widely to the loosely affiliated tissues associated with the lamina propria and epithelium. This occurs through interactions between protein components of MadCAM1 on the post-capillary venule (PCV) and $\alpha^4\beta_7$ on the T cell together with interactions between LFA-1 on the T cell and ICAM-1/ICAM-2 on the PCV within the lamina propria, making this largely an effector compartment. That said, recent studies have suggested that at least in the case of B cells, naïve B cells may be able to home directly to the lamina propria wherein they receive signals from the IEC and dendritic cells (DCs) independently of T cells that cause them to undergo maturation and switching from IgM bearing to IgG and IgA bearing and secreting

B cells [77]. Such a pathway has been proposed to be under the control TNF-related family members such as BAFF and thymic stromal lymphopoietin protein (TSLP) and is further likely regulated by TLRs on epithelial cells [78]. Such a pathway may be particularly important to the generation of commensal bacteria-specific IgA such that the IgA production is induced by commensal bacteria and in turn regulates the composition of the commensal microbiota through secretion of the IgA via the apically directed polymeric Ig receptor [79].

Returning to the conventional pathway of lamina propria population, whereas both $CD4^+$ and $CD8^+$ memory cells and blasts from initial antigen encounters populate the lamina propria, the epithelium is preferentially populated by $CD8^+$ T cells. The basis for this is unclear but presumably reflects differences again in homing properties of these cells, their interactions with secreted factors and cell surface molecules from IECs and consequently in the presumed effector functions of the T cells within the lamina propria and epithelium [80].

Lamina propria lymphocytes (LPLs)

LPLs represent a tightly regulated effector compartment. Most of these T cells express the memory marker, CD45RO (>80%), with a ratio of CD4 to CD8 cells which approximates peripheral blood (2:1) [43,73]. Based on TCR repertoire analysis, the $CD4^+$ LPLs are directed at a broad range of antigens with evidence of a limited number of clonally expanded cell populations [47]. Although the nature of the antigens to which these small numbers of clonal expansions is directed is unknown, it is presumed that they relate to secondary responses to previously remote antigenic encounters. This is consistent with the fact that LPLs, which are responsive to remote enteral viral infections and presumably microbial infections, for example, can be identified [81]. The $CD8^+$ LPLs, on the other hand, exhibit a significantly greater proportion of clonal expansions as defined by complementarity-determining region 3 (CDR3) analyses, suggesting, perhaps, either a larger number of secondary exposures for this subset and/or differences in their susceptibility to activation induced cell death in comparison with the $CD4^+$ T cell subset [47].

The entire LPL compartment, both CD4 and CD8, is highly activated. This activation is manifest by a phenotype which is uniformly L-selectin^{lo} $CD44^{hi}$ $CD69^+$. However, at the same time, the activation state of LPL is restrained since only a limited proportion of these cells express the high-affinity IL-2 receptor α chain, CD25 (<20–25%) [73]. Moreover, LPLs exhibit limited evidence of spontaneous proliferation based on expression of Ki67, a nuclear marker of proliferation or uptake of bromodeoxyuridine. Additionally, LPLs are hypoproliferative to TCR/CD3 complex-mediated signals *in vitro* [82]. However, at the same time, LPLs respond to antigenic signals with significant cytokine production (as measured by IL-2). These characteristics suggest that, under physiologic circumstances, LPLs are chronically stimulated by antigens ubiquitously present in the intestinal environment but which elicit predominantly cytokine production and little proliferation. This maintains tight control on LPL T cell numbers. The cytokines produced are consistent with a predominantly T helper (Th) 2 and/or Th17 tone under normal physiologic conditions, depending upon the location of bowel examined [83,84]. In the physiologic context, it is likely that the antigens responsible for this unique state of T cell activation are derived from the intestinal microenvironment and, presumably, components of the normal microbiota. Interestingly, the sites of the intestine that are normally in a state of exaggerated Th17 tone under normal circumstances may be those that are most susceptible to the development of IBD such as the distal ileum [84]. It is also notable that mice expressing a transgenic T cell receptor specific for ovalbumin in the context of MHC class II I-A^d do not exhibit this activated LPL phenotype when the transgenic animal is back-crossed on to a recombinase activating gene (RAG)-deficient background [85]. In the absence of the RAG gene, the expression of non-allelically excluded T cell receptor (TCR)-α chains is prevented. Hence, taken together, it can be argued that the LPLs are maintained at a certain level of restrained activation by components that are either derived from the host and/or the normal microbiota, poised for a pathologic assault. It is important to mention at this point the mechanisms by which antigens from the lumen gain access to the LPL compartment. These can be either transcellular across the intestinal epithelial cell by either fluid phase transport or receptor-mediated transcytosis via antigen–antibody complexes of IgG with the neonatal Fc receptor [53,54] or IgE with Fcε-receptor [86] or paracellular by either nonspecific bulk transport or via projections from dendritic cells access the lumen through the tight junctions [87].

The loosely affiliated lamina propria also contains varieties of organized structures: lymphocyte-filled villi (LFVs), isolated lymphoid follicles (ILFs) and cryptopatches. LFVs are congregations of predominantly $CD4^+$ $CD45RO^+$ T cells with variable numbers of B cells and MHC class II^+ dendritic cells with an overlying epithelium similar to FAE [88]. ILFs are submucosal lymphoid aggregates that contain a B cell follicle and memory T cells, suggesting a region undergoing a cognate immune response. Finally, cryptopatches, which are present in rodent but not adult human intestinal tissues, consist of lamina propria structures that appear to be the source of extrathymic T cell lymphopoiesis within the intestine. These structures are independent of luminal bacteria (occur in germ-free animals) and a thymus (occur in nu/nu mice) but are dependent on IL-7 and consist of a limited number (2,000–5,000) of c-kit^+ $IL7R^+$ $CD44^+$ $Thy1^{+/-}$ $CD4^{+/-}$ $CD25^{+/-}$ $\alpha^E\beta_7^-$ Lin^-

(lineage markers such as CD3, B220, Mac-1 and Gr-1 associated with T cell, B cell, macrophage and granulocyte development, respectively) cells which give rise to TCR-αβ and -γδ cells [89].

IELs

IELs are predominantly T cells with few, if any, B cells [80]. Although IELs are present in the epithelium during antenatal life as early as 11 weeks of gestation, suggesting an initial antigen independent origin, their numbers increase rapidly post-natally. This massive increase (more than 10-fold) appears to be driven by the luminal microbiota as it is not evident in germ-free mice and rats [90]. Although it is possible that these developmental changes may be due to the induction of phenotypic changes in the epithelium and associated structures, there is some reason to believe that these changes are antigen driven as the effects of the microbiota on IELs are also reflected in changes in TCR repertoire [91].

Most IELs of mouse and human origin are $CD45RO^+$ (memory) $CD8^+$ T cells which express a limited array of αβ TCRs, suggesting restriction to a narrow range of MHC class I-related molecules and their antigenic ligands. Whether these putative antigens are derived from the normal luminal microbiota, thus reflecting important immunoregulatory functions of these cells such as the MHC class I chain-related genes (MICA [92]), or are directed to either noncognate "stress signals" on altered intestinal epithelial cells or remote pathogenic exposures remains to be defined. It is likely that all these possibilities are operative, although their relative contributions at any given time are unknown. IELs have been shown to have immunoregulatory functions associated with oral tolerance, to exhibit recognition of nonclassical MHC-like molecules (see below) and to exhibit antigen-specific, MHC recognition of remote viral antigens, for example [80].

Like LPLs, IELs are in a unique state of activation. They are almost uniformly $CD69^+$, $CD44^{hi}$ and L-$selectin^{lo}$, consistent with previous exposure to an activation signal delivered presumably through the TCR [73]. Given that this phenotype is evident in the "physiologically" inflamed intestine, it seems plausible to suggest that these antigenic signals are derived from the luminal microbiota. Whether the oligoclonality normally associated with human and rodent IELs is related to these same antigenic signals is unknown [93–95].

In addition to these activation markers, IELs also express a unique constellation of homing markers consistent with their mucosal localization within the epithelium ($\alpha^E\beta_7^+$, $CCR9^+$) and natural killer markers [96–98]. The latter are interesting as this group of molecules (killer inhibitory and activating receptors, KIR and KAR, respectively) affect the regulation and activation state of cells in the human intestine, especially under inflammatory conditions [99]. CD94/NKG2D recognizes MICA/MICB (and its mouse homologue RAE), leading to activation of cytolysis by IELs [100]. Such a pathway may be important to the epithelial injury in both celiac disease and IBD [100,101]. The expression of such KIRs and KARs is but one possible mechanism by which IELs may be tightly controlled despite constant exposure to their cognate, perhaps luminal bacterial, antigens as reflected by their anatomic location and activation state. Control of IELs and probably LPLs is also likely mediated by a large array of soluble mediators including TGFβ, IL-10 and unknown factors, many of which are derived from the IEC itself [102]. Whether these factors are directly regulated by the luminal microbiota is unknown.

Regulation of lymphocyte populations by luminal microbiota

Given the apparent central role of the normal luminal microbiota in regulating the development of IBD and that colonization of the mammalian intestine occurs prior to the onset of disease in genetically susceptible animals (rodents and humans), it is important to consider how the phenotype and function of mucosal lymphocyte populations are regulated by such factors. There are two potential roles of the luminal microbiota: promoting the development of the normal GALT and maintenance of the state of physiologic inflammation. Given that both of these are likely operative in the normal mammalian host, it is possible that in the genetically susceptible host, abnormalities of one and/or the other processes may exist. It is therefore important to consider the supporting evidence for these processes in the normal host and what is known in the genetically susceptible host.

Most of the work in this area has been performed with respect to the development and responses of B lymphocytes [103]. Germ-free rodents exhibit functional hypotrophy of the cells and tissues associated with the GALT. The PPs are small, poorly developed structures that primarily contain B cells which exhibit diminished responses to B cell mitogens and antigens. Most of the B cells which are present have a naïve (IgM^+IgD^+) phenotype with few or none IgG^+ or IgA^+ cells, indicating that these cells have switched their immunoglobulin locus under stimulation of cognate antigen. Consistent with this, IgA^+ antibody-secreting plasma cells are markedly diminished in the spleen and lamina propria. In comparison, rodents raised in conventional conditions exhibit PPs with secondary lymphoid follicles with reactive germinal centers as would be observed in an antigen activated peripheral lymph node, high levels of switched B cells (IgG^+ and IgA^+) in the PPs and a significant number of IgA-secreting plasma cells in the spleen and lamina propria. Interestingly, when adult rodents which had previously been raised under germ-free conditions are later colonized with nonpathogenic microorganisms, their immune responses are often augmented [104]. This suggests that if mucosal priming does

not occur either at the correct time of life or with the appropriate microbial antigen, hyper-responsiveness to the microbial antigen may ensue, a phenomenon of obvious relevance to IBD. Whether this is due to an imbalance of effector cells over regulatory cells or vice versa is unknown but these remain valid possibilities.

It is also important to recognize that not all organisms have the ability to drive the development and activation state of the GALT. Some organisms which are components of the normal microbiota elicit no detectable immune response as assessed by the presence of IgA antibodies. Such organisms are considered part of the autochthonous microbiota which presumably have coevolved with the host to such a degree that are truly tolerated nonpathogens. Other organisms, on the other hand, which are also components of the normal microbiota, elicit a nonpathogenic secretory IgA immune response. This immune response is, however, self-limited in that secondary exposures with the same microorganism is muted. At the same time, the immunizing microorganism can be found intraluminally coated with IgA. Thus the normal microbiota of the mammalian intestine stimulates a state of tolerance that is manifest by the production of specific secretory IgA antibodies that likely prevents the further uptake of the relevant microbe [79].

Similar regulation of the development and activation of the cellular immune system has also been elucidated [103]. As noted above, the numbers of TCR-$\alpha\beta^+$ IELs, but not TCR-$\gamma\delta^+$ IELs, increase dramatically with bacterial colonization [90]. Moreover, the spontaneous cytotoxic activity of these IELs, a characteristic feature of these cells, is also markedly upregulated. Thus, the cytotoxicity of IELs obtained from conventionally reared animals is markedly elevated over those observed with animals reared under germ-free conditions. Not only the $CD8^+$ but also the $CD4^+$ compartment is affected. $CD4^+$ T cells from germ-free animals exhibit a diminution in the autologous mixed lymphocyte reaction. Consistent with these functional observations, most PP T cells in germ-free animals are naïve ($CD45RB^{hi}$), similar to the levels observed in a sterile peripheral lymph node. Upon colonization, increased levels of activated or memory ($CD45RB^{lo}$) cells are observed. Given that all of these characteristics are observed in healthy animals, it can only be assumed that the normal microbiota establishes a unique state of inflammation which is associated with the health of the host. Moreover, this healthy state affects not only the physiology of the intestine but extends to the whole host [105].

The intestinal epithelial cell as an antigen-presenting cell

The gastrointestinal tract represents a unique immunologic compartment which subserves an important role in host defense against a wide variety of microorganisms and the regulation of responses to foreign antigen [2,43]. The management of antigen responsiveness is accomplished through complex pathways of antigen uptake and processing by APCs and their subsequent presentation to T lymphocytes. In the intestine, APCs include not only cells of the monocyte–macrophage lineage and B cells, but also potentially nonconventional APCs such as the IEC [106]. The gastrointestinal tract epithelial cells may have a unique role in immunoregulation, since luminal events are likely important in normal gut homeostasis and may be a prerequisite for the initiation and/or perpetuation of inflammation as occurs in IBD. The IECs are the first host cells to come in contact with dietary and microbial antigens and are therefore in a unique position to function as an early host-signaling system to the immune cells located adjacent to and in the underlying intestinal mucosa. Therefore, antigen-presenting molecules that are expressed on the IEC cell surface may function in the regulation of gut immune responses by presenting specific antigen-containing ligands to T cells which function in the direct activation and/or downregulation of local T lymphocytes. In the former case, this activation may be associated with engagement of regulatory T cells which mediate downregulatory signals. These MHC restricted signals which are delivered to local T cells by IECs are geared towards the self-perpetuation of the intestinal barrier, the removal of altered epithelial cells injured by stress (including hypoxia), infection and/or neoplasia and the tight regulation of responses to antigen at the mucosal surfaces [106]. As such, the IEC and local gut T cells within the epithelium and lamina propria utilize the fine specificity provided by MHC-related molecules on intestinal epithelia and the TCR and costimulatory molecules on T cells to regulate barrier function, antigen absorption and processing, immunosurveillance and local immunoregulation.

Antigen presentation

It is now well established that there are two major pathways of antigen presentation: the MHC class I pathway and the MHC class II pathway (reviewed in [44]). T cells recognize processed nominal antigens, usually peptides, derived from proteolytic degradation of a larger polypeptide chain. These processed peptides are recognized in the context of an MHC class I or II molecule on the cell surface of an APC. MHC class I consists of a 43–45 kDa glycosylated heavy chain noncovalently associated with β2-microglobulin which presents a nonapeptide, acquired from the transporter associated with antigen presentation (TAP), to $CD8^+$ T cells. MHC class II consists of a 32–34 kDa αβ heterodimer that presents much longer peptides (14–22 amino acids) to $CD4^+$ T cells. Since MHC class I generally presents an array of peptides derived from the degradation of intracellular proteins and MHC class II from extracellular proteins, these antigen-presenting

pathways have likely evolved to a large extent for the protection of the host from unforeseen intracellular and extracellular deleterious events, respectively.

As described above, T lymphocytes are abundant in the gastrointestinal tract and are implicated in a wide variety of "physiological" immune responses (both oral tolerance and responses to invasive pathogens) and "pathophysiological" responses (for example, the induction/maintenance of chronic inflammation or IBD). T cells are stimulated by foreign protein antigens via specific molecules on their surface (the so-called T cell receptor or TCR) which recognize processed nominal fragments of the antigen. These fragments are "presented" to the T cell by molecules of the MHC complex which are expressed on the surface of so-called antigen-presenting cells (APCs). These APCs also express additional surface molecules (e.g. co-stimulatory molecules such as CD80 and/or CD86) that are required for maximum stimulation of T cells via counter receptor expressed on the surface of the T cell other than the specific TCR. The majority of APCs are bone marrow-derived cells [e.g. dendritic cells (DCs)] with specialized mechanisms for regulated and efficient antigen uptake, processing and presentation. In addition, within specific anatomical contexts, other "nonprofessional" APCs exist. These are cells which express MHC antigens and have a limited, but significant, capacity to process and present antigen to T cells.

One such "nonprofessional" APC relevant to the gastrointestinal tract is the polarized epithelial cell which separates the extremely high concentration of foreign antigen from the underlying lymphoid tissue. Consistent with a potential role in antigen presentation, these IECs are in intimate contact with the various T cell populations previously described. Specifically, IECs have extensive contact along their lateral and basal surface with IELs and contact underlying LPLs via basolateral projections through the semi-porous basement membrane.

As outlined, considerable reactivity against bacterial antigens exists within the T cell compartments of the GALT. The mechanisms underlying the processing of intact bacteria and/or bacterial fragments and the generation of specific bacteria-derived T cell epitopes remain poorly defined, however. The GALT and regional nodes (e.g. mesenteric lymph nodes) are replete with potential APCs, including apparently distinct populations of DCs, which may be involved in processing of bacterial antigens [107]. In addition, the anatomical considerations noted above and the fact that IECs of the colon are exposed to the highest concentration (by several orders of magnitude) of luminal bacteria and bacterial antigens of any cell in the gastrointestinal tract highlight the possibility that these cells may participate in the processing and/or presentation of bacterial (or other) antigens to T cells in the GALT. In this section, we review some of the recent data consistent with this supposition, focusing on several of the unique features of IEC antigen processing and presentation that distinguish these cells from other more conventional APCs.

Antigen presentation by IECs

General features of IECs as antigen-presenting cells

In order for IECs to act as APCs, they must be able to internalize and process antigen. The apical mucous layer and glycocalyx in the intestine can restrict the size of particles capable of being internalized by IECs favoring antigen uptake by the modified "dome" epithelium (M cells) which overlie PPs [108]. The highly specialized M cells facilitate transport of macromolecules without significant "cellular processing" to the underlying lymphoid tissue, which contains several different types of professional antigen-presenting cells including dendritic cells and B cells [109]. However, the surface area of the villous epithelium is extraordinary (the surface area of the human small intestine being approximately equal to that of a tennis court) and IECs have a well-documented role in nutrient and solute uptake. IECs are exposed to a wide variety of antigens of diverse sizes and varying biochemical properties, including some antigens such as gliadin with known pathological significance. In addition, a wide variety of organisms of various sizes (e.g. rotavirus, *Salmonella* spp. and *Helicobacter* spp.) can enter IECs from their luminal (i.e. apical) surface. Collectively, these observations highlight a physiologic role for the IEC in the uptake and processing of luminal antigens and pathogens.

Several unique characteristics related to antigen uptake by the epithelium are worthy of note. First, IECs express a variety of molecules on their apical surface that might serve as "antigen receptors", e.g. FcRn [110,111], DEC-205 [112], ganglioside M1 (GM1), which may enhance antigen processing by directing internalization via receptor-mediated (instead of fluid-phase) endocytosis. The polarized expression of these molecules on IECs is likely to modulate processing of antigens dramatically by enhancing antigen uptake and/or "targeting" antigens to certain intracellular compartments. In addition, the rate and efficiency of antigen uptake and endocytic processing by IECs can be modulated by a variety of inflammatory mediators, such as interferon (IFN)-γ.

Interaction of IECs with $CD4^+$ T cells – MHC class II processing by IECs

$CD4^+$ T cell responses are critical for both the establishment of oral tolerance [113–115] and in several experimental models of IBD [116]. Two distinct populations of $CD4^+$ T cells are present within the intestinal mucosa and in intimate contact with IECs. A limited, but significant, number of IELs also express CD4 (most notably in the colon [117,118]) and approximately two-thirds of LPLs express

CD4 [73]. Because the expression of MHC class II antigens is required on an APC in order to stimulate $CD4^+$ T cells, it is noteworthy that MHC class II expression has been observed on IECs from human, mouse and rat, with elevated levels consistently seen in the context of mucosal inflammation [119–121]. Indeed, using a variety of *in vitro* models, several groups have demonstrated the capacity of IECs to process and present antigen via HLA class II [120,122].

There are marked regional differences in the composition (and presumably the function) of the GALT along the cephalo-caudal axis of the gastrointestinal tract. Given the limitation of the *in vitro* models, it is difficult to address potential distinctions in MHC class II processing between IECs of the small and large intestine. In fact, little information is available regarding specific regional differences (both crypt to villous and small intestine to colon) in the co-expression of MHC class II antigens and other molecules (for example, invariant chain and HLA-DM) essential for efficient class II processing in conventional APCs (reviewed in [123]), which are typically induced along with MHC class II at the transcriptional level following activation of the APC with IFN-γ [124]. Data from IEC cell lines transfected with HLA-DR alone or with Ii and HLA-DM are consistent with the observations that the generation of specific class II peptide epitopes shows a variable dependence on the expression of Ii and HLA-DM in IECs – with some epitopes processed via other class II pathways [125,126]. Several groups have described one such "alternative" class II processing pathway that uses MHC class II molecules recycled from the cell surface, which presumably bind peptide antigens in an early endosomal compartment [127,128]. These alternative pathways may be particularly relevant to MHC class II processing by IECs, especially when MHC class II expression may be limited (i.e. in the absence of inflammation) and the antigen may be denatured or fragmented following luminal "pre-processing".

Influence of IEC polarity on antigen presentation by IEC class II molecules

IECs are highly polarized cells, with distinct apical and basolateral domains with very different physiochemical properties. The highly polarized morphology of IECs highlights several important distinctions in class II processing between IECs and other more conventional APCs. First, the expression of HLA class II antigens *in vivo* and in cell culture models of polarized IECs is mostly restricted to the basolateral membrane (where the cell contacts both iIELs and LPLs) [125,129,130]. Hence antigen presentation occurs in a highly polarized manner. In addition, *in vitro* data suggest that the polarized surface from which antigen is internalized dramatically affects the functional outcome with regards to the generation of T cell epitopes [125]. This notion is consistent with the biochemical differences observed between endocytosis from the apical and basolateral surface of polarized epithelial cells [131,132].

Consequences of MHC class II expression and processing by IECs

There are limited experimental data regarding the *in vivo* function of MHC class II on IECs. MHC class II restricted antigen presentation can be identified in uninflamed small intestinal [133] and colonic epithelium [134] and is increased in colonic epithelium in the setting of inflammation as observed in IL-2-deficient animals [134]. It is therefore not unreasonable to suggest that under pathologic circumstances, when the balance shifts towards uncontrolled inflammation such as in IBD, the IEC functions as an important accessory APC, stimulating mucosal $CD4^+$ T cell responses. Within the setting of the inflamed mucosa, the unique features of polarized MHC class II processing may come into play. In particular, the processing of luminal antigens (such as bacterial or food antigens) normally exposed only to the apical surface might have especially untoward effects when these antigens gain access to the basolateral surface of IECs via "leaky" tight junctions [135]. Conceivably, "pathogenic epitopes" within an antigen (that normally elicits no significant response or a tolerogenic response when processed apically) may be unmasked via internalization and trafficking from the basolateral surface and presented to underlying $CD4^+$ T cells. Whether IECs can stimulate "naïve" $CD4^+$ T cells or CD28-dependent T cells in the intestinal mucosa (if either of these populations exist in this anatomical location) remains a matter for speculation. However, it is now clear that IECs can direct T cell-independent regulation of B cells and their switching to both IgA- and IgG-producing cells as well as the immune deviation of T cells to either Th1 or Th2 cells in response to microbial challenges independently of engagement of TCR engagement [78,136].

$CD4^+$ T cells have been widely implicated in the development of IBD [116]. Hence the possibility exists that the processing of intact bacteria and/or bacterial fragments by HLA class II positive professional (dendritic cells, macrophages) and nonprofessional (intestinal epithelial cells) results in the presentation of specific bacterial T cell epitopes to pathogenic $CD4^+$ T cells. This hypothesis, which does not preclude the potential role of bacteria or bacteria-derived products such as LPS in modulating mucosal immune responses, is an attractive explanation for the dependence of mucosal inflammation on a subset of bacteria present in the lumen of the gastrointestinal tract. Data supporting this hypothesis exist in mouse and humans. It has recently become appreciated that IBD-like pathology can be initiated by $CD4^+$ T cells with a Th1 cytokine profile which are restricted to MHC class II and specific for peptide antigens associated with the normal luminal microbiota [137]. In a corollary manner, $CD4^+$ regulatory T cells with a cytokine profile consistent with

T-regulatory 1 cells can be similarly identified and established which can antagonize these effector Th1 cells in adoptive transfer models [138]. Although their site of origin is unclear, based on studies of oral tolerance induction and work in the TCR-α-deficient animal model of colitis, it can be suggested that these agonist and antagonist cell populations originate within the inductive sites of the distal small intestine (appendix, PPs) and carry out their effector functions at the epithelial barrier of the colon after subsequent reactivation by either homologous or heterologous microbial antigen at this location [2,49]. Whether the subsequent encounters are through interaction with the IECs or professional APCs subjacent to the epithelium remains unclear. Nonetheless, these studies suggest that MHC class II antigen presentation pathways in the context of the immunologic milieu associated with the inductive sites of the GALT lead to the generation of agonist and antagonist (regulatory) T cell populations. Furthermore, it can hypothesized that under normal circumstances, these regulatory populations prevail over the agonist cells leading to homoeostasis. In IBD, however, this tolerance to the antigens of the normal microbiota is not established [13].

Interaction of IECs with $CD8^+$ cells

In the earliest studies assessing the capacity of IECs to act as nonprofessional APCs, the T cells activated were predominantly $CD8^+$, which functionally suppressed immune responses in an antigen-nonspecific fashion [139]. Consistent with the somewhat unusual features of these $CD8^+$T cells stimulated by IECs (i.e. the lack of cytotoxicity and the ability of these cells to suppress a mixed lymphocyte reaction), recent data have emerged revealing atypical characteristics of MHC class I function in IECs. For example, although IEC lines have been shown to be good targets for class I-restricted virus-specific cytotoxic T lymphocytes (CTLs) and appear to express a normal proteasome repertoire, they fail to prime an antiviral CTL response (K. Becker and L. Mayer, unpublished work). These data suggest that MHC class I-mediated processing may be somehow "altered" in IECs compared with professional APCs such as dendritic cells or imply that the lack of conventional costimulation may preclude priming of naïve $CD8^+$ T cell responses [140]. Indeed, it has recently been shown that the immunoproteasome associated with the IEC is unique [141].

Several lines of evidence point to novel interactions between $CD8^+$ T cells and IECs. One stems from the early observation that antibodies specific for HLA class I did not inhibit the IEC-induced proliferation of $CD8^+$ T cells *in vitro* [139]. One intriguing hypothesis proposes that "non classical" or class Ib molecules function in antigen presentation by IECs to $CD8^+$ T cells in the intestinal mucosa. This hypothesis is supported by data demonstrating the expression of these molecules by IECs in mouse and humans, often in a pattern highly restricted to the intestinal epithelium [142,143] (reviewed in [144]). In humans, the list includes CD1d [145], MICA and MICB [146], HLA-E [147] and HFE (which is involved in iron metabolism), all of which are expressed by IECs [148].

CD1d, a novel ligand on IECs

CD1 family of proteins

Recent studies have revealed that, in addition to MHC class I and class II, the host also utilizes a third pathway of antigen presentation which is represented by the CD1 gene family. This pathway (or pathways) functions in specific tasks of immunologic recognition distinct from MHC class I and II, yet draws on structural and functional features of both. First identified on cortical thymocytes, the CD1 gene family encodes a group of proteins which are structurally most similar to MHC class I proteins but have several features in common with MHC class II [45,149]. This suggests that CD1 emerged from an ancient ancestor that diverged into MHC class I, MHC class II and CD1. The human CD1 locus on chromosome 1 contains five genes, CD1A-E [by convention, CD1 genes are in capital letters (A–E) and CD1 proteins in lower-case letters (a–e)]. The human CD1 gene family falls into two groups based upon sequence homology: CD1A–C (group 1), CD1D (group 2) and CD1E (group 3) [150]. CD1a–c subserves a specific role in presenting lipoglycan antigens from mycobacteria (and bacteria) to discrete subsets of $CD8^+$ and double-negative ($CD4^-CD8^-$) T cells [151,152]. These T cells utilize a wide variety of conventional αβ TCRs. CD1a–c are expressed by the majority of immature, double-positive ($CD4^+CD8^+$) thymocytes and professional APCs such as B cells, dendritic cells and activated monocytes. Rodents do not express group 1 CD1 proteins and appear to have deleted these genes [45].

CD1d and natural killer T cells

CD1d is expressed by the majority of thymocytes and at low levels by resting B cells and monocytes and dendritic cells [45,153–155]. In addition, CD1d is expressed by a variety of epithelial cell types including IECs and hepatocytes [153,154]. CD1d functions in the presentation of lipids. These include endogenous antigens such as glycosylphosphatidylinositol, which has been eluted from murine CD1d in transfectants [156], and exogenous lipid antigens from bacteria including α-galactosylceramide (α-GalCer), which has been extracted from marine sponges and may be derived from a contaminating species of *Pseudomonas* [157]. These observations are consistent with the hydrophobic nature of the CD1d groove [158]. Recognition of CD1d appears to be primarily accomplished by a discrete subset of T cells, which share similarities with natural killer (NK) cells [159]. These NK-T cells, which have also been commonly called natural T cells, were initially

described in mouse systems [160]. Mice express the human homologue of CD1d but not CD1a–c [161,162]. These murine CD1d-restricted T cells are characterized by their expression of the NK1.1 marker (NKR-P1C or CD161), a C-type lectin and an invariant TCR-α-chain (Vα14-Jα18), which preferentially pairs with Vβ8.2, 7 or 2 in hierarchical order [159,163,164]. These cells are also either $CD4^+CD8^-$ or double-negative since the forced expression of CD8 in mouse results in the deletion of invariant NK-T cells [165].

NK-T cells represent a major fraction of mature thymocytes, liver-associated T cells and up to 5% of splenic cells [166]. In addition, they have been identified in the epithelium amongst IELs [167] and amongst LPLs at low levels [167]. Although their function is not entirely clear, they have been recognized to be the major source of IL-4 and interferon-γ (IFN-γ) *in vivo* in response to anti-CD3 stimulation, suggesting an important immunoregulatory role during early phases of an immune response [168]. CD1d-deficient mice lack NK-T cells and the early cytokine response associated with anti-CD3 activation but maintain their ability to mount a T helper (Th2) response [169–171]. Murine NK-T cells are also involved in IL-12-mediated pathways as they express IL-12 receptors and, when stimulated by IL-12, exhibit significant cytolytic activity and regulation of IFN-γ responses, responses which are lacking in CD1d-deficient mice [172,173]. These observations with murine NK-T cells suggest that they play an important role in immunoregulation of type 1 and type 2 cytokine responses through their ability to generate high levels of cytokines and immunosurveillance through high cytolytic activity.

Similar populations of NK-T cells are also expressed in humans. Analogous to mouse, these human NK-T cells express an invariant TCR composed of Vα24-JαQ which preferentially pairs with Vβ11, the human homologue of mouse Vβ8 [174,175]. Most human NK-T cells are double-negative while a minority express CD4 [176]. They uniformly express NKR-P1A (CD161), the only human homologue of mouse NK1.1, which may provide crucial costimulatory signals [177]. NK-T cells also express two other C-type lectins, CD94 (the ligand for HLA–E) and CD69, but lack expression of most other NK markers including CD16, CD56, CD57 or killer inhibitory receptors [174]. Similarly to the mouse NK-T cells, human NK-T cells function as potent cytolytic effectors and secrete cytokines with a Th0 phenotype in response to CD1d-transfected APCs [174,177]. These functional activities are significantly enhanced by the lipid antigen α-GalCer isolated from marine sponge [178–180].

Although much is known about the cellular immunology of CD1a–d, little is known about the role of CD1d in human diseases. The information to date points towards a crucial role of CD1d in immunoregulation to microbial antigens given the vigorous cytokine production of CD1d-responsive NK-T cells and observations that NK-T cells are significantly diminished in diabetes mellitus [181], systemic lupus erythematosus [182] and systemic sclerosis [183] and immunosurveillance to microbial antigens functions given the potent anti-tumor responses of NK-T cells in mouse models [184].

The CD1d pathway is particularly relevant to IBD pathogenesis given its biological relevance to the central hypothesis raised above. CD1d is expressed by IECs [153,185] and professional APCs [155] and on IECs is able to present model glycolipid antigens to NK-T cells [186]. This presentation is most efficient basally relative to apically suggesting a role for CD1d in presenting glycolipid antigens from luminal microbial antigens to subepithelial CD1d restricted T cells such as the NK-T cells [187]. Indeed, activation of NK-T cells in a CD1d-restricted manner leads to the amelioration of colitis in a strong Th1 model, the dextran sodium sulfate (DSS) colitis model [187], due to immune deviation of the immune response towards a regulatory and Th2 phenotype [187]. Consistent with this, NK-T cells that are restricted to CD1d are directly involved in the development of a model for human ulcerative colitis (oxazolone colitis) through the secretion of IL-13 [188], which further damages IEC barrier function [60]. Human UC is also characterized by increased function of NK-T cells that secrete IL-13, in contrast to human Crohn's disease, which is characterized by increased Th1 cells [189].

Mechanisms of innate immunity throughout the intestinal mucosa

The intestinal lumen, particularly that associated with the colon, contains a large variety of bacteria and bacterially derived products [190,191]. As a consequence, a number of "broadly specific" defense mechanisms have evolved to guard against the risk of attack from invasive microorganisms and at the same time maintain homeostasis. These factors are important because they can be mobilized rapidly in comparison with the time taken for mobilization of the adaptive responses described in the sections above. These mechanisms can be classified as intrinsic or extrinsic in nature. Intrinsic mechanisms of immunity are derived from the physical presence of an epithelial barrier and are dependent on the unique structural properties exhibited by the IEC. Extrinsic defenses are defined as those processes which act outside of the monolayer to resist microbial interaction with the mucosa. In addition to these two mechanisms, cells constituting the epithelial monolayer have developed an ability to interact with immune cell populations of the underlying intestinal mucosa, thereby alerting the immune system to the presence of luminal pathogens. These three levels of innate immunity are described below.

Intrinsic barriers

The formation of a selectively permeable epithelial barrier is essential in preventing the uncontrolled passage of pathogenic antigens from the external environment to the internal tissue. Establishment of the epithelial monolayer by contributing IECs is dependent on a considerably high degree of intracellular and intercellular organization [192]. In addition to allowing the formation of tight intercellular junctions, IECs exhibit a number of structural and functional features that help physically restrict the passage of potentially harmful microorganisms into the underlying mucosa. Interaction of macromolecules with the apical surface of IECs is reduced by the presence of large, heavily glycosylated proteins or mucins, anchored to the epithelial plasma membrane [193]. In addition, the organization of the apical membrane into microvilli is thought to minimize the contact area available to luminal macromolecular antigens [194]. Cell trafficking processes within the IEC may also reduce the amount of intact microbial antigen crossing the monolayer. The contents of epithelial cell endocytic vesicles are, for example, primarily directed to proteolytic lysosomal compartments. Thus, internalization of many microorganisms is liable to lead to their degradation.

Indeed, numerous derangements either primarily or secondarily in IBD may affect this intrinsic barrier function. In terms of the former, several genetic defects that are associated with increased risk for the development of human IBD may be associated with disruption of the epithelial barrier. These include polymorphisms of NOD2 on chromosome 16, which may disrupt the antimicrobial function of Paneth cells [195], an organic cationic transporter encoded on chromosome 5, which may disrupt the ability of the IEC to regulate toxin export [70], and disc-like gene 5 encoded on chromosome 10, which is an intracellular protein that may disrupt the tight junctions [71]. A primary genetic basis for disruption of intrinsic barrier function is consistent with reports of increased barrier permeability in first-degree relatives of IBD subjects [66]. However, it remains to be proven that genetically imposed, cell autonomous defects in IEC barrier function exist in human IBD. A variety of secondary events also can affect intrinsic barrier function. These include a variety of cytokines that are overproduced in IBD such as interferon-γ, tumor necrosis factor, IL-4 and IL-13, which affect tight junction function or directly affect the balance of electrolyte secretion and absorption resulting clinically in diarrhea [60,196–198]. Activation of myosin light chain kinase intracellularly has also been associated with diminished barrier function, suggesting that intralumenal nutrients may exacerbate barrier dysfunction in the context of inflammation [199]. This is counterbalanced by the enhancement of IEC barrier function by anti-inflammatory cytokines such as IL-10 and tumor growth factor-β [67,200].

Extrinsic Barriers

Mucus

The surface of the intestinal epithelium is lined with a layer of mucous produced as a result of mucin secretion by goblet cells [201,202]. Mucins, a group of glycoproteins of which the mucous lining is comprised, protect the epithelium in several ways. By creating a hydrated viscous layer, they act as a physical barrier, separating IECs from the turbulent luminal environment of the intestinal tract [203,204]. In addition, the carbohydrate moieties present in mucins display the ability to adhere to the surfaces of many microorganisms, preventing microbial association with the monolayer [205–207]. With time, mucous is propelled down the intestinal tract, thereby facilitating the removal of bound microbial components away from the epithelia. The mucous lining is therefore considered to contribute to innate immune responses within the intestine. In support of this role, it has been observed that mucous secretion is induced in the presence of agents potentially harmful to the epithelium, including noxious chemical and bacterial toxins [208–210]. Such a response may act to counter the presence of these agents by reducing their capacity to interact with the monolayer. The importance of mucins as a major extrinsic barrier is the evidence that in the total absence of the MUC2 gene product, mice deficient in this gene develop spontaneous colitis [211]. Similarly, mice that are deficient in intestinal trefoil factor that associated with mucins in the formation of an extrinsic barrier are more susceptible to the development of colitis associated with DSS administration [212]. How these findings relate to human IBD remains unclear.

Defensins

A second extrinsic mechanism of mucosal defense occurs by the secretion of agents that directly exhibit microbicidal or anti-viral activities. Investigations performed on sections of the mouse epithelium have revealed the presence of mRNA encoding cyptdins or α-defensins, molecules sharing a conserved cysteine-rich motif with the family of anti-microbial peptides known as defensins [213]. Expression of cryptdin mRNA and protein is confined to Paneth cells, located within intestinal crypts [214]. These peptides display a high degree of sequence homology to the defensins secreted by polymorphonuclear leukocytes and exhibit potent microbicidal activity on a variety of bacteria, including strains of *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*. The potential importance of cryptdins in conferring resistance to bacterial infection is highlighted by the observation that whereas avirulent strains of *S. typhimurium* are susceptible to the anti-microbial properties of cryptdins, the growth of pathogenic *S. typhimurium* is not affected by these peptides [214]. Although unclear, it remains possible that the loss

of cryptdin sensitivity in the pathogenic strain contributes to its enhanced virulence.

Intestinal epithelial cells also express defensins. Recent studies have revealed that human IECs express two functionally related peptides termed β-defensin 1 and β-defensin 2 (hBD1 and hBD2, respectively) [215]. hBD1 was shown to be constitutively expressed whereas hBD2 was induced following stimulation with the cytokine IL-1. Like the α-defensins, both hBD1 and hBD2 exhibit antimicrobial activity against a range of bacteria, including *E. coli* and strains of *Salmonella* spp. and *Pseudomonas* spp.

Recent information has focused attention on Paneth cells and their production of defensins in the pathogenesis of human IBD. Specifically, mice that are deficient in NOD2 exhibit decreased Paneth cell defensins and susceptibility to *Listeria monocytogenes* challenge [195]. NOD2-deficient mice do not, however, exhibit spontaneous intestinal inflammation or increased susceptibility to DSS administration [216]. Interestingly, human CD patients exhibit decreased Paneth cell defensins, which is only partially related to polymorphisms in the NOD2 gene [217]. This raises the possibility that bacterial dysbiosis may be an important contributor to IBD pathogenesis. Indeed, innate immune defects due to co-deletion of T-bet and RAG2 in mice lead to a profound dysbiosis that is remarkably pro-inflammatory through induction of TNF [15].

Lysozyme and lactoferrin

Lysozyme and lactoferrin represent two proteins, which, like defensins, are thought to contribute to the innate defense of mucosal surfaces [218]. Both lysozyme and lactoferrin are found in exocrine secretions and can be detected within the lumen of the human gastrointestinal tract. Lactoferrin (Lf), a molecule that is thought to facilitate iron transport across the epithelium of suckling infants, is cleaved by gastric pepsin to form a peptide exhibiting potent anti-microbial properties [219]. *In vitro*, Lf inhibits replication of a number of viruses, including Cytomegalovirus (CMV), Herpes simplex virus (HSV) and members of the Rotavirus family [220,221]. Lf interacts with both the lipoglycan-coated cell wall of Gram-positive bacteria and the outer membrane of Gram-negative bacteria, processes that are thought to mediate its bactericidal function. In addition, lactoferrin inhibits the adhesion of enterotoxigenic *E. coli* to human epithelial cells, thereby preventing its colonization of the surface monolayer [222]. Lactoferrin is also capable of inhibiting fungal growth and has been shown to be cytotoxic to several protozoan parasites, such as trophozoites of *Giardia lamblia* and *Toxoplasma gondii* [223,224].

Human lysozyme consists of single polypeptide chain of 129 amino acids, which is cross-linked by four stabilizing disulfide interactions. The polypeptide is a constituent of both saliva and pancreatic juice. Lumenal concentrations within the intestinal tract range from 43 to 106 $\mu g\,ml^{-1}$ [218]. Lysozyme exerts its microbicidal activity at a number of levels. These include the hydrolysis of bacterial cell wall peptidoglycans, the activation of bacterial autolysins, induction of bacterial cell aggregation and the abrogation of bacterial adhesion to host epithelial cells. However, although lysozyme may possess multiple enzymatic activities, it is thought that its cationic properties are primarily responsible for its ability to interact strongly with bacterial membranes and may contribute to the disruption of membrane processes [225]. In many cases, lysozyme alone does not alter bacterial viability but does render the bacteria more susceptible to lysis by other environmental factors. In this regard, a combination of lysozyme with lactoferrin is microbicidal for *Streptococcus mutans* whereas lysozyme alone has no visible effect on the growth of this bacterial strain [226]. Similarly, lysozyme synergizes with peroxides in preventing glucose uptake by *Streptococcus mutans* [218]. *In vivo*, therefore, lysozyme is likely to function in association with additional antimicrobial agents.

Other microbicidal factors that function as innate mechanisms of defense at mucosal surface include peroxidases and components of the complement pathway. Studies using the human epithelial cell line Caco-2 have demonstrated that these cells express and secrete complement components including C3, C4 and factor B [227]. Peroxidases, secreted from exocrine glands on to mucosal surfaces, catalyze the peroxidation of halides such as chloride and bromide and thiocyanide into oxidative products, which exhibit potent anti-microbial activity.

Epithelial cell–immune cell cross-talk

The exposed location of the epithelia implies that IEC are likely to play an important role in alerting components of the mucosal immune system to the presence of foreign antigen. Intestinal epithelial cells coexist with immune cell populations present along the epithelium itself and those localized to the lamina propria. Intestinal intraepithelial lymphocytes reside at the basolateral surface of the epithelium. Immune cell populations within the lamina propria include lymphocytes, macrophages, granulocytes and mast cells. Within this environment, IECs have developed an ability to communicate with regional immune cell populations in a manner proposed to influence directly their growth, migration and state of responsiveness to antigenic stimuli. Some of the methods by which IECs affect the mucosal immune system are categorized and discussed below.

Chemokines

Under conditions that promote infection or insult to the epithelial monolayer, an influx of immune and inflammatory cells into the mucosa is observed as these cells are recruited to the site of injury. It should be noted that the

mechanisms of tissue inflammation and injury are highly similar between infections of the intestines and IBD such that it may be considered that in IBD the gut (including the intestinal epithelium) is responding to the commensal microbiota as if it were a pathogen. This recruitment of leukocytes can occur within hours of infection and is mediated, partly, by a group of chemotactic cytokines known as chemokines [228,229]. Several studies have revealed the ability of IECs to express and secrete chemokines including CXCL8 (IL-8), ENA 78, CXCL1 (gro-α), MIP-1α, CXCL3 (gro) and MCP-1 (CCL-2) to induce chemotaxis of neutrophils [230]. Infection of the epithelial cell lines T84, HT29 and Caco-2 with Salmonella dublin, Yersinia enterocolitica or Shigella dysentariae, for example, selectively induces expression of CXCL8 and CCL-2 (MCP-1) that is responsible for recruitment of neutrophils and macrophages, respectively [231–234]. Following cellular invasion by S. typhimurium, CXCL8 is secreted from the basolateral surface, establishing a chemical gradient and facilitating the migration of neutrophils into the paracellular spaces of the monolayer [232]. CXCL8 expression is also induced in gastric epithelial cells in response to H. pylori infection [235,236]. Taken together, these data demonstrate the ability of IECs to participate actively in recruiting immune cells to sites of infection, through the release of chemotactic factors upon exposure to luminal pathogens.

In the same manner, IECs actively secrete chemokines under basal conditions that maintain homeostatic regulation of lymphocyte and dendritic cell composition of the lamina propria. Secretion of CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (I-TAC) is responsible for the recruitment of Th1 cells [237]. CCL22 (MDC) secretion by IEC induces migration of dendritic cells and Th2 cells [238]. Dendritic cells and memory T cells are induced to migrate into tissues by secretion of CCL20 (MIP3α) by IECs [239]. Finally, some of this secretion is tissue specific, such as through secretion of CCL25 (TECK) by IECs that induces migration of $CCR9^+$ $\alpha4\beta7^+$ T cells in the small intestine [240]. In IBD, many of these same chemokines are increased in expression in response to bacterially induced signals to the epithelium, thus promoting inflammation.

Cytokines

Cytokines are a group of factors that exert a profound influence on the functional state of immune cell populations through inducing an altered pattern of gene expression upon binding to specific cellular receptors [241]. Intestinal epithelial cells are capable of expressing a number of cytokines, thereby possessing the ability to affect local immune responses. The cell lines T84 and Caco-2 constitutively express mRNA for the pro-inflammatory cytokines, IL-1α, IL-1β, IL-15 and TNFα [230,234,242,243]. These cells also express TGFβ, a cytokine thought to play a role both in promoting IEC growth and in regulating IEC barrier function and responses of IELs to antigenic stimuli [244–246]. IL-6, a cytokine thought to be important in generating IgA-secreting plasma cells, has been shown to be expressed in freshly isolated human IECs and can be induced in a variety of epithelial cell lines by cholera toxin secreted from *Vibrio cholerae* [247]. IL-6 release by IECs may therefore contribute to the production of secretory IgA.

IL-10 represents another cytokine expressed by IECs [61]. Like IL-6, it is thought to promote IgA secretion by B cells [248]. In addition, IL-10 acts as a suppressor of Th1 responses through inhibiting macrophage function and cytokine secretion from Th1 cells [249]. Recently, studies have shown that IL-10 secretion from T84 cells can protect the epithelial monolayer from IFN-γ-induced permeabilization [61,250,251]. Both its ability to suppress Th1 responses and to maintain epithelial barrier function implicate IL-10 as a regulator of intestinal inflammation. The production of IL-10 from IECs, therefore, may act to protect the barrier against injury resulting from an influx of inflammatory effector cells.

A number of additional factors secreted from IECs are thought to be important in influencing the growth and development of surrounding iIELs. Mice lacking the functional gene encoding IL-7 exhibit reduced levels of $\gamma\delta^+$ T cells in the epithelia, whereas IL-7 receptor knockout mice do not possess any intestinal $\gamma\delta^+$ T cells [252,253]. IL-7 has also been shown to play a role in activating T cell cytolytic function against various intracellular parasites including HIV [254,255]. Such findings, together with the observation that freshly isolated IEC produce IL-7 [256], suggest that epithelial-derived IL-7 may contribute to maintaining iIEL growth and responsiveness to foreign antigens. In addition to IL-7, two other molecules secreted from epithelial cells are important in influencing iIEL activity. Stem cell factor (SCF) is required for sustaining levels of $\gamma\delta^+$ T-cells in the intestine [257]. Furthermore, *in vitro* studies suggest that SCF can protect IECs from bacterial infection [258]. Murine epithelial cells have also been shown to express thyroid-stimulating hormone (TSH) [259]. Secreted following the action of thyrotropin-releasing hormone on its receptor, TSH is critical in maintaining normal levels of $CD8\alpha\beta^+$ T-cells within IEL populations [259,260].

Intestinal epithelial cells are also able to synthesize a variety of prostanoids. Rabbit colonic IECs constitutively express prostaglandin E2 (PGE2), 6-ketoprostaglandin F1α (6-keto-PDF1α), prostaglandin D2 (PGD2) and prostaglandin F2α (PGF2α) [261]. PGE2 has been shown to reduce IL-3 secretion from lamina propria mononuclear cells, inhibit the effects of local T cells on the epithelial barrier and reduce IL-2 secretion by activated T cell lines [261,262]. Based on such data, it seems likely that the release of PGE2 from IECs functions to regulate leukocyte responses within the intestinal mucosa and promote inflammation. The same IECs participate together with leukocytes in the generation of a novel class of lipids (so-called resolvins) that are derived from eicosapentaenoic

acids derived from fish oils that function in the resolution of inflammation [263]. Resolvins have been identified as anti-inflammatory mediators in animal models of colitis and may provide a rationale for the apparent clinical benefit of fish oils in a limited number of clinical studies [263].

Cytokine receptors

In addition to exhibiting an ability to influence mucosal immune responses, IECs are also able to alter their own phenotype in response to cytokines released from local immune cell populations. Freshly isolated human IECs, and also a number of IEC lines, express mRNA for the common gamma chain and specific alpha chains of the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 [243,244,264]. Northern blot analysis has further revealed the presence of the IL-2 receptor β chain and studies of rat IECs demonstrated the expression of the IL-1 receptor (IL-1R), a molecule homologous to the human type I IL-1R [264,265]. Through the expression of cytokine receptors, the epithelium is sensitive to immunologic changes within its environment and can subsequently respond in a way that either facilitates or regulates those responses. In the presence of IL-4, IFN-γ or TNFα, for example, IECs respond by enhancing levels of the polymeric immunoglobulin receptor, thus facilitating the release of secretory IgA [266,267]. IFN-γ also induces the expression of MHC class II molecules and the epithelial cell adhesion molecule ICAM-1 [268–271]. IFN-γ is capable of stimulating cell surface expression of the costimulatory factor CD86 and IL-6 and IL-1 trigger the release of complement components C3, C4 and factor B from IECs [227,272,273]. Receptiveness of the epithelium to the local cytokine environment, therefore, allows the mucosal immune system to enhance both the epithelial cells' innate capacity for defense and their ability to interact with and influence local immune cell populations.

It is very clear that in IBD, there is a dramatic increase in cytokine and chemokine secretion by the epithelium, which is to a large extent induced by the luminal microbiota [79,231]. Similarly, the intestinal epithelium is exposed to high concentrations of barrier disruptive cytokines as described in detail above. It has not, however, been specifically determined whether cytokines derived from the intestinal epithelium can initiate IBD or whether cytokines derived from subepithelial tissues are responsible for the tissue injury observed through effects on the epithelium. It can be surmised that this occurs, but it requires formal demonstration.

Toll-like receptors

The mucosal immune system represents a dominant interface between the two main arms of the immune system – so-called "innate" and "adaptive" immunity. We have outlined the various cellular components of the adaptive immune system, in particular the diverse T cell populations with somatically rearranged T cell receptors that recognize specific processed protein fragments displayed by histocompatibility molecules on the surface of antigen-presenting cells. Although it is likely that "specific" T cell (and B cell) responses to bacteria and other luminal microorganisms will be increasingly implicated in the pathogenesis of IBD, a growing body of literature suggests that "innate" responses to the mucosal microbiota are likely to have a dramatic influence on the immunological tone at mucosal surfaces.

As detailed, the mucosal microbiota is extremely diverse, with most major classes of bacteria, yeast and, often, helminths represented. In this complex microbiological ecosystem, the challenge of the innate system is daunting. It has become increasingly clear that, in order for the innate immune system to be able to recognize and respond to such a large number of diverse microbial stimuli, it has evolved an intricate system of "pattern recognition" [41]. Specifically, this involves the ability to respond to structural motifs shared by diverse groups of organisms (e.g. lipid A from Gram-negative bacteria, peptidoglycans from Gram-positive bacteria) (Table 9.2).

Recent data on the Toll-like receptors (TLRs) in human and mouse have provided detailed insight regarding the mechanisms by which this diverse pattern recognition occurs [274,275]. The term "Toll-like" derives from structural homology to the gene product of the Toll locus in the fruit fly *Drosophila melanogaster* [276]. Mutations in Toll and genes relevant to the signaling events downstream from this surface receptor (with homology to the receptor for interleukin-1) result in marked defects in innate immunity (particularly to yeast and Gram-positive bacteria). Homologues to *Drosophila* Toll have been found in mouse and humans, with a growing family of proteins with homology to Toll, currently with 10 distinct members comprising the list of TLRs. Interestingly, the different TLRs appear to have distinct ligand specificity, in the cases so far determined, representing structural disparate "bioactive" components of microorganisms. This rapidly changing area of scientific inquiry has been the topic of recent insightful reviews [277,278].

One particular example of TLR biology illustrates both the importance of these molecules in innate immunity and the potential relevance to inflammatory responses of the gastrointestinal tract. First, in an elegant series of genetic studies in mouse strains hyporesponsive to bacterial LPS (e.g. C3H/HeJ) mutations were identified in a conserved region of TLR4 [279]. Subsequent studies from a number of laboratories have demonstrated that (in concert with LPS binding protein, LBP and surface or soluble CD14) TLR4 is the "dominant" surface molecule that confers a signal by LPS [280]. Recent data are emerging that TLR2 may be involved in signaling by LPS of certain bacteria, for example, *Porphomonas gingivalis*. The pattern of expression of TLR4 is variable and includes macrophages and dendritic cells. Interestingly, functional TLR4 has also been

shown to be expressed on nonhematopoietic cells, including IECs [281]. Recently, it has been demonstrated that TLR4 is selectively upregulated on IECs from patients with IBD. Based on these studies, one might predict an "exaggerated" response to LPS (a potent immunomodulatory molecule driving "pro-inflammatory" responses) in IBD. Additionally, these studies highlight TLR4 as a potential target for antagonism therapeutically in IBD. The complexity is likely to increase further as one considers the role of other bacterially derived immunomodulators (e.g. CpG islands in bacterial DNA that signal via TLR9 [282]) and potential interactions between the TLRs within the anatomically varied sites along the gastrointestinal tract. Moreover, not only is it clear that TLRs are responsible for initiating pathologic immune responses, it is also clear that they are engaged in maintaining mucosal homeostasis. Specifically, deletion of the common signal transduction module downstream of most TLRs, MyD88, leads to increased, rather than decreased, susceptibility to DSS administration [42]. Stimulation of TLRs is also likely responsible for initiating Paneth cell secretion of antimicrobial peptides and the induction of these critical antimicrobial pathways within epithelia in the first instance [195]. Finally, TLRs such as TLR9 have been linked to the development of anti-inflammatory regulatory pathways [283]. Hence understanding pattern recognition receptors such as the TLRs will likely be an extremely important area with considerable therapeutic relevance in the coming years.

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Chapter 10
Cytokines and Chemokines in Mucosal Homeostasis

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Summary

- IBD can be seen as a state of imbalance between pro- and anti-inflammatory cytokines.
- In the context of IBD, the study of cytokines and chemokines can allow a global view of the aberrant immune process as cytokines/chemokines are secreted by multiple cell types and each cytokine/chemokine has pleiotropic effects.
- Chemokines drive the recruitment of immune cells to inflammatory or non-inflammatory sites. Elevated pro-inflammatory chemokine levels have been associated with both Crohn's disease and ulcerative colitis.
- Crohn's disease has classically been associated with Th1 cytokine skewing whereas Th2 cytokines have been linked to ulcerative colitis. This paradigm is currently being reconsidered in the light of the recently identified Th17 cytokine family.
- Pro-inflammatory cytokine neutralization and anti-inflammatory cytokine administration are treatment strategies currently being used or under evaluation.

Introduction

The digestive tract contains the largest immune cell repository and also the largest microbial reservoir in the body. Although this combination might be expected to lead to unwanted immune activation and inflammation, complex regulatory networks in the mucosa maintain unresponsiveness to the gut microbiota. This unresponsiveness or tolerance is essential to gut immune homeostasis – with breakdown of tolerance associated with various immune disorders, including inflammatory bowel diseases (IBD).

Regulated immune responses are essential to maintaining intestinal homeostasis and require direct or indirect communication among cells. Communication that occurs among cells in the absence of direct contact is often through the use of cytokines and chemokines. These small molecules are not only secreted by a variety of cells (immune or non-immune), but can also transmit signals to multiple cell types. In the context of IBD, the study of cytokines and chemokines can allow a global view of the aberrant immune process. Indeed, such study has resulted in an improved understanding of IBD and has permitted major advances in IBD therapeutics. In this chapter, we discuss the cytokines and chemokines implicated in the pathogenesis of IBD and the implications for IBD therapeutics.

Cytokines are small, secreted glycoproteins that are produced by a wide variety of immune and non-immune cells and signal through high-affinity binding to specific cytokine receptors. These proteins can act in either a paracrine or autocrine fashion and rarely have systemic (endocrine) effects. Chemokines are a subfamily of cytokines that have the unique ability to direct recruitment and migration of circulating leukocytes to specific tissues. Chemokines have been implicated in many fundamental immune processes, including lymphoid organogenesis, immune cell differentiation, development and positioning.

Cytokines are subdivided into nine families based on their biochemical properties: hematopoietin (type 1 cytokines), interferon (or type II cytokines), interleukin (IL)-12, IL-17, IL-10, tumor necrosis factor (TNF), IL-1, transforming growth factor-β (TGFβ) and the chemokine families. Chemokines are further subdivided into four groups: the C, CC, CXC and CX3C families.

Given the diversity and complexity of cytokine and chemokine function, in this chapter we focus on molecules that have been studied both in human IBD and in animal models (Figure 10.1). For simplicity, we will first discuss pro-inflammatory cytokines and subdivide them into Th1, Th17 and Th2 cytokines. We will then describe

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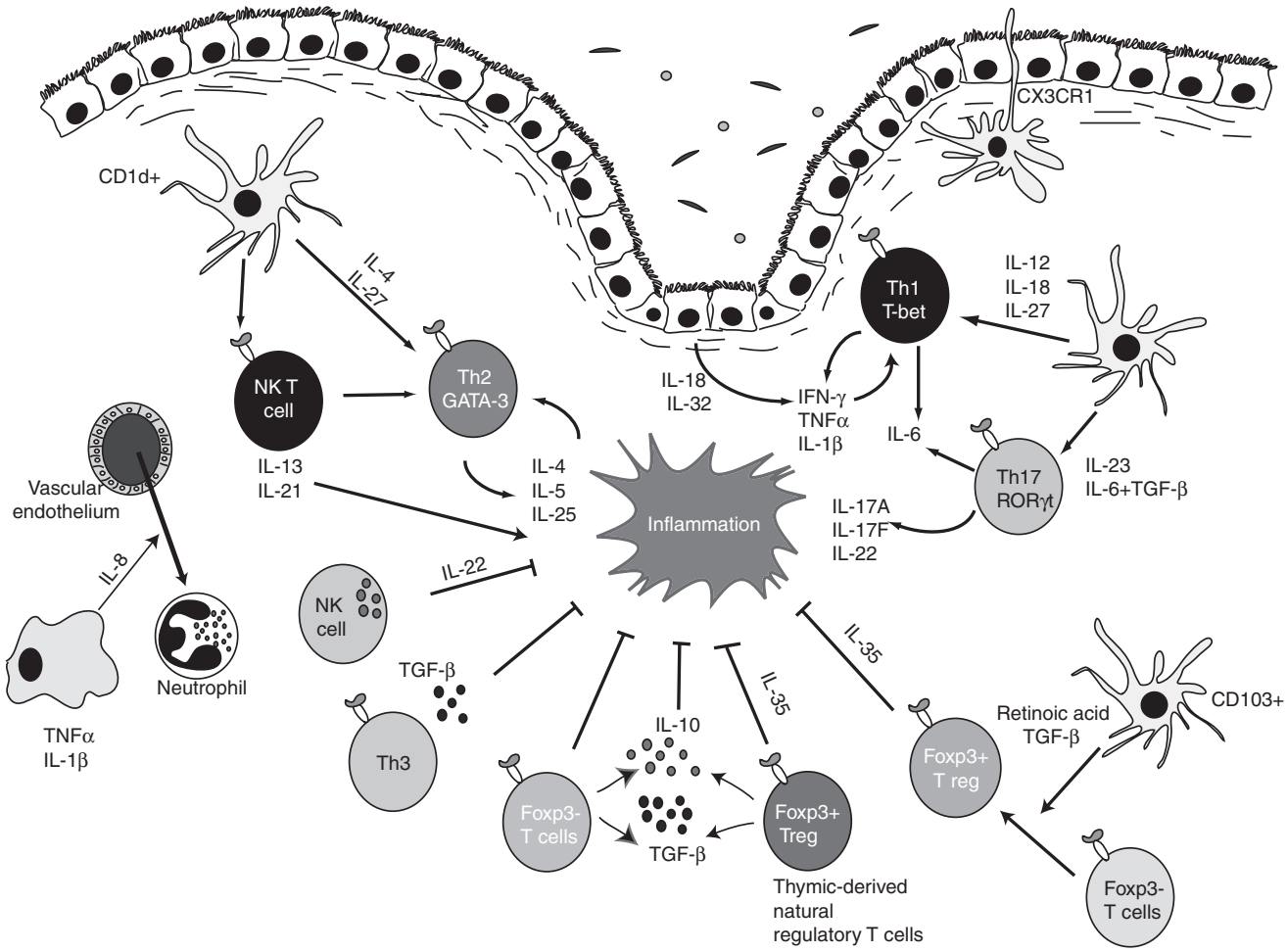

Figure 10.1 Influence of cytokines on gut immune homeostasis. Neutrophil recruitment to the intestinal LP in the early stages of inflammation is induced by IL-8, a chemokine secreted by macrophages. IL-1 production can be stimulated by pro-inflammatory cytokines including TNFα and IL-1β. Luminal antigen sampling by DCs occurs via transepithelial dendrite extensions, a process that depends on the CX3CR1 fractalkine receptor. Antigen-presenting cells including DCs drive Th1, Th17 or Th2 differentiation through the secretion of IL-12, IL-18, IL-27 (Th1) or IL-23, IL-6, TGFβ (Th17) or IL-4, IL-27 (Th2). T effector cells secrete pro-inflammatory cytokines that lead to inflammation. CD1d-restricted NK T cells secrete IL-13 upon activation and lead to Th2 cytokine secretion. Suppression of inflammation can occur through naturally occurring thymic-derived Foxp3$^+$ regulatory cells (Foxp3$^+$ Treg), IL-10 producing T cells or TGFβ secreting T cells (Th3). Suppressive Foxp3$^+$ T cells can also arise from Foxp3$^-$ T cells upon retinoic acid and TGFβ stimulation via CD103$^+$ DCs. NK cells have anti-inflammatory properties through the secretion of the suppressive cytokine IL-22. T-bet, GATA-3, RORγt and Foxp3 are transcription factors involved in Th1, Th2, Th17 and Treg differentiation respectively.

anti-inflammatory cytokines and other unclassified cytokines. Chemokines will be separated into C, CC, CXC and CX3C families. One should bear in mind that in some cases these distinctions are somewhat arbitrary, since overlap exists between the function of various cytokines/chemokines and some inflammatory molecules can have anti-inflammatory properties in specific settings.

Pro-inflammatory cytokines

Gut immune homeostasis can be envisioned as a state of equilibrium between pro- and anti-inflammatory pathways. These pathways are complex, inter-related and sometimes redundant, allowing the flexibility and plasticity that is necessary to respond to pathogens while avoiding undesired immune activation. Pro-inflammatory signals in IBD have long been seen in a dichotomous way with Crohn's disease (CD) being associated with Th1 cytokine skewing and ulcerative colitis (UC) being associated with Th2 cytokine production. The recent discovery of a new effector T cell lineage, Th17, and its associated cytokines has encouraged us to reconsider the current paradigm. In parallel with the discovery of the Th17 cell lineage, an intense effort has been made over the past 10 years to better characterize the gut immune regulatory pathways. The discovery that cytokines can influence the balance between inflammatory and regulatory pathways

has provided new strategies for the development of IBD therapeutics.

For each cytokine discussed below, we first present general information about the cytokine, followed by a review of the current data available on this cytokine in murine and human IBD.

Th1 cytokines

Interleukin-12

IL-12 is a heterodimer formed by a p40 and p35 subunit [1]. IL-12 shares the p40 subunit with IL-23, a newly described member of the IL-12 family. Upon microbial stimulation, IL-12 is synthesized and secreted by antigen-presenting cells (APCs), including monocytes, macrophages and dendritic cells (DCs). IL-12 binds to the IL-12 receptor (IL-12R), which is composed of a β1 and a β2 subunit [1]. IL-12 receptor stimulation promotes IFN-γ and TNFα production by NK cells and T cells. IL-12 is also a growth factor for Th1 cells and stabilizes IFN-γ production [2].

IL-12 is upregulated in several murine models of IBD, including the acute dextran sodium sulfate (DSS)-induced colitis, Gαi2-deficient mice, IL-2-deficient mice, $TNF^{\Delta are}$ mice and IL-10-deficient mice [3–7] (see Table 10.1). In IL-2-deficient animals, the colitis that is triggered by intraperitoneal injection of trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) can be blocked by co-administration of anti-p40 antibodies [4]. Similarly, p40 antibody blockade can prevent/limit disease progression in trinitrobenzene sulfate (TNBS)-treated BALB/c mice, DSS-exposed mice or *Helicobacter hepaticus*-infected IL-10-deficient mice [3,4,8]. Colitis induced by bone marrow transplantation of WT into Tgε26 mice is also blocked by anti-p40 antibodies [9]. $TNF^{\Delta are}$ mice, which have increased TNF expression due to a specific deletion in AU-rich regions inside the TNF gene, are protected from colitis when bred on an IL-12p40-deficient background [6]. Taken together, these studies favor a model where p40 plays a pathogenic role in mucosal immune homeostasis. However, since p40 is shared between IL-12 and IL-23, it is likely that some of the observed effects may reflect IL-23 blockade. Careful studies targeting the IL-12 or 23 unique subunits, p35 and p19, respectively, have shed some light on these data (discussed below).

IL-12 receptor signaling activates STAT4, which is critical for Th1 responses [10]. To address the role of STAT4 in colitis induction, Simpson *et al.* transplanted Tgε26 mice with STAT-4-deficient bone marrow. Whereas wild-type (WT) transplanted Tgε26 mice develop a severe wasting disease, mice receiving STAT-4-deficient bone marrow are largely protected from colitis [9]. Since IL-23 also signals through STAT-4 [11], the precise role of IL-12-dependent STAT4 signaling in this experimental setting remains unclear.

In human IBD, early studies identified IL-12 as a cytokine that was upregulated in the gut of affected individuals [12] (Table 10.2). Based on the encouraging preclinical data discussed above, two clinical trials using anti-IL12p40 antibodies for CD have been published. A higher percentage of CD patients treated with anti-IL12p40 antibodies achieved a clinical response than in the placebo group, suggesting that anti-IL12p40 antibody treatment may be beneficial [13,14] (Table 10.3).

Interleukin-18

IL-18 is a member of the IL-1 family of cytokines (also referred to as IL1-F4) [15]. It is produced as an inactive precursor that requires cleavage by caspase-1 for activation. Caspase-1 is generated upon cleavage of its precursor pro-caspase-1, which is promoted by TLR4 signaling via LPS or Fas–Fas ligand interaction. IL-18 is secreted by a vast array of cells, including macrophages, DCs and intestinal epithelial cells (IECs). The IL-18 receptor shares many similarities with Toll-like receptors and the IL-1 receptor. It has been suggested that IL-18 stimulates both innate and adaptive immunity. IL-18 was first discovered as an IFN-γ-inducing factor (IGIF). In fact, IL-18 can synergize with IL-12 to induce IFN-γ production by T cells through mechanisms that are independent of T cell receptor engagement [16]. Since IL-18 can also promote IL-1β, TNFα and IL-2 secretion, in addition to stimulating IFN-γ production, IL-18 has been classically associated with Th1 cytokines. However, more recent work revealed that IL-18 was also able to stimulate T cells, basophils and mast cells leading to Th2 responses [17]. IL-18 acts on NK cells in addition to $CD8^+$ T cells to upregulate their cytotoxic activity and also promotes IFN-γ and IgG2a production by B cells while inhibiting IgE responses [18].

In the context of IBD, several lines of evidence suggest a role for IL-18 in disease pathogenesis. IL-18 is increased in IECs in colitic mice resulting from the transfer of naïve $CD4^+CD62L^+$ T cells into SCID mice [19] (Table 10.1). Colonoscopic administration of an adenovirus encoding for an IL-18 antisense mRNA led to reduced IL-18 production in the mucosa of diseased animals together with improved endoscopic and histological scores of colitis. This correlated with decreased IFN-γ production in the inflamed mucosa [19]. IL-18 is also upregulated in mice following rectal administration of TNBS [20]. Since IL-18 is mainly produced by macrophages, the authors compared the effects of macrophage depletion (via the administration of an anti-Mac1 antibody coupled to the ribosomal inhibitor saporin) to direct IL-18 antibody blockade. Both anti-Mac1 antibody and anti-IL-18 antibody administration led to reduced weight loss, lower histological scores of colitis and reduced IFN-γ production, leading the authors to conclude that macrophage-derived IL-18 depletion was critical for disease attenuation [20]. These findings were further supported by reduced susceptibility to

Table 10.1 Cytokines/chemokines in murine models of IBD.

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Effect of cytokine blockade (antibody or gene targeting)
Th1 cytokines				
IL-12 (IL-12p40 and p35 heterodimer)	Macrophages, monocytes, DCs	• Th1 differentiation • NK and T cell growth factor	Acute DSS colitis [3]; Gαi2$^{-/-}$ mice [4], IL-2$^{-/-}$ mice [5], TNF$^{\Delta are}$ mice [6], CD45RB transfer [9], Tgε26 mice [9], TNBS-induced colitis [35], IL-10$^{-/-}$ mice [7]	**Colitis amelioration:** TNBS-induced colitis [35], IL-2$^{-/-}$ mice [5], Tgε26 mice [9], CD45RB transfer [9], IL-10$^{-/-}$ mice [8], TNF$^{\Delta are}$ mice [6]
IL-18	Monocytes, macrophages, DCs, intestinal epithelial cells	• Stimulates Th1 and Th2 responses • Promotes cytotoxicity by NK and CD8^{+} cells • Stimulates IgG2a secretion by B cells	CD45RB transfer [19], TNBS-induced colitis [20], IL-2$^{-/-}$ mice [341], DSS-induced colitis [21]	**Colitis amelioration:** CD45RB transfer [19], TNBS-induced colitis [20], DSS-induced colitis [21]
IFN-γ	Th1 T cells, NK cells	• Th1 differentiation • Inhibits Th2 differentiation • Ig class switching (IgG2a) • Macrophage activation (upregulation of class II MHC)	CD45RB transfer [32], TNBS-induced colitis [35], DSS-induced colitis [3]; IL-10 $^{-/-}$ mice [33], TNF$^{\Delta are}$ mice [6], IL-2$^{-/-}$ mice [5,77], bone marrow transplanted Tgε26 mice [9]; Gαi2$^{-/-}$ mice [4], Samp1/Yit mice [52], IL-7^{Tg} mice [271], GFAP-HA^{tg} mice [342], LIGHT^{Tg} mice [343], STAT-4^{tg} mice [344], C3H/HeJBir mice [345], WASP$^{-/-}$ mice [29], CD45RB transfer (nude mice) [189], STAT3$^{-/-}$ in myeloid cells [124], agonist CD40-treated RAG-1$^{-/-}$ mice [34]; OVA–*E. coli*-fed RAG-2$^{-/-}$ mice transferred with Th1-polarized DO11.10^{Tg}/RAG-2$^{-/-}$ T cells [346]	**Colitis amelioration**: CD45RB transfer [32], IL-10$^{-/-}$ mice [8,33], agonist CD40-treated RAG-1$^{-/-}$ mice [34], TNF$^{\Delta are}$ mice [6]; DSS-induced colitis [3] **No effect:** anti-CD3ε-treated mice [53], CD45RB transfer [9], Tgε26 mice [9], TCR-$\alpha^{-/-}$ mice [170], WASP$^{-/-}$ mice [29]
TNFα	Macrophages, monocytes, DCs, B and T cells, basophils, eosinophils, NK cells, neutrophils, mast cells. Non-immune cells	• Promotes tumor necrosis. • Enhances phagocytic activity and production of reactive oxygen species in macrophages and neutrophils. • Positive feedback on the activation of Th1 cells and macrophages	C3H/HeJ mice administered 3% acetic acid [188]; Gαi2$^{-/-}$ mice [4], TNF$^{\Delta are}$ mice [6], Samp1/Yit mice [52], STAT4^{Tg} mice [344], CD45RB transfer [121], IL-10$^{-/-}$ mice [8], WASP$^{-/-}$ mice [29], CD45RB transfer (nude mice) [189], STAT3$^{-/-}$ in myeloid cells [124], agonist CD40-treated RAG-1$^{-/-}$ mice [34]; IL-2$^{-/-}$ mice [77]	**Colitis amelioration:** Samp1/Yit mice [52], anti-CD3ε-treated mice [53], agonist CD40-treated RAG-1$^{-/-}$ mice [34]; TNBS-induced colitis [122]; IL-10$^{-/-}$ mice [122]
IL-2	Activated CD4^{+} cells, CD8^{+} cells, NK cells, NK T cells, DCs	• T cell proliferation and survival • Self-tolerance • Promotes natural regulatory T cell activation, growth and competitive fitness • Macrophage activation	TNBS-induced colitis [35], IL-7^{tg} mice [271], C3H/HeJBir mice [345]	**Spontaneous colitis in IL-2$^{-/-}$ and IL-2R$\alpha^{-/-}$ mice** [69,70]

(*Continued*)

Table 10.1 (*Continued*)

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Effect of cytokine blockade (antibody or gene targeting)
IL-1β	Monocytes, macrophages, DCs, T and B lymphocytes, NK cells	• Fever induction • Positive feed-back on its own secretion • Acute inflammatory phase protein secretion • Induction of IFN-γ secretion • Macrophage activation • Favors leucocyte binding to the gut vascular endothelium	C3H/HeJ mice administered 3% acetic acid [188]; Gαi2$^{-/-}$ mice [4], CD45RB transfer [121], immune complex-induced colitis in rabbits [95], STAT3$^{-/-}$ mice in myeloid cells [124]; DSS-induced colitis (SCID and WT mice) [98,347]; TCR$\alpha^{-/-}$ mice (early stage) [96]; IL-2$^{-/-}$ mice [77]	**Colitis amelioration:** immune complex-induced colitis in rabbits [95]; TCR$\alpha^{-/-}$ mice (early stage) [96]; DSS-induced colitis [98,99]
IL-6	Monocytes, macrophages, neutrophils, Th1, Th17 and B lymphocytes	• Promotes acute inflammatory responses • Involved in Th17 differentiation • Favors T and B cell proliferation • Plasma cell differentiation	C3H/HeJ mice administered 3% acetic acid [188]; Gαi2$^{-/-}$ mice [4], CD45RB transfer [121], TNBS-induced colitis [122], STAT3$^{-/-}$ mice in myeloid cells [124]; IL-2$^{-/-}$ mice [77]; DSS-induced colitis [97]	**Colitis amelioration:** CD45RB transfer [121], TNBS-induced colitis [122], TCR$\alpha^{-/-}$ mice [123], acute DSS-induced colitis [120]
TL1A (tumor necrosis factor-like 1)	Endothelial cells, DCs, monocytes, T cells, kidney, prostate	• Improves T cells responsiveness to IL-2 • Enhances IFN-γ expression in synergy with IL-12 and IL-18 • Promotes Th1 and Th17 responses	Samp1Yit mice [135], TNF$^{\Delta are}$ mice [135], Gαi2 $^{-/-}$ mice [139], DSS-induced colitis [139]	**Colitis amelioration:** Gαi2 $^{-/-}$ mice [139,140], DSS-induced colitis [139]
Th17 cytokines				
IL-23 (IL-12p40 and p19 heterodimer)	Activated DCs, macrophages	• Induces memory T cell expansion • Maintenance, terminal differentiation and expansion of Th17 cells • Protection against extracellular pathogens	*H. hepaticus*-infected RAG-2$^{-/-}$mice [152]; IL-10$^{-/-}$ mice [153], agonist CD40-treated RAG-1$^{-/-}$ mice [34], C3H/HeJ Bir mice [154]	**Colitis amelioration:** *H. hepaticus*-infected RAG-2$^{-/-}$mice [152], IL-10$^{-/-}$ mice [153], agonist CD40-treated RAG-1$^{-/-}$ mice [34]; chronic DSS-induced colitis [139] **Colitis prevention and treatment:** C3H/HeJ Bir mice [154]
IL-17	Th17 cells, NKT cells, CD8^{+} cells, $\gamma\delta$ T cells	• Tight junction fortification • Granulocyte recruitment during inflammation	*H. hepaticus*-infected RAG-2$^{-/-}$mice [152], CD45RB transfer [152], agonist CD40-treated RAG-1$^{-/-}$ mice [34], C3H/HeJ Bir mice [154]	**Colitis amelioration (together with IL-6 blockade):** IL-10$^{-/-}$ mice [153] **Colitis exacerbation:** DSS-induced colitis [159]
Th2 cytokines				
IL-4	Mast cells, T cells, bone marrow stromal cells	• Th2 differentiation • Induces B cell growth and class switching • Mast cell growth factor	TCR-$\alpha^{-/-}$ mice [170], WASP$^{-/-}$ mice [29], oxazolone-induced colitis [169], CD45RB transfer (nude mice) [189], TNBS-induced colitis (BALB/c) [172]; OVA–*E. coli*-fed RAG-2$^{-/-}$ mice transferred with Th2-polarized DO11.10^{Tg}/RAG-2$^{-/-}$ T cells [346]	**Colitis amelioration:** TCR$\alpha^{-/-}$ mice [170], WASP$^{-/-}$ mice [29], oxazolone-induced colitis [169] **No effect:** CD45RB transfer [32]
IL-5	Th2 cells, mast cells, eosinophils	• Stimulates eosinophil recruitment and differentiation • Allergic and anti-helminth reactions	CD45RB transfer (nude mice) [189], SAMP1/Yit mice [175], TNBS-induced colitis (BALB/c) [172], oxazolone-induced colitis [169]	**Ileitis amelioration:** SAMP1/Yit mice [175]

(*Continued*)

Table 10.1 (*Continued*)

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Effect of cytokine blockade (antibody or gene targeting)
IL-13	Activated Th2 cells, NKT cells	• B cell proliferation and class switching • Inflammatory cytokine secretion by monocytes	$WASP^{-/-}$ mice [29], oxazolone-induced colitis [177]	**Colitis amelioration:** oxazolone-induced colitis [177]
IL-25	Th2 cells	• Stimulates IL-4, IL-5 and IL-13 production • Induces IgE, IgA and IgG1 production • Favors eosinophilia	IL-25 injection induces marked colonic changes [179]	$IL\text{-}25^{-/-}$ mice are more susceptible to Helminth infections [180,181]

Immunoregulatory cytokines

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Mucosal effects
IL-10	Th2 cells, Tr1 regulatory cells, B cells, monocytes, DCs, macrophages, neutrophils, endothelial cells	• Inhibit activation and effector function of T cells, monocytes and macrophages • Limit and ultimately terminate inflammatory responses. • Regulate growth and/or differentiation of B cells, NK cells, $CD8^+$ T cells, $CD4^+$ T cells, mast cells, granulocytes and DCs • Differentiation and function of Tr1 regulatory cells	C3H/HeJ mice administered 3% acetic acid [188]; CD45RB transfer (nude mice) [189], $IL\text{-}2^{-/-}$ mice [77]; $WASP^{-/-}$ mice [29]	**Blockade exacerbates colitis:** CD45RB transfer (SCID) [187], $STAT3^{-/-}$ in myeloid cells [124] **Protective:** $TCR\alpha^{-/-}$ mice [192], CD45RB transfer [185]; TNBS-induced colitis [193]; $IL\text{-}10^{-/-}$ mice [194]
TGFβ	T and B cells, NK cells, DCs, macrophages, mast cells neutrophils Non-immune cells	• Cell proliferation, growth, motility and extracellular matrix production, embryogenesis, tissue remodeling, wound healing and immunomodulation	Chronic TNBS-induced colitis [178]	**Protective:** CD45RB transfer [206], TNBS-induced colitis [348], $IL\text{-}2^{-/-}$ mice [204], oxazolone-induced colitis [169], acute DSS-induced colitis [349]
IL-22	$CD4^+$T cells (Th17), NK cells, NK T cells, $\gamma\delta$ T cells, $CD8^+$ cells	• Stimulates acute-phase proteins secretion • Induction of MUC gene expression [229]	$TCR\text{-}\alpha^{-/-}$ mice [229], CD45RB transfer [229], DSS-induced colitis [229]	**Protective:** IL-22 microinjection in $TCR\alpha^{-/-}$ mice [229], aggravation of DSS-induced colitis upon IL-22 neutralization [229]
IL-11	Bone marrow stromal cells	• Stimulates differentiation and proliferation of platelets, B cells and myeloid cells • Inhibits Th1 and favors Th2 responses • Inhibits enterocyte proliferation	Unknown	**Protective:** TNBS-induced colitis in rats [239]; HLA-B27 rats [240]
IL-35	$Foxp3^+$ regulatory T cells	• *In vitro* suppression of T cell proliferation • *In vivo* regulatory T cell-mediated suppression	Unknown	Unknown

(*Continued*)

Table 10.1 (*Continued*)

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Effect of cytokine blockade (antibody or gene targeting)
Unclassified cytokines				
IL-21	Activated $CD4^+$ cells, NK T cells	• T cell proliferation • B cell differentiation • Enhances NK T cell activity • Stimulates MIP-3α secretion by IECs • Enhances MMP secretion by gut fibroblasts	TNBS-induced colitis [247], DSS-induced colitis [247]	**Colitis prevention (IL-21$^{-/-}$ animals):** TNBS-induced colitis [247], DSS-induced colitis [247]
IL-27 (EBI3 and p28 heterodimer)	DCs	• Regulates Th1 and Th2 and Th17 cell differentiation • Stimulates NKT cells		**Colitis prevention**: oxazolone-induced colitis in EBI3$^{-/-}$ mice [251], IL-27R$^{-/-}$ × IL-10$^{-/-}$ mice [252], DSS colitis in IL27R$^{-/-}$ mice [253] **No effect** (EBI–3$^{-/-}$ mice): TNBS-induced colitis [251]
IL-32	Activated lymphocytes NK cells Epithelial cells	• Stimulates TNFα and IL-8 production • Activates NF-κB and MAPK in monocytes/macrophages • Synergizes with NOD1 and NOD2 ligands for IL-1β and IL-6 production		**Colitis exacerbation**: TNBS-induced colitis in mice overexpressing IL-32 in bone marrow [258]
GM-CSF	Myeloid cells, IECs	• Stimulates the growth, differentiation and effector functions of granulocytes, monocytes, macrophages and DCs • Control of chemotaxis, phagocytosis, free radical respiratory burst and bacterial killing in neutrophils and macrophages	DSS-induced colitis [97]	**Colitis prevention:** GM-CSF administration during DSS-induced colitis [261]
IL-7	Non-hematopoietic stromal cells	• T and B cell growth regulation • T cell thymic development and peripheral homeostasis • Intraepithelial lymphocyte development		**IL-7^{tg} mice:** colitis [271] **IL-7 deficiency** protects from colitis in *H. hepaticus*-infected RAG-2$^{-/-}$ mice [272]. **IL-7R neutralization** protects from colitis in TCR$\alpha^{-/-}$ mice [275]
Chemokines/chemokine receptors				
Chemokine/ chemokine receptor	Source of chemokine	Main effects	Murine models of colitis with elevated chemokine levels	Mucosal effects
CCL2 (MCP-1)/CCR2	IECs	• Recruitment of monocytes, DCs and memory T cells during inflammation		**CCL2$^{-/-}$ mice, CCR2$^{-/-}$ mice, CCR2 antagonism:** Protection from DSS-induced colitis [286–288]
CCL3 (MIP-1α), CCL4 (MIP-1β) CCL5 (RANTES)/CCR5	Unclear	• T cells and monocyte migration	TNBS-induced colitis (rats) [296], MDR1a$^{-/-}$ mice [299]	**CCL3 administration** aggravates TNBS-induced colitis (rats) [297]; **CCR5 blockade** ameliorates TNBS-induced colitis (rats) [298]; **CCR5$^{-/-}$ mice**: protected from DSS-induced colitis [287]

(*Continued*)

Table 10.1 (*Continued*)

Cytokine	Source	Main effects	Murine models of colitis with elevated cytokine levels	Effect of cytokine blockade (antibody or gene targeting)
CCL20 (MIP3α)/CCR6	IECs	• Recruitment of T cells, B cells and DCs		**CCR6$^{-/-}$ mice:** less susceptible to DSS-induced colitis, more susceptible to TNBS-induced colitis [305]. **CCL20 neutralization:** ameliorates TNBS-induced colitis [306]
CCL25 (TECK)/CCR9	Thymic and IECs	• Recruitment of T cells and plasma cells to the gut • Intraepithelial lymphocytes recruitment/generation		**CCR9$^{-/-}$** TNF$^{\Delta are}$ mice equally susceptible to colitis as TNF$^{\Delta are}$ mice [350]
CXCL12 (SDF-1)/CXCR4	Widely expressed IECs and microvasculature	• Migration of progenitors during embryonic development • Receptor for HIV entry into T cells	DSS-induced colitis (late stages) [336]	**CXCR4 antagonist:** ameliorates DSS-induced colitis and chronic colitis in IL-10$^{-/-}$ mice [336]

TNBS in IL-18-deficient mice [20]. IL-18 blockade via a recombinant IL-18 binding protein (IL-18bp.Fc) was also efficient in reducing disease in the acute DSS model of colitis (C57BL/6) [21].

Moreover, a pathogenic role for IL-18 was demonstrated when co-administered with IL-12 in mice, leading to weight loss, diarrhea, hemorrhagic colitis, splenomegaly, fatty liver and thymic atrophy. Again, IL-12 and IL-18 appeared to work in synergy since these dramatic clinical features were not observed when cytokines were administered separately [22].

In humans, increased levels of IL-18 were found in the lamina propria (LP) of CD but not UC patients [23,24] (Table 10.2). Treatment of lamina propria mononuclear cells (LPMCs) from CD patients with IL-18 antisense oligonucleotides reduces IFN-γ production [24]. There have been no published clinical trials on IL-18 neutralization strategies for human IBD.

Interferon-γ

IFN-γ is a type 2 member of the interferon family primarily secreted by Th1 T cells and NK cells [25]. It is a potent macrophage-activating factor leading to intracellular pathogen clearance. IFN-γ induces B cell class switching and IgG2a production. Once bound to its receptor, IFN-γ activates STAT1, which promotes Th1 differentiation via the induction of the transcription factor T-bet [26,27]. IFN-γ also antagonizes Th2 differentiation by suppressing the transcription factor GATA-3 – a Th2 master regulator – and inhibits IL-4 signaling [28].

IFN-γ is upregulated in a number of Th1-associated mouse models of IBD listed in Table 10.1. However, elevated IFN-γ has also been observed in some situations where Th2 cytokines are also present, such as in TCR-α-deficient mice, WASP-deficient mice and certain mice strains following oral DSS administration [29–31].

Several studies have assessed whether IFN-γ neutralization can modulate colitis development in mice (Table 10.1). Anti-IFN-γ antibodies are able to ameliorate colitis in the CD45RB transfer model [32], IL-10-deficient mice [8,33], in acute DSS colitis [3] and in anti-CD40-treated RAG-1-deficient mice [34]. Moreover, TNF$^{\Delta are}$ mice are protected from colitis when bred on an IFN-γ-deficient background [6]. While there is a clear interrelationship between IFN-γ and IL-12 as demonstrated *in vivo* by reduced IFN-γ levels upon IL-12 neutralization in various murine models of IBD [5,8,9,35], the efficacy of IL-12 neutralization is not always due to inhibition of T cell-specific IFN-γ secretion. This is exemplified by persisting wasting disease obtained after reconstitution of SCID or Tgε26 mice with T-cells from IFN-γ-deficient donors despite the efficacy of IL-12 neutralization in these models [9,36]. In the SCID transfer model, this could be due to non-T cell-secreted IFN-γ as anti-IFN-γ antibodies are able to block colitis development [32].

In human IBD, many studies have demonstrated elevated IFN-γ in colonic biopsies from CD patients [37–42] (Table 10.2). In contrast, most [37–41,43] but not all [44] studies have found normal IFN-γ levels in the colon of UC patients. Interestingly, early CD lesions may differ from chronic lesions as early lesions exhibit increased IL-4 yet normal IFN-γ levels whereas chronic lesions have increased IFN-γ and low IL-4 levels [45]. One randomized controlled clinical trial assessing humanized anti-IFN-γ antibodies (fontolizumab) for the treatment of moderate to severe CD has been published (Table 10.3). Fontolizumab administration was well tolerated. While response rates between fontolizumab- or placebo-treated patients were

Table 10.2 Cytokines and chemokines in human IBD.

Cytokine	Cellular source	Main effects	Crohn's disease*	Ulcerative colitis*
Th1 cytokines				
IL-12	Macrophages, monocytes, DCs	• Th1 differentiation • NK and T cell growth factor	↑ [12,163]	↑ [163] N [12]
IL-18	Monocytes, macrophages, DCs, intestinal epithelial cells	• Stimulates Th1 and Th2 responses • Promotes cytotoxicity by NK and $CD8^+$ cells • Stimulates IgG2a secretion by B cells	↑ [23,24]	N [23,24]
IFN-γ	Th1 T cells NK cells	• Th1 differentiation • Inhibits Th2 differentiation • Ig class switching (IgG2a) • Macrophage activation (upregulation of class II MHC)	↑ [37–42]	N [37–41,43] ↑ [44]
TNFα	Macrophages, monocytes, DCs, B and T cells, basophils, eosinophils, NK cells, neutrophils, mast cells. Non-immune cells Tumor cells	• Promotes tumor necrosis. • Enhances phagocytic activity and production of reactive oxygen species in macrophages and neutrophils. • Positive feedback on the activation of Th1 cells and macrophages.	↑ [37,41,55,105]	↑ [37,55,105] N [41]
IL-2	Activated $CD4^+$ cells, $CD8^+$ cells, NK cells, NK T cells, DCs	• T cell proliferation and survival • Self-tolerance • Promotes natural regulatory T cell activation, growth and competitive fitness • Macrophage activation	↑ [39,83–87] ↓ [38]	↑ [83] N [39,43,84–87]
IL-1β	Monocytes, macrophages, DCs, T and B lymphocytes, NK cells	• Fever induction • Positive feed-back on its own secretion • Acute inflammatory phase protein secretion • Induction of IFN-γ secretion • Macrophage activation • Favors leukocyte binding to the gut vascular endothelium	↑ [55,56,83,103,104]	↑ [55,83,103,104] N [56]
IL-6	Monocytes, macrophages, neutrophils, Th1, Th17and B lymphocytes	• Promotes acute inflammatory responses • Involved in Th17 differentiation • Favors T and B cell proliferation • Plasma cell differentiation	↑ [55,105,127,128]	↑ [43,55,105,127,128]
TL1a	Endothelial cells, T cells, kidney, prostate	• Improves T cells responsiveness to IL-2 • Enhances IFN-γ expression in synergy with IL-12 and IL-18 • Promotes Th1 and Th17 responses	↑ [141,142]	↑ [141]
Th17 cytokines				
IL-23	Activated DCs, macrophages	• Induces memory T cell expansion • Maintenance, terminal differentiation and expansion of Th17 cells • Protection against extracellular pathogens	↑ [155]	N [155]

(*Continued*)

Table 10.2 (*Continued*)

Cytokine	Cellular source	Main effects	Crohn's disease*	Ulcerative colitis*
IL-17	Th17 cells, NK T cells, $CD8^+$ cells, $\gamma\delta$ T cells	• Tight junction fortification • Granulocyte recruitment during inflammation	↑ [155,161–164]	↑ [162,163] N [164]
Th2 cytokines				
IL-4	Mast cells, T cells and bone marrow stromal cells	• Th2 differentiation • Induces B cell growth and class switching • Mast cell growth factor	↓ [38,44,173]	↑ [44] N [38,174]
IL-5	Th2 cells, mast cells, eosinophils	• Stimulates eosinophil recruitment and differentiation • Allergic and anti-helminth reactions	↓ [38]	↑ [38,174]
IL-13	Activated Th2 cells NK T cells	• B cell proliferation and class switching • Inflammatory cytokine secretion by monocytes	N [174]	↑ [174]
IL-25	Th2 cells	• Stimulates IL-4, IL-5 and IL-13 production • Induces IgE, IgA and IgG1 production • Favors eosinophilia	Unknown	Unknown
Immunoregulatory cytokines				
IL-10	Th2 cells, Tr1 regulatory cells, B cells, monocytes, DCs, macrophages, neutrophils, endothelial cells	• Inhibit activation and effector function of T cells, monocytes and macrophages • Limit and ultimately terminate inflammatory responses • Regulate growth and/or differentiation of B cells, NK cells, $CD8^+$ T cells, $CD4^+$ T cells, mast cells, granulocytes and DCs • Differentiation and function of Tr1 regulatory cells	↑ [41,85]	↑ [41,43,195] ↓ [173]
TGFβ	T and B lymphocytes, NK cells, DCs, macrophages, mast cells neutrophils Non-immune cells	• Cell proliferation, growth, motility and extracellular matrix production, embryogenesis, tissue remodeling, wound healing and immunomodulation	↑ [215,216] ↓ [217]	↑ [215,217]
IL-22	$CD4^+$T cells (Th17), NK cells, NK T cells, $\gamma\delta$ T cells, $CD8^+$ cells	• Stimulates acute phase proteins secretion • Induction of MUC gene expression [229]	↑ [228,232]	↑ [228]
IL-11	Bone marrow stromal cells	• Stimulates differentiation and proliferation of platelets, B cells and myeloid cells • Inhibits Th1 and favors Th2 responses • Inhibits enterocyte proliferation	Unknown	Unknown
IL-35	$Foxp3^+$ regulatory T cells	• *In vitro* suppression of T cell proliferation • *In vivo* regulatory T cell-mediated suppression	Unknown	Unknown

(*Continued*)

Table 10.2 (*Continued*)

Cytokine	Cellular source	Main effects	Crohn's disease*	Ulcerative colitis*
Unclassified cytokines				
IL-21	Activated $CD4^+$ cells, NK T cells	• T cell proliferation • B cell differentiation • Enhances NK T cell activity • Stimulates MIP-3α secretion by IECs • Enhances MMP secretion by gut fibroblasts	↑ [248]	↑ [248]
IL-27	DCs	• Regulates Th1 and Th2 differentiation • Stimulates NK T cells	Unknown	Unknown
IL-32	Activated lymphocytes NK cells Epithelial cells	• Stimulates TNFα and IL-8 production • Activates NF-κB and MAPK in monocytes/macrophages • Synergizes with NOD1 and NOD2 ligands for IL-1β and IL-6 production	↑ [255]	↑ [255]
GM-CSF	Myeloid cells, IECs	• Stimulates growth, differentiation and effector functions of granulocytes, monocytes, macrophages and DCs • Control of chemotaxis, phagocytosis, free radical respiratory burst and bacterial killing in neutrophils and macrophages	↑ [266]	↑ [266]
IL-7	Non-hematopoietic stromal cells	• T and B cell growth regulation • T cell thymic development and peripheral homeostasis • Intraepithelial lymphocytes development	Unknown	Unknown
CC chemokines				
CCL2 (MCP-1)	IECs	• Recruitment of monocytes, DCs and memory T cells during inflammation	↑ [289–291]	↑ [280,289–291,293]
CCL3 (MIP-1α)	Unclear	• T cells and monocyte migration • Receptor is a co-factor for HIV entry into macrophages	↑ [292]	N [292,301]
CCL4 (MIP-1β)	Unclear	• T cells and monocyte migration	↑ [292]	↑ [280,292]
CCL5 (RANTES)	Unclear	• T cells and monocyte migration	↑ [290,293]	↑ [280,290,293,300]
CCL20 (MIP-3α)	IECs	• Recruitment of Tcells/B cells and DCs	↑ [304]	N [304]
CCL25 (TECK)	Thymic and IECs	• Recruitment of T cells and plasma cells to the gut • Intraepithelial lymphocytes recruitment/generation	Unknown	Unknown

(*Continued*)

Table 10.2 (*Continued*)

Cytokine	Cellular source	Main effects	Crohn's disease*	Ulcerative colitis*
CXC chemokines				
CXCL5 (ENA 78)	IECs	• Neutrophil chemoattractant	↑ [323,324]	↑ [280,323,324]
CXCL-8 (IL-8)	Monocytes/macrophages, neutrophils, IECs	• Neutrophil chemoattractant	↑ [292,293,327–330] ↓ (stimulated PBMCs) [333]	↑ [280,292,293,301, 327–332]
CXCL12 (SDF-1)	Widely expressed Intestine: epithelial cells and microvasculature	• Migration of progenitors during embryonic development	N [336]	↑ [336]
CX3C chemokines				
CX3CL1 (fractalkine)	Endothelial cells and IECs	• Transepithelial dendrite formation by intestinal DCs	↑ [337] N [339]	Unknown

*Arrows indicate whether the cytokine is upregulated (↑) downregulated (↓) or unchanged/normal (N) in comparison with healthy controls.

not significant at 28 days after one dose of administration, response rates were significant in patients receiving two doses of fontolizumab.

Tumor necrosis factor-α (TNFα)

TNFα (also called cachectin) was originally cloned as the factor responsible for tumor necrosis during septic shock [46]. TNFα is produced as a 26 kDa transmembrane protein with a transmembrane tail that is further cleaved by the metalloproteinase TNFα-converting enzyme [47]. Following secretion as a 17 kDa soluble protein that forms a trimer, TNFα interacts with two different receptors: TNF receptor 1 (p55) and TNF receptor 2 (p75). TNFα is produced mainly by macrophages and lymphocytes but can also be secreted by a wide variety of other cells. TNFα activates macrophages to enhance both phagocytic activity and reactive oxygen species production. TNFα also can induce apoptosis and T cell proliferation, weight loss and bone resorption [48]. TNFα acts on IECs to promote secretion of TNFα and IL-8 and upregulate the IgA transporter pIgR [49]. TNFα also impedes IEC proliferation, inhibits the expression of peptides important for intestinal restitution (e.g. trefoil factors) and induces cell death upon chronic stimulation [49,50].

TNFα is upregulated in several murine models of IBD (Table 10.1). The critical role of TNFα in the control of gut immune homeostasis is perhaps best exemplified by the Crohn's-like ileitis observed in mice where the AU-rich region in the 3′UTR region of the TNFα gene has been deleted [51]. This deletion leads to increased mRNA stability and a subsequent increase in TNFα expression. In this model, both myeloid cells and T cells are involved in the pathogenesis as conditional deletion of the AU-rich region in either of these cell subtypes can lead to ileitis [6].

A pathogenic role for TNFα has also been demonstrated by colitis amelioration upon TNFα neutralization via anti-TNFα antibodies both in Samp1/Yit mice and in agonist–CD40-treated RAG-1-deficient mice [34,52]. TNFα signaling through TNFR1 appears critical in colitis development as anti-CD3ε-treated TNFR1-deficient mice are partially protected from colonic fluid accumulation [53]. On the other hand, TNFR1/RAG-2-deficient mice are more susceptible than RAG-deficient mice to DSS-induced colitis. These defects were reversed by WT bone marrow transplantation, indicating that TNF-dependent signaling by bone marrow-derived cells protects against DSS colitis [54].

Several investigators have found increased TNFα levels in CD patients [37,41,55] (Table 10.2). A role for TNFα has been less clear in UC as studies have found both increased [37,55] and normal levels of TNFα [41,56]. Following the original publication by Targan *et al.* [57], showing efficacy of anti-TNFα antibody in CD, an explosion of data has been generated fully establishing the beneficial effects of anti-TNFα antibody treatment in both CD and UC. There are currently three FDA-approved commercially available anti-TNFα antibody preparations (Table 10.3). Infliximab, a mouse/human chimeric antibody given intravenously, was the first anti-TNFα antibody with proven efficacy for inducing and maintaining remission in CD and UC [57–61]. The fully humanized anti-TNFα antibody (adalimumab) and the pegylated Fab fragments against TNFα (certolizumab), both administered subcutaneously, also have proven efficacy in CD [62–65]. These compounds are generally well tolerated and are currently the most widely used biological therapies for the treatment of IBD [66,67].

Interleukin-2

IL-2 was originally identified as a T cell growth factor. It is secreted by T cells following T cell receptor activation and leads to *in vitro* proliferation and development of effector T cell function [68]. Other cell types such as DCs, NK and NK T cells produce IL-2, but the biological relevance of these

Table 10.3 Cytokine-directed therapeutic strategies in IBD.

Cytokine	Biological agent	Disease	Efficacy	Side effects	Ref.
TNFα	Infliximab	CD and UC	Efficacy shown in several RCTs	Well tolerated but increased risks of infection including tuberculosis and pneumonia. Increased risk of lymphoma	57–61
	Adalimumab	CD	Efficacy shown in RCTs		62,63
	Certolizumab pegol	CD	Efficacy shown in RCTs		64,65
IFN-γ	Fontolizumab	CD	Increased rate of clinical response and remission	Well tolerated	351
IL-12/ IL-23	Neutralizing anti-IL-12p40 antibody	CD	Efficacy in pilot studies	Well tolerated	13,14
IL-1β	Anakinra (IL-1R antagonist)	CD	Largely unknown. One case report of worsening	Unknown in IBD patients	111
IL-2	Anti-IL-2 receptor (CD25): daclizumab	UC	No effect	Unknown	91
	Anti-IL-2 receptor (CD25): basiliximab	UC	Significant increase in remission rate in a pilot study	Unknown	89
IL-6	Anti-IL-6 receptor antibody	CD	Significant increase in remission and response rates in a small pilot study	Unknown	133
IL-10	Recombinant IL-10 (.i.v)	CD	Safe, significant reduction in CDAI	Fully reversible, dose-dependent decrease in hemoglobin and thrombocyte counts	352
	Recombinant IL-10 (s.c.)	CD	Safe, clinical and endoscopic efficacy	Fully reversible, dose-dependent decrease in hemoglobin and thrombocyte counts	353
	Recombinant IL-10 (s.c.)	CD	Safe, clinical improvement but no difference in remission rate	Fully reversible, dose-dependent decrease in hemoglobin and thrombocyte counts	354
	L. lactis secreting IL-10	CD	Safe, reduced disease activity (phase 1 trial)	Fully reversible, dose-dependent decrease in hemoglobin and thrombocyte counts	196
IL-11	Recombinant IL-11 (s.c.)	CD	Safe, significant increase in remission rate in the IL-11 treated group	Mild injection site reactions. Headache, edema and increased platelet count	243
	Recombinant IL-11 (s.c.)	CD	Significantly lower remission rate when compared with prednisone	Fever, rash, arthralgia/arthritis, nausea/vomiting and headache	244
GM-CSF	Sargromostim	CD	Significant increase in clinical response rates	Mild–moderate injection site reactions. Bone pain	263,264

cellular sources is unclear. IL-2 signals through the IL-2 receptor, which consists of three subunits, IL-2Rα (CD25), IL-2Rβ (CD122) and the common γ chain (CD132). Ligand binding leads to recruitment of JAK3 to the common γ chain and JAK1 to IL-2Rβ, Shc recruitment and induction of downstream signaling pathways [68]. Although T cell proliferation *in vitro* is IL-2 dependent, IL-2 and IL-2-dependent signaling is dispensable for T cell survival *in vivo* – yet critical for self-tolerance. Genetic targeting of the IL-2, IL-2Rα or the IL-2Rβ genes all lead to a similar phenotype that results in a lymphoproliferative disorder and systemic autoimmunity [69–71]. Autoimmunity in these mice is due, at least in part, to defects in naturally occurring regulatory T cells (nTreg cells). These cells express the IL-2Rα chain (CD25) and of the forkhead winged transcription factor Foxp3 [72,73]. IL-2 regulates the growth and competitive fitness of nTreg cells *in vivo* and their suppressive function *in vitro* [74,75]. Adoptive transfer of WT $CD4^+CD25^+$ cells into IL-2Rα-deficient mice rescues the autoimmunity, supporting the notion that defective nTreg cells are critical for the autoimmunity in these mice [76].

Both IL-2- and IL-2Rα-deficient mice develop severe colitis histologically similar to UC [69,70]. This colitis is characterized by upregulation of Th1 pro-inflammatory cytokines such as IL-1β, IL-6, TNFα and IFN-γ and the anti-inflammatory cytokine IL-10 [77]. IECs isolated from IL-2-deficient mice prior to colitis development reveal elevated TGFβ, IL-15 and CD14 expression [78]. $CD4^+$ T

cells are required for colitis development in IL-2-deficient mice since IL-2/MHC classII double-deficient mice are protected from colitis [79]. More recent data suggest that colitis results from overzealous $CD4^+$ T cell activation as IL-2-deficient mice bred on a PKC-theta-deficient background are protected from colitis [80]. The microbial flora is required for colitis development since colitis is abrogated in IL-2-deficient mice raised in a germ-free environment [81]; however, microbial TLR-dependent signals through MyD88 appear not to be required for colitis induction [82].

Most studies have found increased IL-2 mRNA levels in colonic biopsies from CD patients and normal levels in UC patients [39,83–87] (Table 10.2). However, these finding have not been universally appreciated, as one study reported elevated IL-2 levels in UC patients [83] and another study reported reduced IL-2 in CD patients [38].

Consistent with a pathogenic role for IL-2 in IBD is one report of two cases where IL-2 administration aggravated symptoms of CD [88]. Although there have been no published reports on the efficacy of an anti-IL-2 antibody treatment for IBD, there have been three studies performed using anti-CD25 antibodies (targeting IL-2Rα) (Table 10.3). Two open-label studies assessing the role of basiliximab in steroid-dependent UC patients suggested some efficacy [89,90]. Unfortunately, these findings were not corroborated in a placebo-controlled study by Van Assche *et al.* assessing the efficacy of daclizumab, another anti-CD25 antibody which failed to show any therapeutic benefit [91]. There are currently no data on these compounds in CD.

Interleukin-1β

IL-1β, also known as endogenous pyrogen, is the main mediator of fever. This pro-inflammatory cytokine is secreted mainly by macrophages and monocytes but is also secreted by other immune cells including B and T lymphocytes [15]. IL-1β transcription can be induced following stimulation of various Toll-like receptor pathways and also through TNFα- or IL-1β-dependent signaling pathways (by a positive feedback loop) [92]. IL-1β is first synthesized as an inactive 35 kDa precursor (pro-IL-1β), which is then cleaved by caspase-1 (also known as IL-1β-converting enzyme or ICE) to release the active 17 kDa protein. Caspase-1 is also present at steady state in an inactive form, pro-caspase-1, which is activated upon cleavage by a large protein complex called the NALP3 inflammasome [93]. There are two forms of IL-1 receptors (IL-1R): IL-1RI, which is capable of activating cells, and IL-1RII, a negative regulator of IL-1RI signaling. Upon ligand binding, IL-1RI binds IL-1RAcP (IL-1R accessory protein) to form a heterodimer and intracellular adaptors including MyD88, IRAK, TRAF6 are recruited, which activate the NF-κB, AP-1, JNK and MAP kinases signaling pathways [15]. IL-1β induces IL-6 secretion from the vascular endothelium, which in turn leads to the secretion of a variety of acute inflammatory proteins (such as the C-reactive protein) by the liver. IL-1β also acts on the bone marrow either directly or indirectly, through IL-6, to promote neutrophil and platelet mobilization [92] and, in the gut, to promote leukocyte binding to the vascular endothelium [94].

The first animal studies assessing the role of IL-1β in colitis development were performed in the rabbit immune complex colitis model. In this model, colitis results from the administration of immune complexes (human albumin and rabbit serum) after colonic exposure to unbuffered formaldehyde [95]. IL-1β protein levels increase in parallel with the development of colitis. Importantly, this colitis can be ameliorated by administration of the soluble IL-1 receptor antagonist, IL-1RA. IL-1β is also upregulated in a number of murine Th1 models of colitis and also in early colonic lesions of TCR-α-deficient mice [96] (Table 10.1). IL-1β administration induces both IL-1β and IL-6 expression in the gut of normal mice [97]. IL-1β antagonism through the use of an anti-IL1β antibody or recombinant murine IL-1R results in protection from colitis upon DSS administration in mice [97,98]. To address whether ICE plays a pathogenic role in disease induction, Siegmund *et al.* challenged ICE-deficient mice with DSS [99]. Strikingly, mice were almost completely protected even after chronic exposure [99]. This protection from DSS colitis was also observed with administration of either IL-1RA or the ICE inhibitor pralnacasan [99,100]. In TCR-α-deficient mice, anti-IL1-β antibody administration ameliorates mucosal T cell infiltration and epithelial proliferation [96]. Recently, ATG16L1, a CD-associated gene involved in autophagy, was found to be directly involved in IL-1β synthesis. Macrophages from ATG16L1-deficient mice express high levels of ICE and IL-1β upon LPS stimulation. This spontaneous increase in IL-1β synthesis correlates with increased susceptibility to DSS-induced colitis, supporting the notion that this cytokine is critically involved in Th1-mediated colitis [101]. An increase in murine macrophage production of IL-1β is also found when stimulating macrophages carrying the known CD NOD2 risk allele 2939iC with the NOD2 ligand muramyl dipeptide [102]. Moreover, similarly to ATG16L1-deficient mice, Nod2 (2939iC) mice have an increased sensitivity to DSS-induced colitis [102].

IL-1β may be an important factor in IBD pathogenesis in humans as a number of reports have demonstrated increased IL-1β in the inflamed gut of both UC and CD patients [55,56,83,103–105] (Table 10.2). IL-1β secretion appears to be restricted to LP immune cells as IECs isolated from IBD patients secrete levels indistinguishable from IECs isolated from normal controls [106]. Increased ICE expression has also been observed in macrophages from IBD patients, which correlates with increased IL-1β secretion [107]. Stimulation of peripheral blood monocytes from CD, but not UC, patients results in increased IL-1β cytokine production, especially in the active phase of disease

[108]. IBD patients also have increased serum concentrations of IL-1RA [109]. This increase appears to correlate with disease activity [109], prompting some to recommend it as a biomarker of inflammation. An imbalance between IL-1RA and IL-1 was also found in the gut mucosa of IBD patients [103]. Finally, high levels of IL-1β were also found in stools of relapsing CD patients [110].

Despite extensive data showing increased IL-1β in IBD patients, no clinical trials have been published assessing IL-1β-neutralizing strategies for the treatment of CD or UC. However, one case report has described worsening of Crohn's disease after administration of the IL-1RA analogue Anakinra [111]. Given the large number of auto-inflammatory disorders that have responded to IL-1 neutralization (e.g., including rheumatoid arthritis, gout, Muckle-Wells syndrome) [15], further studies may be warranted.

Interleukin-6

IL-6 participates in acute inflammatory responses and induces the secretion of acute-phase proteins by the liver. It is secreted by monocytes and macrophages, neutrophils and Th1, Th17 and B lymphocytes [112]. IL-6 induces plasma cell differentiation and is a growth factor for myeloma cells. IL-6 binds to the 80 kDa ligand-binding chain, IL-6R (also known as CD126), with the 130 kDa signal-transducing protein gp130. IL-6R is expressed on hepatocytes, neutrophils, monocytes–macrophages and some lymphocytes. In contrast, gp130 is ubiquitous and can complex with other cytokine receptors. Signaling through the IL-6R–gp130 complex leads to JAK/STAT3 signaling. In addition, a soluble form of the same receptor can be generated following alternative splicing of the gene encoding IL-6R or through proteolytic cleavage of the extracellular portion of IL-6R. This molecule, called sIL-6R, has the same structure as IL-6R but lacks the transmembrane domain. It can bind to IL-6 and activate cells that only express gp130 (trans-signaling). Finally, a soluble form of gp130 (sgp130) has been reported as a natural IL-6 antagonist that competes with its membrane-bound isoform [112]. Over the past few years, IL-6 has been implicated in the *de novo* generation of Th17 cells. Indeed, studies in mice demonstrated that IL-6 can synergize with TGFβ and lead to Th17 differentiation *in vitro* [113,114]. In addition, TGFβ alone was sufficient to promote expression of the transcription factor Foxp3 and to generate inducible (adaptive) T regulatory cells (iTregs) [113]. Interestingly, the requirements for Th17 and Treg generation in humans appear to be different from those in mice. In humans, combined IL-1β and IL-6 stimulation can lead to Th17 differentiation through processes that are antagonized by TGFβ [115].

Alterations in IL-6-dependent signaling have been associated with several autoimmune disorders, including IBD [116]. IL-6 is secreted by neutrophils attracted in the early phase of inflammation and promotes monocyte–macrophage and also lymphocyte recruitment through chemokines (CXCL1, -5, -6, -8, CCL2, -8, CX3CL1) and adhesion molecules (ICAM-1, VCAM-1, CD62L) expression. IL-6 and sIL-6R trans-signaling regulate T cell and neutrophil apoptosis. In this regard, neutrophils from IL-6-deficient mice are more resistant to apoptosis than WT controls and IL-6 deficiency is associated with increased neutrophil influx and aberrant lymphocyte recruitment [117,118]. Although IL-6 has been implicated in neutrophil apoptosis, it seems to promote survival in lymphocytes. Indeed, *in vitro* studies have shown that IL-6 leads to maintenance of Bcl-2 expression and T cell survival [119]. Since IL-6 regulates the switch between early neutrophil inflitration and later monocyte–lymphocyte recruitment, it may serve as a bridge between innate and adaptive immunity [112].

Increased levels of IL-6 have been observed in a variety of murine models of colitis (Table 10.1). IL-6-deficient mice are less susceptible to colitis following DSS administration [120]. Moreover, IL-6 neutralization via an anti-IL-6R antibody or a soluble gp130–Fc fusion protein has been effective in colitis prevention in the CD45RB transfer model of colitis [121], TNBS colitis [122] and IL-10-deficient mice [122]. IL-6 may also regulate Th2-mediated inflammation as colonic epithelial cells from TCRα-deficient mice bred on an IL-6-deficient background have lower proliferation rates, lower NF-κB activation and lower expression of the TNF receptor type II than colonic epithelial cells from TCRα-deficient mice [123].

Somewhat counterintuitively, STAT3, which is activated following IL-6R stimulation, can have a protective role in intestinal inflammation. In this regard, myeloid-specific STAT3 deficiency results in spontaneous colitis [124]. This is further supported by the spontaneous development of enterocolitis when STAT3 is deleted by conditional targeting in numerous cell types [125]. In contrast, STAT3 has also been shown to play a pro-inflammatory role, as demonstrated by increased susceptibility to DSS in mice bearing a constitutively activated form of STAT3 [120]. Overall, the relevance of these findings to IL-6 biology is controversial since STAT3 is shared with other cytokine signaling pathways such as IL-23 [126].

In human IBD, intestinal IL-6 expression is upregulated in both CD [55,105,122,127,128] and UC patients [43,55,105,122,127,128] (Table 10.2). Plasma IL-6 concentrations appear to be higher in CD than UC patients [129,130]. In contrast, expression of IL-6R is relatively low in both CD and UC LPMCs despite increased activated STAT3 levels. In CD, this paradox may be explained by increased levels of sIL-6R expression, which was specifically upregulated in CD LPMCs, suggesting that IL-6 transactivation may explain the increase in STAT3 activation [122]. IL-6 and IL-6R serum concentrations seem to correlate with clinical disease activity [131,132]

with inactive disease being characterized by reduced IL-6 and IL-6R concentrations and increased soluble gp130 levels. Importantly, LP T cells form IBD patients are relatively resistant to apoptosis compared with healthy donors and apoptosis can be triggered by blocking IL-6 trans-signaling. This further supports the notion that IL-6 plays a critical role in disease pathogenesis [122].

Clinical trials on IL-6 neutralization strategies have shown some promise in the treatment of rheumatoid arthritis and IBD (Table 10.3). In one pilot placebo-controlled study, anti-IL-6R antibody administration led to a dramatic increase in response and in remission rates in CD patients [133].

TL1A (tumor necrosis factor-like 1)

TL1A was identified in a screen for novel TNF-like molecules in endothelial cells [134]. TL1A can be found as a membrane bound protein or processed and expressed in a soluble form. Initial studies have shown that TL1A expression is essentially restricted to endothelial cells but can also be found in the kidney and prostate [134]. Its expression is induced by TNF and IL-1α. TL1A binds to the death-receptor 3 (DR3) and TNF-receptor 6 (TR6), leading to activation of the NF-κB pathway. Transmembrane DR3 expression is upregulated in activated T cells [135]. TL1A is highly expressed on $CD11c^{hi}$/MHC class II^+ cells but also on $CD11c^{lo}$/MHC class II^- cells, suggesting that intestinal DCs may be a main source of TL1A. When LPMCs are stimulated with IL-12 or IL-23, TL1A stimulation enhances IFN-γ or IL-17 production, respectively [135]. TL1A surface expression and secretion are strongly induced in human fresh blood monocytes and monocyte-derived DCs after FcγR stimulation but not after stimulation with TLR agonists [136]. Upon TL1A stimulation, activated T cells are more responsive to IL-2 and secrete large amounts of IFNγ and GM-CSF [134]. TL1A synergizes with IL-12 and IL-18 to induce DR3 expression and strong IFN-γ responses in $CD4^+$ T cells, $CD8^+$ T cells and, to a lesser extent, NK cells [137]. In combination with IL-2 and IL-12, TL1A promotes memory $CD4^+CD45RB^{lo}$ T cell proliferation [135]. TL1A appears to target preferentially CCR9-expressing gut homing T cells, which secrete IFN-γ following cytokine stimulation in a TL1A-dependent fashion [138].

In mice, TL1A and DR3 can be isolated from the inflamed ileum of $TNF^{\delta are}$ mice, Samp1Yit mice [135], mice chronically exposed to DSS and Gαi2-deficient mice [139] (Table 10.1). The pathogenic effects of TL1A are exemplified by studies showing colitis amelioration and improved clinical scores in mice treated with a neutralizing TL1A antibody and concurrently exposed to DSS [139]. Similar data have been obtained with Gαi2-deficient mice [139,140].

In human IBD, TL1A protein is indentified in large quantities in involved and uninvolved intestinal segments from CD and UC patients [141,142] (Table 10.2). Although early reports did not identify TL1A expression in immune cells, FACS analysis of LPMCs from CD patients demonstrated that the increased TL1A expression was originating from T cells [141]. Analysis of membrane-bound TL1A on CD LPMCs reveals higher percentages of $TL1A^+$ cells in inflamed versus uninflamed intestine [142]. In UC, the cellular source may be mainly plasma cells as TL1A-expressing cells also express CD138, a plasma cell marker [141]. Finally, DR3 expression is also upregulated in LPMCs from IBD patients and TL1A stimulation leads to secretion of large amounts of IFN-γ [141,142]. Clinical trials assessing the role of TL1A in IBD are under way (S. Targan, personal communication).

Th17 cytokines

Interleukin-23

IL-23 is a heterodimer containing a p19 subunit, which is unique to IL-23, and a p40 subunit, which is shared with IL-12 [143]. It is mainly secreted by activated DCs and induces the expansion of memory T cells [143]. IL-23 stimulation drives the secretion of IL-17A and IL-17F and can induce naïve cells to differentiate into Th17 cells [144,145]. While initially felt to be required for Th17 differentiation, subsequent *in vitro* studies have implicated IL-23 in the maintenance and expansion of Th17 cells with Th17 differentiation resulting from either combined TGFβ and IL-6 signals in mice or combined IL-1β and IL-6 signals in humans [113–115,146]. However, recent *in vivo* data restricting IL-23R deficiency to T cells suggest that terminal differentiation of Th17 cells depends on IL-23 signals [147]. IL-23 binds a heterodimer composed of the IL-12Rβ1 subunit, shared with the IL-12R, and a unique inducible component called IL-23Rα. IL-23 signaling leads to STAT3 and STAT4 activation, which are also activated by IL-6 and IL-12, respectively [126]. The discovery of IL-23 (and its unique p19 subunit) has expanded the IL-12 family of cytokines to include IL-27 and IL-35 (discussed below) [148].

IL-23 is critically involved in immune homeostasis; transgenic mice constitutively expressing p19 in all tissues exhibit inflammatory disorders in multiple organs including the intestine. This defect appears specific to the immune compartment as p19-overexpressing bone marrow is sufficient to induce disease [149]. IL-23 likely plays a physiologic role in the intestine, as evidenced by its baseline production by DCs in the terminal ileum [150]. One of its likely functions is protection against extracellular bacteria as IL-23-deficient mice are more sensitive to a number of extracellular pathogens including *Citrobacter rodentium* and *Klebsiella pneumoniae* [114,151]. Given their pro-inflammatory activity and the utilization of p40 in both cytokines, the relative contribution of IL-23 and/or IL-12 in IBD pathogenesis has been extensively studied (Table 10.1).

In *Helicobacter hepaticus*-infected RAG-2-deficient mice, Hue *et al.* evaluated the relative expression of IL-12p35 and IL-23p19 in colitic mice. IL-23p19 is upregulated (as is the shared IL-12p40) in the intestine whereas the unique IL-12p35 subunit was expressed in similar levels when compared with controls [152]. Consistent with a role for IL-23p19 in mediating intestinal inflammation, colitis and inflammatory cytokine secretion are reduced in *H. hepaticus*-infected RAG-$2^{-/-}$ mice treated with anti-p19 antibodies. In the CD45RB transfer model, susceptibility to colitis is also reduced in both p19/RAG-2 and p40/RAG-2, but not in p35/RAG-2, double-deficient animals reconstituted with naïve WT $CD4^+CD45RB^{hi}$ cells [152]. Similarly, in IL-10-deficient mice, disease development depends on IL-23 but not IL-12 since IL-10/p19 but not IL-10/p35 double-deficient animals are protected from colitis [153]. Moreover, colitis in IL-10-deficient mice is exacerbated by recombinant IL-23 administration [153]. Protection from colitis in IL-10/p19 double-deficient mice is not dependent upon reduced IFN-γ as IL-10/p19 double-deficient mice have higher systemic production of IFN-γ than control IL-10-deficient mice [153]. In the C3H/HeJBir experimental model of IBD, colitis is induced by transfer of cecal bacterial antigen (CBA)-specific C3H/HeJBir (C3Bir) $CD4^+$ T cells into C3H/HeSnJ SCID mice. Using this model, Elson *et al.* showed upregulation of IL-12 and IL-23 and also increased levels of IFN-γ and IL-17 following adoptive transfer of CBA-specific T cells [154]. Using an *in vitro* system where CBA-pulsed APCs are co-cultured with CBA-specific T cells, the authors demonstrated that IL-17 is driven by IL-23 but not IL-12. To explore the role of the IL-23/IL-17 axis in colitis induction, anti-IL-23p19 antibodies were given to SCID mice at the time of transfer of $CD4^+$ T cells. Abrogation of colitis followed anti-IL23p19 treatment in conjunction with reduced pro-inflammatory cytokines. Moreover, in the same model, anti-IL23p19 antibodies also induced remission of established colitis.

Agonist CD40-treated RAG-1-deficient mice develop a severe systemic and intestinal inflammatory disorder with elevated IL-12p40 and IL-23p19 [34]. Interestingly, p19 was found to control mucosal inflammation, whereas p35 was found to control systemic inflammation. In this regard, agonist anti-CD40 treatment led to severe colitis in p35/RAG-2 double-deficient mice despite normal weight and blood cytokine levels (i.e. TNFα, MCP-1 and IL-6). In sharp contrast, agonist anti-CD40 treatment does not induce colitis in p19/RAG-2 double-deficient mice but leads to pronounced weight loss and elevated blood cytokine levels. As expected, p40/RAG-2 double-deficient mice administered agonist anti-CD40 treatment fail to develop mucosal and systemic signs of inflammation [34]. Another study assessed the efficacy of an anti-IL23R antibody treatment in the DSS colitis model [139]. IL-23R neutralization has minimal effects in mice following *acute* exposure to DSS; however, such antibody therapy led to a reduction in pro-inflammatory cytokine secretion and the severity of colitis and in animals receiving *chronic* DSS exposure.

In humans several lines of evidence strongly support a role for IL-23 in IBD pathogenesis. LPMCs isolated from the gut of CD but not UC patients secrete high levels of IL-23 (and IL-12) (Table 10.2) and anti-IL12p40 antibody administration *in vitro* is associated with decreased IL-12 and IL-23 production [155]. Nonetheless the question remains whether clinical efficacy of anti-IL12p40 is due to a reduction in IL-12 and/or IL-23 [13]. Genome-wide association studies have identified IL-23R genetic polymorphisms and also mutations within known downstream signaling components (e.g. JAK2 and STAT3) as risk alleles for both CD and UC [156,157]. To date, there have been no published studies evaluating the efficacy of IL-23 neutralizing strategies for the treatment of IBD.

Interleukin-17

There are currently six members of the IL-17 family of cytokines: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F [126]. The cytokine classically referred to as IL-17 is IL-17A. IL-17A, which binds and signals through IL-17RA, is secreted by Th17 cells, $CD8^+$ cells, NK cells, γδ T cells and neutrophils. IL-17 expression is induced by the Th17-specific transcription factor RORγt [158]. Th17 cells also secrete IL-17F, the IL-17 family member with the most homology to IL-17A. IL-17D and IL-17E are also known as IL-27 and IL-25, respectively, and are discussed below. Interestingly, about 10% of lamina propria lymphocytes in mice express RORγt under non-inflammatory circumstances, suggesting that Th17 cells play a physiologic role in the uninflamed intestine [158]. This is supported by data showing increased disease susceptibility in DSS-treated animals upon anti-IL-17A treatment [159]. IL-17 might also enhance epithelial barrier function as demonstrated *in vitro* by increased tight junction formation in T84 epithelial cells upon IL-17 treatment [160].

IL-17 and Th17 cells have been isolated in a number of murine models of IBD but its exact role remains elusive (Table 10.1). Hue *et al.* reported increased expression of IL-17 in the colon of *H. hepaticus*-infected RAG-2-deficient mice [152]. As noted above, anti-p19 treatment in these mice results in reduced colitis severity and decreased pro-inflammatory cytokine production, which is accompanied by a decrease in IL-17 production. Colitis in $CD4^+$ $CD45RB^{hi}$ T cell-reconstituted RAG-2-deficient mice is also associated with an increase in lamina propria IL-17 production that is reduced in the absence of IL-23. Interestingly, IL-17-producing cells can still be detected in the gut of $CD45RB^{hi}$-reconstituted p40/RAG-2, p35/RAG-2 and p19/RAG-2 double-deficient mice, suggesting that IL-23 is not completely required for the generation of IL-17-producing cells. Moreover, a significant number of IFNγ/IL-17 double-positive cells are found in

RAG-2 and p35/RAG-2 double-deficient mice with unclear pathogenic role [152]. IL-17 is also elevated in colons from IL-10-deficient mice, but blockade using an anti-IL-17 antibody is only marginally effective unless combined with IL-6 blockade [153]. In the C3H/HeJBir experimental model of IBD, CBA-specific Th17 cells efficiently transfer colitis to C3H/HeSnJ SCID mice [154]. However, the role of IL-17 in colitis generation in these studies remains unclear since Th17 cells secrete a number of other potent proinflammatory cytokines that could be involved in disease pathogenicity (i.e. IL-6, IL-1β, TNFα, IL-21 and IL-22).

In humans, IL-17 and Th17 cells have been isolated from inflamed intestinal segments from patients with CD [155,161–163] or UC [162,163] (Table 10.2). In one study, a greater increase in IL-17F transcripts was found in segments from inflamed compared with uninflamed CD segments. This trend was not found in UC patients. Intriguingly, IL-17F production was globally higher in the colon of UC than CD patients [164]. Another recent study described the presence of a large number of IFN-γ/IL-17 double-positive cells in the inflamed gut of CD patients [161]. These cells (termed Th1/Th17) have features of both Th1 and Th17 cells since they express both T-bet and RORγt, IL-12Rβ2 and IL-23R. Both Th17 and Th1/Th17 cells express CCR6 but only Th17 cells respond to CCL20 (CCR6 ligand) with increased calcium fluxes [161]. Finally, Th1/Th17 cells are able to downregulate RORγt and upregulate T-bet upon IL-12 stimulation, leading to increased IFN-γ secretion [161]. Taken together, these data suggest that significant overlap between Th1 and Th17 cells appears to exist in the intestine and further studies will be needed to characterize these lineages fully. To date, there have been no published studies evaluating the efficacy of IL-17 neutralizing strategies for the treatment of IBD.

Th2 cytokines

Interleukin-4

IL-4 is the prototypical Th2 cytokine and was first identified as a mediator of B-cell class-switching (favoring IgE responses). IL-4 is also a T cell and mast cell growth factor [165]. IL-4 inhibits Th1 responses such as IgG2a production, macrophage activation and delayed-type hypersensitivity. Since the effects of IL-4 frequently are antagonistic to Th1 cytokines, IL-4 has long been considered an immunoregulatory cytokine. Although immunoregulatory in some inflammatory models, IL-4 has a pathogenic role in some animal models of inflammation, including IBD. Moreover, IL-4 upregulation has been associated with a variety of allergic disorders and also in response to helminth infection. IL-4 is mainly secreted by Th2 cells but it can also be secreted by mast cells and bone marrow stromal cells. IL-4 signals through its own receptor, which is a heterodimer of IL-4Rα and the common γc chain [166]. Ligand binding leads to activating signals that induce the JAK/STAT pathway. STAT6 is one of the specific adaptors downstream of IL-4 as STAT6-deficient cells fail to develop IL-4-producing capacities *in vitro* [167]. IL-4 production is driven by the Th2 master transcription factor GATA-3 [168]. IL-4 induces IL-4 and also IL-4Rα expression in a positive feedback loop and inhibits IFN-γ and Th1 differentiation.

Although Th1 models of colitis are numerous, few have been associated with Th2-cytokine skewing and IL-4 production (Table 10.1). Colitis induction following exposure to the hapten oxazolone was the earliest described Th2-associated model of IBD [169]. The resulting colitis is superficial and shares some histologic properties with UC. The inflamed intestine has increased IL-4 and IL-5 expression and colitis induction can be prevented via antibody-mediated IL-4 blockade [169]. The TCRα knockout mouse was the first spontaneous mouse model of IBD reported to develop a chronic Th2-associated colitis [170]. Although levels of both IL-4 and IFN-γ are increased in the colon of diseased mice [30], only IL-4 appears to be critical for colitis development [170]. Colitis in Wiskott–Aldrich syndrome protein (WASP)-deficient mice is another chronic Th2-mediated model of colitis. The Wiskott–Aldrich syndrome (WAS) is an X-linked recessive human primary immunodeficiency in which the majority of patients develop autoimmunity, including an IBD-like disease in some patients [171]. The majority of WASP-deficient mice develop colitis with increased IL-4 levels found in unmanipulated inflamed intestine and also in stimulated LPMCs *in vitro* [29]. Although colitis can be ameliorated in WASP-deficient mice treated with neutralizing antibodies to IL-4, colitis development is not absolutely dependent upon IL-4 since WASP/IL-4 double-deficient animals can develop intestinal inflammation [29].

Another model of colitis that is associated with elevated IL-4 production has been described when OVA-specific DO11.10 TCR transgenic T cells are transferred to RAG-2-deficient mice fed an OVA-producing *E. coli*. Transfer of either *in vitro* skewed DO11.10 Th2 or Th1 TCR transgenic cells (but not naïve $CD4^+$ T cells) leads to severe colitis in RAG-2-deficient mice fed OVA-producing *E. coli*. The Th2-associated colitis is associated with elevated IL-4 and IL-10 colonic tissue concentrations. The cytokine profile found in the colon of some strains of mice depends strongly on the specific strain used. For example, as noted above, the transfer of naïve $CD4^+CD45RB^{hi}$ T cells into RAG-2-deficient or SCID mice (which lack both T and B cells) results in a Th1-associated colitis, whereas the colitis induced by transfer of $CD4^+CD45RB^{hi}$ T cells into nude mice (which lack only T cells) results in elevations of Th2 cytokines (IL-4, IL-5 and IL-10) and Th1 cytokines. Colitis in Balb/c mice administered TNBS is another example of a mixed Th1 and Th2 disease. Dohi *et al.* reported that the Th2 cytokines IL-4 and IL-5 are upregulated in WT Balb/c

mice challenged with TNBS and that the colitis was at least in part IL-4 dependent [172].

In human IBD, IL-4 has been found to be downregulated in the gut of CD patients compared with healthy individuals [38,44,173] (Table 10.2). In contrast, IL-4 concentrations are normal or elevated in UC patients [38,44,174]. Despite consistent preclinical data showing efficacy of IL-4 neutralization strategies in the treatment of Th2-mediated colitis, there are currently no published clinical trials that have assessed the efficacy of anti-IL-4 treatment for UC.

Interleukin-5

IL-5 is a potent stimulator of eosinophil recruitment and differentiation. It is produced by Th2 cells, mast cells and eosinophils. IL-5 signals through the IL-5 receptor, which is composed of a low-affinity α subunit and a non-binding β chain, shared with the IL-3R and the GM-CSF receptor. IL-5 has been classically associated with allergic and anti-helminth immune responses.

Elevated levels of IL-5 have been described in several experimental models of IBD where IL-4 has been elevated (Table 10.1). One study specifically addressed the role of IL-5 in ileitis development in SAMP1/Yit mice [175]. Elevated IL-5 transcript levels are found in the inflamed ileum of SAMP1/Yit mice which correlates with the accumulation of eosinophils. Intraperitoneal transfer of SAMP1/Yit $CD4^+$ T cells to SCID mice is sufficient to induce ileitis. Importantly, anti-IL-5 administration at the time of transfer (and also 3 weeks following transfer) results in decreased ileal inflammation, weight loss and eosinophil recruitment. These data suggest that IL-5 plays a non-redundant role in disease pathogenesis in SAMP1/Yit mice.

In humans, elevated IL-5 has been reported in colonic extracts from UC but not CD patients [38,174] (Table 10.2). There have been no published trials to date assessing IL-5 neutralizing strategies in UC.

Interleukin-13

IL-13, secreted by $CD4^+$ Th2 cells and NK T cells, shares many functional similarities with IL-4, including the promotion of B cell proliferation, differentiation and IgE class switching. IL-13 binds to its cognate receptor, which is comprised of the IL-4Rβ chain (an alternative form of the IL-4Rα) and the IL-13Rα chain leading to JAK1/Tyk2 and then STAT3 or STAT6 activation [176]. IL-13 has been implicated in the pathogenesis of asthma, atopic dermatitis and other allergic disorders.

Oxazolone-induced colitis has provided the most data on the role of IL-13 in colitis development. Indeed, LPMC isolated from inflamed gut segments of oxazolone-treated animals produced large amounts of IL-13 upon anti-CD3/CD28 stimulation [177]. NK T cells in mice express an invariant T cell receptor composed of the Vα14Jα281 chain (Vα24Jα18 in humans) and a restricted repertoire of β chains. This TCR specifically recognizes the MHC class I-like molecule CD1 (CD1d in mice). The natural ligand for CD1 has yet to be identified but CD1-mediated signals can be mimicked by the glycolipid α-galactosylceramide (α-GalCer). Using this strategy, Heller *et al.* showed that NK T cells are the predominant cellular source of IL-13 in oxazolone colitis as LPMC stimulated with CD1-transfected L cells loaded with α-GalCer secreted large amounts of IL-13. A pathogenic role of NK T cells in disease development is further supported by data showing protection from oxazolone-induced colitis in mice depleted of NK T cells and in CD1d-deficient mice. Reduced IL-13 likely contributes to the observed effects of colitis protection in these studies as antibody-mediated IL-13 blockade could also protect mice from oxazolone-induced colitis [177]. Elevated IL-13 has also been associated with the Th2 colitis found in WASP-deficient mice. LPMC isolated form these mice produce large amounts of IL-13 upon *in vitro* stimulation [29].

IL-13 has also been linked to TGFβ1-dependent tissue fibrosis upon chronic inflammatory challenge. Chronic TNBS administration in Balb/c mice (where collagen deposits can be found in the exposed colons after 6 weeks) leads to an early increase in the Th1 cytokines IL-12 and IFN-γ with the subsequent development of a mixed Th17 and Th2 pattern (elevated IL-23, IL-17, IL-25, IL-4, IL-13 and TGFβ1) [178]. In this model, IL-13 production by colonic LPMCs is dependent on IL-25 and IL-23 as antibody blockade of these two cytokines leads to reduced IL-13 production. IL-13 synthesis induces expression of the IL-13Rα2 receptor, which subsequently stimulates TGFβ1 production. Blockade of IL-13Rα2 signaling via a soluble IL-13Rα2–Fc fusion protein results in a dramatic decrease in IL-13 production. Using a plasmid encoding IL-13Rα2–Fc fusion protein and also IL-13Rα2 siRNA knockdown strategies, TGFβ1 expression was reduced and tissue collagen contents were decreased. Taken together, these data demonstrate that IL-13 leads to IL-13Rα2 expression, which favors TGFβ1 production and fibrosis upon ligation.

In humans, LPMCs isolated from the colon of UC but not CD patients produce large amounts of IL-13 upon anti-CD2/CD28 stimulation [174]. NK T cells have been implicated as the source of IL-13 secretion in these studies since LPMCs from UC patients are cytotoxic and can be induced to secrete IL-13 by B cells expressing CD1d. However, these are not the classical NK T cells as CD1d-tetramer positive cells could not be detected by FACS in LPMCs from UC or CD patients. In contrast, the percentage of NK1.1 positive cells was higher in UC and CD LPMC than in healthy controls. Since activated T cells also express this marker, these differences could be explained by increased numbers of activated T cells and not NK T cells. There have been no published trials to date assessing IL-13-neutralizing strategies in IBD.

Interleukin-25

IL-25 was identified in 2001 in a BLAST search for ESTs that share sequence homology with IL-17A [179]. This 17.5 kDa protein shares only 16% homology with IL-17A. It is a member of the IL-17 cytokine family and is also called IL-17E. It is uniquely expressed by Th2 cells *in vitro* and is mainly restricted to mucosal compartments *in vivo* [179]. Importantly, *in vivo* administration of IL-25 in mice recapitulates most of the Th2-associated phenomena, i.e. eosinophilia and increased IgE, IgA and IgG1 production. IL-25 also induces expression of IL-4, IL-5 and IL-13. IL-25 treatment results in marked epithelial hyperplasia with eosinophilic inclusions in the stomach and esophagus of recipient mice. In the small and large intestine, IL-25 induces mucus production and goblet cell hyperplasia. A similar phenotype is seen in the lung of IL-25-treated mice. These changes do not occur in IL-4Rα-deficient mice despite conserved eosinophilia showing that the morphologic features associated with IL-25 administration depend on IL-4Rα signaling.

IL-25 is required for efficient anti-parasitic immune reactions as IL-25-deficient mice fail to clear the *Nippostrongylus brasiliensis* helminth [180]. This appears to be due to the absence of a non-B/non-T (NBNT) c-kit$^+$, FcεR1$^-$ cell population that produces IL-4, IL-5 and IL-13. IL-25 also has anti-inflammatory properties as IL-25-deficient mice fail to mount an efficient type 2 immune response and develop severe infection-induced intestinal damage upon *Trichuris muris* infection [181]. This is accompanied by increased IL-17 and IFN-γ expression in intestinal lesions. Importantly, IL-12 and IFN-γ blockade rescues type 2 responses upon *Trichuris* infection in IL-25-deficient mice. These findings suggest that type 2 responses can be obtained in the absence of IL-25 when type 1 responses are blocked.

Anti-inflammatory cytokines

Interleukin–10

IL-10 was first identified as a factor produced by Th2 cells and capable of antagonizing cytokine production by Th1 cells [165]. In addition to Th2 cells, IL-10 can be secreted by many other cell types, including Th1 cells, B cells, mast cells, eosinophils, macrophages, DCs and many subsets of T cells such as CD8$^+$ T cells, CD4$^+$CD25$^+$Foxp3$^+$ naturally occurring regulatory T cells and Tr1 cells [182]. IL-10 has immunosuppressive effects through inhibition of the antigen-presentation efficiency of macrophages and DCs. IL-10 also enhances class II expression on B cells, favors IgA production and enhances CD8 and NK cell cytolytic activity. IL-10 signals through its cognate receptor, a member of the IFN receptor family, which is composed of the IL-10R1 (ligand binding) and the IL-10R2 subunits (necessary for signaling). Upon IL-10 binding, the IL-10 receptor signaling cascade activates the tyrosine kinases JAK1 and Tyk2 leading to STAT3, STAT1 and STAT5 (in non-macrophage cells) activation [182].

IL-10 plays an important role in gut immune homeostasis, as evidenced by the spontaneous development of colitis in IL-10-deficient mice [183]. Colitis in IL-10-deficient mice has been linked to the lack of Tr1 cells (or IL-10-producing regulatory T cells) and is dependent on bacterial signals [8]. MyD88-dependent signals are required for colitis development in IL-10-deficient mice as IL-10/MyD88 double-deficient mice are protected from colitis [82]. Tr1 cells can be obtained by *in vitro* stimulation of naïve polyclonal T cells with IL-10. Tr1 cells are able to block colitis induced by CD45RB^{hi} cells into SCID mice [184]. This effect is, at least in part, IL-10 specific since recombinant IL-10 administration is sufficient to prevent colitis development in SCID mice reconstituted with CD45RB^{hi} cells [185]. Further, CD45RB^{hi} cells isolated from IL-10 transgenic mice fail to induce colitis in SCID mice upon transfer [186]. Finally, IL-10-deficient CD45RB^{lo} cells inefficiently control colitis induced by CD45RB^{hi} cells [187]. IL-10 has also been identified in other Th1 models of colitis such as IL-2-deficient mice [77] and C3H/HeJ mice administered 3% acetic acid [188] (Table 10.1). Th2 models of colitis including WASP-deficient mice [29] and nude mice transferred with CD4$^+$CD45RB^{hi} cells [189] are also associated with elevated colonic IL-10 production. Taken together, these data suggest that IL-10 has anti-inflammatory properties in gut immune homeostasis. However, it is still unclear whether Tr1 cells are responsible for this protective effect. Indeed, whereas several studies have characterized Tr1 cells *in vitro*, *in vivo* studies have been limited by the lack of specific surface markers. Data using IL-10 reporter mice have nonetheless allowed *in vivo* "tracking" of these cells [190,191]. These studies show that under normal physiologic conditions, about one-third of the CD4$^+$ cells in the colonic lamina propria produce IL-10 and have suppressive properties. It is still unclear whether these cells are induced in the periphery (for example, upon bacterial signals) or whether they are thymic derived. Indeed, while both Foxp3$^-$ and Foxp3$^+$ IL-10-producing CD4$^+$ cells were found in the small intestinal lamina propria, all CD4$^+$IL10$^+$ cells were also Foxp3$^+$ in the colonic lamina propria [191].

Other studies looking at molecules involved in IL-10-dependent signaling are consistent with a role for IL-10 in protection from IBD. Indeed, mice rendered deficient for STAT3 in macrophages spontaneously develop severe colitis. These mice have impaired IL-10 responses leading to aberrant control of Th1 responses and excessive production of Th1 cytokines [124]. Apart from T-cell-derived IL-10, IL-10-producing B cells appear to play a protective role in TCR-α-deficient mice. Mizoguchi *et al.* reported that the MHC class I-like molecule CD1d is upregulated on B cells isolated from inflamed colons from TCR-α-deficient

mice [192]. This correlates with increased disease susceptibility in TCR-α/CD1d double-deficient mice. Importantly, $CD1d^+$ B cells isolated from TCR-α-deficient mice produce IL-10 and can suppress colitis in TCR-α/Igμ double-deficient mice, a mouse line that lacks B cells and has increased disease susceptibility compared with TCR-α-deficient mice. The protective effects of transferred $CD1d^+$ B cells can be blocked by concomitant anti-IL-10 administration, suggesting that IL-10 is required for disease protection. This was further confirmed by lack of protection upon transfer of TCR-α/IL-10 double-deficient B cells to TCR-α/Igμ double-deficient mice [192].

Because of its protective effects, IL-10 administration has been evaluated as a potential treatment strategy for IBD. In mice, intravenous administration of an IL-10-encoding adenovirus was effective in reducing colitis severity after TNBS administration [193]. Interestingly, an orally administered IL-10-producing probiotic bacterial strain (*Lactococcus lactis*) can efficiently reduce colitis severity in IL-10-deficient mice [194].

In human IBD, exaggerated inflammatory responses may result from aberrant immune regulation, leading to the hypothesis that IL-10 might be reduced in the mucosa of IBD patients. However, only one study [173] has shown reduced IL-10 expression in UC and all other studies have demonstrated elevated IL-10 in both CD [41,85] and UC [41,43,195] (Table 10.2). Since most studies compare IBD patients with healthy individuals, there are currently no data assessing whether increases in IL-10 are also present in self-limited intestinal inflammatory conditions (such as acute diverticulitis or infectious colitis).

IL-10 may have protective effects in human IBD. Several studies have suggested clinical efficacy of recombinant IL-10 administration in CD patients (Table 10.3). Further, as in mice, an orally administered genetically modified bacterium (*L. lactis*) for mucosal delivery of IL-10 is safe and had some efficacy in a phase 1 trial in active CD patients [196].

Transforming-growth factor β (TGFβ)

The TFGβ family of cytokines, including TGFβ1, TGFβ2 and TGFβ3, are involved in cell proliferation, growth, motility and extracellular matrix production. They have been implicated in various physiological processes, including embryogenesis, tissue remodeling, wound healing and immunomodulation [197]. TGFβ can be secreted by T and B lymphocytes, NK cells, DCs, macrophages, mast cells and neutrophils and also in non-immune cells [198]. Abundant in the normal intestine, TGFβ1 is an important mediator of epithelial cell differentiation [199] and IgA class switching [200]. It was one of the first factors identified to mediate oral tolerance. Indeed, induction of TGFβ1-secreting T cell clones can be obtained after oral administration of myelin-based protein, a peptide that leads to autoimmune encephalomyelitis following systemic administration [201]. These TGFβ1-secreting T cells were subsequently referred to as Th3 cells [202]. This seminal work was then followed by studies assessing the specific role of TGFβ in gut immune homeostasis. Gorelik *et al.* showed that mice expressing a dominant negative form of the TGFβ receptor expressed in T cells develop colitis [203]. In the Th2-associated oxazolone colitis model, anti-TGFβ1 antibodies worsened colitis development [169]. Similar data were obtained when anti-TGFβ1 antibodies were administered to IL-2-deficient mice [204]. To address the role of epithelial-derived TGFβ1, TGFβ signaling was inactivated in mouse intestinal epithelial cells by expressing a dominant negative mutant form of the TGFβ type II receptor under the control of the mouse intestinal trefoil peptide (ITF)/TFF3 promoter. These mice have increased sensitivity to DSS administration [169]. Together, these data suggest that TGFβ1 plays a role in both the epithelium and adaptive immune cells.

Since Th3 cells do not have specific cell surface markers, several studies have tried to identify whether Th3 cells and natural $CD4^+CD25^+Foxp3^+$ T regulatory cells overlap. Indeed, $CD4^+CD25^+$ cells isolated from TGFβ-deficient neonatal mice do not protect from colitis induced by WT $CD4^+CD45RB^{hi}$ cells in SCID mice [205]. Similarly, suppression of colitis induced by WT $CD4^+CD45RB^{hi}$ cells by WT $CD4^+CD45RB^{lo}$ cells can be abrogated when an anti-TGFβ-neutralizing antibody was administered [206]. However, *in vitro* suppression by $CD4^+CD25^+$ cells is preserved in the absence of TGFβ [207]. Since TGFβ-deficient mice die early in life from lymphoproliferative disorders and systemic autoimmunity, isolation of $CD4^+CD25^+$ from these mice is limited to the neonatal period. As natural T regulatory cell development and Foxp3 expression typically occur early in life [208], it was still unclear whether TGFβ-deficient $CD4^+CD25^+$ cells express Foxp3. To circumvent these issues, Fahlen *et al.* bred TGFβ-deficient mice with mice expressing a TCR transgene specific for chicken ovalbumin (i.e. $DO11.10^{Tg}$ mice) [209]. These mice are protected from autoimmunity and have similar numbers of $Foxp3^+$ regulatory T cells to WT mice. Using these mice, the authors showed that $DO11.10^{Tg}$ TGFβ-deficient $CD4^+CD25^+$ cells were able to block colitis induced by WT $CD4^+CD45RB^{hi}$ cells, suggesting that nTregs, deficient in TGFβ production alone, are not impaired in their capacity to mediate suppression of colitis.

Over the past few years, emerging data have suggested a key role for TGFβ in the peripheral induction of Foxp3 in $CD4^+Foxp3^-$ cells. *In vitro* studies show that whereas TGFβ alone is sufficient to induce Foxp3 expression, the combination of TGFβ and IL-6 results in the differentiation of Th17 cells [113]. More recently, a role for TGFβ in the induction of Foxp3 expression in the intestinal LP has been demonstrated. This effect requires a specialized subset of intestinal DCs that express the $\alpha^{e7}\beta$ [7] integrin (CD103) [210]. This cell subset seems to play a major role

in gut immune homeostasis since WT $CD4^+CD25^+$ cells cannot control colitis induced by WT $CD4^+$CD45RBhi cells in $CD103^{-/-}$ SCID mutant mice. In addition, $CD103^+$ DCs are able to induce expression of the gut homing chemokine receptor CCR9 on $CD4^+$ cells [210,211]. Expression of gut homing molecules is also mediated and dependent on signals delivered by the vitamin A metabolite retinoic acid (RA) [212,213]. RA cooperates with TGFβ to induce Foxp3 in peripheral T cells and inhibit Th17 differentiation [214].

In human IBD, TGFβ transcripts are upregulated in colonic extracts from both active CD [215,216] and active UC [215] (Table 10.2). In one study, LPMCs from CD patients had reduced protein secretion upon CD2 and CD28 stimulation compared with both healthy donors and UC patients [217]. Increased numbers of TGFβ-expressing immune cells can be found in proximity to the gut lumen [215], but IEC-specific TGFβ expression was unchanged compared with healthy donors [218]. Changes in TGFβ receptor I and II expression have also been observed. More specifically, early fibrosis is characterized by expression of both receptors on fibroblasts, whereas receptors expression was undetectable in advanced fibrosis [219]. This hypothesis is further supported by increased TGFβ1 expression in strictures from CD patients [220] and murine data implicating TGFβ1 in tissue fibrosis [221,222]. Aberrant TGFβ1 signaling has also been attributed to changes in downstream signaling molecules (SMADs). Indeed, SMAD7, a downstream inhibitor of TGFβ1, is upregulated in colonic extracts from CD and UC patients and is associated with reduced phosphorylation of SMAD3. SMAD3 phosphorylation can be rescued by SMAD7 inhibition by an antisense RNA. This in turn led to the inhibition of production of several pro-inflammatory cytokines including TNFα and IFN-γ [223].

There have been no published clinical trials testing the efficacy of direct TGFβ1 administration in IBD patients.

Interleukin-22

IL-22 is a member of the IL-10 family of cytokines. It is preferentially expressed by Th17 cells [224] in addition to activated NK cells, NK T cells, $CD8^+$ T cells and γδ T cells [225]. IL-22 signals through a heterodimer containing IL-22R1 and IL-10R2 leading to STAT3 activation. IL-22R1 expression is found in the kidney, keratinocytes and hepatocytes [226]. IL-22 stimulates acute-phase protein secretion and amyloid production in the liver [227]. In the digestive tract, IL-22R1 is expressed in both the small intestine and the colon [226]. Although human data suggested that receptor expression was limited to subepithelial myofibroblasts [228], more recent findings suggest that intestinal epithelial cells also respond to IL-22 [229,230]. While IL-22 is protective in a T cell-mediated mouse model of hepatitis [231], it has been implicated in the pathogenesis of psoriasis in the skin because of its pro-inflammatory properties in this system [224].

There are currently two detailed studies on the role of IL-22 in IBD (Table 10.1). The first study, by Sugimoto *et al.*, shows increased IL-22 expression in the inflamed colon of TCR-α-deficient mice, in SCID mice transferred with WT $CD4^+CD45RB^{hi}$ cells and in mice exposed to DSS [229]. Using a local gene delivery system, direct IL-22 injection in inflamed intestine dramatically improves colitis in TCR-α-deficient mice through upregulation of mucus-associated proteins. The protective effects of IL-22 are further corroborated by increased weight loss and colitis exacerbation in DSS-exposed mice following administration of neutralizing anti-IL-22 antibodies. IL-22 transcription and protein expression are found to be upregulated in RAG-2-deficient mice transferred with WT $CD4^+CD45RB^{hi}$ cells [230]. Transfer of WT $CD4^+CD45RB^{hi}$ cells into RAG-2/IL-22 double-deficient mice results in increased severity of colitis compared with RAG-2-deficient mice, suggesting a critical role for innate immune cell production of IL-22. Since NK cells are the only cellular source of IL-22 present in RAG-2-deficient mice, the authors determined that chemokines involved in NK cell recruitment are increased in the course of WT $CD4^+CD45RB^{hi}$-mediated colitis (CXCL9, CXCL10 and CXCL11) and that this correlates with an accumulation of NK cells. Moreover, IL-22-deficient mice are more susceptible to DSS-induced colitis, a model that is dependent on innate immune signals. Overall, these studies suggest that IL-22 is an important regulatory cytokine that may have a protective role in the pathogenesis of IBD in murine models.

A protective role for IL-22 in human IBD remains uncertain. IL-22 expression is upregulated in the mucosa of both CD and UC patients and appears to be secreted by $CD4^+$ T cells and to induce inflammatory gene expression in subepithelial myofibroblasts [228] (Table 10.2). This effect is dependent on the NF-κB and MAP kinase pathways and can be enhanced when IL-17 or IL-19 is used in combination with IL-22. Further, IL-22 serum levels in IBD patients correlate well with CD activity [232]. An increase in IL-22 serum concentration is also found in CD patients carrying the risk-associated IL-23R variant compared with carriers of the risk-protective IL-23R variant.

Interleukin-11

IL-11 is a pleiotropic cytokine that is primarily secreted by bone marrow stromal cells. It stimulates the differentiation and proliferation of platelets, B cells and myeloid cells [233]. It has anti-inflammatory properties as demonstrated by decreased IL-12p35 and IL-12p40 production by activated monocytes/macrophages upon exposure to IL-11 [234]. It also has anti-inflammatory effects on T cells and upregulates Th2 cytokines while inhibiting Th1 cytokine secretion inluding IFN-γ [235]. Recombinant IL-11 administration can ameliorate the course of disease in a murine T-cell mediated experimental liver injury model [236]. In the gut, the IL-11 receptor is expressed on

enterocytes and ligand binding inhibits proliferation, thereby influencing enterocyte turnover [237,238].

Recombinant IL-11 administration leads to disease reduction in TNBS-induced colitis in rats [239] and in HLA-B27 transgenic rats [240]. In the latter model, molecular analysis demonstrated that disease amelioration is associated with reduced colonic RNA expression of IFN-γ, TNFα, IL-1β and IL-12p40 [241].

In humans, rIL-11 has shown clinical benefits in various inflammatory disorders [235] (Table 10.3). Recombinant IL-11 reduces expression of pro-inflammatory cytokines including iNOS, IFN-γ, IL-8, IL-12, TNFα and IL-1β in psoriatic lesions [242]. In human IBD, safety and efficacy of recombinant IL-11 administration have been evaluated in two independent studies for CD. In the first randomized controlled study, subcutaneous recombinant IL-11 administration was safe and led to a significant increase in disease remission rates in mild to moderate CD patients at the dose of 15 $\mu g\,kg^{-1}$ weekly [243]. In the second randomized controlled trial, early efficacy of recombinant IL-11 was compared with prednisone in active CD. This study did not have a placebo arm and recombinant IL-11 was found to be less effective than prednisone [244].

Interleukin-35

IL-35 is a novel member of the IL-12 family of cytokines [245]. It is a heterodimer between the IL-12p35 subunit and the Epstein–Barr virus-induced gene 3 (EBI-3). It appears to be selectively expressed by $Foxp3^+$ regulatory T cells. EBI-3- or IL-12p35-deficient regulatory T cells do not suppress T cell proliferation *in vitro* and fail to protect RAG-2-deficient mice efficiently from colitis induced by naïve T cell transfer. Recombinant IL-35 is sufficient to suppress proliferation *in vitro* and suppressive properties can be induced in effector T cells by retroviral gene transfer of IL-35 [245].

Elevated levels of IL-12p35 have been found in the gut of IBD patients [163]. However, no studies have assessed the role of EBI-3 or IL-35 in IBD patients.

Unclassified cytokines

Interleukin-21

IL-21 is a pro-inflammatory cytokine that is secreted by activated $CD4^+$ T cells and NK T cells. The IL-21 receptor is composed of a heterodimer containing a specific IL-21 receptor (IL-21R) subunit and the common γ-chain. It induces T cell proliferation and B cell differentiation and enhances NK T cell activity. It also has effects on non-immune cells and stimulates IECs to produce MIP-3α, a T cell chemoattractant. IL-21 also enhances matrix metalloproteinase (MMP)-1, -2, -3 and -9 secretion by gut fibroblasts [246].

IL-21 is upregulated in the colon of mice exposed to DSS or TNBS [247] (Table 10.1). This cytokine has a pathogenic role in disease development as IL-21-deficient mice are largely protected from colitis in both models. Moreover, cytokine expression analysis demonstrates that Th17 cytokines are absent in the gut of diseased IL-21-deficient mice. This is corroborated by *in vitro* data showing that naïve IL-21-deficient T cells fail to differentiate into Th17 cells under Th17-skewing conditions and that WT T cells could be differentiated into Th17 cells upon stimulation with IL-21 and TGFβ [247].

In human IBD, IL-21 is present in inflamed intestine of IBD patients, with CD patients having the highest concentrations [248] (Table 10.2). IL-21 production by LPLs from healthy individuals can be enhanced *in vitro* by exogenous IL-12. In IBD patients, IL-21 blockade inhibits Th1 and TH17 responses resulting in reduced IFN-γ and IL-17 production by CD LPLs [248].

Interleukin-27

IL-27 is a member of the IL-12 family of cytokines that is mainly produced by DCs. It is a heterodimer containing EBI-3 and the IL-27 specific subunit p28. The IL-27 receptor is composed of WSX-1, an orphan-type 1 receptor expressed by lymphocytes and gp130 [148]. IL-27 is involved in Th1 cell differentiation and IFN-γ production presumably by increasing naïve T cell sensitivity to IL-12 signals. Transgenic expression studies demonstrate that IL-27 promotes IFN-γ production by cytotoxic $CD8^+$ T cells, which increases their anti-tumor effects. Although these data suggest that IL-27 is purely pro-inflammatory, it also appears that IL-27 signaling may modulate immune signals as exemplified by exaggerated Th1-immune responses to intracellular pathogens (e.g. *Toxoplasma gondii*, *Trypanozoma cruzi*, *Leishmania donovani* and *Mycobacterium tuberculosis*) in IL-27R-deficient mice [148]. These effects are not specific to Th1 responses as IL-27 can also inhibit overzealous Th2 responses to *Trichuris muris* and *Leishmania major* [249]. To add to this complexity, IL-27 is critical in suppressing the generation of IL-17-producing T cells [250].

In the gut, studies suggest that IL-27 supports both Th1- and Th2-mediated pathologies (Table 10.1). EBI-3-deficient mice are less susceptible than WT mice to oxazolone-induced colitis [251]. IL-10-deficient mice bred on an IL-27R-deficient background have lower disease prevalence [252] and IL-27R-deficient mice are partially protected from DSS-induced colitis [253]. In contrast, EBI-3-deficient mice are equally susceptible to TNBS colitis as WT mice [251]. Data generated in EBI-3-deficient mice must be interpreted with caution as the precise contribution of IL-27 versus IL-35 in this context is not yet clear.

Interleukin–32

IL-32 is produced by activated lymphocytes, NK cells, immature DCs and IECs [254–256]. IL-32 production is

stimulated by IL-12 and IFN-γ [246]. Through mechanisms that have not yet been elucidated, IL32 activates the NF-κB and MAP kinase pathways in monocytes and macrophages to induce production of TNFα and IL-8. Importantly, IL-1β and IL-6 production is stimulated by IL-32 through synergy with NOD1 and NOD2, a mechanism lost in CD patients bearing the 3020insC NOD2 mutation [257]. Mice overexpressing IL-32 only in bone marrow-derived cells are more susceptible to TNBS than non-transgenic animals [258]. Although these data suggest a role for bone-marrow-derived IL-32, it does not address the role of epithelial-derived IL-32.

Epithelial-derived IL-32 may be of clinical relevance, as one study in human IBD demonstrated that epithelial IL-32 expression was increased in the inflamed mucosa of both UC and CD patients. Further, IL-32 expression could be induced by IL-1β, IFN-γ and TNFα through NF-κB-dependent pathways in IECs [256].

Granulocyte macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a 23 kDa protein, which is produced by fibroblasts, endothelial cells, stromal cells, macrophages, smooth muscle cells and osteoblasts [259]. GM-CSF acts through binding of a heterodimeric receptor (GM-CSFR or CSF2R) composed of an α-chain specific for GM-CSF and a β-chain shared by the GM-CSF, IL-3 and IL-5 receptors. GM-CSFR is expressed primarily on myeloid cells, but some reports suggest expression on epithelial cells [260]. GM-CSF primarily stimulates the growth, differentiation and effector function of granulocytes, monocytes, macrophages and DCs, with a key role in the control of chemotaxis, phagocytosis, free radical respiratory burst and bacterial killing in neutrophils and macrophages [259].

GM-CSF administration prevents colitis in DSS-exposed mice and leads to decreased production of TNFα and IL-1β [261] (Table 10.1). This protection is lymphocyte independent as DSS-treated RAG-2-deficient mice benefit equally from GM-CSF administration and this protection can be reversed by antibody depletion of plasmacytoid DCs. Further, type 1 IFN expression is stimulated by GM-CSF and is likely one key mechanism for disease protection as IFN-β administration could ameliorate DSS-induced colitis. Another study showed increased susceptibility to DSS in GM-CSF-deficient mice [262]. Interestingly, the colonic bacterial content in GM-CSF-deficient mice exposed to DSS was increased compared with WT controls.

It has been hypothesized that IBD could result from an innate immune disorder and that GM-CSF (sargramostim) therapy might be beneficial for the treatment of CD. In an open-label trial, sargramostim led to a clinical response in 12 out of 15 CD patients with moderate-severe active disease and a clinical remission in eight patients [263]. In a phase II trial assessing the impact of GM-CSF therapy on active CD, sargramostim resulted in a decrease in disease severity and improved quality of life in patients with active CD [264]. An open-label study assessing the efficacy of G-CSF (filgrastim) administration to patients with active CD yielded similar data [265]. Interestingly, GM-CSF can be isolated from involved colonic mucosa of CD and UC patients [266]. In CD, elevated mucosal GM-CSF correlated with disease activity [267]. Current data do not allow us to differentiate whether these latter findings correspond to a pro-inflammatory signal or an inefficiently activated regulatory pathway.

Interleukin-7

IL-7 is a member of the type 1 cytokine family that is predominantly secreted by non-hematopoietic stromal cells (thymus, lymphoid organs, skin, intestine and liver) [268]. IL-7 signals through a receptor composed of the common γ chain and IL-7Rα. IL-7 plays a critical role in T and B cell growth regulation, as demonstrated by the nearly complete absence of lymphocyte development in IL-7- or IL-7Rα-deficient mice. One of the primary functions of IL-7 is the regulation of T cell thymic development and peripheral homeostasis. IL-7 is present in small amounts in basal conditions and is mainly involved in maintaining normal T cell turnover. In contrast, in lymphopenic conditions, high amounts of IL-7 will lead to massive T cell expansion in a process known as homeostatic proliferation. IL-7 also plays a fundamental role in the generation of extra-thymically derived intraepithelial lymphocytes (IELs) [269]. IL-7Rα-deficient mice contain only TCRαβ and no TCRγδ IELs. These effects could be rescued in these mice by transgenic IL-7 expression restricted to IECs supporting the notion that IEC-dependent IL-7 expression mediates extra-thymic development of TCRγδ IELs [270]. TCRαβ IELs have different signaling requirements since complete loss of all IEL populations is observed in γc-chain-deficient mice, which have signaling defects in multiple cytokine receptors including IL-2 and IL-15.

A pathogenic role for IL-7 in the intestine was suggested by studies in IL-7 transgenic mice which spontaneously develop severe colitis [271]. Although under physiologic conditions IL-7 expression appears limited to non-immune cells, IL-7 expression in transgenic mice appears to originate from LPLs and, to a lesser extent, IECs. Non-lymphocyte-derived IL-7 production appears to exaggerate immune responses to intestinal microflora, as RAG-2-deficient mice, but not RAG-2/IL-7 double-deficient mice, exposed to *H. hepaticus*, develop severe colitis [272]. The pathogenic role of non-lymphocyte-derived IL-7 is further supported by lack of colitis development in RAG/IL-7 double-deficient mice transferred with either total naïve $CD4^+$ cells or colitogenic IL-7Rα^{high} $CD4^+$ T cells from diseased T cell-transferred RAG-2-deficient mice [273]. IL-7Rα expression is also induced in $CD4^+$ LPLs in inflamed segments in TCRα-deficient mice as well as in RAG-2-deficient mice transferred with $CD4^+$ T cells.

These IL-7Rα^{high} cells are colitogenic when transferred to RAG-2-deficient mice and colitis can be exacerbated by IL-7 administration and ameliorated by IL-7R neutralizing strategies [274]. Anti-IL-7R antibody treatment ameliorates established colitis in TCRα-deficient mice [274,275]. To determine whether systemic IL-7 or tissue-derived IL-7 is required for colitis induction, Tomita *et al.* designed a parabiosis system where the skin on the opposing flanks of RAG-2-deficient mice and RAG-2/IL-7 double-deficient mice are surgically attached to allow circulating cell exchange. Adoptive transfer of naïve T cell to the RAG-2-deficient parabiont leads to colitis in the RAG-2/IL-7 double-deficient parabiont. This suggests that systemic IL-7 signals can drive colitogenic T cell development and that intestinal cell secretion of IL-7 is not required for colitis development [276].

Chemokines

Chemokines are small 8–12 kDa cytokines that can direct the recruitment and migration of circulating leukocytes and play a critical role in the differentiation of secondary lymphoid organs. They can either be constitutively secreted or induced under conditions of inflammation. There are approximately 50 known chemokines and 20 known chemokine receptors. A single chemokine can interact with several different chemokine receptors and a single chemokine receptor can respond to multiple chemokines.

Chemokines are classified in four groups based on the pattern of their cysteine residues: The CC family of chemokines contains two adjacent cysteine residues. The CXC family has two cysteine residues separated by a non-cysteine amino acid, whereas the CX3C family has two cysteine residues separated by three non-cysteine amino acids. The C family has only one cysteine residue. Although the original name of a chemokine is still often used in practice, the official chemokine name consists of its family name followed by L (for ligand) and a unique number. Receptors follow the same official nomenclature with the family name followed by an R (receptor) and a number. Chemokine secretion is induced by reactive oxygen species production and calcium influx, a process recently described as being dependent on the calcium-permeable channel TRPM2 [277]. Chemokine receptors are G-protein-coupled receptors that upon binding lead to calcium influx and activation of several downstream targets including the PI3 kinase pathway [278].

In general, a given inflammatory stimulus is accompanied by upregulation of a large panel of chemokines. For example, quantitative PCR analysis showed an induction of IP-10, MCP-1, MDC, MIG, TARC, RANTES, CCR4 and CCR5 in the early stages of colitis in the CD45RB transfer model of colitis and the induction of MIG, RANTES, lymphotactin, MIP-3α, TCA-3, TARC, MIP-3β, LIX, MCP-1 and MIP-1 β and the receptors CCR4, CCR6 and CCR2 in the colon of IL-10-deficient mice [279]. Some of these chemokines are influenced by pro-inflammatory cytokines, as IL-12 blockade led to their reduction [279]. Such chemokine diversity has also been demonstrated in IBD with both CC and CXC chemokines being upregulated in inflamed UC colon biopsies [280].

Here we will review the chemokines that have been investigated in the context of IBD. In this section, we will combine the discussion of several chemokines based on their predominant receptor usage.

The CC family of chemokines

CCL2 (MCP-1) and CCR2

CCR2 and its ligands MCP-1, -2, -3 and -4 are involved in the recruitment of monocytes, DCs and memory T cells [281,282]. In the intestine, MCP-1 is produced by IECs. MCP-1 expression in the intestine is downregulated by the Th2 cytokines IL-4, IL-13 and IL-10 [283] and upregulated by the Th1 cytokines TNFα and IFN-γ [284]. MCP-1 appears to play a pathogenic role in the colon as injection in the colonic wall of an adenovirus encoding MCP-1 led to increased collagen deposition and fibrosis and an upregulation of TGFβ [285]. Moreover, mice deficient in MCP-1 are protected from hapten-induced colitis, as demonstrated by reduced histological scores of colitis and lower IL-1β, IL-12p40 and IFN-γ production relative to WT mice [286]. Further, DSS-exposed CCR2-deficient mice had lower histological scores of colitis in addition to reduced mucosal ulcerations [287]. The chemokine receptor antagonist TAK-779, which blocks CCR2, CCR5 and CXCR3, can protect mice from DSS-induced colitis, further suggesting that CCR2 may be involved in the recruitment of pathogenic cells to the intestine [288]. Thus, CCR2 and its ligands seem to be required for the inflammation associated with several animal models of IBD.

In human IBD, MCP-1, MCP-2 and MCP-3 have been isolated from involved intestine from CD [289–292] and UC [280,289–293] patients (Table 10.2). CCR2-positive $CD4^+$ LPLs have also been shown to be recruited to the small bowel of CD patients [294].

CCL3 (MIP-1α), CCL4 (MIP-1β) CCL5 (RANTES) and CCR5

CCR5 and its ligands are involved in the migration of T cells and monocytes [281]. CCR5 has been extensively studied in the context of HIV infection since this receptor serves as a co-receptor for HIV entry in macrophages [295].

CCL3 is upregulated in the colon of rats exposed to TNBS. This coincides with massive neutrophil influx which can be blocked by neutralizing antibodies to CCL3 [296]. In addition, TNBS colitis is exacerbated by CCL3 administration, which is accompanied by massive cellular

influx into the colon and induction of the pro-inflammatory cytokines TNFα and IFN-γ [297].

Similarly, RANTES expression is induced by TNFα and IFN-γ [284] and, together with its receptors CCR1 and CCR5, is upregulated in the chronic phase of TNBS-induced colitis in rats. This correlates with an accumulation of macrophages and monocytes in the diseased colons. Administration of a CCR1/CCR5 antagonist to TNBS-exposed rats at the time of RANTES upregulation suppressed this cellular influx and ameliorated colitis [298]. RANTES expression together with MIP-2/CXCL2, KC/CXCL1, MIP-1α/CCL3, MCP-1/CCL2 is also increased in the inflamed colons of MDR1a-deficient mice [299]. Further, CCR5-deficient mice are less susceptible to DSS-induced colitis and the inflammation that occurs in CCR5-deficient mice is characterized by increased $CD4^+$ T cell and $NK1.1^+$ cell influx together with an upregulation of the Th2 cytokines IL-4, IL-5 and IL-10 [287].

Whereas CCL4 [280,292] and CCL5 [280,290,293,300] are upregulated in both CD and UC, CCL3 expression is increased in the colon of CD but not UC patients [292,301] (Table 10.2). Interestingly, RANTES was found to be expressed in non-caseating granulomas of CD patients by *in situ* hybridization with surrounding $CD4^+$ T cells expressing the CCR5 and CXCR3 receptors. This staining was specific for granulomas as lymphoid aggregates in CD and lymphoid follicles in control patients did not express RANTES [302].

CCL20 (MIP-3α) and CCR6

CCL20 mediates chemotaxis of T cells, B cells and DCs [281]. In the intestine, CCL20 is secreted by IECs and TNFα, IL-1α or enteric pathogens can induce its expression [303]. In human IBD, CCL20 protein and RNA expression was increased in CD but not in UC patients [304].

CCR6 deficiency in mice leads to decreased susceptibility to DSS but increased susceptibility to TNBS [305]. On the other hand, MIP-3α neutralization leads to disease amelioration upon TNBS administration [306]. Thus, whereas CCR6 is beneficial in TNBS-induced colitis, its ligand is pathogenic. The fact that CCR6 is protective might be due to its role in innate immune responses as CCR6-deficient mice are also resistant to experimental peritonitis [307].

Intravital microscopic analyses have shown that CCR6 blockade on T and B cells reduced their adherence to mucosal and submucosal microvessels in the course of DSS colitis [308]. Using CCR6-GFP knockin mice, Salazar-Gonzalez *et al.* demonstrated that the majority of DCs found in the Peyer's patches are in fact CCR6 positive. They further showed that these cells are recruited to the subepithelial dome and activate pathogen-specific $CD4^+$ T cells upon exposure to *S. typhimurium* [309].

Recently, CCR6 has been identified as a key modulator of Th17 cell recruitment to the intestine [310]. CCR6-deficient T cells that have been skewed towards Th17 cells can lead to colitis when transferred into SCID mice. This colitis is characterized by increased Th1 but decreased Th17 and $Foxp3^+$ T cells. Finally TGFβ seems to induce CCR6 expression whereas IL-2 leads to the opposite effect [310].

Taken together, these data show that (1) CCL20 and CCR6 have a chemotactic effect on T and B cells under inflammatory conditions in the colon and (2) CCR6 expression defines a specific DC subtype localized in the small intestinal Peyer's patches.

CCL25 (thymus-expressed chemokine, TECK) and CCR9

CCL25 is constitutively expressed by thymic epithelial cells and IECs in the small intestine but not in the colon [311]. CCL25 binds to the CCR9, which is expressed on T cells and IgA^+ plasma cells [312]. CCR9 is selectively expressed on integrin $\alpha4\beta7^+$ T cells in the mesenteric lymph nodes and *in vivo* neutralization of CCL25 impairs recruitment of antigen-specific T cells to the small intestine [313]. In the intestine, CCR9 is expressed by both αβ and γδ $CD8\alpha\alpha^+$ intraepithelial lymphocytes (IELs) and these cells migrate towards CCL25 in *in vitro* chemotaxis assays [314]. CCR9-deficient mice have a vast reduction of γδ IELs [311] and anti-CCL25 antibody administration to young mice leads to decreased αβ and γδ CD8αα IELs, suggesting that the CCL25–CCR9 pathway is involved in the early recruitment/generation of these cells [314].

Over the past few years, several reports have shed light on the mechanisms involved in the induction of gut-homing molecules. MLN and Peyer's patch DCs but not PLN DCs are able to induce CCR9 and α4β7 integrin expression on T cells [315,316] and this gut-homing imprinting can be induced by the vitamin A metabolite retinoic acid [212]. DCs poised with gut-homing imprinting capacities express the α^e integrin (CD103) [317]. These cells appear to be derived from the LP as mice deficient in CCR7, a molecule critically involved in homing to lymph nodes, have conserved numbers of $CD103^+$ DCs in the LP but reduced numbers in the MLNs [317,318]. Importantly, $CD103^+$ DCs also play a tolerogenic role in gut immune homeostasis. Indeed, $CD4^+CD25^+$ nTreg cells cannot block colitis induced by transfer of WT $CD4^+CD45RB^{hi}$ cells to CD103/RAG-2 double-deficient mice [210,319]. Further studies have implicated TGFβ signaling and also retinoic acid in the generation of $Foxp3^+$ T cells via $CD103^+$ DCs [210,211,213,214]. Taken together, these data demonstrate that $CD103^+$ DCs both influence T cells to induce CCR9 expression and a gut homing phenotype and peripherally induce the generation of $Foxp3^+$ T cells.

There appears to be a reduction of $CCR9^+$ T cells in affected CD small bowel segments, which correlates with an increase in $CCR9^+$ T cells in the peripheral blood [320].

These cells have an activated phenotype and produce high levels of the pro-inflammatory cytokines IFN-γ and IL-17. Cytokine production is enhanced when TL1a is added to stimulating conditions [321]. Taken together, these data suggest that $CCR9^+$ T cells may play a role in IBD pathogenesis, yet it remains unclear whether $CCR9^+$ cells are pathogenic or protective.

The CXC family of chemokines

CXCL5 (ENA-78) and CXCR2

Epithelial cell-derived neutrophil-activating peptide-78 (ENA-78) is a potent neutrophil chemoattractant. It is produced by IECs and its secretion is induced by LPS and the pro-inflammatory cytokines IL-1β or TNFα [322,323]. ENA-78 shares sequence homology with CXCL-8 (IL-8), another neutrophil chemoattractant, and both are downregulated upon IFN-α and IFN-γ stimulation of human monocytes [322]. Like ENA-78, CXCL-8 (IL-8) can bind CXCR2, although its affinity for the receptor is lower.

ENA-78 expression is induced in the colon of IBD patients. In UC, ENA-78 is highly upregulated in IECs [323,324] and this correlates with an increase of other CXCR2 chemokine ligands such as IL-8, GROα, GROβ and GROγ in addition to CC chemokines [280]. Induction was maximum in mild–moderate disease compared with severe disease [323,324]. ENA-78 has also been found in the colon of CD patients [323,324].

CXCL8 (IL-8)

IL-8 is also a neutrophil chemoattractant that is produced by macrophages, fibroblasts, epithelial cells, hepatocytes and endothelial cells [325]. Like ENA-78, IL-8 production by epithelial cells (HT–29) is enhanced by IL-1β and TNFα [323]. Muramyl dipeptide (MDP) stimulation of NOD2 leads to IL-8 production and this induction is lost in the presence of the CD-associated NOD2 variant Leu1007fsinsC [326].

Several studies have shown upregulated IL-8 in the gut of both CD [292,293,327–330] and UC [280,292,293,301,327–332] patients and IL-8 production appears to correlate with histological severity of disease [328,329]. In addition, one study found reduced IL-8 secretion upon stimulation of PBMCs from CD patients [333].

CXCL12 (SDF-1) and CXCR4

CXCL12 and its receptor CXCR4 are widely expressed. Both factors play essential roles in the migration of progenitors during embryonic development as CXCL12- or CXCR4-deficient mice die during embryogenesis [334, 335]. CXCR4 has been identified as a co-factor for HIV entry into T cells and has been implicated in tumor metastasis and hematopoiesis [281].

In the intestine, CXCL12 expression has been reported in IECs and the microvasculature. Expression of its ligand CXCR4 on the same cell types suggests paracrine or autocrine stimulation. CXCL12 is induced in the late stages of acute DSS colitis and appears to be expressed by reticular cells adjacent to the endothelium [336]. There is a parallel increase in the percentage of $CXCR4^+$ cells in PMNs, $CD4^+$ and $CD8^+$ T cells from DSS-exposed mice. The CXCL12/CXCR4 chemotactic pathway appears to be involved in colitis induction as colitis can be partially ameliorated by administration of the CXCR4 antagonist TF14016. Interestingly, this treatment strategy was associated with reduced pro-inflammatory cytokine production in the MLN and did not impact IL-10 expression or $Foxp3^+$ regulatory T cell recruitment to this site [336]. The efficacy of CXCR4 blockade was also observed in IL-10-deficient mice [336].

In human IBD, CXCR4 expression on peripheral blood T cells is increased in UC patients during the active phase and is comparable to healthy donors in the inactive phase. The magnitude of expression correlates with disease activity. There is no increase in expression in patients with CD [336].

CX3CL1 (fractalkine) and CX_3CR1

Fractalkine is a member of the CX3C chemokine family. It is synthesized as a type I transmembrane protein and a soluble form can be generated by proteolytic cleavage. CX3CL1 is expressed by endothelial cells and IECs. IL-1β stimulation leads to increased expression and release from the cell membrane [337]. In the terminal ileum, DCs form dendrites that can directly extend through the epithelium to sample luminal antigens. Using a Cx_3CR1 GFP-knock-in approach, Niess *et al.* demonstrated that most LP DCs in the terminal ileum of $Cx_3CR1^{GFP/+}$ mice are GFP positive and express Cx_3CR1 and that these cells can sample luminal antigens [338]. Importantly, Cx_3CR1 is necessary for the complete formation of these dendrites as they are diminished in mice that do not express Cx_3CR1. This impaired dendrite formation leads to decreased uptake of commensal and enteropathogenic bacteria, which results in impaired immune responses against these pathogens [338].

While increased fractalkine expression has been reported in active CD patients [337], another report suggested that there is no alteration in expression [339]. One study in CD showed that human intestinal microvasculature endothelial cells express CX3CL1 and that this could be modulated by TNFα and IFNγ stimulation. Further, circulating T cells and also LP T cells from active CD patients contained a higher proportion of $CX3CR1^+$ cells than CD patients with inactive disease or healthy subjects. The CX3CL1–CX3CR1 pathway was then shown to induce leukocyte adhesion through stimulation of active β1-integrin expression [340].

Conclusion

The complexity of the events that occur during IBD development is directly reflected by the high diversity of cytokines and chemokines that are found in affected individuals. The pattern of cytokine/chemokine expression is influenced by many factors, including disease duration, disease activity, genetics and therapeutic interventions. Cytokines and chemokines are multifaceted: they can be pro- or anti-inflammatory, they can affect gut epithelium/barrier integrity, innate immune defenses and adaptive immune responses (Figure 10.1). Because these molecules coordinate many key processes, one can readily appreciate why cytokines/chemokines are considered as central targets for future drug development. As we further our understanding of the pathogenesis of IBD, the panel of cytokines/chemokines available for therapeutic intervention is also growing. Because of the complex inter-relationships among cytokines/chemokines, targeting one specific cytokine might have considerable effects on a large number of others. Nevertheless, the clear therapeutic benefits obtained with anti-TNFα therapies clearly demonstrate that cytokine-directed therapy can be feasible, safe and efficacious. Emerging data on the targeting of alternative pathways certainly provides hope for effective new therapies in the treatment of IBD.

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Chapter 11
The Role of the Vasculature in Chronic Intestinal Inflammation

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Summary
- Chronic intestinal inflammation results from a dysregulated immune response to enteric bacterial antigens, thereby enhancing the production of different effector T cell- and leukocyte-derived inflammatory mediators within the intestinal tissue.
- Increased production of inflammatory mediators promotes platelet and leukocyte adhesion to post-capillary venular endothelium, induces the recruitment and activation of leukocytes and initiates pathogenic angiogenesis.
- Leukocyte- and/or effector T cell-derived mediators promote the formation of powerful vasoconstrictors (e.g. thromboxane, endothelin) that reduce blood flow, amplify the stimulus for blood vessel proliferation and enhance tissue injury.
- Uncontrolled inflammation activates the coagulation cascade leading to microthrombus formation within the inflamed colon, systemic thromboembolism and amplification of the inflammatory response.

Introduction

Inflammation is defined as the reaction of a tissue and its *vasculature* to a pathogenic insult designed to dilute, destroy or wall off the injurious or pathogenic organism. There is an accumulating body of evidence suggesting that the chronic intestinal inflammation observed in the inflammatory bowel diseases [IBD; Crohn's disease (CD); ulcerative colitis (UC)] results from a dysregulated immune response to normal enteric antigens resulting in the overproduction of a number of different effector cell- and leukocyte-derived inflammatory mediators within the intestinal tissue [1–3]. Uncontrolled production of these mediators will result in adhesion of platelets and leukocytes in post-capillary venules, followed by recruitment and activation of leukocytes and platelets. In addition to their effects on leukocyte/platelet adhesion to endothelial cells, certain tissue- and leukocyte-derived mediators will initiate angiogenesis and the coagulation cascade. A dysregulated angiogenic response will result in abnormal blood vessel formation that enhances leukocyte adhesion to the newly formed blood vessel endothelium, thereby perpetuating colonic inflammation. In addition, leukocyte and/or platelet adhesion to activated venular endothelium promotes the formation of powerful vasoconstrictors (e.g. thromboxane, endothelin), which reduce blood flow, amplify the stimulus for blood vessel proliferation and enhance tissue injury. Accompanying these local inflammatory responses is the activation of the coagulation cascade by locally released inflammatory mediators, which leads to microthrombus formation within the inflamed colon and also systemic thromboembolism. Furthermore, dysregulation of the natural anticoagulant mechanisms results in the formation of additional mediators that amplify the inflammatory response and create a vicious cycle of coagulation and inflammation. The objective of this chapter is to provide an overview of the role of the vasculature in the pathophysiology of chronic intestinal inflammation. An outline of the inter-relationships among T cell trafficking, angiogenesis, microvascular blood flow, coagulation and intestinal inflammation is presented in Figure 11.1.

Lymphocyte trafficking in chronic gut inflammation

The tissue vasculature plays a critical role in the induction and perpetuation of chronic inflammation by virtue of its ability to regulate the trafficking of naïve and pathogenic lymphocytes into lymphoid and target tissue, respectively. No animal model more dramatically illustrates the importance of T lymphocyte (T cell) trafficking in the induction and perpetuation of chronic intestinal inflammation

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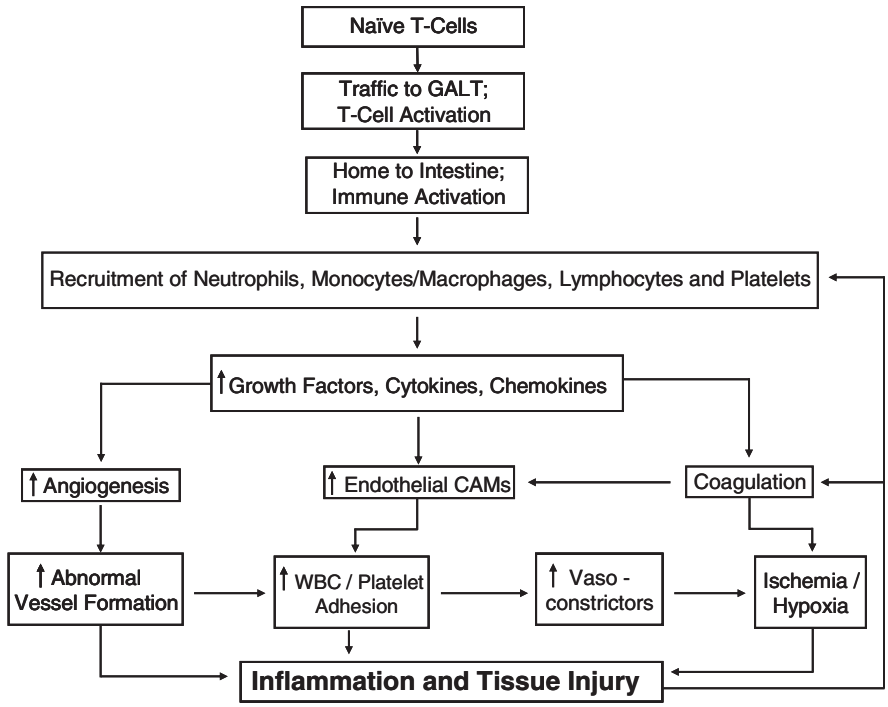

Figure 11.1 Interrelationships among T cell trafficking/activation, angiogenesis, microvascular blood flow, coagulation and intestinal inflammation. Dysregulated immune responses to enteric antigens result in mucosal immune system activation, recruitment of leukocytes and platelets and the overproduction of a variety of pro-inflammatory cytokines, chemokines and growth factors. Some of these mediators will enhance endothelial cell adhesion molecule (CAM) expression and initiate pathogenic angiogenesis, resulting in abnormal blood vessel formation, thereby enhancing leukocyte adhesion to the newly formed blood vessel endothelium and perpetuating intestinal inflammation. In addition, leukocyte and/or platelet adhesion to activated venular endothelium promote the formation of powerful vasoconstrictors (e.g. thromboxane, endothelin), which reduce blood flow, amplify the stimulus for blood vessel proliferation and enhance tissue injury. Accompanying these local inflammatory responses is the activation of the coagulation cascade by locally released inflammatory mediators, which leads to microthrombus formation within the inflamed intestine and also systemic thromboembolism.

than does the "T cell transfer model" of chronic gut inflammation. Adoptive transfer of naïve T cells into immuno-deficient recipient SCID or recombinase-activating gene-1 or -2-deficient mice induces chronic small bowel inflammation and colitis 6–8 weeks following transfer [2,4,5]. Histopathologically, intestinal inflammation (and colitis) resembles CD in that the inflammation is transmural in nature and exhibits erosions, epithelial cell hyperplasia adjacent to areas of epithelial cell injury, goblet cell depletion and massive infiltration of polymorphonuclear leukocytes (PMNs), monocytes and lymphocytes [4,5]. The intestinal inflammation is associated with the differential expansion of Th1 cells (20–30-fold accumulation in the colon) which is driven by normal enteric bacteria [4;5].

It is thought that induction of chronic intestinal inflammation begins with the migration of naïve T cells from the blood to the gut-associated lymphoid tissues (GALT) such as the Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) followed by *enteric antigen*-driven activation, polarization and expansion of the T cells within these lymphoid tissues to produce colitogenic effector cells such as Th1 and/or Th17 cells. These effector cells then exit the lymphoid tissue via the efferent lymphatics, enter the systemic circulation and home to the gut interstitium, where they initiate intestinal inflammation (Figure 11.1). Because much of the current evidence demonstrates that antigen-loaded dendritic cells (DCs) are transported to the MLNs from the intestinal lamina propria *and* PPs, investigators have suggested that MLNs may function as the primary GALT where naïve T cells encounter enteric antigens and are activated to disease-producing effector cells. Thus, it has been proposed that naïve T cells must traffic (migrate) to and become activated within the MLNs to produce colitogenic effector cells such as Th1 and/or Th17 cells [6]. Trafficking of intravascular T cells to lymphoid and target tissue is a complex process that is controlled by a sequence of three molecularly distinct adhesion and signaling steps that include tethering of T cells to the endothelial surface, rolling along the endothelial cell surface and finally activation-induced firm adhesion of the lymphocytes to the endothelium (Figure 11.2).

Although a wealth of information has been generated suggesting that naïve T cells must first be activated (and polarized) to effector cells within secondary lymphoid tissue, it is still not clear whether the GALT (i.e. MLNs

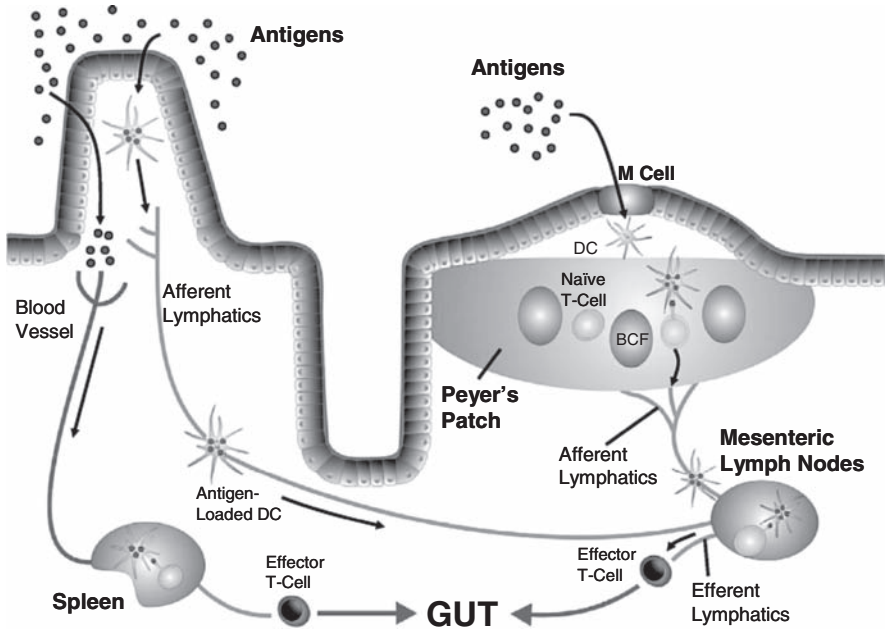

Figure 11.2 Transport of enteric antigens from the lumen to the gut-associated lymphoid tissue (GALT). Enteric antigens may be transported by the M cells which overlie Peyer's patches (PPs), where they are endocytosed by dendritic cells (DCs) that reside within the subepithelial dome of the PPs. These antigen-presenting cells may bind to naïve T cells within the PPs, thereby initiating an immune response, or the antigen loaded DCs may be transported to the mesenteric lymph nodes (MLNs) by way of the afferent lymphatic vessels. Effector cells produced by T cell–DC interactions within the MLNs enter the circulation where they will home to gut. Alternatively, enteric antigens may gain access to the gut interstitium by paracellular diffusion, where they are endocytosed by tissue DCs and transported to the MLNs by the afferent lymphatic vessels. Again, T cell–DC interactions within the MLNs will produce effector T cells that home to the gut. Finally, free antigen within the intestinal tissue may be cleared into the circulation by the microvasculature where they enter the spleen and endocytosed by splenic DCs. T cell–DC interactions within the spleen may also produce gut homing effector T cells. Adapted by permission from Macmillan Publishers Ltd: Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 2003; **3**(4):331–41.

and/or PPs) is required for the generation of colitogenic T cells. Although there is very good evidence demonstrating that MLNs are required for the induction of mucosal immune responses (and tolerance), there is much less known about the role of PPs for mounting immune responses or for inducing tolerance to luminal antigens. One report suggests that MLNs and/or PPs may actually function to limit or suppress the acute colonic inflammation induced by dextran sulfate sodium (DSS) [7]. Another study, using LT-$\alpha^{-/-}$ × RAG$^{-/-}$ double-deficient mice as recipients, suggests that neither MLNs nor PPs are required for the induction of chronic inflammation as transfer of naïve T cells into these GALT-deficient recipients resulted in chronic colitis [8]. It should be pointed out, however, that interpretation of these studies is complicated by the fact that although these mice lack all secondary lymphoid tissue they also lack the ability to produce LT-α. It may be that in the presence of this cytokine, certain lymphoid tissues are in fact required for induction of disease. Further experiments will be required to define definitively the role of the GALT in experimental IBD.

Much of what is known regarding the molecular determinants and adhesive interactions of T cells with the vasculature of secondary lymphoid tissue has come from studies on peripheral lymph nodes (PLNs). Much less is known about the requirements for T cell trafficking to MLNs and PPs [9]. It is known that the migration of naïve T cells from the blood and into secondary lymphoid tissues is thought to occur exclusively by way of high endothelial venules (HEVs), which are composed of specialized post-capillary venular endothelial cells in the these tissues. HEVs associated with PPs contain mucosal addressin cell adhesion molecule-1 (MAdCAM-1) whereas HEVs within MLNs express both MAdCAM-1 and peripheral node addressin (PNAd). It is thought that T cell-associated L-selectin binds to both MAdCAM-1 and PNAd to tether the lymphocytes to the HEVs to initiate T cell rolling. Although the L-selectin–MAdCAM-1 and L-selectin–PNAd interactions are thought to mediate much of the T cell tethering and rolling in the MLNs, $\alpha 4\beta 7$ may be used by naïve T cells to tether themselves to MAdCAM-1, thereby providing additional tethering capacity for these cells in both PPs and MLNs (Figure 11.2) [9]. Indeed, we have recently demonstrated that T cell-associated CD62L (L-selectin) is *not required* for induction of chronic colitis in the T cell transfer model in mice [5]. Interaction of T cell-associated chemokine receptor CCR7 with secondary lymphoid tissue chemokine

(SLC; CCL21) and/or Epstein–Barr virus-induced gene-1 ligand chemokine (ELC; CCL19) presented on the luminal surface of the HEVs activates lymphocyte function-associated antigen-1 (LFA-1) and α4β7 on the naïve T cells to bind to ICAM-1 (or ICAM-2) and MAdCAM-1, respectively. These interactions promote firm adhesion and lymphocyte arrest which ultimately lead to T cell extravasation into the MLNs and PPs [9]. We have recently determined that T cell-associated CD18 and LFA-1 are required for the induction of chronic colitis in the T cell transfer model [5;10].

Once naïve CD4+ T cells enter the GALT, they may encounter their cognate antigens presented on the surface of dendritic cells (DCs) in association with major histocompatibility complex class II (MHC II) (Figure 11.2). In a process that is not well understood, T cells move into close proximity of the DCs, ultimately binding to the MHC–Ag complex via its T cell receptor (TCR), thereby initiating cell activation. During this initial activation process, the T cell will proliferate, shed its L-selectin and enhance surface expression of certain adhesion molecules such as LFA-1 (CD11a/CD18), α4β7, VLA-4 (α4β1), VLA-5, VLA-6 and CD44 [9]. Data obtained from previous studies using different animal models of chronic gut inflammation suggest that a dysregulated immune response to the enteric antigens will result in the polarization of naïve T cells to *colitogenic (Th1) effector cells*. More recent studies suggest that naïve T cells may give rise to an additional population of disease-producing T cells that produce large amounts of the pro-inflammatory cytokine IL-17 [11]. We, and others, have found that IL-17 is expressed in large amounts both in experimental models of IBD and in human disease [12]. One T cell-associated adhesion molecule that has received a substantial amount of attention related to its role in cell action is LFA-1. Using different *in vivo* and *in vitro* models, the interaction of T cell-associated LFA-1 with ICAM-1 (or -2) on APCs has been demonstrated to constitute a major co-stimulatory pathway for T cell activation. For example, LFA-1 has been suggested to facilitate the interaction of APCs to promote T cell activation, polarization and proliferation *in vitro*. T cells require two signals; the first signal is antigen-dependent involving the interaction of antigen–major histocompatibility complex (MHC) on an antigen-presenting cell and the T cell receptor (TCR), whereas the second signal is antigen independent, involving multiple T cell surface molecules and their ligands on APCs. In particular, LFA-1–ICAM interactions are thought to be important for optimal T cell activation by mediating and stabilizing the T cell–APC contact referred to as the immunologic synapse. LFA-1 on T cells is thought to lower the threshold level of activation and promote proliferation of T cells. We, and others, have demonstrated that CD11a-deficient (i.e. LFA knockout) T cells have defects in alloantigen (MLR)- or Con A-stimulated proliferation *in vitro*. In addition, it has been shown that blocking the interaction of LFA-1 with ICAM-1 expressed on APCs inhibits Th1 polarization and cytokine production. In fact, blocking of both ICAM-1 and ICAM-2 in the interaction of naïve T cells with APCs induces a >100-fold increase in the production of Th2 cytokines IL-4 and IL-5, which will downregulate Th1-dependent inflammation [13]. Our data support an important role for T cell-associated CD18 and LFA-1 in the pathogenesis of chronic colitis in mice [5,10].

Following this initial priming/activation step in the GALT, effector T cells enter the systemic circulation via the efferent nodal lymphatics where they home to the gut (Figure 11.2). This new pattern of homing is thought to be mediated by the interaction between lymphocyte-associated P-selectin glycoprotein ligand-1 (PSGL-1), LFA-1, $\alpha_4\beta_7$ and CD44 with venular P/E-selectin, ICAM-1 (and ICAM-2), hyaluronate and MAdCAM-1, respectively [9]. However, we have found that PSGL-1 is not required for the induction of colonic inflammation in the T cell transfer model [10]. The mechanisms responsible for "imprinting" the recruitment of T cells to the gut following antigen stimulation within the GALT are not known with certainty; however, it is thought that effector T cells are exposed to specific signals resulting from subtle differences in antigen–T cell interactions in addition to the local environment of the lymphoid tissue which imprint gut-specific homing receptors [9]. For example, it is well appreciated that T cells activated within PPs (and possibly MLNs) express high levels of α4β7 and also the chemokine receptor CCR9 which bind to their respective ligands MAdCAM-1 and TECK (CCL25), both of which are constitutively expressed on intestinal endothelial cells and are thought to promote homing of T cells to the gut [9].

Once the effector T cells enter the gut interstitium, they re-encounter their specific antigen presented on a wider range of APCs, such as macrophages and B cells, and DCs. This secondary antigen-specific interaction promotes the production of large amounts of IFN-γ, IL-17, TNFα and IL-2 (Figure 11.3). IL-2 promotes the clonal expansion of T cells and enhances the function of helper T cells and B cells, whereas IFN-γ interacts with and activates APCs and macrophages to produce additional IL-12. IFN-γ, TNFα and IL-17 will activate endothelial cells and enhance endothelial cell adhesion molecule (ECAM) expression on the post-capillary venular endothelium. In addition, IFN-γ-activated macrophages produce large amounts of pro-inflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, IL-12, IL-17 and IL-18, reactive oxygen and nitrogen metabolites (e.g. superoxide, hydrogen peroxide, nitric oxide) and a variety of pro-angiogenic growth factors and chemokines (Figure 11.3). The net result of this uncontrolled production of Th1/Th17- and macrophage-derived inflammatory is the recruitment and activation of additional leukocytes (e.g. PMNs, monocytes, macrophages) in the gut tissue, leading to the induction of angiogenesis (Figure 11.3).

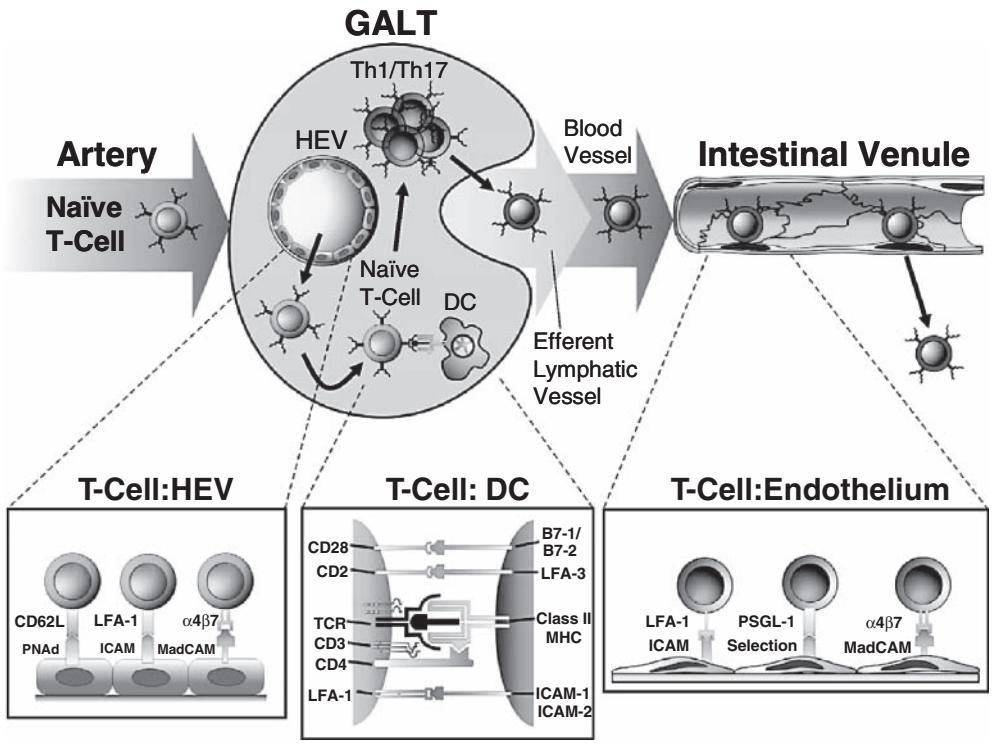

Figure 11.3 Cellular determinants for lymphocyte trafficking and activation. Naïve T cells enter the GALT by way of the high endothelial venules (HEV) via the interactions between T cell associated CD62L (L-selectin), LFA-1 and/or $\alpha_4\beta_7$ and endothelial cell counter receptors peripheral node addressin (PNAd), ICAM-1 or -2 and mucosal addressin cell adhesion molecule-1 (MAdCAM-1), respectively. Within the GALT, naïve T cells interact with DCs containing their cognate antigen, resulting in activation, polarization and expansion of effector T cells such as Th-1 and/or Th-17 cells. This activation process is mediated by a variety different T cell and DC cell adhesion molecules and accessory proteins. Once activated, the effector cells exit the GALT via the efferent lymphatic vessels and enter into the systemic circulation, where they home to the intestine. Extravasation of intravascular effector cells is thought to be mediated by the interactions of T cell LFA-1, platelet glycoprotein-1 (PSGL-1) and $\alpha_4\beta_7$ with post-capillary venular ICAM-1 or -2, P/E-selectins and MAdCAM-1, respectively. Adapted from Abbas AK, Lichtman, AH. *Cellular and Molecular Immunology*, 5th edn, Philadelphia: Elsevier Saunders, 2005, pp. 16–39.

Angiogenesis and IBD

Angiogenesis plays an important role in several chronic inflammatory diseases and directly contributes to pathophysiological processes which exacerbate disease (Figure 11.4). Recent findings, both clinical and experimental, indicate that angiogenesis also plays a crucial role in IBDs, consisting of CD and UC [14,15]. Development of new vasculature during chronic inflammation of the bowel may play a negative "pathological" role by contributing to increased inflammatory responses due to dysfunctional new vessel architecture and increases in the recruitment of inflammatory cell types. Importantly, we have recently discussed in detail numerous studies that implicate angiogenic activity during IBD and experimental colitis and refer the reader to that report for more detailed information [14]. Here we broadly address the process of angiogenesis during IBD which is beginning to define our understanding of microvascular dysfunction and pathophysiology during colitis.

Vascular changes in IBD were noted as early as 1954 and throughout the late 1950s and 1960s; however, descriptions of these vascular changes and the interpretations of what they meant varied widely. In 1970, Brahme and Lindstrom reported increases in vascularity in active Crohn's disease which they showed by radiography of vascular castings [16]. Recent forays into human and experimental colitis have further indicated an important role for the microvasculature in IBD. Increased microvascular density in CD and UC has been shown clinically with increased vascular density being similar to that observed in experimental colitis [17]. Importantly, microvascular dysfunction during IBD shows a temporal relationship with tissue pathology based on observed microscopic alterations in tissue morphology. These data provide evidence that vascular changes, in the form of angiogenesis, are critical to the disease process.

The development of new blood vessels is a necessary part of life from its earliest stages. Vasculogenesis is the initial process during embryonic development by which

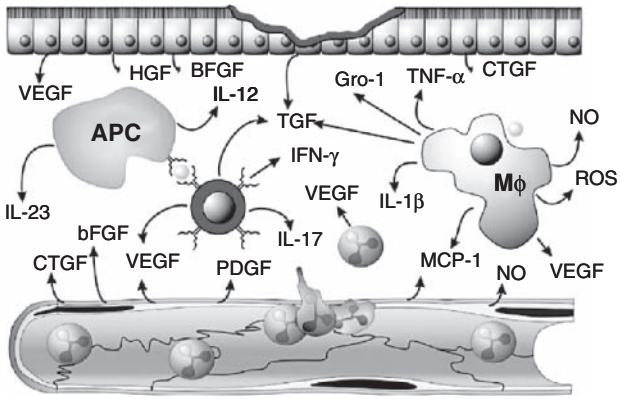

Figure 11.4 Production of pro-inflammatory and angiogenic mediators in the inflamed gut. Invading and resident inflammatory cell leukocytes and also epithelial and endothelial cells release a variety of pro-inflammatory cytokines and mediators of angiogenesis during active inflammation. VEGF, BFGF, CTGF, PDGF and HGF represent vascular endothelial growth factor, basic fibroblast growth factor, connective tissue growth factor, platelet-derived growth factor and hepatoctye growth factor, respectively. NO and ROS are nitric oxide and reactive oxygen species, respectively.

the blood supply for the forming organism is created. After vasculogenesis, the formation of new vessels is known as angiogenesis, which is critical for numerous physiological processes. New vessels are produced primarily by one of two routes, vascular sprouting or intussuception. Vascular sprouting is the process whereby new vessels occur due to sprouting off of existing vessels and the second, known as intussuseptive growth, is the process whereby existing vessels divide into two distinct parallel vessels through a multi-step process. Normal wound healing angiogenesis, known as physiological angiogenesis, is closely controlled by multiple growth and tissue factors, resulting in minimal changes in microvascular permeability, proteolysis and inflammation. However, abnormal or pathologic angiogenesis, such as that observed in IBD, is characterized by its abnormal vasculature, which exhibits torturous architecture, increased permeability and increased inflammatory and thrombogenic potential. It is important to note that there still is some debate concerning the importance of angiogenesis for ulcerative healing during IBD versus its role in facilitating chronic inflammation. However, the majority of tissue alterations which occur during IBD are not fully corrected in remission. Thus, the role of the microvasculature in inflammatory angiogenesis is important for sustaining chronic inflammation, differential regulation of which between angiogenic phenotypes remains poorly understood.

An appreciation of vascular changes during angiogenesis in inflamed tissues is necessary to clarify the role of the microvasculature during chronic inflammation. It is thought that the different microvascular segments, arterioles, capillaries and venules all have specific roles in this process through their various interactions with angiogenic mediators. Importantly, venules act as the site of most activity in the development of inflammation and through recruitment of cell types which produce angiogenic mediators. The multi-step process of angiogenesis begins with the production of various angiogenic cytokines which are released from inflamed tissue and bind endothelial cell surface receptors, initiating intracellular signaling which in turn causes dilation of vessels, increased vascular permeability and degradation of the underlying basement membrane (Figure 11.3). Angiogenic chemokines and cytokines then stimulate endothelial cell proliferation and directional migration. Various integrins, matrix metalloproteinases (MMPs) and additional mediators are then involved in remodeling the extracellular matrix (ECM) and incorporating migrating endothelial cells within the reorganizing ECM. Endothelial cells which have migrated into the ECM then undergo the processes of tube or lumen formation and junctional complex maturation as new vessels begin to form. The vascular tubes produced at this stage will then anastomose with other sprouting vessels and undergo arteriolar–venular differentiation. Once this has occurred, endothelial cell proliferation and cell migration cease and smooth muscle cells and pericytes attach to stabilize the new vasculature, thus completing the process. We have recently determined that differential regulation of pro- versus anti-angiogenic gene expression plays a key role in governing pathologic angiogenesis through expression differences between regulatory factors (i.e. upregulation of pro- over anti-angiogenic factors or relative downregulation of anti-angiogenic factors) [17]. Thus, the angiogenic gene expression profile is different between various experimental colitis models, which is reflected in the angiogenic responses described above.

Angiogenic mediators in IBD and experimental colitis

Growth factors

VEGF-A has been shown to be elevated in the tissue and serum of patients with CD and UC and in distal colon tissue from the $CD4^{+}CD45RB^{high}$ model of colitis [14,15]. Specifically, VEGF-A serum levels have been examined in CD and UC patients and found to be significantly elevated with active disease, but not during remission [15]. However, platelets are major producers of VEGF-A and *ex vivo* release of VEGF by platelets (due to handling artifacts) may give a false reading of VEGF levels [15]. Similarly, VEGF-A serum levels in children and young adults are elevated during active CD. Finally, elevated tissue levels of VEGF-A have been reported in mucosal tissue extracts from CD and UC patients. We have reported

that elevated concentrations of VEGF-A, as seen in IBD, stimulate inflammatory activation of colon microvascular endothelial cells leading to neutrophil and T cell adhesion [18]. Lastly, placenta-like growth factor (PlGF), an evolutionary predecessor of VEGF, is also upregulated during experimental colitis [17]. These data demonstrate that VEGF-A expression is involved in IBD-associated angiogenesis, which may be important for disease pathology.

Additional angiogenic growth factors such as PDGF, b-FGF, HGF and TGF-β have also been observed in IBD. IBD patient serum and mucosal tissue levels of b-FGF were reported to be elevated in both active CD and UC, while TGFβ serum levels were elevated in those with active CD [14]. Additional studies found elevated plasma b-FGF in UC patients and elevated serum b-FGF in children with CD. PDGF expression is also significantly increased in inflamed colonic mucosa but not in non-inflamed mucosa, suggesting that PDGF may be important for the maintenance of damaged vasculature during IBD and possibly an autocrine mediator of inflammatory angiogenesis. Production of TGFβ has been examined in cells cultured from active CD and UC patients. Interestingly, in intestinal cells cultured from CD patients, less TGFβ was produced than in control cells, which is opposite to that in cells from UC patients. This indicates differential regulation of angiogenesis in CD and UC similarly to what we have observed in experimental colitis models [17]. HGF expression is also upregulated during IBD. However, the administration of human HGF and HGF gene therapy decreases disease severity in experimental colitis, an effect that is the opposite of that of most growth factors during IBD. These findings demonstrate that several growth factors facilitate inflammation induced angiogenesis in experimental and human IBD.

Adhesion and matrix molecules

PECAM-1 (CD31), an adhesion molecule which is highly involved in angiogenesis, is abundantly expressed on endothelium (concentrated at intercellular junctions) and platelets and to a lesser extent on leukocytes. PECAM-1 interacts with itself in a homotypic manner and with $\alpha_v\beta_3$-integrin, whose expression is upregulated on activated endothelium during experimental colitis and in CD and UC [1,4]. PECAM-1 facilitates angiogenesis through its involvement in tube formation and inhibition of PECAM-1 activity attenuates angiogenesis [19]. During vascular remodeling $\alpha_v\beta_3$-integrin is associated with angiogenesis by stimulating endothelial cell proliferation and stabilizing endothelial–matrix interactions partially through its interaction with PECAM-1. Importantly, PECAM-1 expression is not upregulated during inflammation; however, new vasculature constitutively expresses PECAM-1, making it an ideal marker for measuring angiogenic activity and vascular density during IBD and experimental colitis [14,17,20,21]. The adhesion molecule VE-cadherin is also associated with angiogenesis and is upregulated during experimental colitis [17]. VE-cadherin is expressed on endothelial cells and is involved in angiogenesis primarily as a regulator of VEGF receptor function and vessel maturation. An important angiogenic role for VE-cadherin has been identified through its regulation of endothelial cell–cell adhesion during vessel development and its involvement in lumen formation through directing vacuole fusion [22,23].

MMPs, specifically MMP-2 and -9, are elevated during experimental colitis and are involved in different aspects of angiogenesis [17]. MMP-1 (interstitial collagenase), MMP-2 (gelatinase A) and MMP-9 (gelatinase B), produced by endothelial cells, are upregulated and have been shown to be crucial for tissue remodeling associated with angiogenesis. These MMPs are zinc-dependent enzymes, secreted as zymogens which are activated in the ECM that can be inhibited by tissue inhibitors of metalloproteinases (TIMPs). These molecules dissolve fibrin in the basement membrane and have collagenolytic activity, important in the process of cell migration. Increased expression of MMPs and TIMPS during ulcerative colitis has been reported, suggesting that MMPs may facilitate angiogenesis in inflammatory bowel disease [24].

Anti-angiogenic gene expression and therapeutics in IBD and experimental colitis

The first concepts of anti-angiogenic drugs were introduced for cancer treatment. However, with our increased understanding of the involvement of angiogenesis during inflammatory diseases, these drugs can be expanded for use in other pathological conditions that involve angiogenesis. Recent studies have investigated anti-angiogenic treatment of experimental colitis models and IBD patients in an effort to attenuate disease. Several anti-angiogenic compounds have been successful in inhibiting large increases in vascular density while reducing the overall pathology of disease. The anti-angiogenic and immunomodulatory drug thalidomide has been reported to induce complete remission of inflammatory bowel disease over a prolonged course of therapy [20,24]. We have recently shown that thalidomide significantly attenuates disease histopathology and increases in vascular density during experimental colitis [16].

Other anti-angiogenic therapies are currently being tested in experimental colitis and IBD. Use of a novel anti-angiogenic agent, ATN-161, in the $CD4^+CD45RB^{high}$ T cell transfer colitis model decreases tissue histopathology and blood vessel density [16]. ATN-161 specifically antagonizes $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin function, which blocks tumor angiogenesis and endothelial cell growth in matrigel.

ATN-161 has also been reported to decrease disease activity in the IL-10 experimental colitis model concomitant with significantly lower histopathology scores and blood vessel density [18]. Additionally, anti-TNFα along with other biological therapies (e.g. IL-10, IL-12 and IFN-γ) may also serve to alter angiogenic responses during IBD and are under consideration as additional therapeutic targets in IBD. Hence targeting pathophysiological angiogenesis with anti-angiogenic therapies has the potential to revolutionize the way we treat IBDs.

Thrombosis and inflammatory bowel disease

In addition to promoting inflammation and angiogenesis, certain inflammatory mediators (e.g. cytokines) and signaling pathways (e.g. TLR-4 and CD40/CD40L) can lead to a reduction in the activity of natural anticoagulant pathways, impair the fibrinolytic system and initiate blood clotting. Inflammation appears to shift the hemostatic mechanisms in favor of thrombosis by exerting an influence on the three dominant anticoagulant pathways, i.e. the heparin–antithrombin system, the tissue factor pathway inhibitor (TFPI) system and the protein C anticoagulant pathway. Indeed, an association between thrombosis and IBD has long been appreciated and thromboembolic disease is gaining recognition as a significant cause of morbidity and mortality in patients with CD and UC [25–28]. The hypercoagulable state that accompanies IBD is manifested both within the inflamed bowel and at extra-intestinal sites. Within the chronically inflamed gut mucosa, microinfarctions are often detected by histology and resin casts of the intestinal vasculature in IBD also reveal evidence for intravascular fibrin deposition and complete thrombotic occlusion [25–28]. Intravascular fibrin deposits, which stain positively for platelet glycoprotein IIb/IIIa, appear to occur at sites of granulomatous destruction of mesenteric blood vessels. It has been reported that 60% of patients with UC exhibit fibrin clots in capillaries and venules of biopsy samples of the rectal mucosa [29]. Similar descriptions of fibrin clot formation in mucosal capillaries have been reported for animal models of colitis [30]. In addition, intestinal microvascular endothelial cells (HIMECs) derived from patients with UC or CD express lower levels of endothelial protein C receptor and thrombomodulin than HIMECs harvested from control subjects [31]. Intravascular platelet aggregates have been detected in mucosal biopsies of patients with UC and there is an increased number of circulating platelet aggregates in the mesenteric venous circulation draining the inflamed bowel in UC [25–27,32]. We have also obtained evidence for the accumulation of platelet–leukocyte aggregates (PLAs) in the colonic microvasculature of mice with colitis induced either by DSS or T cell transfer into immunodeficient mice. Although the pathophysiological consequences of PLA in IBD remain unclear, there is evidence that the formation of these aggregates, particularly platelet–monocyte aggregates, enhances the production of intravascular tissue factor [25], which increases the likelihood of thrombus formation.

The IBD-associated hypercoagulable state is also manifested in systemic blood and in extra-intestinal vascular beds. Although no single consistent coagulation abnormality has been identified, blood samples from patients with active IBD have revealed thrombocytosis, accelerated thrombin generation and increased circulating levels of fibrinogen, von Willebrand factor, thrombin–antithrombin (TAT) complexes and clotting factors V, VII and VIII. Antithrombin III, protein C, protein S, plasminogen-activating inhibitor (PAI) and tissue plasminogen activator (tPA) levels are often reduced in patients with IBD. These hemostatic abnormalities are accompanied by functional changes in circulating platelets that exhibit hyperactivity, hyperaggregability and a propensity to form platelet–leukocyte aggregates [25–27,33–35].

A consequence of the hypercoagulability of blood in IBD patients is extra-intestinal thromboembolism (TE). The results of recent clinical studies indicate that the incidence of TE events in IBD patients is ~6.5%, with a threefold increase in risk for TE complications compared with the general population [27]. Post mortem studies reveal a sixfold higher incidence of systemic TE compared with clinical studies, suggesting that many cases are not detected [27]. Both the arterial and venous circulations appear to be involved in the TE that occurs in IBD patients; however, venous complications occur more frequently. TE is usually manifested as deep vein thrombosis (DVT) or pulmonary embolism (PE), although thromboses have been detected in other regional circulations, including brain, retina and liver. Studies on UC and CD patients who experienced TE events have revealed that the risk of TE may correlate with disease severity and the extent of colonic involvement [27]. However, large clincal studies have noted that up to one-third of TE complications occurred during disease quiescence, suggesting that the procoagulant tendency is independent of disease activity [25–27]. We have recently obtained evidence in a murine model of DSS colitis that demonstrates an increased propensity for thrombus formation in skeletal muscle arterioles and (to a lesser extent) venules subjected to light/dye-induced injury, when compared with the same vessels in normal (non-colitic) mice [36].

Interdependence of inflammatory and hemostatic mechanisms in IBD

There is emerging evidence that supports an intimate connection between inflammatory and hemostatic mechanisms in different inflammatory diseases, including IBD [25,28]. Indeed, the evidence supporting a link between

inflammatory and thrombotic processes suggests that thrombosis is involved, if not in the initiation of IBD, at least in the progression and/or maintenance of the inflammatory process. A role for coagulation mechanisms in the pathogenesis of IBD is supported by a study of 9000 patients with bleeding disorders, wherein a protective effect of inherited defects of coagulation, i.e. hemophilia and von Willebrand's disease, was shown against the occurrence of both CD and UC [25–27]. This possibility is also supported by reports describing how different components of the coagulation–anticoagulation pathways can regulate inflammation. Thrombin, for example, has been shown to increase the expression (via transcription-independent and -dependent mechanisms) of adhesion molecules on endothelial cells and to promote leukocyte–endothelial cell adhesion [28]. Furthermore, the thrombin inhibitor argatroban has been shown to reduce the macroscopic and microscopic (histologic) damage, elevated tissue myeloperoxidase activity (neutrophil accumulation) and mucosal LTB_4 levels in rats with TNBS-induced colitis [30]. We have shown that DSS colitis is associated with increased circulating thrombin–antithrombin (TAT) complexes and that immunoneutralization of tissue factor largely prevents the DSS-induced TAT elevation [36].

Tissue factor (TF) has also been implicated in inflammation [28]. The engagement of TF with its ligand (factor VIIa) activates the protease-activated receptors PAR 1–4, which elicits the production of pro-inflammatory cytokines (TNFα, IL-6) and promotes leukocyte rolling in venules. Mice that lack the cytoplasmic domain of TF exhibit an attenuated recruitment of rolling, adherent and transmigrating leukocytes in post-capillary venules after LPS challenge. Similarly, a small molecule inhibitor of the TF–VIIa complex (BCX-3607) has been shown to attenuate the LPS-induced production of IL-6 and IL-8 *in vitro* (by HUVEC) and IL-6 *in vivo*. [28] We have obtained evidence that strongly implicates tissue factor in the recruitment of leukocytes and platelets into colonic venules and in the development of tissue injury that is associated with DSS-induced colitis [36].

There is also evidence that the natural anticoagulant activated protein C (APC) exerts anti-inflammatory actions. APC has been shown to inhibit the production of adhesion molecules (VCAM-1, ICAM-1) and cytokines in endothelial cells, and also agonist-induced leukocyte activation and LPS-induced production of TNFα and other cytokines by cultured monocytes/macrophages [28,30]. Mice with single-allele targeted disruption of the protein C gene [heterozygous protein C deficient ($PC^{+/-}$) mice] have higher levels of circulating cytokines, including a fourfold increase in TNFα, after endotoxin challenge. The findings of a recent preliminary report indicate that administration of recombinant APC in mice with DSS-induced colonic inflammation effectively blunts disease activity index, weight loss and tissue damage [31]. Although there are several lines of circumstantial evidence suggesting that impairment of the major anticoagulant pathways (heparin–antithrombin, tissue factor and protein C) could contribute to the perpetuation and/or maintenance of the inflammatory response in IBD, there have been few systematic attempts to test for the involvement of these anticoagulant pathways in the genesis of experimental IBD, nor has much effort been made to determine how these alterations in the anticoagulant pathways promote inflammation *in vivo*.

The interdependence of inflammation and hemostasis is also evidenced by findings suggesting that inflammation shifts the hemostatic mechanisms in favor of thrombosis [28]. Evidence derived from patients with acute (e.g. sepsis) or chronic (e.g. IBD) inflammatory diseases and from animal models suggest that inflammation favors coagulation. This influence of inflammation on coagulation likely reflects the ability of a variety of inflammatory mediators to decrease the activity of natural anticoagulant mechanisms, impair the fibrinolytic system and initiate clotting. Inflammation is often associated with elevated fibrinogen levels, increased tissue factor expression, reduced antithrombin and protein C activities, decreased thrombomodulin and endothelial cell protein C receptors and increased platelet count and reactivity [25–28]. All of these responses of the coagulation–anticoagulation pathways to inflammation favor a hypercoagulable state and likely contribute to the coagulopathy that is associated with IBD. Our own data from mice with DSS colitis reveal an altered hemostatic mechanism that favors thrombosis in distant tissues (skeletal muscle) [30].

Although the identity of the inflammatory mediators that elicit the hypercoagulable state in IBD remains unknown, several candidate molecules can be proposed based on the literature, including tumor necrosis factor α (TNFα), bacterial lipopolysaccharide (LPS) and CD40 ligand (CD40L). All of these molecules have been implicated in the pathogenesis of IBD and each mediator has been shown to promote thrombus formation in both large and small blood vessels [25–28]. These mediators also appear to affect different components of the coagulant–anticoagulant pathways in a manner that favors thrombosis. For example, TNFα has been shown to (1) increase plasma levels of von Willebrand's factor, (2) induce tissue factor expression on endothelial cells, (3) decrease protein C mRNA and protein C activation in endothelial cells and (4) deplete tissue factor pathway inhibitor and downregulate thrombomodulin [25]. The CD40–CD40L pathway has been implicated in the pathogenesis of IBD and in vascular thrombosis. Ligation of CD40 on endothelial cells with CD40L elicits tissue factor-dependent procoagulant activity. sCD40L is also a ligand of GP IIb–IIIa and is involved in thrombus stabilization and platelet activation. We have obtained evidence that mice deficient in either CD40 or CD40L exhibit an

attenuated accumulation of platelets that bind directly to vascular endothelium and also platelets that form aggregates with leukocytes in inflamed colonic venules [37]. Similarly, we have shown that the trapidil (triazolopyrimidine), which is used clinically to prevent restenosis after vascular injury and considered to act as an inhibitor of the CD40–CD40L pathway, also attenuates the colonic inflammatory and tissue injury responses to DSS [37].

Microvascular blood flow in intestinal inflammation

A major consequence of uncontrolled production of effector cell- and phagocyte-derived inflammatory mediators is the adhesion of platelets and leukocytes to the post-capillary venules of the intestinal microcirculation. Leukocyte and/or platelet adhesion to activated venular endothelium is known to promote the formation of powerful vasoconstrictors (e.g. thromboxane, endothelin), which may have profound effects on the microvasculature, including reducing blood flow and amplifying the stimulus for blood vessel proliferation in addition to enhancing tissue injury. Because the microcirculation is intimately linked to the events associated with inflammation, knowledge of the microvascular changes that occur during the onset of intestinal inflammation is helpful to understand key issues of fluid filtration and blood cell delivery to the diseased tissue. Each of the three segments of the microcirculation (venules, capillaries and arterioles) is likely to be involved in the progression of IBD. As with many diseases of inflammatory origin, leukocyte and/or platelet adhesion to the venular endothelium not only plays a critical role in potentially detrimental consequences of inflammation, but also contributes to the beneficial regulatory component of the immune response. Upstream from the venules, fluid filters from the capillary barrier, which may have become hyperpermeable. An alternative explanation for the excessive capillary filtration in acute IBD is an arteriolar hyperemia that allows elevated hydrostatic pressure into the capillary bed. However, the time course over which hyperemia exists in IBD has not been investigated fully and, in fact, there is a growing body of evidence in support of the reverse, that arteriolar constriction (or impaired vasodilation) functions as an integral part of the disease process. The following discussion summarizes the evidence supporting the possibility of phases of arteriolar constriction and/or reduced perfusion in human IBD or in corresponding animal models of IBD.

Although certain periods of inflammation that occur with IBD may include hypervascularity and angiogenic appearance of tortuous capillaries, evidence reviewed by Hatoum *et al.* [26] demonstrates a lack of perfusion in advanced cases of human IBD. Specifically for CD, arterioles have been noted to be constricted and, as reviewed by Thornton and Solomon [38], evidence suggests that ischemia precedes and mediates the acute inflammatory events that occur in CD.

Thromboxane, endothelin-1 and angiotensin II in intestinal inflammation

Thromboxane is a vasoconstrictor produced by the cyclooxygenase pathway of arachidonic acid metabolism. In this pathway, prostaglandin H_2 (PGH_2) is converted to one of several downstream products, including TxA_2 (thromboxane), PGD_2, PGE_2, PGF_2 or PGI_2 (prostacyclin). The balance of TxA_2 (a vasoconstrictor) and PGI_2 (a vasodilator) is known to influence arteriolar tone and an imbalance towards TxA_2 is found in many inflammatory states.

A study by Carty *et al.* [39] demonstrated a dramatic upregulation of thromboxane synthase in mucosal biopsies of patients with active IBD, with endoscopic and histologic scores positively correlated with the number of cells staining positive for thromboxane synthase. Animal models of IBD similarly implicate thromboxane; for example, in models of immune complex- and dinitrochlorobenzene-induced colitis, thromboxane appears to contribute to inflammation and increased vascular resistance. In the commonly used model of trinitrobenzenesulfonic acid (TNBS)-induced colitis, Appleyard *et al.* [40] found an eightfold increase in TxA_2 in rabbits: despite a simultaneous increase in vasodilating prostaglandins, the investigators noted a 60% reduction in the number of flowing vessels 48 h following TNBS administration.

In experiments in our own laboratory, we have performed an intravital microscopy study of submucosal arterioles in a DSS model in mice [41]. We found constriction of arterioles that were in close proximity to venules in which platelets were adhering to the vessel wall. The platelets may have been releasing significant amounts of thromboxane, considering that the thromboxane synthase inhibitor ozagrel acutely reversed the constriction seen in closely venule-paired arterioles.

Only a few clinical studies have investigated the potential pharmacological intervention of blocking thromboxane, and unfortunately these have been limited to thromboxane synthase inhibition. The limitation of this strategy is that blocking thromboxane synthase potentially can result in an increased concentration of the upstream prostaglandin PGH_2, which not only is a vasoconstrictor, but also binds the same receptor (thromboxane–prostanoid TP receptor) as does TxA_2. Therefore, future studies would be predicted to use either TP receptor antagonists alone or in conjunction with synthase inhibition.

Increases in the potent vasoconstrictor endothelin-1 have been found in intestinal tissue samples and in plasma of patients with UC and CD. Blockade of endothelin-1 has been an effective treatment strategy in DSS and TNBS models of colitis. Kanazawa *et al.* [42] and Murch *et al.* [43] suggested that endothelin-1-induced vasoconstriction

leads to hypoxia, which induces VEGF formation. However, it should be considered that in addition to inducing angiogenesis, VEGF can be a vasodilatory mediator, which would work in the opposite direction to endothelin-1 with respect to vascular tone. In the Kanazawa study, endothelin-1 and VEGF production from patients with active ulcerative colitis and CD were greatly elevated and may have produced an ongoing reciprocal action among vasoconstriction, hyperemia and angiogenesis.

In a recent study, Inokuchi *et al.* [44] found that TNBS-induced colitis was attenuated in angiotensinogen knockout mice and that angiotensin II blockade with losartan decreased the production of inflammatory cytokines. A role for this vasoconstrictor may extend to human IBD inasmuch as increased colonic mucosal levels of angiotensin II have been found in CD patients. Whether the role of angiotensin II is dependent on or independent of thromboxane and endothelin-1 is uncertain based on studies showing that the effects of angiotensin II are mediated partially by endothelin-1 and partially by binding of the thromboxane/prostanoid receptor. Moreover, both angiotensin II and thromboxane have been implicated in elevations of oxidative stress, the consequence of which can be inhibited endothelium-dependent vasodilation irrespective of the direct vasoactive actions of the two constrictors.

Defective endothelium-dependent dilation and perfusion in intestinal inflammation

Arterioles have been dissected from human intestinal submucosa of patients undergoing clinical bowel operations. These arterioles were studied in an experimental protocol in which the response to acetylcholine (an endothelium-dependent vasodilator) was measured: in the patients suffering from chronic IBD, the arterioles demonstrated significantly attenuated dilation. The same type of information has been gathered from an animal model of IBD: Mori *et al.* [45] induced intestinal inflammation in mice with DSS and measured submucosal arteriolar diameters (via intravital microscopy) before and after dose–response exposure to the endothelium-dependent dilator bradykinin. Dilation was attenuated by more than 50% in the DSS mice compared with controls; however, the deficient dilation was virtually normal when DSS was given to transgenic mice overexpressing superoxide dismutase. Therefore, superoxide may play a major role in the deficient arteriolar vasodilation in IBD.

Experimental colitis can be induced by colonic instillation of acetic acid, with the inflammation at least partially dependent on the ensuing period of hypoperfusion: the inflammation can be mimicked by a model of ischemia–reperfusion that uses a period of ischemia similar to that induced by acetic acid. In another chemical-induced model of IBD, TNBS produces arteriolar narrowing and regions of poor intestinal perfusion. Heparin treatment improves the outcome in the TNBS model. The mechanism by which heparin intervened was not clear, but the anticoagulant is known to have an inhibitory effect on the biosynthesis and release of endothelin. Indomethacin also has been used to mimic aspects of IBD and results in reduced perfusion. In a model of mitomycin C-induced colitis, Kruschewski *et al.* [46] found only mild inflammation until 7 days, at which time microvascular flow decreased significantly: flow remained low throughout the entire 6 week protocol and, at the conclusion, flow was ~50% of normal and histological scores in the proximal colon were significantly elevated.

An alternative hypothesis to the preceding studies is that IBD is a disease of hypervascularity, involving hyperemia and angiogenesis. However, these two contrasting views are not mutually exclusive. One of several possible scenarios that could accommodate both viewpoints is that time-dependent and/or local ischemia could lead to time-dependent and/or local hyperemia and angiogenesis. As described by Hatoum *et al.* [26], the chronic phase of IBD seems to be characterized by hypoperfusion and attenuated endothelium-dependent vasodilation, whereas acute phases of the disease involve hyperemia. The dual aspects of this hypothesis are similar to findings reported by Hulten *et al.* [47] over 30 years ago, where increased vascularity was found in acute human UC and CD, but normal or reduced vascularity was seen in chronic phases. One possibility is that reduced perfusion could lead to hypoxia and hypoxia-inducible factors, which in turn would lead to accumulating levels of VEGF or other inflammatory agents, the consequences of which could at least partially explain the hyperemia, increased vascular density and edema associated with acute flare-ups of the disease.

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Chapter 12
Biological Basis of Healing and Repair in Remission and Relapse

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Summary
- Inflammation and repair are interrelated processes mediated by common cytokines and growth factors.
- Mesenchymal–epithelial interactions and cross-talk are probably important in maintaining intestinal integrity and repair.
- Repair processes may involve exposure of previously inaccessible growth factor receptors or redistribution of receptors within surviving cells.
- A large number of growth factors have shown promise using *in vitro* and *in vivo* animal studies. However, the majority have failed to translate into benefit when tested in clinical trials.
- Despite the failure of many of the early clinical trials, our greater understanding of the fundamental processes underlying the inflammatory and repair pathways is likely to be translated into novel therapies within the next few years.

Introduction

Maintenance of structural and functional integrity of the intestinal mucosa depends on its ability to defend itself against noxious luminal agents and to effect repair when injury occurs. Defensive mechanisms include a high rate of cellular turnover, an efficient mucosal blood flow, a continuous adherent mucus layer (in the stomach and colon) and the presence of regulatory peptides that can directly stimulate repair and also influence all of the other protective factors.

Ulceration/injury occurs when the dynamic balance is disrupted due to additional aggressive factors or decreased mucosal defense or when there is a combination of the two. It is important to note that aggressive factors and defense mechanisms are not independent and the net effects of some processes depend upon the biologic context. For example, an immune response is normally beneficial to eradicate infectious organisms and to assist in cellular clearing, but an excessive -immune response, such as occurs in autoimmune diseases and inflammatory bowel disease (IBD), ultimately contributes to tissue injury.

Repair after intestinal injury usually involves a cascade of events: stimulation of cell migration, proliferation and differentiation in association with upregulation of the immune system, leukocyte accumulation and activation. Angiogenesis and extracellular matrix formation also contribute to mucosal tissue remodeling. These usually occur in a stereotypic sequence.

Re-establishing epithelial surface continuity is the first requirement of mucosal wound healing. This is initially accomplished by rapid migration of intestinal epithelial cells from the wound margin, a process termed restitution. This process is not dependent on proliferation. Rho subfamily GTPase proteins appear to play a key role in cytoskeletal reorganization processes involved in the restitution process [1]. Restitution prevents deeper mucosal damage and effects closure of shallow defects of the mucosal epithelium within minutes to hours, a much shorter time frame than that required of cell proliferation. Cell migration depends on coordinated extension of lamellipodia and filopodia formation and breaking of focal contacts at the leading edge of the cell in addition to cytoskeletal-mediated retraction at the trailing edge. Following restitution, an increase in cell proliferation accomplishes replacement of lost epithelial cell populations.

While resealing surface epithelial continuity is a priority, injury associated with IBDs and other intestinal disorders is typically accompanied by deeper damage. The processes that reconstitute normal intestinal architecture in the context of transmural inflammatory injury remain incompletely understood. However, it is clear that heterogeneous populations of connective tissue fibroblasts, myofibroblasts and smooth muscle cells, and also other cell types present in the intestine, also make

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a substantial contribution to mucosal wound healing. Of note, eventual downregulation of the inflammatory responses, including factors such as metalloproteinases, that are associated with mucosal injury is necessary to prevent ongoing injury and subsequent pathological fibrosis which may lead to the clinical manifestations of intestinal stenosis or stricture formation.

Numerous murine models established through genetic manipulation have complemented previous cell culture approaches and models of *in vivo* injury induced by administration of exogenous agents to yield insights into the importance of various mucosal homeostatic peptides. This chapter summarizes recent advances in the understanding of the regulation of mucosal repair processes and also intrinsic protective key mechanisms essential for maintaining mucosal surface integrity and mucosal function in the intestine.

Factors regulating mucosal repair and protecting from injury in the intestine

Over the past several years, it has become clear that the factors that play a critical role in epithelial healing after injury are essentially the same as those protecting the intestinal mucosa from damage. Growth factors are generally considered to be peptides, which act via specific receptors to trigger intracellular secondary messengers and result in cell proliferation. However, in the interest of the complex interactions that control the growth and differentiation of mucosal cell populations, the classification of a molecule based on an individual function can be arbitrary and somewhat misleading as it is now clear that such factors have multiple effects, e.g. "epidermal growth factor" promotes proliferation but also has properties comparable to those of regulatory proteins classified as cytokines. Although they are often considered separately, the distinction between cytokines and growth factors is often semantic, e.g. "cytokine" interleukin-8 (IL-8] has been shown to stimulate migration of human colonic epithelial cells, a process normally associated with "growth factors", and also serves as a potent chemotactic factor placing it also among chemokines [2]. Similarly, molecules such as glutamine or butyrate, which have been generally considered to be simple energy sources, and vitamins, such as A and D, which were at one time thought to have limited biological functions, are now known to influence multiple "new" cellular activities, such as development, differentiation and proliferation (e.g. [3]). To complicate matters even further, assumptions that peptides that stimulate repair must be "growth factors" appear oversimplified. Although this is true in the majority of cases, certain peptides appear to stimulate the repair process without influencing proliferation, the trefoil peptides being a notable example. These peptides are therefore not *growth factors* as such but, nevertheless, play an important physiologic role and offer potential targets for the development of new therapeutic strategies.

In a simplified view, repair promoting regulatory factors can be classified as non-peptidyl, classic cytokines and peptide growth factors and extracellular matrix and integrin molecules.

Non-peptidyl factors

Nucleotides have been shown to influence proliferation and restitution, with synergistic responses being seen when mixtures of nucleotides are used [4]. Similarly, polyamines stimulate cell growth and migration of intestinal epithelial cells [5]. The polyamine putrescine is not itself essential for intestinal epithelial migration and growth, but is effective after conversion into spermidine and/or spermine [6].

Prostaglandins and short-chain fatty acids also appear to promote intestinal epithelial restitution since selective COX-2 inhibitor significantly delayed growth factor-mediated restitution *in vitro* [7]. Of interest, both COX-1 and COX-2 appear to be important for protection of the intestinal mucosa since mice deficient in either of the two isoforms were more susceptible to dextran sodium sulfate (DSS)-induced colonic epithelial injury than wild-type mice [8]. Although several studies suggest that non-steroidal anti-inflammatory drug (NSAID) ingestion is associated with early clinical relapse of quiescent IBD [particularly ulcerative colitis (UC)], the benefits of using specific COX-2 inhibitors rather than non-specific COX-1 and COX-2 dual inhibitors in patients with UC remains unclear (for a review, see [9]).

Peptide growth factors and cytokines

There are at least 30 different peptide "growth factors" that appear to contribute to intestinal mucosal homeostasis. Space precludes a detailed description of each of these but the discussion below describes key peptides that highlight relevant principles.

EGF receptor ligand family

This group of polypeptides, with the common property of binding to the EGF receptor (also known as the c-erb1 receptor) and related receptor family members, includes EGF itself, transforming growth factor alpha (TGFα), amphiregulin, betacellulin and heparin-binding EGF (for a general review of these peptides, see [10]).

Epidermal growth factor (EGF)

EGF is a 53 amino acid peptide produced by the adult salivary glands and also the Brunners glands of the

duodenum, where it is secreted into the gut lumen. In the mature, intact gastrointestinal tract, the deployment of EGFR appears to be limited to basolateral membranes of enterocytes and colonocytes [11]. It is likely, therefore, that luminal EGF influences growth and repair only at sites of injury when it can reach it receptor. This has led to the suggestion that EGF acts as a "*luminal surveillance peptide*" readily available to stimulate the repair process at sites of injury [12]. It is important to note that luminal EGF might gain access to basolateral receptors in the immature neonatal gut.

Transforming growth factor alpha (TGFα)

TGFα is a 50 amino acid molecule produced within the mucosa throughout the gastrointestinal tract, particularly in the epithelial cells [13]. Like EGF, systemic administration of TGFα stimulates gastrointestinal growth and repair, inhibits acid secretion, stimulates mucosal restitution after injury and increases gastric mucin levels [14].

Within the small intestine and colon, TGFα expression occurs mainly in the upper (non-proliferative) zones, which suggests that its physiological role may be to influence differentiation and cell migration rather than cell proliferation. TGFα may, therefore, play a complementary role with TGFβ (see below) to control the balance between proliferation and differentiation in the intestinal epithelium [15]. Upregulation of TGFα expression has been shown to occur in a rat TNBS colitis model and upregulation of TGFα binding sites has been observed in an experimental rabbit model of colitis induced by a combination of immune complex and formalin [16]. Enhanced TGFα expression has also been found in the colonic mucosa of patients with inactive UC compared with active UC, Crohn's disease (CD) or normal controls [17]. TGFα null mice have a relatively normal phenotype under control conditions but an increased sensitivity to colonic injury [18]. Conversely, mice over-expressing TGFα showed reduced susceptibility to DSS-induced colitis [19].

The transforming growth factor beta family

This family of molecules are structurally distinct from TGFα and, in most systems, actually inhibit proliferation. There are least five different isoforms of TGFβ and their major sites of expression in the normal gastrointestinal tract are in the superficial zones where they may function to inhibit proliferation once the cells have left the crypt region [15]. TGFβ has many diverse functions including being a potent chemoattractant for neutrophils and stimulating epithelial cell migration at sites of wounds. It appears to be particularly important in stimulating restitution and may also act as a "second messenger" to stimulate restitution following release by cells in response to factors such as EGF [20].

TGFβ expression is increased in patients with both UC and CD with active disease [17] and increased TGFβ$_1$ expression precedes development of colitis in IL-2-deficient mice [21]. In addition to its effects on cell migration, proliferation, differentiation and extracellular matrix production, TGFβ has chemotactic proinflammatory and immunoregulatory effects. Targeted disruption of the TGFβ$_1$ gene in mice results in multifocal inflammatory disease and early death [22].

Evidence for the functional importance of TGFβ in IBD comes mainly from animal models. In mice with Th1 T cell-mediated colitis induced by the haptenizing reagent 2,4,6-trinitrobenzenesulfonic acid (TNBS), TGFβ-producing cells inhibit the inflammatory response and anti-TGFβ antibodies aggravate the disease [23]. Furthermore, disruption of the TGFβ pathway in intestinal epithelial cells, by targeted expression of a TGFβ dominant negative receptor II, resulted in decreased wound healing *in vitro* [24]. As in other organ systems, TGFβ appears to be involved in the pathogenesis of intestinal fibrosis and muscle hypertrophy found in IBD. The role of other members of the TGFβ superfamily in mucosal inflammation, repair and protection in the intestine is still uncertain.

Vascular endothelial growth factor (VEGF, "vasculotropin")

VEGF is a homodimeric 34–42 kDa heparin-binding glycoprotein with potent angiogenic, mitogenic and vascular permeability-enhancing factors which is related to platelet-derived growth factor (PDGF). Specific receptors for VEGF have been identified on the apical membranes of the human colonic cell line Caco-2 [25] and on the human H-4 cell line. Although VEGF bound to these cell lines; it did not induce a proliferative response [25] and the pathophysiological role of VEGF remains unclear.

Fibroblast growth factor family

The FGF family comprises more than 20 ligands, which bind to at least five distinct receptors. In humans, each of the receptors also has splice variants that, in some cases, have been associated with varying pharmacological profiles. The FGF ligands all share a conserved central domain of about 120 amino acids that bind heparin, which is involved in the formation of stable FGF ligand–receptor interactions. *In situ*, FGFs bound to heparin (or other glucosaminoglycans) within the extracellular matrix may serve as a reserve "depot" of growth factors.

Keratinocyte growth factor (KGF)

KGF, also known as fibroblast growth factor (FGF)-7, is the most abundant member of the large family of FGFs. It is produced by mesenchymal cells of the gastrointestinal tract and binds to a splice variant of the FGF 2

receptor located on the intestinal epithelium, suggesting that it normally functions in a paracrine manner. It is a potent stimulator of proliferation of a variety of epithelial cell lines *in vitro* and stimulates mucosal proliferation in a concentration-responsive manner in rats when administered via continuous intravenous infusion [26]. KGF expression is substantially increased in mesenchymal cells in the inflamed intestine of patients with either CD or UC [27,28]. KGF mRNA expression has also been shown to be greater in UC than in CD due to increased production by mucosal myofibroblasts [29]. Intraepithelial lymphocytes (IELs) can also express KGF, although the amounts found in IELs in inflamed IBD tissue appear to be low.

Studies using animal models of injury suggest that KGF homologues may be of value in the treatment of chemotherapy and radiation-induced gastrointestinal injury and short-bowel syndrome. Although animal studies with a homologue of KGF-1, keratinocyte growth factor-2 (repifermin) showed a beneficial effect in preventing DSS-induced colitis, results from a phase I/II dose escalation clinical trial were not encouraging [30]. However, it is notable that KGF-1 has demonstrated a significant benefit in patients developing diffuse gastrointestinal tract mucositis due to chemotherapy received in the context of bone marrow transplantation [31] and has been approved for that indication in the USA.

Basic FGF (bFGF)

bFGF is a heparin-binding factor with potent angiogenic properties. In a small pilot clinical trial, on NSAID-induced gastric ulcers, recombinant, acid-stable basic fibroblast growth factor (bFGF CS-23] appeared to stabilize the mucosa [32]. Equivalent studies in IBD have not been reported.

Hepatocyte growth factor (HGF, scatter factor)

HGF is a potent mitogen and morphogen. Both HGF gene expression and its receptor c-*met* are increased after induction of injury in various animal models. The timing of the response varies according to the method used but, collectively, these studies suggest that this signaling system may have relevance in both the early and late phases of repair. HGF mRNA is located in stromal cells between the regenerative glands and in the arterial vessels in the submucosa, whereas c-*met* mRNA is located in the epithelial cells of the regenerative glands. This system therefore provides an example of epithelial–mesenchymal cross-talk. HGF is secreted from stromal fibroblasts as a single chain biologically inactive precursor (pro-HGF). This is converted to an active heterodimeric protein by a novel serine protease (HGF activator). This proteolytic process is probably essential for HGF to exert its mitogenic activity. Interestingly, HGF is only converted to its active heterodimeric form in injured tissue, suggesting that selective activation of HGF by HGF activator is a mechanism by which HGF action is localized to damaged tissues. Although it appears plausible that HGF also plays a role in protection and repair of the intestinal epithelium, its role in IBD is largely unknown. Increased blood levels of HGF have been detected in mice after acetic acid-induced colitis and in active UC [33].

Gastrointestinal hormones as growth factors

The bowel shows a remarkable ability to respond to changes in dietary intake. Cross-circulation experiments support the concept of circulating trophic factors influencing intestinal growth although the identity of such factor(s) remains unclear. Amidated gastrin probably plays a role as a trophic factor for mucosal growth within the stomach. Non-amidated gastrin may promote epithelial proliferation elsewhere in the gastrointestinal tract though the importance of this remains uncertain. Although early studies suggested that peptide YY may act as a trophic agent, recombinant peptide infusion experiments have failed to show a pro-proliferative effect.

Glucagon-like peptide-2 (GLP-2]

GLP-2 is secreted by intestinal epithelia and enhances proliferation and inhibits apoptosis of intestinal epithelial cells *in vitro*. In addition, systemic infusion of GLP-2 has been shown to cause a general trophic response within the gut [34]. This has led to the suggestion that GLP-2 ligands may be useful to stimulate growth of the bowel in conditions such as short bowel syndrome and to treat conditions such as IBD. GLP-2 plasma levels have been found elevated in patients with either active CD or UC [35].

Insulin-like growth factors (somatomedins)

IGF-I and IGF-II promote cell proliferation and differentiation and are similar in structure to pro-insulin. It is possible that they also exert insulin-like effects at high concentrations. The liver is a major site of IGF synthesis; IGF-I and II are also expressed at high levels in the developing human fetal stomach and small intestine with expression reaching a maximum soon after birth. Increased IGF-I expression has been found in several animal colitis and injury models and administration of IGF-I has been shown to reduce the severity of colitis [36]. In pediatric CD patients, reduced IGF-I serum concentrations have been found in active disease, while serum levels increased with corticosteroid treatment [37]. Besides its wound healing promoting effects, IGF-I increases type I collagen synthesis

in rat intestinal smooth muscle cells. Interestingly, IGF-I was detected in intestinal lavage fluid in a high percentage of CD patients with strictures, while IGF-I was found in only a few patients without strictures [38]. In addition to IGF-I, fibroblast-derived IGF-II may also play a role in IBD although that role still remains to be defined.

Trefoil peptides, trefoil factor family (TFF)

The trefoil peptide family in mammals consists of a group of small proteins, each containing one or two copies of the trefoil motif. The trefoil motif comprises a three-loop (trefoil) configuration with six highly conserved cysteine residues allowing three inter-chain disulfide bridges. Although they appear to have little, if any, pro-proliferative activity, they are considered in this "growth factor" section as they have potent protective/healing activity. For a fuller review, see [39].

In humans, the trefoil peptides are found in the mucus-producing epithelium in the stomach and small and large intestines. Trefoil factor family 1 (TFF1) (also known as pS2) and TFF2 (also known as spasmolytic polypeptide) are predominantly found in the stomach, whereas TFF3 (also known as intestinal trefoil factor) is predominantly found in the small intestine and colon. All members of the TFF family are secreted on to the luminal surface and are relatively resistant to both acidic and enzymatic degradation. The trefoil peptides are thought to have two distinct functions in the gastrointestinal tract: under basal circumstances, they may play a role in mucus stabilization, and when an acute injury occurs, their rapid upregulation is important in stimulating the repair processes, particularly that of restitution.

The importance of this family of peptides has been supported by results from the development of transgenic overexpression and knockout models. In these models, disruption of the TFF2 and -3 genes (knockout animals) has little effect on baseline mucosal homeostasis but does increase the animal's susceptibility to injurious agents [40,41]. Similarly, transgenic animals overexpressing TFF1 or -3 in the intestine gave a phenotype of normal baseline morphology or morphometry but with an increased resistance to noxious agents in TFF-expressing regions [42,43]. Surprisingly, the phenotype of TFF1 knockout mice is markedly different, exhibiting the development of gastric adenoma and carcinomas [44], suggesting that TFF1 plays an additional role as a tumor suppressor gene.

Upregulation of trefoil peptides at sites of ulceration is not confined to the specific trefoil peptide normally present in that region of the gastrointestinal tract but all three may be induced, a form of "molecular metaplasia" [45]. In gastric epithelial cell lines, trefoil peptides were capable of auto- and cross-induction. Trefoil-mediated transcriptional cross-regulation requires activation of the Ras/MEK/MAP kinase signal transduction pathway and EGF receptor activation, as shown by tyrosine phosphorylation of EGF receptor after trefoil peptide stimulation [45]. Since EGF receptor expression is itself strongly induced after mucosal damage, the trefoil–EGF receptor relationship may play a key role in generating and maintaining mucosal repair. In support of this idea, marked synergistic healing effects are seen when TFF and EGF peptides are co-administered.

Cytokines

Lymphocyte recruitment from the local vasculature into inflamed areas forms an important component of the normal defense process. As many of the molecules involved in this process are expressed on circulating cells or on the surface of the vascular epithelium, they are attractive targets for manipulation in the treatment of IBD. A detailed review of the inflammatory process goes beyond the scope of this chapter and is discussed elsewhere. Nevertheless, some key elements of relevance to the repair process are discussed briefly below.

Over the last few years, the importance of exaggerated Th1 and Th2 responses has been a major focus in the study of the pathogenesis of excessive inflammation in the gut. Mucosal inflammation is almost always mediated by one of two pathways – an excessive Th1 cell response that is associated with increased secretion of IL-12, interferon-γ (IFN-γ) and TNF, or an excessive Th2 cell response that is associated with increased secretion of IL-4, IL-5 and IL-13. CD has been considered as a classic example of a Th1-mediated process. In addition to the actions of Th1 and Th2 cells, there has recently been interest in the function of IL-17-producing T cells (Th17 cells) and their more proximate importance to tissue injury. Th17 cells appear to arise as a separate Th lineage from Th1 and Th2 T cells, through differentiation of naïve T cells via IL-23-dependent mechanisms. Although IL-17 secretion is probably limited to T lymphocytes, the IL-17 receptor is widely distributed in various cell types [46].

The IL-23–IL-17 axis appears to have major significance in the pathophysiology of IBD. IL-23 is a major factor in the pathogenesis of autoimmune destruction in experimental allergic encephalomyelitis, collagen-induced arthritis and IBD. IL-23 drives the development of autoreactive IL-17-producing T cells and promotes chronic inflammation dominated by IL-17, IL-6, IL-8 and tumor necrosis factor and also neutrophils and monocytes [47].

The adaptive immune process underlying CD is currently best understood as an activation of lamina propria macrophages and dendritic cells, driven by intestinal luminal bacterial antigens, leading to Th1 lymphocyte proliferation. This may be enabled by altered innate

immune function as one of the most important mechanisms of the IBDs. TNF features prominently in the early stages of the inflammatory chain reaction and as a clinical correlate, TNF concentrations are known to be elevated in the blood, stool and intestinal tissues of patients with CD. Furthermore, the clinical effectiveness of TNFα-neutralizing antibodies is now well established. These immune pathways and their demonstrated activation of inflammatory mediators provide the quintessential example of the two-sided dimensions of host defense mechanisms. These processes are fundamentally homeostatic, enabling the host to respond to challenges, particularly infectious agents, but these same processes also effect the tissue damage that gives rise to many if not most of the clinical manifestations of IBD. Accordingly, strategies that abrogate immune activation and inflammatory responses, broadly speaking, promote tissue repair. Although a major focus in clinical IBD research has been around TNFα neutralization, disruption of other cytokine pathways is being actively explored, including p75 and p55 TNF receptors, anti-leukocyte trafficking, anti-IL2 receptor antibodies, anti-IL12p40 and anti-IFN-γ.

Extracellular matrix (ECM) molecules

Tissue cells and their underlying mesenchyme constantly turn over as part of the normal homeostatic mechanisms. Mesenchymal cells and stroma not only act as a supporting scaffold but are probably also of relevance for mucosal differentiation via epithelial–mesenchymal cross-talk. Alterations in the amount and composition of the mesenchyme are therefore likely to be of high importance in understanding mucosal breakdown in IBD.

Basement membranes are composed predominantly of laminin, type IV collagen, nidogen/entactin and heparin sulfate proteoglycans [48]. In addition, fibronectin has been found in the basement membrane of the intestine. Although intestinal fibroblasts, including pericryptal fibroblasts, are presumed to be the principal source of basement membrane in the intestine, intestinal epithelial cells have also been shown to contribute to basement membrane synthesis. Non-transformed rat intestinal crypt epithelial cells express fibronectin, laminin β and laminin γ_1 transcripts and proteins in addition to low levels of collagen IV (α_1/α_2), but not laminin α_1 [49].

Cell restitution activity *in vivo* is likely to be highly dependent on the composition of its underlying ECM. TGFβ_1 stimulates ECM expression by intestinal epithelial cells as well as mesenchymal cells. Thus TGFβ may mediate its restitution-enhancing effects, at least in part, through enhanced expression of fibronectin and collagen type IV and perhaps other ECM components in intestinal epithelial cells. Although heterotrimeric laminin ($\alpha_1/\beta_1/\gamma_1$) does not appear to play a significant role in restitution, it may contribute to other processes involved in tissue repair, e.g. differentiation and organization of intestinal and colonic epithelial cells, as found using model small intestinal and colonic epithelial cell lines (IEC-6, Caco-2) and fetal intestinal epithelial cells *in vitro* (e.g. [50]).

To counterbalance the production of ECM, mechanisms to aid removal are also important; the family of matrix metalloproteinases (MMPs) may play a key role. MMPs comprise a large family of Zn^{2+}-dependent peptides involved in normal tissue remodeling. Three of the most important members of this family comprise interstitial collagenase (MMP-1), stromolysin 1 (MMP-3) and gelatinase B (MMP-9). A dynamic equilibrium normally exists between the presence of these MMPs and their natural antagonists called tissue inhibitors of metalloproteinases (TIMPs [51]). However, in conditions such as IBD, this balance is perturbed with an increase in several MMPs and a corresponding reduction in TIMPs, resulting in an alteration in the protease–anti-protease balance. The molecular signaling involved in changes in MMP/TIMP activity are unclear although T cell activation pathways may be relevant [52]. Interestingly, the oral administration of a broad-spectrum matrix metalloproteinase inhibitor resulted in significantly reduced tissue injury and inflammation in a rat colitis model [53].

Regulation of mucosal repair by subepithelial cell populations and stroma

Lamina propria cell populations are present in close proximity, subjacent to the epithelium. Despite increasing knowledge of the effects of regulatory factors, there is currently limited information available on regulation of epithelial wound healing by subepithelial cell populations. In addition to the "indirect" effects of subepithelial cell populations on surface repair (mediated by production of soluble factors and ECM molecules), these cells may also have direct involvement through numerous discrete, uniformly distributed pores of 0.2–3.3 μm in the intestinal basement membrane of the small and large intestine [54]. *In vitro* studies have demonstrated that various lamina propria cells can migrate through pores and tunnels of intestinal basement membrane (e.g. [54]).

Intestinal mesenchymal cells encompass a family of heterogeneous cells, which serve as integral components of a complex network of immune and non-immune cells in the intestinal mucosa. It is now appreciated that intestinal fibroblasts, in addition to maintaining a structural role, also have a substantially broader spectrum of mesenchymal cell functions such as modulating epithelial repair. For example, mesenchymal cells such as stromal fibroblasts and myofibroblasts affect the recruitment, retention and activation of immune cells, through their synthesis of growth

factors such as HGF, IGF-II, TGFβ and FGF, cytokines, chemokines, eicosanoids and ECM components [55].

Gastrointestinal stem cells and the stem cell "niche"

Stem cells are undifferentiated primitive cells that exist within a tissue throughout the lifetime of an organism. They divide asymmetrically both to undergo self-replication and to produce committed daughter cells that can differentiate to form all adult lineages within a cell compartment. The gastrointestinal epithelial stem cells are located and maintained within a mesenchymal niche, a specialized microenvironment that provides an optimal milieu for stem cell survival and function, believed to be created and maintained by the mesenchymal cells of the underlying subepithelial region. Although morphologically indistinct, adult epithelial stem cells can be defined functionally by this potential for asymmetric division and are characterized by their residence within a stem cell compartment or "niche" situated toward the center of the gastric gland and near the base of the intestinal crypts [56].

The contribution of bone marrow-derived cells to the intestinal epithelium has been a subject of speculation for many years. Most recent studies, using XX–XY mismatch bone marrow transplantation followed by Y-probe analyses, suggest that the intestinal subepithelial myofibroblasts population may be contributed to by circulating pluripotential bone marrow stem cells, whereas this appears not to be true for actual enterocytes/colonocytes [55,56].

Novel approaches to stimulating repair

In addition to facilitating the development of factors that can influence the inflammatory process, advances in recombinant peptide technology have allowed the production of pure "growth factor" peptides to be tested in a variety of models and clinical trials. There are a number of luminal gastrointestinal pathologies where novel therapies might prove important, including use for multi-organ failure [57], necrotizing enterocolitis [58], short bowel syndrome and IBD.

In the most severe cases, short bowel syndrome requires long-term parenteral (intravenous) feeding or, in a few selected cases, small bowel transplantation, both options being associated with high cost and morbidity. Stimulating growth of the residual bowel is, therefore, an attractive option and several peptides, usually administered systemically, have given positive results in a variety of animal models including EGF, glucagon-like peptide 2 (GLP-2), KGF and IGF-1. Clinical translational studies have been performed on a subgroup of these and two peptides worthy of further mention are GLP-2 and growth hormone (GH).

Treatment with GLP-2 has been shown to improve intestinal absorption and nutritional status in patients with short bowel resulting from ileal and colonic resection [59]. In addition to possible actions on cells already present, GLP-2 enhances enterocyte proliferation. GLP-2 could, therefore, be useful clinically to enhance small bowel regeneration and adaptation in patients with short bowel.

Similarly, beneficial effects were seen when low-dose GH was administered to adult home parenteral nutrition-dependent patients with short bowel syndrome. In this study, 3 weeks of low-dose GH significantly improved intestinal absorption in HPN-dependent patients who were on a hyperphagic Western diet [60].

Inflammatory bowel disease

Several growth regulatory peptides have shown promise in animal studies, including EGF, platelet-derived growth factor, TGFβ, IGF-I, KGF, trefoil peptides and combination therapy of multiple peptides in the form of bovine colostrum. Several of these have advanced to stage I/II clinical trials and the results from these studies are summarized in Table 12.1. A subgroup which demonstrate some general principles are discussed below.

Studies of the effect of GM-CSF on animal models of IBD had given favorable results. Optimism for the use of this peptide was reinforced by a small clinical trial in which eight patients achieved clinical remission and retreatment of subjects who had responded but subsequently relapsed responding a second time with reduced CDAI scores [61]. Unfortunately, a subsequent randomized trial of synthetic GM-CSF (sargramostim) 6 $\mu g\,kg^{-1}\,day^{-1}$ s.c. ($N = 124$ total) showed no significant difference from placebo in the primary end point (CDAI score reduction of 70 points or more) on day 57, although some of the secondary end points gave significant results [62]. Enthusiasm for the study of this product in the clinical setting has therefore diminished.

Similarly, keratinocyte growth factor analogues were shown to be capable of reducing inflammation in various animal models of colitis, prompting a clinical trial of Repifermin (KGF-2) for ulcerative colitis. Eighty-eight patients with active ulcerative colitis were enrolled in a randomized placebo-controlled dose escalation study. Repifermin (1–50 $\mu g\,kg^{-1}$ i.v.) was well tolerated but did not show a positive result compared with placebo [63]. These studies demonstrate the limitations of the current animal models of inflammatory bowel disease and the need for caution in extrapolating from the *in vivo* to the clinical arena.

A small clinical trial of GH ($N = 37$) has shown promising results where 4 months of s.c. treatment with growth hormone or placebo was compared [64]. Although there was some mismatch in baseline CDAI scores between the

Table 12.1 Current situation regarding peptide growth factor therapy for inflammatory bowel disease.

Peptide	Animal model results	Indication	Clinical trial stage
TGFβ	Oral $TGF\beta_2$ reduced colitis in IL10 knockout mice	UC/CD	?
IGF-1	Systemic IGF-I attenuated DSS colitis	UC/CD	?
KGF-2	KGF-2 reduced small bowel ulceration caused by NSAIDs	Systemic therapy of UC	Negative result on dose finding study of repifermin [63]
Trefoil peptides	Success in systemic, enema and *Lactobacillus* delivery systems in IBD models	UC/CD	Small phase I/II trial did not show effect [78]
Multiple peptides in colostrum	Success in IBD models (and NSAID gut injury)	Left-sided UC Enema therapy	Small phase I/II trial gave positive result [69]
Multiple peptides in cheese whey	Success in oral administration in IBD models	UC	?
GMCSF	Systemic administration improved gut barrier function after burn and cecal puncture injury in mice	CD	No effect seen in phase III [62]
EGF	Success in UC models	Left-sided UC Enema therapy	II [67]
GH	GH overexpressing mice had improved repair following DSS colitis	CD	Phase I/II mixed results regarding improvement in CDAI [64]

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two groups (287 ± 134 vs 213 ± 120, $p = 0.09$), at 4 months the CDAI score had decreased by a mean of 143 ± 144 points in the GH group, as compared with a decrease of 19 ± 63 points in the placebo group ($p = 0.004$). Edema was a common side effect in the GH-treated group.

While quality of life and CDAI scores are key elements in adults with IBD, maintaining growth in children with IBD is an important additional consideration in this patient group. An initial study of GH on children (N = 10) with prednisolone-dependent inflammatory bowel disease analyzed effects on growth and CDAI scores. Although it did not appear to influence CDAI, positive changes in body composition, bone metabolism and linear growth, without deterioration of carbohydrate tolerance, were reported [65]. Unfortunately, a subsequent study of GH on seven children with CD and growth failure found that GH treatment did not stimulate growth [66].

Although most clinical trials have used systemic growth factors (either s.c. or i.v.), luminal therapy may have value, particularly when colitis is restricted to the left side of the colon (which is within reach of enema therapy). This approach also has the advantage of reducing the systemic exposure of cells to the mitogenic activity of such peptides. As an example, in a randomized, double-blind clinical trial 12 patients with mild-to-moderate left-sided UC received daily enemas of 5 μg of EGF in 100 ml of an inert carrier and 12 received daily enemas with carrier alone for 14 days. All also began to receive 1.2 g of oral mesalamine per day or had their dose increased by 1.2 g per day. After 2 weeks, 10 of the 12 patients given EGF enemas were in remission, compared with one of 12 in the control group (83 vs 8%, $p < 0.001$). At the 2 week assessment, disease-activity scores, sigmoidoscopic score and histologic scores were all significantly better in the EGF group than in the placebo group ($p < 0.01$ for all comparisons) and this benefit was maintained at 4 and 12 weeks [67].

Although most clinical studies have used single growth factors, rather than combinations, there are potential advantages to using combination therapy as, for example, synergistic responses have been shown in animal models when trefoil peptides and EGF are used together [68]. There is also much interest by the general public in using natural-based therapies or bioactives to treat and prevent disease. One example of such an approach was shown in a study examining the effects of bovine colostrum, a rich source of nutrients, antibodies and multiple growth factors. In a randomized, double-blind, controlled protocol, 14 patients with mild to moderately severe distal colitis received colostrum enema (100 ml of 10% solution) or placebo (albumin solution) b.d. for 4 weeks. Both groups also received mesalazine ($1.6\,g\,day^{-1}$) or, if already taking it, had a dose increment of $1.6\,g\,day^{-1}$. After 4 weeks, the colostrum group showed a significantly greater reduction in symptom scores and histologic scores than the control group [69]. Further work in the area of functional foods (nutriceuticals, bioactives) is ongoing.

Downstream mechanisms

In general terms, migration involves a tightly controlled spatial and temporal interaction of multiple factors, which include extracellular molecules such as soluble factors such as growth factors and cytokines and matrix components (e.g. collagen, laminin, fibronectin). The signaling molecules activated by the interaction of these factors with cell surface receptors then cascade through an amplification process involving, among others, protein kinases, phospholipases and low molecular weight GTPases. These signals are also transmitted to other cells in the vicinity such as regulating adhesion to other cells (e.g. E-cadherin) and to matrix components (e.g. integrins, hyaluronic acid receptors). Stimulation of the migratory process also requires regulation of detachment from the extracellular matrix by factors such as urokinase-type plasminogen activator and matrix metalloproteinases and alteration in molecules which regulate cytoskeletal function resulting in the formation of lamellipodia (for a general review, see [70]).

Our understanding of the downstream pathways involved in these processes has markedly increased in the last few years. Importantly, many regulatory peptides share the same post-receptor pathways and can be influenced together. Mitogen-activated protein (MAP) kinase pathways are a key controlling system by which cells transduce extracellular signals and several distinct MAP kinase cascades have been identified. Among the extracellular signal-regulated Erk, c-Jun and p38 kinase pathways, Erk-1 and Erk-2 are known to be activated by several growth factors, including TGFα, EGF, TGFβ, FGF and HGF. Erk-1/-2 kinases are activated by MAP kinase 1 (MEK-1) through Ras- or Raf-dependent mechanisms [71]. Subsequently, Erk-1 and Erk-2 phosphorylate various downstream substrates including ternary complex factor-1/Elk, fos and early growth response-1 (Egr-1) nuclear phosphoprotein, resulting in activation of transcription factors that control cellular growth, differentiation, transformation and development. As many growth factors, such as TGFα, EGF, HGF and TGFβ activate both the Erk-1 and Erk-2 MAP kinases, this signaling pathway may well have relevance to the healing process. The importance of Erk-1 and Erk-2 MAP kinases in epithelial wound healing in the gut is consistent with the finding of increased Erk-1 and Erk-2 activation and enhanced fos and Egr-1 nuclear phosphoprotein mRNA expression in wounded IEC-6 cells [72]. Further support for the importance of these pathways was shown in the same studies where the pro-restitutive response was seen to be blocked by the addition of the MAP kinase inhibitor PD98959 or by the transfection of dominant negative EGr-1 construct [73].

Collectively, these data support the concept that MAP kinase activation and the resulting downstream events are pivotal to the cell migration process as well as the later proliferative phase. Other pathways such as c-Jun-N-terminal protein kinase-1 (JNK-1) and p38 MAP kinases have also been shown to be activated following injury, although their importance is less well defined.

While important information can be obtained from considering gut epithelial cells in isolation, there is added value in also examining models that look at mesenchymal–epithelial cell signaling, reflecting the complex *in vivo* situation. As an example, some recent studies examined signaling pathways involved in epithelial–mesenchymal interactions associated with mechanical strain-induced restitution. Mechanical strain stimulated wound closure on fibronectin in Caco-2 and IEC-6 cells and caused activation of myosin light chain (MLC) and extracellular signal-regulated kinase (ERK). Migratory effects could be blocked by inhibiting MLC or ERK phosphorylation. Intracellular localization studies showed that phosphorylated MLC was redistributed to the leading edge of migrating cells and phosphorylated ERK redistributed to the lamellipodial edge [74].

Perturbation of the repair process leading to fibrosis

Although ECM contributes to re-establishing surface epithelial continuity and reconstruction of normal mucosal architecture after transmural intestinal injury, excessive ECM production may result in pathological fibrosis and scarring. Reports have demonstrated increased collagen content and relative amounts of type III and V collagen content in intestinal strictures of patients with CD [75]. These changes were associated with typical thickening of the bowel wall and increased proliferation of smooth muscle cells.

Increased collagen type III synthesis has been documented in fibroblasts isolated from strictures of patients with CD [76] and increased collagen production has also been observed in intestinal smooth muscle cells of IBD patients. Why this occurs is still uncertain, although human intestinal mucosal mesenchymal cells (muscularis mucosae cells and fibroblasts) proliferate in response to many pro-inflammatory cytokines including IL-1β, IL-6 and TNFα. Similarly, stimulation of intestinal mucosal mesenchymal cells with pro-inflammatory cytokines resulted in increased mRNA encoding their pro-inflammatory gene products [77]. Therefore, intestinal mucosal mesenchymal cell populations may not only facilitate mucosal healing and intestinal tissue remodeling by release of growth factor and ECM production, but may also amplify intestinal inflammation by increased expression of pro-inflammatory cytokines and increased proliferation, which may eventually result in excessive fibrosis and scarring. Increased IGF-I and TGFβ expression in IBD mucosa may further stimulate myofibroblast and smooth muscle cell proliferation and production of ECM, most notably collagens. Some observations suggest that intestinal subepithelial myofibroblasts in different regions

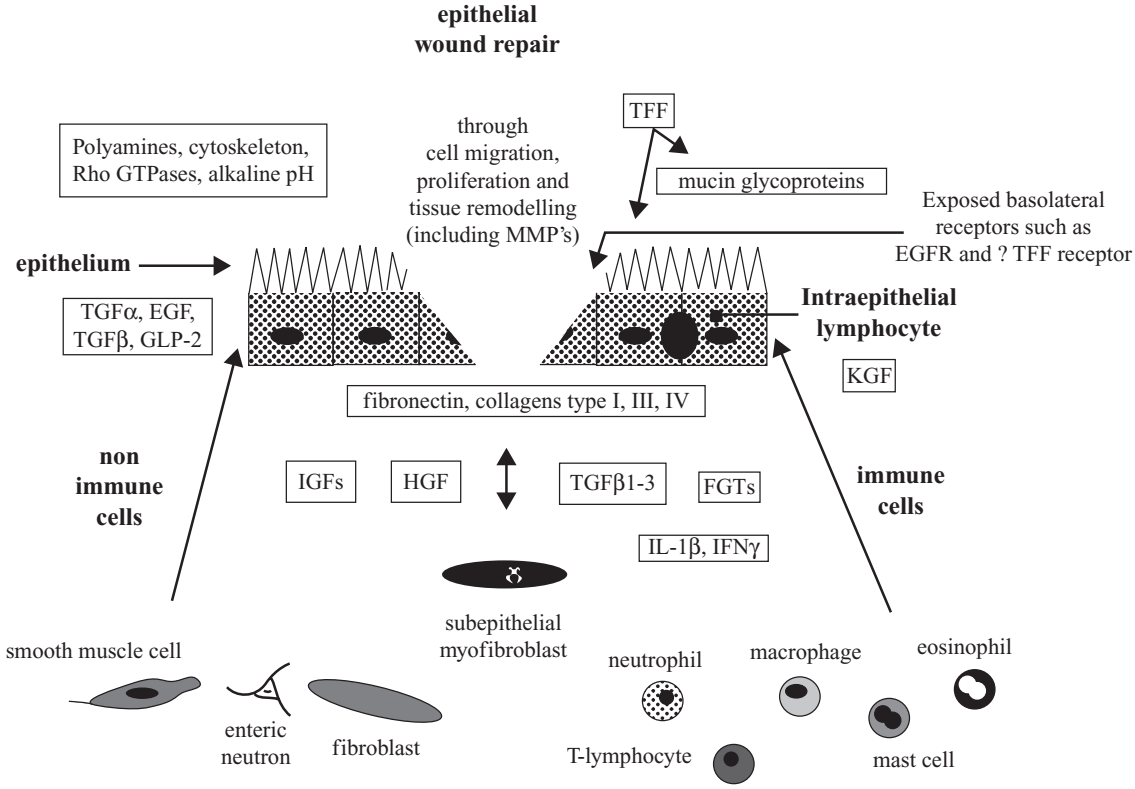

Figure 12.1 Healing of the intestinal epithelium is regulated by cytokines, growth factors, epithelial mesenchymal interactions and luminal factors. When damage occurs, growth factor and other receptors may become exposed to luminal agents through either direct exposure of basolateral receptors or redistribution of receptors within the surviving cells. While intestinal myofibroblasts appear to be an important for epithelial repair, the roles of many other subepithelial populations need to be defined.

of the intestine exhibit distinct phenotypes. For example, myofibroblasts isolated from the distal ileum and colon differ in their relative expression of HGF and TGFβ. While TGFβ is the predominant product of myofibroblasts from the distal ileum, HGF was the predominant factor expressed by colonic myofibroblasts. Myofibroblasts expressing more HGF were more effective in promoting epithelial growth, whereas those expressing more TGFβ did not support epithelial cell growth. However, the latter myofibroblasts may stimulate cellular ECM production more effectively.

Conclusions

Our understanding of the processes underlying mucosal injury and controlling repair have rapidly expanded over the last decade and it is becoming clear that, rather than being distinct elements, inflammation and repair are interrelated processes mediated by common cytokines and growth factors. Similarly, mesenchymal–epithelial interaction and cross-talk are likely to be important in maintaining intestinal integrity and in stimulating repair (Figure 12.1). The next few years should see the transition of these insights into clinical care through the development of novel therapeutics aimed at targets formed by an understanding of these processes.

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Chapter 13
The Bidirectional Relationship of Gut Physiological Systems and the Mucosal Immune System

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Summary
- Epithelial cells are contributors to the inflammatory responses.
- The sensory and motor systems of the gut are affected by the inflammatory process and are responsible for symptom generation in IBD.
- The integrity of the enteric nervous system is critical for host defense and for modulating inflammatory responses in the gut.
- The bidirectional gut–brain axis is involved in intestinal inflammatory processes.
- The involvement of the gut–brain axis explains the linkages between depression and IBD.

Introduction

The clinical expression and the natural history of chronic intestinal inflammation reflect interplay between physiologic systems that include the nervous system, the mucosal immune system. This chapter explores the bidirectional nature of this relationship. First, it examines the impact of immune activation and mucosal inflammation on the physiologic systems as a basis for symptom generation. Second, it evaluates emerging data supporting a role for these systems, the nervous system in particular, in determining susceptibility to intestinal inflammatory stimuli and modifying the natural history of chronic intestinal inflammation.

Part I. The effect of immune activation and inflammation on gastrointestinal physiology

Symptoms of IBD arise as a result of alterations in gut physiology. For example, diarrhea reflects changes in epithelial transport fluids and electrolytes in addition to changes in gastrointestinal motility. These changes occur as a result of the actions of inflammatory mediators on target cells that include the epithelial cell, smooth muscle cell and nerves. The altered physiology may also arise as a result of phenotypic shifts in these cells induced by inflammation or immune activation; such shifts enable these cell types to produce mediators that act in an autocrine or paracrine fashion, altering the physiologic role of the cell; an example is the production of cytokines by smooth muscle or epithelial cells.

Gut physiology is highly integrated and is controlled via a hierarchy of mechanisms that include cell-to-cell contact, release of local factors that act in a paracrine fashion such as prostaglandins and regulatory peptides, the endocrine secretion of peptide hormones such as cholecystokinin (CCK) and 5-hydroxytrptamine (5-HT) or through neural networks. The last factor may be intrinsic to the gut or may involve extrinsic nerves including those of the autonomic or central nervous systems.

Finally, gut physiology may be perturbed indirectly through inflammation-induced structural changes in the gut. Examples include the effects of bacterial overgrowth resulting from strictures or fistulae or the effects of obstructing strictures on the physiology of cells in the prestenotic regions of the gut.

It follows, therefore, that inflammation, albeit restricted to one region of the gut, may produce widespread perturbations of gut function by altering a variety of cell types, in both non-inflamed and inflamed regions of the gut, through the involvement of endocrine or neural networks.

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The effect of inflammation on epithelial cells

Clinical observations

The intestinal epithelium is responsible for the absorption of nutrients and the regulation of water and ion transport. Changes in these functions contribute to the diarrhea associated with IBD. The net accumulation of fluid into the intestinal lumen leads to increased stool number and volume. Inflammation and ulceration of the epithelium lead to loss of the digestive enzymes required for the breakdown and absorption of protein and fat and loss of the large surface area required for nutrient absorption. The clinical result is diarrhea, weight loss, malnutrition and changes in fluid and electrolyte homeostasis. The inflammatory destruction of the mucosa is also associated with blood and protein losses manifest by iron deficiency anemia and, in severe cases, hypoalbuminemia. In addition, disruption of the integrity of the intestinal epithelial layer, an important barrier to macromolecules, allows for stimulation of the local immune system by luminal antigens [1,2]. The issue of whether all intestinal permeability changes are acquired or inherited remains controversial [3].

Chronic mucosal inflammation has a significant influence on the physiologic function of the intestinal epithelium. In addition, the intestinal epithelial cell is actively involved in regulating and influencing the inflammatory process and the mucosal immune function of the intestine. Indeed, there is now a new appreciation of the direct effect of the non-pathogenic gut flora on epithelial cell function. These findings are supported by the clinical observation that broad-spectrum antibiotics may have therapeutic value in specific clinical situations in patients with inflammatory bowel disease (IBD) [4]. The advances made in our understanding of the mechanisms underlying these observed changes in epithelial cell function in IBD are critical to our management of the disease.

Insights into underlying mechanisms from animal studies

Epithelial cell growth, differentiation and apoptosis

Epithelial cell (EC) proliferation, differentiation and death are significantly altered during inflammation. In the small bowel of rodents and humans, parasite-induced inflammation can lead to villous atrophy and crypt hyperplasia. The loss of the large mucosal surface area provided by the intestinal villi leads to loss of normal absorptive capacities [5]. Similar changes can be initiated by immune activation with food antigen in patients with celiac disease [6]. The immune activation leads to release of mediators and cytokines that participate in the initiation of mucosal damage [7]. In fact, direct T cell activation *in vivo* in mice leads to loss of normal mucosal structure with loss of villi and crypt epithelial cells due to apoptosis [8]. T cell activation in human fetal intestinal explants also caused loss of villi and increased proliferation of crypt cells [9]. Although cytokines such as tumor necrosis factor alpha (TNFα) can directly cause these changes *in vivo* [10], T cell-mediated damage involves the interplay of perforin and Fas/FasL, with TNFα playing a non-essential role [8]. Other factors influencing epithelial cell turnover include cytokines such as transforming growth factor beta (TGFβ) and interferon-γ γ and growth factors such as growth hormone, glucagon-like peptides and trefoil factor peptides) [11–15]. It is evident that these molecules influence the normal growth and development of intestinal epithelial cells in addition to participating in the immune-mediated damage of the mucosa.

Permeability changes

An important function of the epithelial layer in the intestine is that of a barrier to macromolecules. Inflammation alters the barrier function of the intestine, as can be measured by change in permeability or leakiness of the mucosal epithelium to radiolabeled macromolecules of varying sizes [16,17]. The mechanisms underlying this loss of barrier function have been examined in a number of animal models of inflammation and also in humans. Antigenic challenge of sensitized rats with increases in mucosal mast cells numbers leads to a localized anaphylactic reaction associated with an increase in epithelial permeability [18–20]. Mediators released from mucosal mast cells, which include histamine, serotonin, proteases and various cytokines, contribute to these changes in epithelial barrier function [21]. The molecular elements that contribute to the altered permeability include interferon gamma (IFN-γ) and TNFα. These cytokines synergistically alter the integrity of epithelial tight junctions through mechanisms that involve direct changes on tight junction [22–24]. *In vitro* incubation of an epithelial cell line monolayer, T84, with neutrophils leads to permeability changes of the monolayer that is correlated with neutrophil transmigration [25]. These studies identify cellular and molecular mechanisms by which immune cells alter intestinal epithelial barrier function.

Fluid and ion transport

Epithelial cell chloride secretion is reflected by measured changes in the short-circuit current generated across intestinal mucosa mounted in an Ussing chamber [26]. Ion transport measured in this way reflects the ability of intestinal epithelial cells to regulate fluid and electrolyte absorption and secretion. Rodents undergoing inflammatory events such as infection with parasites such as *Nippostrongylus brasiliensis* and *Trichinella spiralis*, and also food antigen-induced hypersensitivities (e.g. egg albumin and cow's milk protein), have altered chloride secretion [27]. *In vitro* studies extend our understanding of how inflammatory cells and cytokines can alter epithelial cell ion transport (7,27–30]. Studies in human tissue confirm

many of these findings, e.g. TNFα induces chloride secretion [16]. Specific changes that occur in patients with IBD are now being defined [16,27,31].

Epithelial cells and cytokines

As described, a number of cytokines such as TNFα and IFN-γ can alter chloride secretion in the intestine [30,32–34]. Thus, immune cells such as lymphocytes and mast cells, rich in cytokines, influence epithelial cell function [35]. Intraepithelial lymphocytes, which lie between epithelial cells, also produce cytokines including interleukin (IL)-2, -3, -5, -6, TGFβ and IFN-γ that may influence chloride secretion [36]. These observations highlight the importance of lymphocyte-derived cytokines and lymphocyte–epithelial cell interactions in altering the intestinal epithelial function.

Effect of inflammation on the sensory-motor apparatus of the gut

Clinical observations

It has long been recognized that motility is altered in IBD. Initial reports were restricted to studies on patients with active colitis and showed a generalized decrease in contractile activity [37]. However, the pharmacological sensitivity of the colon to opiates was exaggerated and the authors believed that this was associated with the development of toxic megacolon [38]. However, the normal physiologic response to a meal is suppressed in patients with active colitis [39,40]. Under normal conditions, contractions in the proximal colon are largely segmental and serve to retard transit, allowing time for water absorption and solidification of the stool. In ulcerative colitis, these contractions are reduced and there is an increase in propagated contractile activity [41,42], resulting in increased colonic transit, particularly in the distal colon. There is also a reduction in anal sphincter function in the presence of active colitis, this may contribute to episodes of fecal incontinence seen in some patients [41]. Changes in contractility are not restricted to the colon in ulcerative colitis. Changes have also been observed in the small intestine [43] and in the gallbladder of patients after colectomy, a finding which may result [44] in increased gallstone formation in these patients [45]. In severe colitis, a loss of motor activity, which is likely mediated by increased nitric oxide generation, results in toxic megacolon and may lead to multiple organ failure [46,47].

Motility changes in ulcerative colitis

Studies on tissue from (passive range of motion) patients with ulcerative colitis provide some insight into mechanisms underlying the reduction in motility in patients with active disease. *In vitro*, there is evidence of impaired contractility of smooth muscle from ulcerative colitis patients [48]. Immunohistochemical studies have identified a dominance on inhibitory nerves, including nerves containing vasoactive intestinal peptide (VIP), and a reduction in excitatory transmitters, including substance P [49]. This is corroborated by functional studies on muscle strips from patients with colitis; there is a large neural inhibitory component compared with responses from non-inflamed tissues and these responses could be blocked through inhibition of nitric oxide synthase (NOS), implicating nitric oxide as the mediator [50]. Similarly, the responses to the excitatory neurotransmitters such as substance P were reduced by 17–33% in muscle from patients with ulcerative colitis compared with controls [51]. There is an increase in inducible NOS (iNOS) in the nerves of the myenteric plexus and also in smooth muscle cells in the colon in ulcerative colitis [52], and these are the likely sources of nitric oxide. Prostanoids and leukotrienes may also contribute to altered motility in IBD as inducible cyclooxygenase (COX-2) has been identified in colonic nerves and muscle cells of patients with active colitis [44]. The cell types involved in the altered motor pattern include smooth muscle and enteric and autonomic nerves in the gut wall and interstitial cells of Cajal [53].

Motility changes in Crohn's disease

Because of limited access, studies on small intestinal motility are few, but there is evidence of altered interdigestive motility in the small intestine in Crohn's disease in almost 80% of patients studied [54]. This may result in changes in oro-cecal transit that could lead to bacterial overgrowth [55] or to altered drug delivery in these patients [56]. A reduction in gastric emptying has been identified in patients with non-obstructive Crohn's disease using radioscintigraphy [57], but not using real-time ultrasonography [58]. Gall bladder emptying is decreased in patients with Crohn's disease but, unlike ulcerative colitis, it is not related to colectomy [59].

Effect of inflammation on smooth muscle contraction

In vitro studies have shown an increase in the contractile response of intestinal muscle to agonists such as acetylcholine and histamine in Crohn's disease [60]. In ulcerative colitis, there is a decrease in muscle contractility due in part to altered neural input to the gut from both enteric and autonomic nerves [61,62].

Mechanisms underlying inflammation-induced altered muscle contraction have been reviewed [63]. From work performed largely in models of acute inflammation, in many cases based on nematode infection or hapten-induced colitis, the following concepts have emerged. Changes in muscle contractility occur with superficial inflammation and without overt infiltration of the neuromuscular layers. [64]. Changes in muscle function occur at non-inflamed sites distant from the site of inflammation [65]; this is important considering the extensive motility

changes that have been identified in IBD described above. Changes may persist after resolution of the inflammation [66]. Numerous cell types and their products have been shown to influence muscle contractility, including polymorph leukocytes [67], mast cells [68] and T lymphocytes[69]. Th1 and Th2 cytokines appear to have opposing effects on smooth muscle contractility [70], but this concept needs to be examined in models of Th1- and Th2-mediated inflammation. Certainly, in Th2-driven inflammation associated with nematode infection, there is hypercontractility of muscle similar to that seen on exposure to IL-4 or -13, is dependent on signal transducer and activator of transcription factor 6 (STAT-6), which is necessary for the effect of many Th2 cytokines [71].

More recent work has examined the impact of chronic inflammation on muscle contractility and have shown changes in ileal muscle evident after 12 weeks of inflammation induced by *Schistosoma mansoni* in mice [72], and time-dependent changes have also been shown in a rat model of colitis [73]. Others have examined the effects of repeated episodes of acute inflammation and have shown that the impact of an acute inflammatory response occurring in a naïve intestine differs from that occurring in a previously inflamed intestine [74,75]. These findings are important in our understanding of motility changes seen in chronic relapsing IBD in humans and merit further investigation.

Several mechanisms contribute to altered muscle contractility. Although some changes are receptor mediated, post-receptor mechanisms are likely to be more important given the broad range of agonists involved. Thus, described changes in the sodium–potassium ATPase [76] and in contractile proteins [77] are the probable basis for hypercontractility, whereas changes in ion channels may contribute to hypocontractility of muscle [78].

Effect of inflammation on efferent nerves

The enteric nervous system plays a crucial role in regulating and coordinating gut physiology. Inflammation-induced changes in enteric nerves are likely to have a widespread effect on gut function and this is evident from studies in animal models. The human literature contains numerous reports of changes in the structure, appearance or neurotransmitter content of the gut in IBD, but the data are often conflicting (for a comprehensive review, see [79]). These discrepancies may be attributed to two factors. The first is the wide difference in techniques used to identify and quantitate nerves in inflamed tissue, the second is the fact that the involvement of nerves by the inflammatory process is patchy and depends on the severity and probably the nature of the inflammatory infiltrate [49,80–82]. Studies in animal models provide clear demonstrations that various cell types in the inflammatory response confer different changes in neuromuscular function [83,84]. It is therefore difficult to generalize about the profile of neurotransmitter changes in IBD. There appears to be an increase in inhibitory nerves in ulcerative colitis, resulting in the observed decrease in motor activity. Whereas reports on the role of vasoactive intestinal peptide (VIP) are conflicting, there appears to be some agreement that nitric oxide is an important mediator of this increased inhibition [50]. With respect to excitatory neurotransmitters, there appears to be agreement on a reduction in cholinergic innervation, in agreement with animal models, but changes in other transmitters such as substance P (SP) are unclear. There are conflicting reports of decreases [49] or increases [85] in SP, and also of increased SP binding sites in IBD [86].

Neurally mediated alterations in gut physiology in IBD may also be due to remodeling rather than injury to nerves. There is growing appreciation of the plasticity of the nervous system and the ability of inflammatory or immune mediators to modulate this [87]. Work on animal models suggests that this occurs during intestinal inflammation [88] and there are a number of observations that provide functional correlation of this plasticity, an example of which is provided in the post-inflammatory remodeling of the enteric nervous system following experimental colitis in the rat [74].

Effect of inflammation on enteroglia

There is increasing acknowledgment of the role of glial cells in mediating neural changes in inflammatory processes in the gut. This was prompted by the report of Geboes *et al.* showing MHC II expression by glial cells in the enteric nervous system in IBD, suggesting immune-mediated injury [88]. Studies using isolated enteroglia demonstrate their ability to respond to, and also produce, cytokines [89,90]. This cell type is therefore strategically important in mediating neuro-immune interactions in the inflamed gut.

Effect of inflammation on sensory nerves in IBD

Although abdominal pain is common in IBD patients in the absence of obstructing lesions, underlying mechanisms are unclear. Although inflammation is a generally accepted mechanism for the induction of hyperalgesia in a variety of diseases, is readily demonstrable in animal model systems, the literature with respect to IBD is conflicting. The inability of patients with active colitis to tolerate balloon distension of the rectum is a longstanding observation [91] and has been interpreted to represent visceral hyperalgesia. However, more recent work from one group using graded distension protocols have identified increased thresholds for pain perception in both the intact colon and in patients with ileo-anal pouches [92–94]. In addition, IBD patients exhibit a greater tolerance of somatic pain [95] and do not have features of chronic widespread pain [96]. The basis for this apparent discrepancy between demonstrations of visceral hyperalgesia and increased

visceral pain thresholds is not immediately clear but may reflect differences in experimental protocol and also the fact that hyperalgesia was demonstrated in patients with active disease whereas more recent studies have focused on patients with less active chronic disease. The authors interpreted the increased pain threshold on the basis of increased descending spinal inhibitory pathways [94]. It is also entirely possible that certain types of inflammation may reduce pain sensitivity [97], via the production of endorphins at the site of injury [98,99]. This is supported by recent studies in comparing pain responses to balloon distension of the colon in mice with acute or chronic colitis induced by oral dextran sodium sulfate (DSS). In acute colitis, there is evidence of hyperalgesia and the underlying inflammatory response is predominantly granulocytic. In chronic DSS colitis lasting over 3 weeks, the acute inflammatory infiltrate is no longer dominant and is replaced by lymphocytes and plasma cells. In this environment, sensory nerve function is suppressed and there is hyporesponsiveness to balloon distension [100]. Further investigation showed that lymphocytes produce the analgesic β-endorphin [101] and that this almost certainly accounts for the hypo-responsiveness seen in the chronic colitis model. Interestingly, in mice with severe combined immune deficiency (SCID) there is hyperalgesia in the absence of inflammation. This could be normalized by reconstituting SCID mice with $CD4^+$ cells, thus confirming an anti-nociceptive role of T lymphocytes in the gut [102].

Effect of inflammation on the autonomic and central nervous systems

The autonomic nervous system modulates all aspects of gut physiology and available evidence indicates that this regulation is altered in IBD. First, there are morphologic data demonstrating changes in sympathetic and parasympathetic nerves in IBD (for a review, see [79]). Second, functional studies have shown that in Crohn's disease there is sympathetic dysfunction whereas in ulcerative colitis there is evidence of vagal dysfunction and consequent sympathetic dominance [103–105]. With respect to the central nervous system, there is a report of small structural defects in the white matter in some patients with IBD [106]. The nature and the significance of this finding remain unclear, however. There is nevertheless growing awareness of the role of the nervous system to modulate intestinal inflammation (see Part II).

Effect of inflammation on interstitial cells of Cajal

It is apparent that the interstitial cells of Cajal (ICCs) play an important role in the control of gastrointestinal motility; these cells serve as pacemaker cells [107]. The absence of these cells results in a major disruption of motility in animals [108,109] and in humans [110]. In animal models of intestinal inflammation, there is evidence of structural damage and functional impairment of these cells [111]. Recent observations have shown that there is structural damage to the ICCs in the colon of patients with ulcerative colitis and this almost certainly contributes to altered colonic motility seen in these patients [112]. Because of their strategic role in the control of motility, ICCs would be ideal targets for therapy aimed at directly correcting motility patterns in IBD.

Effect of inflammation on entero-endocrine cells (EECs)

Local and systemically produced hormones constitute another mechanism by which gut physiology is regulated. Important among these hormones is serotonin, the highest concentration of which is found in the gut, in the colon in particular. One report in patients with ulcerative colitis suggests a significant decrease in the enterochromaffin cells in the colonic mucosa [113].There is a single report of an increase in enterochromaffin cells in an animal model of colitis [114]. Recent reports of "ischemic" colitis occurring in patients receiving serotonin antagonists for the treatment of irritable bowel syndrome raise the possibility of a linkage between serotonin and gut defense. Recent studies have shown that the upregulation of EE cells and 5-HT secretion during intestinal inflammation is T cell mediated [115,116], influenced by the Th1 and Th2 bias of the immune response [117].

Part II. The role of physiological systems in modulating intestinal inflammation

Epithelial cell as a participant in the immune response

The discussion above illustrates how the inflammatory and immune response can alter intestinal epithelial cell function, contributing to the pathophysiology of the clinical syndrome of IBD. At the same time, it has become clear that the intestinal epithelial cell can contribute to the local immune and inflammatory response in the intestine.

Antigen presentation

Intestinal epithelial cells express the MHC class II molecule in both rodent and humans [118,119]. This cell surface molecule is associated with the ability of cells such as macrophages, B cells and dendritic cells to present antigen to T cells. *In vitro* evidence suggests that intestinal epithelial cells can serve in Ag presentation [120,121]. MHC class II expression on intestinal epithelial cells is stimulated by IFN-γ [122]. Intestinal epithelial cells preferentially stimulate CD8 T cells, i.e. the suppressor/cytotoxic T cell subset. One can speculate that in this way epithelial cells help downregulate the mucosal immune response [123]. Epithelial cell interaction with CD8 T cells leads to CD8-associated p56lck activation [124]. The CD8

binding ligand is a 180 kDa glycoprotein that has homology with carcinoembryonic Ag [125]. In patients with IBD, the type of MHC class II molecules expressed are altered [126], as is the antigen-presenting ability of epithelial cells. IBD-derived epithelial cells preferentially stimulate CD4 helper T cells, which might contribute to or enhance the inflammatory response [127]. Other accessory molecules identified on colonic epithelial cells may contribute to this stimulation of CD4 T cell proliferation [128]. Therefore, in IBD the epithelial cell contributes to the initiation and possibly the perpetuation of the local inflammation.

Interaction between epithelial cells and lymphocytes

Mucosal mast cells have unique mediator content and function differently to connective tissue mast cells [129]. Mucosal tissue also attracts unique subsets of T cells such as those bearing the γ/δ T cell receptor [130,131]. In addition, mucosal B cells also include unusual populations that preferentially produce and secrete IgA selectively recognizing gut flora-related Ag [132]. These unusual phenotypes are a result of local influences in which cytokines and growth factors favor the development of immune effector cells specially adapted to the mucosa. For example, epithelial cells express cell surface markers that allow for specific interactions with local lymphocytes. In addition to MHC class II molecules, these include CD1 [121,133] and adhesion molecules, such as ICAM-1 [134]. Furthermore, the IEL adhesion molecule ligand CD105 serves to maintain epithelial integrity [135,136]. In addition to the ability of epithelial cells to influence local lymphocyte traffic and function, epithelial cells also influence local T cell differentiation. The gut mucosal immune system has long been considered a primary lymphoid organ, primarily for B cell development [137]. It is also evident that interactions between epithelial cells and lymphocytes derived from Peyer's patches induce the differentiation of epithelial cells into M cells [138]. This is in keeping with the notion that the intestine is an important site for T cell development [139–141]. Evidence has shown that intestinal T cell subsets can develop extra-thymically [131,141] and that *in vitro* epithelial cell lines can influence bone marrow-derived T cell differentiation [142]. The presence of intestinal T cells with unique phenotypic characteristics in humans suggest that a similar thymus-independent lineage exists in humans [143] The significance of an intestinal-derived T cell lineage is that the intestinal environment controls the development of the T cell repertoire. Changes in this environment might then alter T cell repertoires with potential increases in autoreactivity.

Cytokine production

Epithelial cells produce and secrete a myriad of cytokines, which could influence the local immune response. Cytokine production by epithelial cells of the thymus, lung, nasal passageways and kidney suggests a functional potential of epithelial cells in general. Some of these cytokines are produced in response to inflammation or normal bacterial products and others reflect constitutive abilities. The list of cytokines produced by gastrointestinal epithelial cells include IL-1α, ILβ, TNFα [144,145], granulocyte macrophage colony-stimulating factor (GM-CSF), G-CSF and IL-6 [146] and IL-8 [147,148]. The ability of EC to produce IL-8 has been of particular interest because IL-8 serves as a chemotactic factor for neutrophils. It is probable that this cytokine is involved in the pathogenesis of crypt abscess and also lamina propria inflammation. Other cytokines produced by epithelial cells include IL-7, an important regulator of mucosal inflammation [149]. Loss of IL-7 function in mice leads to the development of colitis [150]. Clearly, epithelial cells have a significant potential to influence the local inflammatory and immune response.

Immunoglobulin receptors

Secretory component (SC) is an epithelial cell receptor for IgA and IgM and functions in the directional transport of polymeric Ig from lamina propria to the intestinal lumenal side of epithelial cells. SC is upregulated by IFN-γ [151] and TNFα [152]. Presumably, the increased SC expression would allow for increased transport of protective IgA into the intestinal lumen in response to inflammation [153]. More recent work has shown that other Ig receptors on epithelial cells can serve as Ag-specific receptors and lead to selective and specific recognition and uptake of luminal proteins [21].

Epithelial–bacterial interactions

Invasive bacteria induce the expression of ICAM-1 on epithelial cells, leading to an increase in neutrophil adhesion [154]. In addition, bacterial infection leads to an increase in the expression of a host of epithelial-derived cytokines [155]. Bacterial pathogens such as *Helicobacter pylori* induce epithelial cell production of cytokines such as IL-8 [147,156]. The induction of cytokine production by epithelial cells is in part a result of direct bacterial adhesion and in part due to soluble bacterial factors such as bacterial chemotactic peptide *N*-formylmethionylleucylphenylalanine (FMLP). Receptors for FMLP have been identified in the subepithelial layer of the gastrointestinal mucosa [157]. Studies have showed that pathogens such as Hp and *Escherichia coli* strains can inject bacterial proteins via type IV secretory mechanisms in host epithelial cells [158]. The injected protein, in the case of Hp cagA, undergoes tyrosine phosphorylation within the host cells, allowing for changes in host cell intracellular signaling [158].

Human and animal studies suggest that non-pathogenic gut bacteria may be involved in the pathogenesis of IBD [159,160]. Intestinal epithelial cell responses to lumenal bacterial flora may contribute to inflammation through the

release of proinflammatory cytokines [161,162]. Epithelial cells constitutively express Toll-like receptors (TLRs), pathogen pattern receptors which are key regulators of innate immune response to bacteria. Changes in the profile of these receptors in patients with IBD may explain differences in responses to normal gut flora in patients with IBD [163]. Mice lacking TLR accessory signaling molecule MyD88 are unable to signal in response to TLR agonists and are more susceptible to colitis induced by dextran sodium sulphate, suggesting that these innate immune molecules play an important role in gut homeostasis in response to commensal flora [164]. More recently, mutations in the NOD-2 gene, responsible for an intracellular receptor for bacteria-derived products (MDP) that can induce NF-κB activation, has been identified as the IBD1 locus gene in patients with Crohn's disease [165,166]. How changes in this gene alter the innate immune response of epithelial cells to normal gut flora and leads to the chronic inflammation seen in patients with Crohn's disease is the subject of intense investigations [167]. Other epithelial-derived mechanisms involved in the innate response to gut flora include defensins, a series of antibacterial peptides produced by Paneth cells and other epithelial cells [168–170]. Future studies are required to define the role of these peptides in the pathogenesis of IBD.

Clinical implications of altered epithelial function in IBD

The mechanisms by which cells of the immune system and the intestinal epithelial cell interact have given us new avenues to explore in our attempt to understand the pathophysiology of IBD and also in our attempt to design new forms of treatment. The challenge is to define which alterations in cytokines are important for the development of IBD (reviewed previously [171]). Such studies will allow for the development of new therapeutic strategies. The potential for treatment of IBD patients with biological reagents such as monoclonal Ab infliximab or adalimumab (anti-TNFα) has already altered the way we treat IBD patients with the most severe disease. For example, work on the epithelial-derived cytokine IL-18 in Crohn's disease [172–175] has led to the development of neutralizing Ab for clinical studies [176]. More recent work that brings together genetic studies in patients with studies focusing on cytokines shown to be critical for colitis induction in animal models, i.e. IL-12/IL-23, have now generated new biological agents with clinical efficacy in patients with Crohn's disease [177]. It is clear that these studies are beginning to extend our developing insight into mechanisms of inflammation to new highly targeted and effective therapies for IBD patients.

The neuro-motor apparatus as a participant in the immune response

It is important to recognize that there is bidirectional communication between the nervous and immune systems and, although it is clearly established that nerves are altered by inflammatory processes, it is also now evident that nerves may influence the inflammatory response. Perhaps the most dramatic example of this comes from a case report in which a patient with previously stable ulcerative colitis underwent implantation of a stimulating electrode into the dorsal horn of the spinal cord to control peripheral neuropathic pain. Stimulation of the cord reliably induced flares of the previously quiescent ulcerative colitis [178]

Data from animal models provide clear demonstrations of the role of several components of the nervous system in modulating intestinal inflammation, in either a deleterious or protective manner. For example, sensory neural circuits are protective, as reflected by the deleterious effect of ablation of primary afferent nerves using capsaicin [179,180]. In contrast, sympathetic nerves appear to be pro-inflammatory, as deflected by the amelioration of inflammation following chemical sympathectomy[145]. The latter observation may explain the reported benefit of clonidine in colitis [181]. The apparent benefit of local anesthetics in colitis [182] is not, however, explained on the basis of animal studies showing a protective role of sensory nerves [183]. More recent work has shown that the integrity of enteroglial cells is a critical component of host defense as the genetic ablation of these cells resulted in a fulminant enterocolitis in mice [184].

The autonomic nervous system

Recent work suggests that sympathetic dominance or parasympathetic impairment in ulcerative colitis is more prominent in patients with limited distal disease [105]. It is possible that the apparent benefit of nicotine in ulcerative colitis is due to balancing of autonomic input to the inflamed colon in the face of sympathetic dominance, as data from experimental models suggest that sympathetic nerves play a pro-inflammatory role [185]. The benefit of nicotine might be more apparent in studies in which patients are identified on the basis of their autonomic profile.

The counter-inflammatory action of the vagus

Evidence has emerged showing that the vagus nerve inhibits the release of pro-inflammatory cytokines by intestinal macrophages and attenuates the response to systemic LPS [186,187]. In further work, it has been shown that the vagus works as a tonic inhibitory modulator of intestinal inflammation, rather than as a short-lived reflex response [188]. The molecular basis for this effect is via the release of acetylcholine which interacts with the α7 sub-unit of the nicotinic acetylcholine receptor on macrophages. These findings offer a new therapeutic pathway for treating IBD by the use of α7 agonists, particularly in those IBD patients with demonstrable autonomic imbalance and parasympathetic impairment.

The role of depression

Depression is common in IBD, particularly in Crohn's disease, where it is either a result of the debilitation of the disease or a coexisting primary disorder [189]. Either way, depression may influence the natural history of IBD. Recent animal-based work shows that in a model of depression induced in offspring by maternal separation early in life, there is increased susceptibility to intestinal inflammatory stimuli which is mediated, at least in part, by a reduction in mucosal barrier function. Interestingly, the vulnerability to inflammation was attenuated by tricyclic antidepressants, which, in this study, had no counter-inflammatory properties in non-depressed mice with colitis [190]. This work was extended using adult mice in which depression was induced by the intra-cerebroventricular administration of reserpine. The increased vulnerability to colitis could be attenuated by tricyclic antidepressants but only if the vagus nerve was intact. It could also be attenuated with nicotine. These findings indicate that depression impairs the counter-inflammatory effects of the vagus, resulting in greater secretion of pro-inflammatory cytokines by macrophages [191].

The role of stress

Stress is often linked to relapses of IBD, particularly ulcerative colitis [192,193]. There is evidence that the central nervous system also influences intestinal inflammation in the context of stress. It is generally acknowledged that stress plays a role in relapses of IBD [194,195] and recent work in animal models shows that stress may enhance inflammatory responses [196] or reactivate inflammation in mice recovered from previous colitis [197]. The susceptibility to reactivation by stress could be adoptively transferred to naïve mice via $CD4^+$ lymphocytes and was associated with a reduction in mucosal barrier function.

Taken together, these observations indicate that close monitoring and management of stress and depression in IBD patients may result in a more stable clinical course of their inflammatory disease.

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Chapter 14
Extraintestinal Consequences of Mucosal Inflammation

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Summary
- Constitutional symptoms such as fatigue, anorexia, fever and arthralgias are the result of the systemic effects of proinflammatory cytokines produced in the IBD mucosa.
- Anemia frequently complicates the course of IBD and is usually multifactorial with iron deficiency, vitamin B_{12} deficiency, anemia of inflammation, medications or combinations of these being the most common causes.
- IBD is characterized by a hypercoagulable state and patients are at risk of developing thromboembolic events.
- IBD carries a higher risk of hematologic malignancies, particularly lymphoma, in patients receiving immunosuppressive or anti-TNF treatment.
- Amyloidosis may rarely complicate the course of IBD.

Introduction

Changes in the concentration of many plasma proteins, known as the acute phase proteins, and several neuroendocrine, metabolic and hematopoietic alterations, collectively termed the acute phase response, comprise the systemic response to inflammation [1]. Tissue injury, such as trauma, ischemia, burns, infections, malignancy and autoimmune diseases, characterize the acute phase response. These symptoms correlate with molecular changes in the levels of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6, although other cytokines and chemokines also mediate the acute phase response. Constitutional symptoms directly or indirectly attributed to the side effects of these proinflammatory cytokines include anorexia, malaise, fatigue, fever, myalgias, arthralgias, night sweats, weight loss and cachexia.

TNF, IL-1 and IL-6 are secreted during inflammation in that order [2,3]. Although many cytokine effects are predominantly paracrine and autocrine, they do mediate systemic effects [4]. For example, central infusion of TNF led to predominant anorexia whereas peripheral production of TNF produced predominant metabolic losses of protein [5,6].

TNF, a 17 kDa protein, is produced by cells of hematopoietic lineage in response to several stimuli such as bacterial pathogens and lipopolysaccharide. It is first produced as a membrane-bound protein of 26 kDa, which is cleaved to the mature form by the TNFα-converting enzyme. It has several biological effects, depending on the amount and the rapidity by which it is produced in response to a specific stimulus. Shock and tissue injury, vascular leakage syndrome, acute respiratory distress syndrome (ARDS), gastrointestinal necrosis, acute tubular necrosis, adrenal hemorrhage, disseminated intravascular coagulation and fever result from high levels of TNF that are produced acutely. Chronic low-dose exposure to TNF leads to acute phase protein release and endothelial activation, weight loss, anorexia, protein catabolism, lipid depletion, hepatosplenomegaly and insulin resistance [6–8].

The IL-1 family consists of three proteins, IL-1α, IL-1β and the IL-1 receptor antagonist (IL-1ra). IL1ra acts as an inhibitor of IL-1 signaling [9,10]. IL-1α and IL-1β are synthesized as precursors and are cleaved to the mature forms by the action of ICE (caspase-1]. IL-1 functions as a lymphocyte activating factor by enhancing the production of IL-2 and IL-2 receptors by T lymphocytes. IL-1 stimulates early bone marrow hematopoietic progenitor cell proliferation by synergizing with various colony-stimulating factors. IL-1 and TNF share numerous biologic activities and frequently act synergistically. In joints, IL-1 stimulates synovial cell proliferation, cartilage and bone resorption and collagen deposition and it stimulates the catabolism muscle, contributing to the myalgias and arthralgias associated with illness. Many of the proinflammatory activities of IL-1 relate to the generation of small

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mediator molecules, frequently in synergy with TNF, such as platelet-activating factor and leukotrienes, prostanoids, nitric oxide and chemokines. IL-1 has several proinflammatory activities, such as induction of fever, slow wave sleep, anorexia and neuropeptide release. Overwhelming expression of IL-1 and other proinflammatory cytokines can lead to hypotension, myocardial suppression, septic shock and death [11]. Humans injected with IL-1 experience fever, headache, myalgias and arthralgias, each of which is reduced by the co-administration of COX inhibitors [12].

IL-6, a 26 kDa protein produced by a wide variety of cells, is one of the principal mediators of the clinical manifestations of tissue injury, including leukocytosis, thrombocytosis, increased plasma levels of acute phase proteins, decreased plasma levels of albumin fever and cachexia. It is a pleiotropic cytokine with both proinflammatory and anti-inflammatory properties. IL-6 also stimulates plasmacytosis and hypergammaglobulinemia and activates the hypothalamic–pituitary–adrenal axis [2]. In addition to its immunologic/inflammatory role, IL-6 may play an important role in bone metabolism, spermatogenesis, epidermal proliferation, megakaryocytopoiesis and neural cell differentiation and proliferation. In addition to differentiating B cells, IL-6 stimulates proliferation of thymic and peripheral T cells. Along with IL-1, IL-6 induces T-cell differentiation to cytolytic T cells and activates natural killer cells. These observations emphasize the importance of IL-6 in both innate and adaptive immunity. Lymphoproliferative disorders, multiple myeloma, osteoporosis and Alzheimer's diseases have been linked to the age-associated rise in IL-6 [13]. IL-6/IL-6R signaling has been shown to be crucial in liver regeneration following hepatectomy [14,15]. In addition to IL-6 itself, the IL-6 family of cytokines comprises IL-11, ciliary neurotrophic factor, cardiotropin, oncostatin M, leukemia inhibitory factor and neurotrophin 1/B cell stimulating factor 3, all of which share the common signal transducer gp130 as part of their receptors [15].

Elevated mucosal and serum levels of several proinflammatory cytokines, including IL-1 and TNF, have been observed in patients with Crohn's disease (CD) and ulcerative colitis (UC). IL-6 serum levels have been reported to be elevated in active CD, but not in UC, whereas elevated circulating levels of IL-6 receptor (IL-6R) have been detected in active stages of both diseases [16]. Increased serum levels of IL-8 have also been reported in active UC but not in CD [17]. A triad of features that are persistent and progressive characterize about 90% of patients with CD, namely diarrhea, abdominal pain and weight loss [18]. About 40% of patients with UC experience noticeable weight loss [19]. Cachexia, the loss of body mass that occurs in severe chronic inflammatory disease, results from decreases in skeletal muscle, fat tissue and bone mass [20]. Cytokines such as IL-1, IL-6, TNF and IFN-γ contribute to these processes [21]. Investigators have found a link between inflammatory cytokines and muscle damage. TNFα and IFN-γ are both activators of NF-κB in muscle. Activation of NF-κB results in the decreased expression of MyoD, a transcription factor that is essential for repair of damaged skeletal muscle. Thus, the cachexia that develops in patients with cancer and other high TNFα states may be explained by the defective muscle repair, which is the net effect of TNFα and IFN-γ is defective muscle repair. Although cachexia is characterized by hypermetabolism, defined as an elevation in resting energy expenditure, in CD patients without malabsorption, short-term weight change is more closely related to decreased caloric intake than to increased resting energy expenditure. Although some patients hesitate to sit due to anticipated abdominal pain and this may contribute to weight loss, it more often relates to the severity of anorexia. Anorexia is one of the most common symptoms associated with acute illness and results from proinflammatory cytokine activity and has both central and peripheral elements [4]. Several cytokines affect food intake directly or indirectly, with effects on other mediators such as corticotrophin-releasing hormone, serotonin, cholecystokinin, neuropeptide Y, insulin or leptin. Binding sites for cytokines are contained by a number of hypothalamic nuclei involved in eating behavior [22]. In animals, endotoxin increases the plasma levels of leptin and white fat leptin mRNA, suggesting that leptin may be a mediator of anorexia in inflammatory states [4,23]. Altered gastric emptying, decreases in intestinal blood flow, changes in small bowel motility and cellular proliferation and altered ion fluxes have been ascribed to proinflammatory cytokines [4].

Fever, usually low grade, affects a significant percentage of patients with active CD and UC [19,24]. Several cytokines, including IL-1α, IL-1β, TNF, lymphotoxin α (LTα), IFN-α and IL-6, are intrinsically pyrogenic in that they act directly on the hypothalamus without the requirement for the formation of another cytokine to produce a rapid-onset fever. Several other cytokines that use the gp130 signal transducer as part of their receptor, as mentioned earlier, may also contribute to the febrile response [25]. Pyrogenic cytokines released during the inflammatory response interact with a rich vascular network close to the cluster of neurons in the preoptic/anterior hypothalamus called the circumventricular organs or organum vasculosum laminae terminalis (OVLT), possess little if any blood–brain barrier. It is likely that endothelial cells lining the OVLT either offer no resistance to the movement of pyrogenic cytokines into the brain or release arachidonic acid metabolites which then may diffuse into the preoptic/anterior hypothalamic region to induce fever. Alternatively, prostaglandin E2 (PGE2) and other prostaglandins may be produced by the endothelial cells, which, in turn, induce a neurotransmitter-like substance, such as cAMP, that acts to raise the set-point [25].

Acute phase proteins

Acute phase proteins are those for which plasma concentrations increase (positive acute phase proteins, such as CRP and SAA) or decrease (negative acute phase proteins, such as albumin) by at least 25% during inflammation, resulting from changes in their production by hepatocytes [20]. Operating both as a network and a cascade, inflammatory cytokines stimulate the production of acute phase proteins include IL-6, IL-1β, TNF, IFN-γ, TGFβ and IL-8 [20]. IL-6 is a major hepatocyte stimulator and produces a variety of acute phase proteins in the liver of experimental animals and in cultured human hepatocytes, SAA and CRP being most induced [26]. IL-6 and glucocorticoids act synergistically in stimulating the production of acute-phase proteins. Synthesis of acute phase proteins in IL-6 knockout mice is greatly impaired in response to nonspecific irritants, such as turpentine, but is normal when bacterial lipopolysaccharide is the inflammatory stimulus [27]. Inactivation of gp130 in adult mice decreases the ability of these mice to synthesize acute phase response proteins similarly to the IL-6 knockout mice [28]. Several components of the complement system are included among the acute phase proteins and are involved in the accumulation of phagocytes at an inflammatory site and the killing of microbial pathogens. CRP activates the complement system, binds various pathogens and materials from damaged cells and promotes opsonization of these materials and activates the complement system. CRP enhances opsonization and phagocytosis of apoptotic cells by macrophages associated with the expression of the anti-inflammatory cytokine TGFβ. The classical complement components and CRP act in concert to promote noninflammatory clearance of apoptotic cells [29]. In this context, production of acute phase proteins by proinflammatory cytokines can be viewed as a protective host defense mechanism that limits tissue injury [2]. Acute phase protein production, however, is not uniformly beneficial. Secondary amyloidosis is a harmful consequence of elevated SAA concentrations in some patients with chronic inflammatory conditions, including inflammatory bowel disease (IBD) [30]. Several acute-phase proteins that correlate with disease activity have been measured in IBD, including CRP, oromucoid (α_1-acid glycoprotein), serum and fecal α_1-antitrypsin, β_2-microglobulin, phospholipase A2 and the ESR [16].

Inhibiting the action of several proinflammatory cytokines further demonstrates their action in human diseases. For example, patients with lymphoma who experience fever, weight loss and night sweats (B symptoms) exhibit significantly higher levels of serum IL-6 levels than patients without B symptoms [31]. Anti-TNF treatment in CD and rheumatoid arthritis clinical improvement has been associated with a decrease in the levels of CRP and IL-6, respectively.

Table 14.1 Systemic effects of proinflammatory cytokines.

Inflammatory mediator	Systemic manifestations
TNF	Fever, cachexia, anorexia, protein catabolism, insulin resistance, activation of HPA axis
IL-1	Fever, myalgias, arthralgias, bone resorption, anorexia, induction of acute phase proteins, activation of HPA axis
IL-6	Fever, fatigue, leukocytosis, thrombocytosis, induction of acute phase proteins, activation of HPA-axis

The systemic effects of key pro-inflammatory cytokines overproduced in IBD are summarized in Table 14.1.

Hematologic consequences of intestinal inflammation

Anemia

In patients with IBD, anemia is multi-factorial and can contribute to poor quality of life [32,33]. Some 30–50% of patients with IBD are anemic [34–36] and the anemia may occasionally pre-date the development of gastrointestinal symptoms, especially in children [37–39]. Studies performed in CD patients with anemia reveal that correction of the underlying anemia improves quality of life scores, especially the feeling of wellbeing, mood, physical ability and ability to perform social activities, supporting the idea that anemia is an important cause of constitutional symptoms [33]. This section focuses on the effect of chronic intestinal inflammation on red blood cell homeostasis and treatment of the common causes of anemia.

In IBD, the distinction between iron deficiency anemia and the anemia of chronic disease (ACD) is important, since both conditions typically overlap. The identification of hepcidin, an iron-regulated acute phase protein that is composed of 25 amino acids, helped to shed light on the relationship of the immune response to iron homeostasis and ACD. Hepcidin expression is induced by lipopolysaccharide and IL-6 and is inhibited by TNFα [40]. Transgenic or constitutive overexpression of hepcidin results in severe iron deficiency anemia in mice [41]. Inflammation in mice that are hepcidin deficient did not lead to hypoferremia, a finding that suggests that hepcidin may be centrally involved in the diversion of iron traffic through decreased duodenal absorption of iron and the blocking of iron release from macrophages that occurs in ACD [40,42]. The induction of hypoferremia by IL-6 and hepcidin occurs within a few hours and is not observed in IL-6 knockout mice that are treated with turpentine as a model of inflammation, a finding that suggests that hepcidin may be central to anemia of chronic disease [43]. A recently

identified gene, hemojuvelin, may act in concert with hepcidin in inducing these changes [44].

Intestinal ulceration whether related to UC or CD is associated with increased intestinal blood loss, both microscopic and macroscopic, and iron deficiency anemia [35]. Iron deficiency anemia can be assessed by measuring iron and total iron binding capacity (TIBC) levels which should reflect low iron saturation, <15%. Ferritin levels may be falsely elevated because of inflammation but low levels (<5 $\mu g\ l^{-1}$) are indicative of iron deficiency [45]. In patients with active IBD, certain cytokines or hepcidin may reduce iron absorption, retain iron within cells of the reticular–endothelial system and inhibit erythropoiesis. ACD is likely if the serum ferritin is >100 $\mu g\ l^{-1}$ and TIBC >15%. In mice that are injected with the proinflammatory cytokines IL-1 and TNFα, both hypoferremia and anemia develop [46,47]; this combination of conditions has been linked to cytokine-inducible synthesis of ferritin, the major protein associated with iron storage, by macrophages and hepatocytes [48]. In chronic inflammation, the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis [49] and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1) [50]. IFN-γ, lipopolysaccharide and TNFα upregulate the expression of DMT1, with an increased uptake of iron into activated macrophages [51]. These proinflammatory stimuli also induce the retention of iron in macrophages by downregulating the expression of ferroportin, thus blocking the release of iron from these cells [51]. Ferroportin is a transmembrane exporter of iron, believed to be responsible for the transfer of absorbed ferrous iron from duodenal enterocytes to the circulation [52]. Moreover, anti-inflammatory cytokines such as IL-10 can induce anemia through the stimulation of transferrin-mediated acquisition of iron by macrophages and by translational stimulation of ferritin expression [53]. Poor iron absorption in the proximal gastrointestinal tract can contribute to the anemia of patients with CD and rapid intestinal transit in both CD and UC can impair oral iron replacement therapy. In IBD, non-absorbed ferrous iron has the potential to worsen IBD symptoms and may aggravate intestinal inflammation through Fenton's reaction $\{FN^{*}\}$, which releases reactive oxygen species [54,55].

In an open-label study performed in patients with UC and anemia, defined as hemoglobin <10.5 $g\ dl^{-1}$, investigators administered iron saccharate over an 8 week period [56]. Mean hemoglobin increased by 3.6 $g\ dl^{-1}$. Only four patients out of 22 did not respond to intravenous iron. This latter group was given erythropoietin and achieved a mean hemoglobin increase of 3.3 $g\ dl^{-1}$. Similar results have been observed in patients with CD. Specifically, patients with CD and anemia who fail oral iron replacement or are intolerant of oral iron who are then given intravenous iron have a 75% response rate with mean hemoglobin increases of 3.3 $g\ dl^{-1}$ [33]. Intravenous iron, especially iron dextran, is associated with a low (<1%) but significant risk of anaphylactoid reaction, which manifests during the first few minutes of an infusion and resembles a type I (IgE-mediated) allergic reaction [57,58]. More commonly, up to 30% of patients given iron dextran develop arthralgias and fever within 24–48 h of initiation of intravenous iron therapy. Iron is an essential nutrient for proliferating microorganisms and the sequestration of iron from microorganisms or tumor cells into the reticuloendothelial system is believed to be a potentially effective defense strategy to inhibit the growth of pathogens [59]. A study investigating predictors of bacteremia among patients undergoing hemodialysis who are receiving iron parenterally showed that transferring saturation above 20% and ferritin levels >100 $ng\ ml^{-1}$ were significantly associated with a higher risk of developing bacteremia [60]. This could be explained by the fact that iron has an inhibitory effect on cellular immune function that can be traced back to downregulation of IFN-γ-mediated immune effector pathways [61]. In addition, iron therapy in a setting of long-term immune activation promotes the formation of highly toxic hydroxyl radicals that can cause tissue damage and endothelial dysfunction and may increase the risk of acute cardiovascular events [59,62,63].

Ileal disease and resections in patients with CD result in impaired vitamin B_{12} absorption, leading to a megaloblastic anemia. Indeed, up to 60% of patients with ileal CD who have never had an intestinal resection have evidence of vitamin B_{12} deficiency and megaloblastosis [64,65]. As little as 30 cm (1 ft) of terminal ileum resected can lead to vitamin B_{12} malabsorption, but in general more than 60 cm of terminal ileum must be resected for vitamin B_{12} malabsorption [66,67]. These patients require monthly injections of vitamin B_{12} (cyanocobalamin) (1000 μg) or weekly nasal delivery of topical vitamin B_{12} once stores have been replenished [68]. Rarely, patients with CD and short bowel who are dependent on total parenteral nutrition (TPN) may develop anemia and pancytopenia as a result of copper deficiency [69].

In IBD, anemia is generally mixed and may have features of anemia of chronic disease, which can be attributed to the increased production of the cytokines that contribute to the underlying IBD including TNFα, IL-1 and the interferons [70,71]. These cytokines have the effect of shortening red cell survival, blunting the erythropoietin response to anemia, impairing erythroid colony formation in response to erythropoietin and abnormal mobilization of reticuloendothelial iron stores. The degree of anemia in patients with IBD correlates with underlying disease activity and systemic levels of IL-1β [72]. Patients with IBD have inappropriately low levels of erythropoietin for their degree of anemia and therefore cannot utilize iron appropriately [70,72]. Erythropoietin is a renally produced hormone that regulates red blood cell mass by preventing apoptosis of erythroid precursors [73]. The reason for

low erythropoietin levels in patients with IBD may be elevated systemic inflammatory cytokines, especially TNFα, IL-1, IL-6 and the interferons, which have been shown to decrease mRNA expression of erythropoietin [74–76]. In addition to causing lower than expected levels of erythropoietin, systemic inflammation leads to relative hyporesponsiveness to erythropoietin [77,78]. A study performed in UC and CD patients failing oral iron therapy showed that supplemental erythropoietin (150 U per kilogram of body weight twice per week) in combination with oral iron replacement (100 mg day^{-1}) resulted in an average hemoglobin increase of 1.7 g dl^{-1} over a 12 week period whereas the placebo-controlled, iron-only group experienced a decline in hemoglobin levels (–0.9 g dl^{-1}) [72]. Another study performed in patients with CD and anemia showed that intravenous iron alone or in combination with erythropoietin is effective at increasing hemoglobin levels [33]. Patients receiving erythropoietin had greater increases in hemoglobin levels than the placebo group (4.9 compared with 3.3 g dl^{-1}). Importantly, patients whose anemia responded to therapy had an improved quality of life, suggesting that anemia does contribute to the constitutional symptoms experienced by these patients. Therefore, in patients with IBD and anemia who have not responded to oral iron replacement, iron levels should be evaluated. Erythropoietin is not effective in patients whose iron stores are not replete and is the most common cause of erythropoietin resistance [79]. The erythropoietic response to iron is considered appropriate if the hemoglobin concentration increases by at least 2 g dl^{-1} or reaches normal within 4 weeks of treatment [80]. If iron stores are normal, patients should be treated empirically with weekly erythropoietin. A response to oral iron with or without supplemental erythropoietin is generally obtained within 8 weeks of therapy. If iron stores are reduced, intravenous iron can be used alone or in combination with erythropoietin to achieve a clinical effect. Using a transferrin saturation (TfS) >50% as a guide to stop therapy, 3600 mg of iron sucrose has been administered safely in controlled trials without liver damage or iron overload. The risk of iron overload can be considered very low in a population with ongoing blood loss [81,82]. Various intravenous iron products are currently available with differences in biochemical characteristics, side effects, dosing and country-to-country availability. High molecular weight iron dextran is obsolete, because of its potential to cause severe anaphylactic shock and associated mortality [83]. If oral iron is used, no reliable data exist to choose any one compound over another. Slow-release products should be avoided as they are released beyond the area of iron absorption and may impact or cause ulceration at Crohn's strictures [84]. The optimal dose of oral iron has still not been established. Since a maximum of 10–20 mg of oral iron can be absorbed per day, higher doses are questionable. Low-dose iron (100 mg elemental iron daily) is effective in other causes of iron deficiency [85,86].

The treatment of IBD may also result in anemia. Antimetabolites such as azathioprine and 6-mercaptopurine are purine antagonists and are thought to exert their beneficial effects by interfering with DNA synthesis. These drugs can cause a megaloblastic anemia that is generally dose related [87]. Both can cause dose-dependent bone marrow suppression by inhibiting DNA and RNA synthesis. Thiopurine methyltransferase (TPMT) converts 6-mercaptopurine to 6-methylmercaptopurine. TPMT is inherited in an autosomal co-dominant fashion such that patients that are heterozygous (14% of Caucasians) or homozygous (1% of Caucasians) for a mutant TPMT gene have diminished or absent ability to metabolize azathioprine or 6-mercaptopurine and are therefore at increased risk for developing hematologic toxicity [88–90]. In addition to an inherited inability to metabolize 6-MP/AZA, certain mesalamine-containing compounds have been described to inhibit the TPMT enzyme leading to bone marrow suppression [91–93]. For this reason, patients who are initiating 6-MP/AZA therapy or who are on a stable dose but have a recent addition of a 5-ASA product should be followed for the development of hematologic toxicity.

Methotrexate and sulfasalazine can interfere with folate metabolism, a requirement for nucleotide synthesis. For patients taking either sulfasalazine or methotrexate, folate supplementation is indicated. Methotrexate acts by inhibiting dihydrofolate reductase. 5-Formyl tetrahydrofolate (calcium leucovorin, citrovorum factor or folinic acid) administered 24 h after the administration of MTX can rescue normal cells from the effects of MTX by providing a reduced form of folic acid to the cells. Folate deficiency has been detected in patients receiving sulfasalazine for IBD and may be due to impaired absorption of folate [94]. Red cell folate levels correlate inversely with the dose of sulfasalazine [95]. Patients who are slow acetylators are also at increased risk for sulfasalazine-induced anemia [96]. In addition to the anti-metabolite effect of drugs for IBD, mesalamine and sulfasalazine have been associated with rare cases of aplastic anemia, thrombocytopenia and neutropenia [97–100].

Hemolytic anemia is perhaps the least common type of anemia in patients with IBD [101–103]. Hemolytic anemia develops in an animal model of UC in which animals are genetically unable to produce IL-2 (IL-2 –/–). Whereas the colitis in these animals is mediated by T cells, B cells are required for the anemia [104]. Although there are many case reports in the literature of UC associated with hemolytic anemia, it is not clear whether this complication is more common in patients with IBD than the general population [101,105]. In the largest series of patients reported, only eight out of 1150 hospitalized patients with UC (0.7%) were found to have autoimmune hemolytic anemia [101]. The highest incidence of hemolytic anemia was found

Table 14.2 Evaluation of anemia in patients with IBD.

Cause of anemia	Characteristics of anemia	Disease prevalence	Diagnostic tests	Treatment
Increased intestinal blood loss	Iron deficiency/microcytic	UC > CD	Serum Fe, TIBC ratio <15%; Ferritin <5 $\mu g\,l^{-1}$ (may be confounded by acute inflammation)	Oral iron replacement (ferrous sulfate 300 mg t.i.d.); if no response within 4 weeks, parenteral iron replacement*
Diminished iron absorption	Iron deficiency/microcytic	CD >UC	Serum Fe, TIBC ratio <15%	Parenteral iron replacement*
Drug-induced	Ineffective erythropoiesis/megaloblastic	Both	Normal iron stores, vitamin B_{12} and folate; drug withdrawal or dose adjustment improves anemia within 4 weeks	Reduce dose of anti-metabolite; supplement with folate (sulfasalazine or MTX); leucovorin (MTX)
Anemia of chronic disease	Normocytic or microcytic if mixed	CD > UC	Serum Fe, TIBC ratio >15% (serum erythropoietin levels are not useful)	Supplement with recombinant erythropoietin (150 U kg t.i.w. for up to 12 weeks or 10,000 U q week subcutaneously)
Vitamin B_{12} deficiency	Megaloblastic	CD (ileal disease or ileal resection)	Vitamin B_{12} levels	Monthly i.m. vitamin B_{12} injections 1000 μg dose, vitamin B_{12} nasal spray one spray (500 μg) administered once weekly
Anti-RBC Abs	Hemolytic anemia	UC > CD	Coombs' test positive	Corticosteroids, usually self-limited

*Parenteral iron dextran replacement is based on the following formula: 0.0476 × (normal hemoglobin – observed hemoglobin in g dl^{-1}) (±) 1 ml/5 kg body weight (up to a maximum of 14 ml) which accounts for storage iron = total dosage of iron dextran in ml.

in a prospective study performed in 302 Greek patients with UC in which 1.7% developed autoimmune hemolytic anemia [106]. Some studies have found a correlation of the hemolysis with disease activity [106], but others have not [101]. The reasons for hemolytic anemia include idiopathic immune-mediated destruction or drugs, especially sulfasalazine [107–109]. Mononuclear cells extracted from the colon of a patient with severe hemolytic anemia and UC, but not peripheral blood cells, were able to transfer IgG with anti-red cell activity, suggesting that in certain cases the colon is the source of hemolysis-inducing antibodies [110]. Characteristics of hemolysis including high LDH and low haptoglobin levels should prompt a Coombs' test for the presence of anti-RBC antibodies. The hemolysis generally responds to steroids but may require colectomy or splenectomy in patients who fail medical therapy [101,106,111].

Hemolytic anemia secondary to sulfasalazine may be due to glucose-6-phosphate dehydrogenase deficiency or can occur as a result of immune-mediated hemolysis associated with Coombs' positivity [107,108,112]. Patients who develop Coombs' positive hemolytic anemia secondary to sulfasalazine may have been taking sulfasalazine for several years prior to the development of this type of anemia [107]. In addition to Coombs' testing, agglutination studies may be carried out wherein sulfasalazine can be added to the patient's serum in the presence of normal erythrocytes and may demonstrate abnormal agglutination.

Table 14.2 summarizes the causes of anemia in IβD and diagnostic and therapeutic implications.

Disorders of coagulation

An estimated one in 1000 people in the general population experience a thrombotic episode [113] compared with approximately 1–7% of patients with IBD [114–118]; hence hypercoagulability is an important systemic consequence of IBD (Figure 14.1). In addition to deep venous thromboses or life-threatening pulmonary emboli, other manifestations of hypercoagulability in patients with IBD include cerebral venous thromboses [119–125], portal vein thromboses [126,127], hepatic vein thromboses (Budd–Chiari) [127–131] and arterial thromboses [132]. Inherited disorders of coagulation and acquired disorders of coagulation often due to inflammation-associated changes in hemostatic factors are among the reasons for hypercoagulability. Most thrombotic events occur in association with disease flares [114,116]. In a study of 52 patients who experienced thromboembolic events, 45% of patients with UC and 89% of patients with CD had active disease at the time of the event [117].

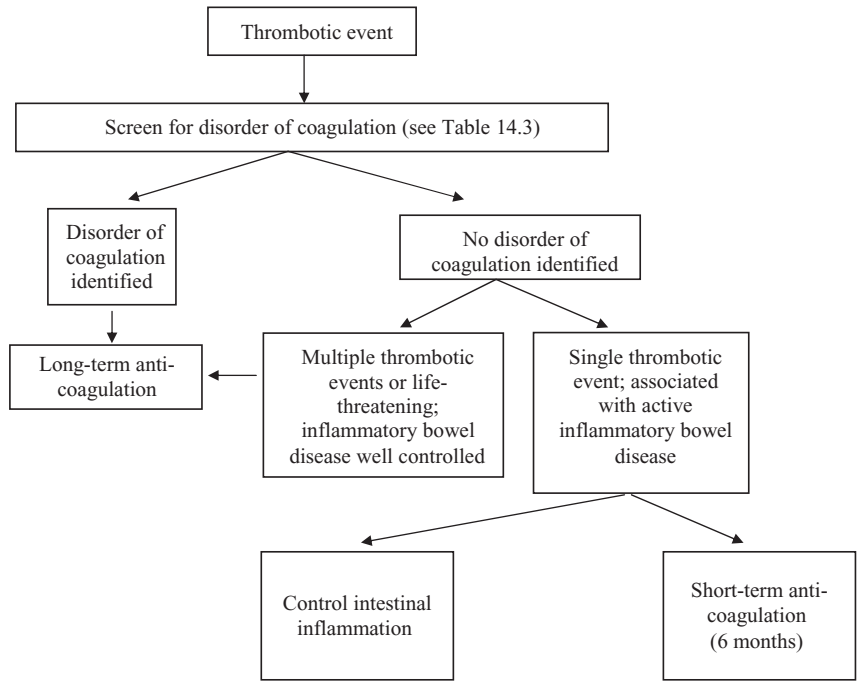

Figure 14.1 Evaluation of the IBD patient with a thrombotic event.

Changes in hemostatic factors associated with active disease may lead to hypercoagulability. Increases in coagulation factors are known risk factors for the development of thromboembolic disease [133]. The most common hemostatic abnormality identified in IBD patients is thrombocytosis, which correlates with underlying bowel disease activity [134]. In a study of 92 thromboembolic events occurring in 7199 (0.3%) patients with IBD, 60% of patients had thrombocytosis and 73% had elevated ESRs, suggesting active IBD [116]. Patients with IBD have increased levels of thrombin generation (prothrombin fragment 1 + 2 and thrombin-antithrombin III complex) compared with control populations and these levels correlate with disease activity [134–137]. Fibrinogen, factor V and factor VIII are also commonly elevated in patients with IBD, whereas the anticoagulant factors antithrombin III and proteins S and C are decreased, which may contribute to the propensity for thrombosis [116,134,137–146]. Other platelet-related abnormalities identified in patients with IBD are increased spontaneous and induced platelet aggregation, which correlated with a history of thromboembolism in seven of eight patients [134,147]. Surgical resections in patients with CD results in significant decreases in platelet counts, fibrinogen levels and spontaneous platelet aggregation, supporting the concept that the underlying bowel disease contributes to the hypercoagulable state [148]. Increased platelet mass in IBD is due to systemic increases in thrombopoietin and IL-6 [149]. Patients with active disease have significantly increased levels of thrombopoietin compared with patients in remission and this increase in thrombopoietin is associated with increased platelet counts. Increased megakaryocyte maturation occurs in response to endogenous production of IL-11 and increases with IL-11 therapy for IBD [150]. Acute inflammation can also result in increased hepatic production of fibrinogen and increased platelet aggregation, which is another risk factor in thrombosis. A study of thrombotic risk factors found that IBD patients had increased plasma factor VII coagulant activity (a marker of thrombin generation), lipoprotein (a) and fibrinogen concentrations compared with a normal population [151].

Anti-cardiolipin antibodies or the lupus anticoagulant are associated with an increased risk of thromboembolic events and have been found with increased frequency in patients with IBD [134,147,152]. Studies examining the prevalence of anti-cardiolipin antibodies in patients with IBD found that both CD and UC patients had increased antibody titers compared with a control group, but the presence of anti-cardiolipin antibodies was not associated with a higher risk of thromboembolic events in these patients [114,153]. The presence of a lupus anticoagulant has been described in the setting of severe thrombotic events such as dural sinus thrombosis or hepatic vein thrombosis in patients with IBD [121,131,154]. Based on these studies, a screen for a lupus anticoagulant should be part of the evaluation of a patient with IBD and a recognized thrombotic event, but has little predictive value in a patient without demonstrated hypercoagulability.

Patients with hypercoagulability and IBD may have inherited disorders of coagulation that may manifest in combination with inflammation-associated changes in hemostasis [155]. Activated protein C resistance from a mutation in the prothrombin gene (factor V Leiden) is the most common inherited disorder leading to thrombosis

and accounts for 30–40% of episodes of idiopathic venous thrombosis [155–157]. Other inherited causes of hypercoagulability include hyperhomocysteinemia and mutations in the prothrombin gene [158]. Since the description of the relatively common factor V Leiden mutation as an inherited cause of thrombophilia, several groups have investigated the frequency of this mutation in IBD patients with and without a history of thromboembolism. A study evaluating the prevalence of factor V Leiden, methylene tetrahydrofolate reductase (resulting in hyperhomocysteinemia) and prothrombin gene mutations in IBD patients without thrombosis compared with an age-matched control group found no increase in these inherited disorders [159]. Several adult and pediatric studies examining the prevalence of activated protein C resistance or factor V Leiden mutations in adults or children with IBD and without a history of thrombosis have not found an increased prevalence of these mutations compared with healthy controls [146,160,161]. A large Greek series identified factor V Leiden mutations in 8.3% of IBD patients, which was not significantly different when compared with a healthy control group with a 4.9% mutation rate [138]. Two studies found a slightly increased allelic frequency of factor V Leiden mutations in UC [162] or CD patients [163], but the sample sizes were limited. Although the prevalence of factor V Leiden mutations or resistance to activated protein C does not appear to be increased in an unselected population of IBD patients, the frequency of this mutation is increased in those IBD patients with a history of thromboembolic events. In an Austrian study of IBD patients without a history of thromboembolism, the frequency of activated protein C resistance was similar to that of healthy controls (7 versus 5.9%) [164]. By contrast, 31.3% of IBD patients with a history of thromboembolism had activated protein C resistance. These results suggest that patients with thromboembolism and IBD are just as likely to have activated protein C resistance as patients with thromboembolism in the general population [146]. Similar results were observed in an American study which found that four of 11 IBD patients (36%) with thrombosis and two of 51 IBD controls (4%) were heterozygotes for the factor V Leiden mutation [165]. In a group of 20 patients with IBD complicated by thrombosis, a screen for the most common inherited and acquired disorders of coagulation including protein S, protein C levels and antithrombin III levels, anti-phospholipid antibodies and activated protein C resistance were negative with only one patient found to be heterozygous for factor V Leiden mutation [117]. There is an increased prevalence of hyperhomocysteinemia, a risk factor for thrombophilia, which can be corrected by the administration of folate, cobalamin and pyridoxine [166]. Data also suggest that patients with IBD have increased prevalence of the C677T variant of the methylene tetrahydrofolate reductase gene (17 versus 7.3% in healthy controls), which is associated with thromboembolic disease [167]. In a retrospective study of 231 IBD patients, hyperhomocysteinemia was more prevalent in patients than in healthy controls but was not higher in IBD patients with a history of thromboembolic disease [168]. Thus, the majority of patients with thromboembolic events and IBD have the same risk as the rest of the population for inherited disorders of coagulation but the majority will have no identifiable risk factor except active inflammation. Although the risk of inherited disorders predisposing to thrombophilia is not increased in IBD patients, there is an epidemiologic study suggesting that hemophilia and Von Willebrand's disease are underrepresented in the IBD population [169]. It is likely that additional patients with IBD and hypercoagulability will be recognized to have an inherited disorder of coagulation as the molecular mechanisms further defined.

Management of thromboses in patients with IBD requires a multi-faceted approach (see Figure 14.1). Even in patients with active IBD, a search for an underlying disorder of coagulation is required. If active IBD is the only identifiable risk factor for the thrombosis and it is a single thrombotic event, short-term anticoagulation combined with treatment of the underlying IBD is appropriate [170]. Heparin therapy can generally be given safely and has a small therapeutic benefit in patients with UC [171]. Thrombolytic therapy may be given cautiously in IBD patients with extensive thromboses or life-threatening thrombotic events [172,173]. In patients with recurrent thrombotic episodes or life-threatening thromboses, long-term anti-coagulation is required. This may be difficult in the setting of active IBD because of associated intestinal bleeding.

Table 14.3 summarizes the causes of hypercoagulability in patients with IBD.

In addition to hypercoagulability due to inherited genetic mutations in coagulation factors or inflammation, there are multiple case reports of disseminated intravascular coagulation in patients with UC generally associated with a flare of the UC [175–177]. Thrombocytopenia may also complicate IBD and may be immune-mediated [178,179] or associated with drug-induced bone marrow suppression as described above. Cases of sulfasalazine-induced and mesalamine-induced immune-mediated thrombocytopenia have been reported [180].

Leukocytosis

As with inflammation of any type, leukocytosis is often present in patients with IBD [149]. Elevated leukocyte counts prior to surgical resection for CD are associated with an increased risk of recurrence [181]. White blood cell counts above 18,000 or the presence of an elevated band count should prompt an investigation for a septic process. Neutrophil and monocyte maturation and release from the bone marrow is regulated by granulocyte

Table 14.3 Causes of hypercoagulability in patients with IBD [155,174]

Inherited causes of hypercoagulability	Acquired causes of hypercoagulability
Activated protein C resistance (Arg506 changed to Gln in Factor V) (Factor V Leiden mutation)	Thrombocytosis (active inflammation)
Prothrombin G20210A polymorphism (results in increased levels of prothrombin)	Increased platelet aggregation
Methylene tetrahydrofolate reductase mutations (hyperhomocysteinemia)	Elevated factor VIII plasma levels
Deficiency or mutations in protein C	Elevated fibrinogen levels
Deficiency or mutations in protein S	Anti-cardiolipin antibodies
Antithrombin III deficiency	Deficiency in protein S Secondary hyperhomocysteinemia (due to folate, pyridoxine or cobalamin deficiency)

colony-stimulating factor (GCSF) and monocyte colony-stimulating factor (MCSF), respectively. These factors are derived from bone marrow stromal cells and monocytes which are activated by IL-1, TNFα and LPS to release these trophic factors. In addition, GM-CSF is derived from activated T lymphocytes and IL-1/TNFα-activated stromal cells and monocytes. In patients with active IBD, leukocytosis correlates with serum concentrations of IL-6 and thrombocytosis [149]. The presence of leukocytosis in patients with active IBD is thus related to increased circulating levels of proinflammatory cytokines or a septic complication such as a microperforation, frank perforation or abscess. Corticosteroids also lead to leukocytosis secondary to down regulation of intercellular adhesion molecule expression and granulocyte demargination.

Hematologic malignancies

Lymphoma occurring in the setting of IBD was first described by J. Arnold Bargen in 1928 [182]. In spite of multiple case reports of leukemias or lymphomas associated with IBD, it remains controversial whether patients with IBD are at increased risk for the development of hematologic malignancies as a result of either chronic inflammatory disease or its treatment [183–185]. Other chronic inflammatory disorders such as rheumatoid arthritis, Sjögren syndrome or celiac disease do pose an increased risk of hematologic malignancies [186,187].). The majority of lymphomas occurring in the gastrointestinal tract of patients with IBD have appeared in areas of active inflammation [188–190], indicating that chronic active inflammation may increase the potential for malignant change in the bowel lymphoid tissue.

Based on several case series and population-based studies, it seems that patients with CD are at a slightly increased risk for the development of non-Hodgkin's lymphomas than the average population search. A study based on records for Olmstead County, Minnesota, USA found a standardized incidence ratio of lymphomas of 2.4 (95% confidence interval 0.1–13) in patients with CD and no cases of lymphomas occurred in patients with UC [183]. Only three patients out of 61 who developed lymphomas were receiving purine analogues. Few patients with IBD in this study were on immunomodulator therapy, hence this may represent the true incidence of lymphomas in IBD patients in the absence of immunosuppressive therapy. There was no increased risk of leukemia in these patients. In another large series of IBD patients, five lymphomas occurred among 1156 patients (0.43%) with UC and four lymphomas among 1480 patients (0.27%) with CD, and the risk of lymphomas correlated with disease duration in patients with CD [191]. A population-based study performed in Florence, Italy reported a nine-fold increased incidence of Hodgkin's lymphoma in patients with UC compared with the general population [192]. The same study found a reduced risk (standardized incidence ratio 0.6) of respiratory tract cancers in patients with UC and increased risk in patients with CD. Severity of the IBD has not been established as an independent risk factor in the development of lymphomas.

Immunosuppressive therapy has been implicated as a risk factor in the development of hematologic malignancies, but a causal role of immunosuppressive therapy in the development of lymphomas associated with IBD has not been established [193,194]. To date, there have been three published cases of brain lymphomas (one each from three large series) in patients with IBD on long-term azathioprine or 6-mercaptopurine in a total of 1701 patients [184,193,195]. Although the overall risk of brain lymphomas is low in patients with IBD treated with purine anti-metabolites, immunosuppression is clearly a risk factor for this otherwise rare malignancy [196]. In a series of 550 patients with IBD on long-term 6-mercaptouprine therapy, two patients developed non-Hodgkin's lymphoma, one leukemia and one brain lymphoma [193]. The overall rate of these malignancies was not higher than in the general population. Two other large series of 755 and 396 patients found no excess in the risk of any hematologic malignancies in IBD patients treated with azathioprine or 6-mercaptopurine [184,195]. A decision analysis comparing the efficacy of azathioprine at maintaining remission in IBD compared with the risk of non-Hodgkin's lymphoma found that azathioprine was favorable and resulted in improved quality of life [197]. In spite of the encouraging data in patients with IBD, children treated with 6-mercaptopurine for acute

lymphoblastic leukemia have an increased risk of secondary myelodysplasia or acute myeloid leukemia (5/493) and this risk is associated with low TPMT enzymatic activity and high erythrocyte 6-thioguanine nucleotides [198]. 6-Mercaptopurine and azathioprine have also been associated with rare cases of acute myeloid leukemia in patients with autoimmune diseases including IBD treated for prolonged periods of time [199,200]. In all the cases, a prolonged pancytopenic phase preceded the onset of the leukemia, suggesting an antecedent myelodysplastic phase. In a recent meta-analysis by Kandiel *et al.* [201], an approximate four-fold (4.18) increased risk of lymphoma was shown in IBD patients treated with azathioprine/6-nercaptopurine.

Immunocompromised hosts, especially patients who have undergone solid organ transplantation, are at significantly increased risk for the development of B-cell lymphomas related to Epstein–Barr virus (EBV) infection [202,203]. The risk in patients post-transplantation is thought to be high because of the use of multiple immunosuppressive drugs and also the long-term nature of the immunosuppression. In a series of four patients with gastrointestinal Hodgkin's lymphoma and IBD, all four lymphomas were found to be EBV mediated with only two patients on immunomodulatory therapy [204]. An additional case of an EBV-driven lymphoproliferative disorder in a CD patient on long-term azathioprine regressed after discontinuation of the azathioprine [205,206]. In the context of the use of infliximab in IBD therapy, *in vitro* data suggest that this increased risk for EBV related lymphomas does not seem to correlate with a direct action of infliximab on apoptosis or proliferation on B cells but rather to the concomitant impairment of T-cell immune surveillance.

TNFα antagonists are well established therapeutic options for IBD. Among the two large already published population-based studies, the role of anti-TNFα agents in the development of lymphoma is also controversial. In a recent population-based Swedish cohort study [208], all patients treated with infliximab between 1999 and 2001 in Stockholm County were evaluated. Among 217 patients [191 CD patients, 22 UC patients and four patients with indeterminate colitis (IC)], three developed lymphomas (one NK, two B cell lymphomas) with an annual incidence of 1.5% suggesting an increased risk of lymphoma. There are no such studies for the risk of lymphoma with the use of adalimumab, certolizumab or other biologic agents in IBD. These results are in contrast with data from a recent multicenter matched pair Italian study [209], in which 404 CD patients treated with infliximab (CD-IFX) were matched with 404 CD patients who had never received infliximab (CD-C). At the end of the study, each CD patient treated with infliximab was matched with one CD control who had never been treated with infliximab, followed up in the same study period in the same center, according to the following criteria: age (±5 years), sex, follow up period in the same center (±5 years), immunosuppressant use and duration, CD site and CD duration (±5 years). Among the 404 CD-IFX, neoplasia was diagnosed in nine patients (2.22%), whereas among the 404 CD-C, seven patients developed neoplasia (1.73%) [odds ratio 1.33 (95% confidence interval 0.46–3.84); $p = 0.40$], while none of infliximab patients had developed a lymphoma whereas one case of non- Hodgkin's lymphoma was reported in the control group. Hepatosplenic T cell lymphomas (HSTCLs) are rare cancers (less than 100 published cases worldwide) and comprise 5% of peripheral T cell lymphomas. As of 5 October 2006, the FDA's Adverse Event Reporting System has received eight cases of HSTCL in young patients using infliximab to treat IBD (six of the eight cases had a fatal outcome). All eight patients were receiving concomitant immunosuppressant therapy (e.g. azathioprine, prednisone) and seven presented with hepatosplenolegaly [210].

Amyloidosis in patients with inflammatory bowel disease

An uncommon but morbid systemic consequence of IBD is the development of amyloidosis [30,211–213]. Amyloidosis is a group of diseases characterized by the extracellular deposition of pathologic insoluble fibrillar proteins in organs and tissues [214,215]. There are three principal forms of amyloidosis. Systemic AL amyloidosis is associated with blood cell dyscrasias and monoclonal gammopathies. There are multiple familial forms of amyloidosis or type ATTR amyloidosis. As with other chronic inflammatory states, such as rheumatoid arthritis, IBD may rarely result in secondary systemic amyloidosis type AA [216,217]. In general, rheumatologic diseases are more commonly associated with amyloidosis than IBD and CD is more often complicated by amyloidosis than UC [211,214,215,218]. In a large series of IBD cases followed at the Mount Sinai Hospital, amyloidosis occurred in 15 of 1709 patients with CD (0.9%) and one of 1341 patients with UC (0.07%) [30]. Amyloidosis was more often associated with CD of the colon than with pure small bowel disease.

The diagnosis of amyloidosis is made by appropriate pathology demonstrating Congo Red-positive amyloid deposits on fat pad biopsy, rectal biopsy or renal biopsy [214,215]. Scintigraphy has been developed as a noninvasive and quantitative alternative to histology. A radioactive tracer ^{123}I-labeled serum amyloid P component is injected and specifically targets amyloid deposits *in vivo*. The technique has almost 100% sensitivity for systemic AA amyloidosis. In four patients with CD and amyloidosis, ^{123}I-labeled serum amyloid P nuclear medicine scanning demonstrated the increased amyloid content and correlated with disease improvement following renal transplantation [216].

In patients with CD and AA amyloidosis, proteinuria is the most common presentation [30,211,213,215,216,219]. The overall 5 year mortality for AA amyloidosis is 50% [214,215]. In one series, one of four patients died prior to renal transplantation, demonstrating the high morbidity and mortality from this complication [216]. Nephropathy is the most common lethal manifestation of IBD-associated amyloidosis. Nephrotic syndrome was responsible for 10 deaths out of 25 patients with IBD-associated amyloidosis in a large series [30].

Therapy for type AA amyloidosis is directed at limiting the acute phase response generating the amyloid protein [214,215]. Unfortunately, this type of amyloidosis is generally advanced by the time of diagnosis, with extensive amyloid deposits and renal failure [211]. Only limited case report information is available on the management of IBD complicated by amyloidosis. Several cases of UC complicated by AA amyloidosis have been effectively treated with colchicine, which led to a reduction in proteinuria [218,220–223]. Azathioprine and colchicine combination therapy has also been effective in improving amyloidosis-induced renal failure and controlling CD in one patient [224]. An elemental diet has been reported as effective in preventing on going renal damage in a CD patient with amyloidosis [225]. Regression of amyloidosis has also been reported in patients with CD after bowel resection or after treatment with dimethyl sulfoxide (DMSO) [226,227]. Based on the limited data, periodic tests of renal function are warranted in patients with IBD to assess nephrotoxicity from medications and also the rare occurrence of amyloidosis.

IBD and metabolic syndrome

A concomitant rise in the prevalence of both metabolic syndrome and IBD in the Western world generates questions about common factors contributing to both diseases and the effect of metabolic syndrome on IBD, if any. Little is known, however, about the prevalence of metabolic syndrome in IBD patients and its relationship with the duration of the disease or its activity. A positive correlation with disease activity in patients with rheumatoid arthritis has already been established [228]. Adiponectin, a 30 kDa peptide with a terminal domain structure similar to that of TNFα [229], is a member of adipocytokines, a group of cytokines that are secreted from the white fat tissue. Adiponectin increases insulin sensitivity and thus has a protective role against obesity, type 2 diabetes and cardiovascular disease. High levels of adiponectin and other adipocytokines are present in plasma and tissue of patients with IBD [230] and other chronic inflammatory diseases such as systemic lupus erythematosus, type 1 diabetes and rheumatoid arthritis [231]. In the context of IBD, higher adiponectin expression was observed in inflamed compared with noninflamed adipose tissue, indicating that the presence of an ongoing inflammatory reaction correlates with high adiponectin expression [232]. In colonic epithelial cells, adiponectin exerted proinflammatory effects by inducing chemokine production [233], which seems to be mediated at least in part by TNFα, as it was shown in human synovial fibroblasts [234]. Nevertheless, a clear role of adiponectin in IBD pathogenesis and autoimmunity has not yet been determined. Interestingly, therapy with infliximab improves the lipidemic profile in patients with inflammatory arthritis and thus may potentially reduce cardiovascular risk in these patients [235]. In patients with IBD, infliximab therapy lowers serum resistin levels, another 12.5 kDa cysteine-rich adipokine, but has no influence on serum adiponectin levels [236].

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Chapter 15
Ulcerative Colitis and Ulcerative Proctitis: Clinical Course and Complications

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Summary

- The diagnostic criteria for ulcerative colitis (UC) need to be reassessed to ensure that they adequately reflect the chronic nature of the disorder.
- Consensus on key aspects of UC natural history including the Montreal classification (disease extent and severity) are required to allow accurate comparisons between adequately powered studies and meta-analyses.
- Differentiation between UC and colonic Crohn's disease requires further detailed study and an international consensus statement.
- The natural history of pediatric UC requires further study, in particular treatment regimes and response to therapy.
- Further work on patient education, self-management and clinical outcomes utilizing e-health strategies may assist this predominantly young and independent patient population deal with their disease more effectively.

Introduction

Ulcerative colitis (UC) is a chronic disease that characteristically has a relapsing and remitting course. Questions concerning the course that an individual patient's disease may take, the prognosis and any associated complications are of paramount importance for the patient and the treating physician. The answers to the above questions would not only help to guide treatment but would also assist the patient in planning their future.

The prognosis of UC today is very different from the prognosis 50 years ago. This is largely attributable to the introduction of safer and more effective medications and better surgical procedures. The course and prognosis of UC have been the subject of many clinical research studies. Early data obtained from tertiary referral centers reported significant morbidity and mortality; however, this is now thought to be due to a referral bias. This chapter focuses on key aspects of UC that influence or potentially influence the disease course, including the risks of colectomy and death. Information has been drawn from recent studies of this disease given the changes in clinical practice over the past 30 years. There are multiple excellent descriptions of the natural history of UC with a greater historical perspective.

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Potential confounders in UC natural history studies

There are multiple biases that may influence data gathered from both population-based and specialist-based studies of UC. These include ascertainment, diagnostic criteria, the source population, investigation of disease extent, treatment and statistical analyses [1]. A list is provided in Table 15.1. Population-based cohort studies are considered the basis for the best available evidence in the study of prognosis as they provide an unbiased assessment [2]. There are several such cohorts drawn from populations within Europe and the United States. For example, the Copenhagen cohort [3] provides comprehensive information on the long-term natural history of treated UC, using well-established medical and surgical approaches. However, as with any chronic illness, there are differences in the clinical approach to patients in each cohort, particularly with respect to treatment. These differences are usually seen most clearly in the management of patients with severe disease, including the threshold for the introduction of immunosuppressants and the threshold for

Table 15.1 Potential confounders in natural history studies of UC.

- Diagnostic criteria
- Ascertainment methods
- Source population
- Methods of investigation
- Changes in treatment
- Local medical and surgical "culture"
- Extent of follow-up
- Statistical analyses

colectomy. The European Collaborative Study on Inflammatory Bowel Disease (EC-IBD) is a more recently assembled cohort from several centers across Europe with incident cases being recruited prospectively during 1991–1993 [4,5]. These studies demonstrate clear changes in patient outcomes compared with previously assembled cohorts and significant regional differences in these outcomes.

The diagnostic criteria for UC are worthy of further discussion as they underpin all studies of disease natural history. The majority of studies use those criteria from Langholz *et al.* [6] or Lennard-Jones [7]. Of four criteria, three need to be satisfied, of which the first is either "typical case history with diarrhea and/or blood and/or pus in the stools for more than a week or in repeated episodes" [6] or "a history of diarrhea and/or blood or pus in the stools" [7]. The other criteria then focus on "typical" sigmoidoscopic and histologic appearances and the absence of any signs of Crohn's disease [7].

One of the cardinal features of UC is its chronicity, with repeated attacks followed by remissions. However, a number of population studies describe a significant proportion of patients who have only ever experienced one attack or do not have inflammatory bowel disease at all upon reassessment 12 months after the initial diagnosis [6,8]. In addition, follow-up colonoscopy on a number of these patients indicates no histological evidence of previous or current UC [9]. This issue of diagnostic certainty is of critical significance to subsequent data analysis. As the "denominator" swells with this benign subgroup of "cases", so the risk of potential complications such as need for colectomy and colorectal carcinoma is seen to decrease. This issue has been highlighted more recently by genetic studies in this field where case diagnostic certainty is again of importance, as indicated by Silverberg *et al.* [10]. The absence of small bowel (ileal) involvement in unoperated UC and the presence of ileal involvement in at least 70% of Crohn's disease case series improve diagnostic accuracy for Crohn's disease and may help to explain, at least in part, the enormous success in finding *ileal* Crohn's disease genes and not genes for colonic Crohn's disease [11]. Subgroup analysis of UC has started to clarify this point in further recent genetic studies [12].

Disease extent

Previous studies have been influenced by two major confounders: the use of barium enema to determine proximal disease extent, as opposed to colonoscopy, and the use of different criteria to define extent, including the terms "distal colitis" and "substantial colitis". This makes comparisons difficult. For example, "substantial colitis" has been used to describe the subgroup of patients with either left-sided disease together with colitis cases extending beyond the splenic flexure, but not including total colitis. This makes it impossible to determine whether there are differences in natural history between patients with disease limited to the left colon compared with those with disease extending to involve the right side. Significant differences in disease extent have been found between colonoscopy (plus histology) and barium enema, with 40–45% of patients having more extensive disease based on colonoscopy compared with a contrast study [13]. In order to facilitate understanding of the natural history of UC, subclassification of disease extent has been considered essential.

The Montreal Working Party [14] highlighted the critical relevance of a subclassification system for UC that incorporates an assessment of disease extent. The Montreal classification allows extent to be defined into three subgroups: ulcerative proctitis, left-sided colitis (disease extending proximally beyond the rectum but no further than the splenic flexure) and extensive UC (including total colitis) (see Table 15.2). This subclassification is felt to have clear biological relevance in terms of response to medical therapy in addition to forming a structure to evaluate better the natural history and prognosis for patients presenting with UC. There are also several serologic and genetic markers that have been associated with extensive colitis, making this subset of particular importance [15,16].

Extent of UC at diagnosis and follow-up

Based predominantly on colonoscopic studies carried out in recent years, the UC subgroups are relatively evenly

Table 15.2 Disease extent in UC – Montreal classification.

Extent	Disease	Anatomy
E1	Ulcerative proctitis	Involvement limited to the rectum
E2	Left-sided UC (distal UC)	Involvement limited to a proportion of the colorectum distal to the splenic flexure
E3	Extensive UC (pancolitis)	Involvement extends proximal to the splenic flexure

Table 15.3 Classification of extent of colitis at diagnosis.

Study	N	Extensive (%)	Left-sided (%)	Proctitis (%)
Jess (2006) (US data) [17]	378	47	35	17
Farmer (US, 1960–83)* [18]	1116	37	17	46
Park (South Korea, 1989–2005) [19]	304	33.2	22.7	44.1
Moum (Norway, 1990–93) [9]	399	34.8	32.6	32.6
Jess (Danish data, 1991–93) [20]	89	25	16	60
Jess (Danish data, 2003–4) [20]	326	27	42	31
Hoie (2007) [5]	781	29	41	30

*Definition of proctitis included those with proctosigmoiditis. Barium studies used to determine proximal extent in some patients.

represented amongst similar Caucasian populations, as shown in Table 15.3. Studies have not been included where the disease extent subgroups have not or cannot be split into those given in Table 15.3. Data from South Korea demonstrate a similar disease distribution to some of the Caucasian studies [17–20].

Disease extent at diagnosis may be a useful predictor of subsequent course. Farmer *et al.* demonstrated a significantly increased risk of complications including severe colitis and colectomy in those with total colitis compared with distal colitis cases (proctitis and proctosigmoiditis combined) (see Table 15.4) [18]. Similarly, total colitis was associated with a greater risk of extraintestinal manifestations in the skin and liver and pediatric growth retardation, compared with distal colitis. Of interest from a genetic perspective, the frequency of family history was also significantly greater in the total colitis group compared with the other two groups ($p < 0.04$), whereas age at diagnosis was younger at 27.6 ± 15.0 for total colitis versus 35.8 ± 15.9 for distal colitis [18].

There are only a limited number of other recent studies specifically addressing the influence of disease extent at diagnosis on outcome and risk of complications. In the study by Langholz *et al.* [6], which, like Farmer *et al.*, relied upon a mixture of barium enema and colonoscopy to document extent, no association was found between extent at diagnosis and changes in disease activity. However, with the same inception cohort of 1161 cases diagnosed between 1962 and 1987, Langholz *et al.* documented an increased probability of colectomy within the first 5 years after diagnosis in those with total colitis (35%), compared with cases of proctosigmoiditis (distal colitis, 9%) and "substantial" colitis (19%, $p < 0.00005$). Similar data, but with higher colectomy rates, have been published on a Swedish patient cohort diagnosed between 1955 and 1984 [21].

With more recent data from Norway, using a well-characterized cohort [8,9], Moum *et al.* [22] demonstrated that those patients with extensive disease at diagnosis required more aggressive medical therapy (e.g. oral steroids, 41%) than those with proctitis (oral steroids, 7%) and also carried a significantly higher risk of colectomy ($p = 0.011$) than those with distal disease (proctitis and left-sided disease).

Table 15.4 Frequency of complications related to extent of disease.

Complication	Overall (%)	Total colitis (%)	Left-sided colitis (%)	Proctitis (%)
Severe colitis	12.7	24	12.6*	3.7*
Toxic dilatation	10.7	21	9.5*	2.9*
Bleeding	16.7	25	17.9	9.5*
Surgery	37.6	61	52	14.2*

*Percentage of total colitis patients with the complication significantly higher ($p < 0.002$) than for patients with left-sided colitis or proctitis.

The dynamics of disease extent

Disease extent in UC can both progress and regress or the disease may "disappear" altogether. The Montreal classification proposes that the maximum extent of involvement in the course of the disease be the critical parameter [14]. The problem with this approach is that it does not help to give a prognosis at initial diagnosis. Again, these types of studies are best performed using well-characterized population-based cohorts followed by sequential colonoscopy and biopsy. A recent example comes from a Norwegian cohort of 496 cases diagnosed over 4 years, of whom 384 (78%) were available for a second colonoscopy and biopsy after an interval of 12 months [9]. This study reported on differences in both macroscopic and histologic appearances over time, as shown in Table 15.5. In total, two-thirds of the cases showed changes in disease extent. Up to 20% of cases showed disease extension, 22–24% regressed and 24% had completely normal histologic findings. The closest correlation between the two modalities of disease assessment – colonoscopic findings and histology – was found in those with total colitis (99% at diagnosis, 88% at follow-up). The finding of 24% with normal histology is perhaps surprising, but underlines the significance of appropriately stringent diagnostic criteria and adequate follow-up. Regression or

Table 15.5 Correlation between colonoscopic and histologic findings at diagnosis and follow-up.

	Diagnosis (%) ($n = 408$)	Follow-up (%) ($n = 384$)
Agreement	78 ($n = 318$)	60 ($n = 230$)
Histology > colonoscopy	4 ($n = 17$)	28 ($n = 108$)
Colonoscopy > histology	18 ($n = 73$)	12 ($n = 46$)

normalization of extensive colitis in up to 72% of cases also raises the question of cancer risk and whether these patients should follow a similar surveillance program to those who progress from left-sided disease to extensive disease. Recent studies suggest that ongoing disease activity may be of greater risk in terms of complicating cancer and therefore patients who show true disease regression are likely to fall into a much lower risk group [23].

Using a combination of sigmoidoscopy and barium enema, comparative data from Langholz *et al.* [24] for changes in extent at 1 year were 10% for progression and 24% for regression. Life-table analyses indicated that the probability of further progression of proctosigmoiditis (evaluated by sigmoidoscopy and radiology) was 53% after 25 years. The progression rate was highest in the first year of disease, after which it was steady for the next 10 years. Twenty-eight percent of the progressing patients underwent colectomy. This is much lower than the 58% reported by Farmer *et al.* [18], once again reflecting differences in ascertainment and the potential problem of referral bias. Multivariate analysis showed that the occurrence of the symptoms of abdominal pain and diarrhea [relative risk of 5.5 ($p = 0.0018$) and 3.6 ($p = 0.001$), respectively] were prognostically unfavorable with regard to progression from proctosigmoiditis to more extensive disease [24]. These data suggest that patients experiencing these symptoms should be observed more closely and treated more aggressively to prevent further progression and possible colectomy. Excluding those patients who underwent colectomy, the probability of regression was 76.8% for left-sided colitis and 75.7% for total colitis after 25 years.

Farmer *et al.* similarly determined changes in the extent of disease over time but ascertainment of cases was heavily influenced by referral bias [18]. The disease progressed to total colitis in 34% of those with an initial diagnosis of proctosigmoiditis and 70.4% of those with an initial diagnosis of left-sided colitis. Of the 253 patients in whom the disease extended, 147 (58.1%) required surgery. Progression occurring despite medical treatment illustrates the aggressive potential of the disease. In terms of predicting extension of disease, Farmer *et al.* found that there were multiple clinical factors that are associated with extension of disease, as displayed in Table 15.6. Gender, race and family history were not significantly associated with disease extension.

A slight but significant influence of increasing age on the potential for regression from initially extensive disease was found by both Farmer *et al.* [18] and Langholz *et al.* [24] {hazard ratio (HR) 1.13 [95% confidence interval (CI) 1.01–1.25], $p = 0.04$}, suggesting that UC occurring later in life may have a more benign prognosis than UC with an early onset. However, not all studies agree with this. Russel *et al.* [25] found that total colitis was significantly overrepresented in the youngest (<25 years) and oldest (>60 years) age groups at diagnosis, compared with those aged 25–60 years ($p < 0.001$), in a large EC-IBD study involving 1317 UC cases.

In contrast to these data from Caucasian populations, Park *et al.* found a significantly greater probability of proximal disease extension in their proctitis patients (60% at 10 years) compared with those with initial left-sided disease (21% at 10 years, $p < 0.001$) [19]. Importantly, these findings were based on colonoscopy and histology, with 69–71% of these patients undergoing follow-up investigations. The clinical significance of this is not clear at this stage given that the colectomy rate in this large series from South Korea was much lower, 3.3% at 5–15 years, than that seen in "Western" UC patients [19].

Table 15.6 Factors associated with extension of disease.

Clinical factor	Adjusted OR	*p*-Value
Toxic or severe colitis	14.8	<0.0001
UC extent at diagnosis (left-sided/proctitis)	2.5	<0.0001
Joint symptoms (yes/no)	3.7	0.0008
Age at diagnosis (per decade)	0.886	0.06
Severe bleeding (yes/no)	1.7	0.07

Severity of attacks of UC

To provide further guidance for clinical researchers in this field, the Montreal Working Party [14] also included a simple classification for severity of relapse made up of four categories (see Table 15.7). However, the timing of this assessment of severity is more difficult in practice. It may be simplest to carry this out at the time of diagnosis. However, this may not correlate with long-term prognosis. Some patients sustain their worst attack at diagnosis (S3) but have a benign disease course thereafter [6]. Further large-scale studies may be required to

Table 15.7 Classification of severity of UC.

Severity	Disease	Definition
S0	Clinical remission	Asymptomatic
S1	Mild UC	Passage of four or fewer stools per day (with or without blood), absence of any systemic illness, normal inflammatory markers (ESR)
S2	Moderate UC	Passage of more than four stools per day but with minimal signs of systemic toxicity
S3	Severe UC	Passage of at least six bloody stools daily, pulse rate of at least 90 beats per minute, temperature of at least 37.5 °C, hemoglobin of less than 10.5 g per 100 ml and ESR at least 30 mm h^{-1}

confirm the inclusion of relapse severity into the UC classification system and both the timing and frequency of its implementation.

Acute severe UC (ASUC)

ASUC is a potentially life threatening condition. The lifetime risk of a severe exacerbation requiring hospital admission is estimated to be between 8 and 15% [26,27]. However, this question is very rarely addressed at a population level. The definition of ASUC has been debated, but consensus appears to have been reached [28]. ASUC is defined as bloody stools $\geq$6 per day and at least one of the following: pulse rate >90 beats per minute, temperature >37.8 °C, hemoglobin <10.5 g dl^{-1} or an ESR >30 mm h^{-1} [28–30].

Continued improvements in the management of severe attacks of UC have changed the natural history of the disease in this patient subgroup. Whereas the mortality from severe attacks of UC was about 75% (16/21) for first attacks in 1933, by 1950 this had dropped to 22% and by 1955, with the introduction of steroid therapy, mortality of severe colitis had dropped further from 24% to 7% [29]. A figure of 1% is now quoted, but applies to specialist inflammatory bowel disease (IBD) centers and not a population [31]. Nevertheless, the response of acute severe cases to intravenous steroids has remained unchanged for 50 years [32,33].

The short term colectomy rate in severe colitis has remained stable over the last 30 years. In a systematic review of 32 trials of steroid therapy for acute severe colitis [32] involving 1991 patients from 1974 to 2006, the overall response to steroids (i.v. hydrocortisone, methylprednisolone or betamethasone) was 67% (1429/1991, 95% CI 65–69%) and mortality was 1% (22/1991, 95% CI 0.7–1.6%) and none of these outcomes changed between 1974 and 2006. Only a minority (100/1991) of patients received cyclosporin. These data are supported by serial results (1955, 1974 and 1996) from a single center [28–30]. When complete response to steroids was defined as a stool frequency $\leq$3 per day without visible bleeding on day 7, 41–42% had a complete response, 27–31% had a partial response and the remainder (28–32%) came to colectomy on that admission [29–31]. This center's figures most closely match the mean colectomy rate of all studies [32] and are similar to those from a prospective study of 116 patients in 29 hospitals enrolled over 3 months [34].

The proportion of patients with ASUC who eventually undergo colectomy is high, especially after an incomplete response to intensive medical therapy. It is necessary that patients, their physicians and surgeons understand this. To determine the outcome of patients admitted with severe colitis who avoided colectomy on the index admission, a prospective cohort of patients from a single center was examined after 15 years [35]. The main outcome measure was colectomy-free survival, time to colectomy and duration of steroid-free remission. There was 92% follow-up of the 49 patients. Of all patients admitted with severe UC, about two-thirds will undergo colectomy within 15 years. Eight of 22 (36%) complete responders underwent colectomy, compared with 8/10 (80%) incomplete responders. Median time to colectomy was 33 months (95% CI 12.6–67.1) for complete responders versus 6.0 months (95% CI 0.9–17.7) for incomplete responders ($p = 0.033$). This means that just 1 week after admission with severe UC, incomplete responders can be advised that the chance of colectomy is around 60% at 1 year and 80% within 5 years. The maximum duration of remission in complete responders is more than five times longer than incomplete responders, but one-third still require colectomy.

The long-term colectomy rate has been unchanged despite the introduction of calcineurin inhibitors and infliximab. Studies of cyclosporin or tacrolimus therapy in corticosteroid-refractory patients showed that colectomy could be avoided in the short term in roughly 70–80% of cases [36]. In contrast, Turner *et al.* found a pooled 51% (95% CI 41–60%) short-term success, which suggests that the real-life success rate is lower [32]. Moreover, it appears that cyclosporin has not gained popularity in the treatment of patients with intravenous corticosteroid failure, as the actual proportion of those receiving cyclosporin is low. Therefore, it is not surprising that the relative benefit of cyclosporin has not translated into a reduced overall colectomy rate. The reasons for the limited use of cyclosporin are probably related to its potential toxicity. More recently, there has been the option of prescribing infliximab for rescue or maintenance in UC [37,38]. The implications of infliximab therapy are yet to be reflected in the literature. Since a complete response to steroids occurs in only 40% of patients with acute severe colitis, evaluation of rescue therapies is appropriate, but awaits appropriately powered randomized controlled trials.

At a population level, the long-term implications of an acute, severe attack of UC have not been adequately addressed. In the study by Langholz *et al.* [6], systemic symptoms (fever and weight loss) at diagnosis were associated with a quiescent course in the longer term, provided that the patient responded to medical therapy and hence avoided colectomy. However, this study did not specifically identify cases who sustained an acute, severe attack. With continuing improvements in treatment, an increasing number of patients will avoid colectomy for either acute or severe disease or for chronic refractory UC. These changes in natural history will require a greater vigilance on the part of the clinician with respect to the frequency of complications, in particular colorectal cancer. Prospective longitudinal studies will be needed to determine whether these "rescued" patients carry a greater risk of cancer than patients with lower levels of disease activity. Thus far,

the data suggest that they will carry this increased risk if disease activity persists [23].

Course of the disease

There are five major outcomes with respect to disease course in UC: a single attack; a chronic, intermittent course; a chronic, continuous course; colectomy; and death. Previous accounts of disease patterns have relied heavily upon the data from the Copenhagen study [6], and there are limited other population-based studies. In a thorough set of actuarial analyses, the authors were able to determine the percentage of patients following three of these outcome patterns over a 25 year period and established that the most important predictor of relapse is disease activity in the previous year. However, the patients in this study had variable lengths of disease duration and there are limited data on the total number of patients available for analysis at some of the key time points. Whereas 23% had only one disease episode during the study period, the cumulative probability of remaining relapse free after 25 years was 10.6% – a potentially important fraction of patients. Reassuringly, this subgroup did not differ in "clinical appearance" at diagnosis from patients with intermittent disease activity.

The predominant disease course was one of intermittent disease activity, a cardinal feature of UC as stressed earlier and with a probability in this study of 90% at 25 years. A subgroup of 600 patients from this cohort who had been followed for seven complete years following diagnosis, *and who had not undergone colectomy*, were the subject of further analysis to identify independent prognostic variables for disease course. These are provided in Table 15.8. Specifically, a greater number of relapses from the time of diagnosis increased the relapse risk in future years ($p < 0.00001$); those patients diagnosed earlier in the study (1962–1968) had a higher relapse rate at 26% compared with those diagnosed later (14%, 1969–1976; 15%, 1977–1980; $p = 0.006$) and, as indicated above, systemic symptoms of fever and weight loss were associated with a quiescent long-term course *in patients who avoided colectomy* ($p = 0.02$). Perhaps surprisingly, age, extent of disease, initial treatment given and extraintestinal manifestations did not correlate with relapse. The investigators did not look at appendectomy or smoking as confounders and a number of patients with extensive disease will have been excluded from this cohort by requiring colectomy earlier in their disease course. Treatment options were limited compared with current approaches, with a lack of steroid-sparing therapy offered to patients with more severe disease.

In a more recent population-based study of UC in a Norwegian cohort, Henriksen *et al.* [39] described relapse rates and the disease course in 454 patients over 5 years. Over this period, 78% of patients experienced at least one relapse, with an increased risk found in females (83.5 vs 73.5%, $p = 0.01$) and younger patients (mean age 38.5 vs 46 years, $p < 0.001$), but no relationship with disease extent or smoking status at diagnosis. In terms of disease patterns, by far the majority of patients experienced either a reduction in disease severity with time (59%) or intermittent relapses with time (31%), compared with a small minority with progressively increasing disease severity (1%) or chronic, continuous symptoms (9%). As with the data on disease course from Denmark [6], these different patterns did not show any association with initial disease extent.

Ulcerative proctitis – a separate entity?

There has been much debate as to whether ulcerative proctitis (UP) should be considered as part of the UC spectrum of disease. A number of large studies have been unable to address this accurately because of the issue of diagnostic criteria for UP in terms of disease extent. Studies from Copenhagen [6], the Cleveland Clinic [18] and Birmingham [40] have included patients with rectal and rectosigmoid disease as one group. UP only includes patients who have macroscopic mucosal involvement up to 15 cm from the anus and not beyond this. It is clear that a percentage of patients demonstrate disease extension from within the UP subgroup. This ranges from a cumulative rate of 11% 1–10 years after diagnosis, up to 30–31% 20–23 years after diagnosis [41–43]. However, a significant number of these UP patients may have only limited disease extension confined to the left colon and hence a lower risk of needing surgery. This point was well illustrated by Meucci *et al.* [44] in one of only a very limited number of recent studies specifically on UP [45,46]. In this study, the cumulative rate of proximal extension was 20% at 5 years, but only 4% for extension beyond the splenic flexure. Corresponding rates at 10 years were 54 and 10%, respectively. In an analysis of risk factors for extension, "refractory disease" (defined as at least one of chronically active disease, need for systemic steroids or more than three relapses per year) was identified as the only independent variable predictive of overall extension and extension beyond the splenic flexure. Need for systemic steroids or immunosuppression was the only factor independently associated with extension beyond the rectosigmoid junction.

Histological differences within rectal mucosal biopsies have also been noted when comparing those patients with extensive UC with those with distal UC, including UP [46]. The mean total cellular content within the lamina propria of those with UP and distal colitis was almost twice that of cases with extensive disease (including total colitis) ($p < 0.001$). The difference was not explained by other factors including age, gender, disease activity and treatment. This study may provide us with some histological evidence of disease heterogeneity that supports more recent genetic data [12], indicating potential

differences in disease pathogenesis between UP and extensive UC. Clearly, patients with UP should ideally be offered specialist follow-up at least initially, to determine disease severity [6,44] and hence their potential risk of disease extension.

Pediatric and adolescent UC

There are a limited number of studies that provide prospective data on the younger age group of UC patients. However, there are some important common themes, some of which have been alluded to in the preceding text. As with other issues regarding classification, in each of these studies a slightly different age category for inclusion was chosen, ranging from <15 years [47] to <19 years [48]. Using an age cut-off of <15 years, Langholz *et al.* [47] identified that 7% (80 cases) of their total UC cohort of 1161 fell into this subgroup. Importantly, there was a median delay of 1 year between symptom onset and diagnosis (cf. 4 years for Crohn's disease), with a median age at diagnosis of 12 years (range 0–14 years). The disease was significantly more extensive at diagnosis in children compared with adults from the same population: 29% of children had total colitis and 25% had proctosigmoiditis, compared with 16 and 46%, respectively, in adults ($p < 0.0003$). However, colectomy rates were not significantly different between these two age groups. Although the cumulative probability of distal disease progression was much higher in children (70%) than adults (39%) at 15 years, this did not achieve significance, possibly influenced by the relatively small size of this pediatric cohort. Similar data on "young-onset" (symptom onset before 21 years) proctosigmoiditis disease progression and an increased colectomy rate compared with adults have been published by the Cleveland Clinic [49].

Young-onset UC patients have similar requirements for corticosteroid therapy to adult cases [48]. In a small series of 36 UC patients diagnosed <19 years in Olmsted County, Minnesota, USA, between 1940 and 2001, 39% of the cases had required corticosteroids by the end of 1 year of follow-up, and 14% were steroid dependent. These figures are similar to adult data from the same population [50]. In a large North American pediatric registry, figures for steroid use in the first year and steroid dependence were 78 and 26%, respectively [51]. These differences likely reflect differing referral patterns and temporal changes with respect to case ascertainment.

Other factors that may influence UC natural history

Appendectomy

UC has been considered as a confluent disease process extending proximally from the rectum. Discontinuous appendiceal involvement was first described in 1974 [52] and has been demonstrated in 15–86% of colonic resection specimens from patients with UC without cecal involvement [53–55]. Prospective colonoscopic analysis of UC patients found segmental inflammation and appendiceal involvement in 75% of cases with isolated distal colonic disease [56] and indicate that this skip lesion is most closely associated with distal UC rather than extensive disease. Although appendiceal orifice inflammation may be highly specific for underlying "appendiceal colitis" (100%), it appears to be insensitive (15%), with a number of patients having appendiceal involvement without appendiceal orifice inflammation in an open study of laparoscopic appendectomy for refractory distal UC [57]. Whether patients with appendiceal orifice inflammation differ in their disease course from those without this marker remains inconclusive [58,59].

Recent studies have also analyzed the potential influence of prior appendectomy on the course of UC. Appendectomy prior to UC diagnosis was associated with a delay in disease onset and with a clinically milder course, as manifested by a reduction in need for immunomodulatory therapy [odds ratio (OR) 0.15] and proctocolectomy (OR 0.33) in a large Australian cohort [60]. Independent studies from France [61] and Japan [62] support these beneficial effects, with less frequent episodes of disease activity in patients with previous appendectomy and a subsequent reduced risk of colectomy. Naganuma *et al.* [62] demonstrated a significantly lower relapse rate at 57.1% in the appendectomy group compared with those who did not undergo appendectomy (78.6%, $p < 0.05$). One study did not find any protective effect on the natural history of UC [63]. However, the analysis may have been limited by the small number of cases in the UC–appendectomy group ($n = 12$) and by a highly specialized patient subgroup followed up by a single practitioner.

The effect of appendectomy on disease course after an established diagnosis of UC remains controversial. In a population-based analysis in Scandinavia, appendectomy post-diagnosis had no significant beneficial effect on admission rates to hospital compared with UC patients with their appendix *in situ* [64]. The decline in admissions for UC relapses observed in the appendectomy group was similarly seen over a comparable time period in those with an appendix. However, the majority of patients with UC do not require hospital admission for their disease, being treated for the majority of relapses with oral or topical therapy in the community or in clinics. In smaller clinical studies of elective appendectomy in patients with distal UC, the results have been more promising but open to potential biases [57,65]. Although appendectomy may represent a novel therapy for patients with poorly controlled distal disease, randomized controlled trials are

required before firm conclusions concerning efficacy can be drawn.

Smoking

The relationship between smoking and UC is a curious but well established one. Smoking is negatively associated with UC, with UC being largely a disease of non-smokers. The epidemiological support for the association is very strong, with a number of studies showing that patients with UC are predominantly ex-smokers or non-smokers [66–69].

Studies of the effect of smoking on the clinical course of UC are difficult to conduct, for obvious reasons. When examining the clinical course of the disease, it becomes clear that cigarette smoking may ameliorate UC but the studies are not all concordant. Mokbel *et al.* [70] found a reduced need for steroids (52 vs 63%), a lower cumulative colectomy rate (32 vs 42% at 10 years) and reduced progression from distal to extensive disease (14 vs 26%) in smokers compared with non-smokers, which just reached statistical significance ($p = 0.04$]. Supporting this are data from Israel showing that UC smokers had less extensive disease than non-smokers ($p < 0.02$), fewer hospitalizations ($p = 0.01$) and fewer operations ($p = 0.025$) [71]. Two studies suggest that ex-smokers carry the highest hospitalization and colectomy rates, followed by non-smokers and then current smokers [72,73], but a number of studies have not found any associations between smoking status and either disease extent, treatment or colectomy [25,74,75]. A major issue in some of these studies was the very small number of smokers available for analysis, making statistical comparisons largely irrelevant. Of interest, smokers also have a lower incidence of pouchitis [76].

Even with the above information, patients with UC should be advised not to smoke because of the multiple other health risks associated with smoking. Patients should be clear about the relationship of smoking and UC and advised to make their own decision based upon their physician's recommendation together with a sound knowledge of all the facts.

Pregnancy

Only one major study has addressed the potential influence of pregnancy on disease course in UC [77]. This European cohort consisted of 2201 IBD patients diagnosed between 1991 and 1993 and followed up prospectively for 10 years. Of these, 777 were available for interview at 10 years, including 206 women with UC. There were 109 pregnancies conceived in these 206 women after the diagnosis of UC, of which 94 were successfully completed. The only effect of pregnancy on UC was a reduction in the number of relapses per year in the 3 years after the pregnancy period, compared with the years prior to conception (0.34 flares per year before versus 0.18 after, $p = 0.008$). Whether this is because a more intensive effort is made to keep the patient in remission or whether it is a direct effect of the pregnancy is unknown. Similar results were found for Crohn's disease.

The use of non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors

The cyclooxygenase pathway leads to the production of prostaglandins with both pro- and anti-inflammatory properties. There are two known isoforms of cyclooxygenase: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Conventional NSAIDs inhibit both cyclooxygenase isoforms.

NSAIDs are relatively contraindicated in patients with UC for fear of more frequent and early clinical relapse of quiescent UC. This relative contraindication is a problem for those patients who suffer from arthritis. Controlled clinical trials have shown that COX-2 inhibitors have fewer gastrointestinal side effects than the traditional NSAIDs. The mechanism of intestinal damage is not completely understood; however, it is thought that the exacerbation in IBD appears to be due to dual inhibition of the COX enzymes [78].

The safety of COX-2 inhibitors in patients with UC in remission is unknown. A placebo-controlled pilot trial to evaluate the safety of celecoxib has been performed on patients with UC in remission. A total of 222 patients with UC were randomized to receive oral celecoxib 200 mg or placebo twice daily for 14 days. Therapy with celecoxib did not have a greater relapse rate than placebo [79]. Takeuchi *et al.* [78] also found that selective COX-2 or selective COX-1 inhibition appears to be well tolerated in the short term in those with UC in remission.

Despite the above, patients with a history of UC should avoid using NSAIDs whenever possible. When symptoms warrant the use of NSAIDs, non-NSAID analgesics, such as paracetamol, should be considered as a first alternative. Only then should NSAIDs be considered for treatment. If an NSAID is used, it should be a COX-2 NSAID, and it should be prescribed on a short-term basis (less than 2 weeks) only.

Colectomy

In UC, colectomy is potentially curative and is therefore an important endpoint when looking at prognosis. It is also one of the patient's major concerns and much time is spent discussing colectomy with patients. Indications for and timing of surgery are very much dependent on where a patient is treated. Looking at UC, regardless of type, Table 15.8 displays the overall rate of surgery (as a

Table 15.8 Cumulative colectomy rates in UC.

Study	Year*	*N*	5 yr	10 yr	15 yr	25 yr
Ritchie [80]	1979	269	8	15		
Leijonmarck [21]	1990	1586	20	28		45
Farmer [18]	1993	1116	24.2	34		43.7
Langholz [6]	1994	1161	20	23.7	29.9	32.4
Henriksen [39]	2006	454	7.5			
Park [19]	2007	304	3.3		3.3	
Hoie [5]	2007	781		8.7		

*Year of publication of study.

percentage of the total population under study, *n*) and also displays this at 5, 10, 15 and 25 year intervals [5,6,18,19,21,39,80]. Clearly, there are potentially multiple confounders, including disease extent and duration, age at diagnosis (pediatric and adult cohorts combined versus adult cohorts alone), cumulative smoking status and relative "aggressiveness" of medical therapy at each unit versus "surgical influence".

The relationship between one of these confounders – disease extent – and rates of surgery in UC is shown in Table 15.9. The colectomy rate is much higher in those patients who present initially with total colitis compared with those who initially present with proctosigmoiditis [6,18]. Not surprisingly, high disease activity at diagnosis manifest by systemic symptoms such as weight loss and fever also correlated with colectomy. At the time of surgery, most patients with UC have a total colitis. Surgery rates were also estimated for total colitis that developed from proctitis, total colitis that developed from left-sided colitis and those cases that were total colitis from diagnosis [18]. The highest colectomy rate was in those in who had progressed from left-sided to total colitis [18]. Table 15.9 clearly illustrates significant differences in colectomy rates between studies, with the lower rates generally seen in the population studies and those carried out more recently, compared with those from tertiary centers [18], such as the specialized Cleveland Clinic. The latter also has a longstanding reputation as a center of excellence for colorectal surgery. This issue has become increasingly relevant since the introduction of the ileoanal pouch as a very realistic and popular alternative to a permanent ileostomy, making surgery a more acceptable option to both patient and clinician.

Table 15.9 Overall rates of surgery in UC relative to disease extent.

Study	*N*	Total colitis (%)	"Substantial" (%)	Proctosigmoiditis (%)	Overall (%)
Langholz [6]	1161	35	19	9	20.2
Farmer [18]	1116	61	53	14.2	37.6
Henriksen [39]*	454	22	3		7.5

*Extent at the time of surgery.

Mortality

Previous studies from multiple geographic areas are not in complete agreement as to whether UC is a risk factor for mortality [81–84] or not [85–89]. Many of these studies were hospital based or retrospective or covered periods of many years. Hence their results need to be viewed with caution as they used different criteria for selection of patients and as both treatment and disease change over time [90]. It was the older studies that reported a poor prognosis in UC [26,84]; however, death from IBD is now rare, with fewer than 400 deaths per year now being certified as due to IBD in the United Kingdom [91]. Also, over 75% of deaths occur in those patients over 70 years old, with IBD having its greatest prevalence in those less than 40 years old.

A population-based cohort study in the United Kingdom of 8301 patients with UC with appropriately matched controls found a hazard ratio for death in UC of 1.44 [92]. This is consistent with the data from Ekbom *et al.* [83] and Persson *et al.* [81]; however, these studies included data from as early as the 1960s. The majority of other population-based studies have shown normal or even improved survival among UC patients compared with the general population [85–89]. In particular, the proportion of deaths directly attributable to IBD has decreased, as have deaths from the complications of surgery and malnutrition [93].

A population-based cohort from Copenhagen County comprising 1160 patients confirmed that UC patients have an overall normal life expectancy. Approximately 10% of deaths were caused by complications or co morbid conditions to UC. The patients older than 50 years of age at diagnosis and with extensive colitis showed an increased mortality within the first 2 years because of UC-associated causes [86]. In a European-wide population-based cohort of 781 patients, higher mortality was not found in patients with UC 10 years after disease onset [5]. This study had a short inclusion period, patients were selected from a range of centers across Europe and Israel and uniform diagnostic criteria were used. The values for survival in this cohort were almost identical with those expected throughout the entire 10 years, as determined by the WHO Mortality Database.

In the most recent population-based study of IBD patients in North America, overall survival was similar to that expected in the US white population, with an almost unchanged pattern of survival over the past six decades [17]. Older age and male gender were associated with

increased mortality. A total of 62 deaths occurred in 378 UC patients, compared with 79.2 expected. Median age of death was 81 years in women and 71 years in men. Observed mortality was less than expected in all age strata, but this was not as prominent among patients more than 50 years old at diagnosis. Overall, it is now understood that survival in UC is slightly greater than expected due to a decreased mortality from cardiovascular diseases and possibly smoking-related deaths such as lung cancer [17,85,94]. This is possibly explained by a lower systolic and diastolic blood pressure in UC patients and a lower incidence of smoking.

Most of the above studies were carried out prior to the introduction of immunosuppressive agents or biological agents for UC and therefore the effect of immunosuppression on outcome of UC has not been well studied. Jess *et al.* [17] showed that only 20 and 3% of patients studied between 1980 and 2001 and between 1960 and 1979, respectively, received immunosuppressive or biologic agents. Among these 46 patients there were 3 deaths versus 2.3 expected. This is difficult to interpret as it is generally the more severe cases in whom immunosuppression is used. We do not as yet know the effect of biologic therapy on natural history. Patients at the more severe end of the spectrum are the subject of a record linkage study from England [95]. Roberts *et al.* used data from the Oxford region (1968–1999) and from England (1998–2003) to study mortality in those IBD patients undergoing elective colectomy compared with the groups undergoing either emergency colectomy or no colectomy for their disease [95]. The results are somewhat alarming in that they indicate significantly higher mortality rates in those undergoing emergency colectomy [13.2 (95% CI 11.0–15.8)] or no colectomy [13.6 (95% CI 12.8–14.5)] compared with elective colectomy [3.7 (95% CI 2.7–4.9), $p < 0.001$] for UC. The data are similar for Crohn's disease. Although the study lacks detailed clinical data on history, severity and treatment of individual cases, the implication is that the threshold for elective surgery in IBD in England is currently set too high [31,96,97].

Colorectal cancer in UC

The actual risk for colorectal cancer (CRC) in UC patients is unknown. The first report of intestinal cancer occurrence in IBD was published over 80 years ago [98]. Since then, numerous studies have addressed this issue, but the true risk of malignancy remains uncertain. The magnitude of risk observed in studies from referral centers [99,100] generally exceeds the risk reported in population-based studies [101–104], and in some population-based studies the overall risk of CRC is even comparable to the background population [105,106]. A meta-analysis in 2001 summarized the available data on CRC in UC patients and showed an increasing cumulative probability of CRC during the disease course [107]. However the meta-analysis included a variety of studies with different designs. A recent population-based study from North America [108] showed that the CRC risk among UC patients overall was similar to that expected in the general population. This is in accordance with a North American study from the 1980s [109] and with recent data from Denmark where the standardized morbidity ratio for CRC in UC was 1.05 (95% CI 0.56–1.79) [106]. On the other hand, population-based studies in Canada [107], Israel [102] and Sweden [101,103] have shown increased relative risks of CRC in UC, ranging from 1.4 to 6. Maintenance treatment with 5-aminosalicylic acid (5-ASA) agents has been advocated in both North America and Denmark and the colectomy rates are also higher in these countries. Whether the low cancer risk observed in North America and Denmark is due to maintenance treatment with mesalamine, surgery, close follow-up or other factors remains to be investigated. It is important to note that surveillance colonoscopy is not performed as part of the standard follow-up regime in Denmark.

We do know that there are factors that increase the risk of CRC in UC. These include longer disease duration, greater proportion of colonic involvement, younger age at diagnosis, coexistence of primary sclerosing cholangitis (PSC), family history of CRC and evidence of ongoing active colonic inflammation.

Disease duration

Chronologically longer colitis yields higher rates of CRC [110]. Most investigators used the time of diagnosis as the start point, others use onset of symptoms. In Eaden *et al.*'s meta-analysis [107], incidence was found to increase with the passage of each successive decade: incidence was 2 per 1000 patient years at 10 years and 11 per 100 patient years at 30 years. The overall cumulative CRC risk starts meaningfully to exceed that of the general population by 8–10 years and therefore most clinicians will initiate surveillance colonoscopy once this threshold has been reached.

Proportion of colonic involvement

The length of the involved colon also correlates with cancer risk: the greater the extent of the colitis, the greater is the cancer risk. Ekbom *et al.* [101] demonstrated an impressive gradient of risk as one moves from proctitis [standardized incidence ratio (SIR) = 1.7] to left-sided colitis (SIR = 2.8) and to total colitis (SIR = 14.8). Many other studies have shown similar trends. As is discussed above, extent of mucosa affected is a dynamic process. It would appear sensible to take the maximum extent of disease during the entire disease course to risk stratify patients.

This will most likely overestimate some patients' risk, but will capture all patients at risk.

Coexistence of primary sclerosing cholangitis (PSC)

Most studies support an association of UC/PSC patients and CRC, with ORs ranging between 9 and 16 [111–114]. There was initially some skepticism with respect to this association, given the potential confounder of disease extent and the difficulty in establishing the year of diagnosis of the colitis in some patients with PSC [115]. Back-wash ileitis has also been put forward as another potential risk factor for CRC [116] and is often a feature of PSC–colitis [117].

Family history of CRC

Family history of CRC in UC patients must not be ignored. Several studies have suggested an increased risk for CRC in UC when a positive family history was documented, with ORs ranging from 2.3 to 5.0. [118–120]. Whatever the absolute magnitude, it is very likely that a family history confers an increased risk of CRC in UC.

Colitis-associated cancers differ in substantial ways from sporadic CRC. They have a reputation for being more aggressive than their sporadic counterparts – colitis patients develop cancer at a younger age and not uncommonly develop multiple tumors that are often found in the rectum, tending to be mucinous and poorly differentiated [121–123]. The molecular genetic changes in colitis-associated cancers also appear to differ in timing and frequency from sporadic CRC. Development of aneuploidy clones and a p53 mutation appear to be early events in colitis-associated CRC compared with sporadic CRC [124–127]. Ki-ras, APC and B-catenin are also mutated later and less frequently in colitis-associated neoplasia compared with sporadic CRC [128,129]. CRC in UC may also be related to the presence of an unstable genome in association with telomere shortening. The reported incidence of micro satellite instability (MSI) in UC-associated CRC is highly variable between studies. Overall, it seems that the incidence of MSI is not overrepresented in colitis-associated lesions as compared with sporadic CRC. Michael-Robinson *et al.* [130] found that the incidence of MSI, especially MSI-high, is lower in colitis-associated lesions than in sporadic CRC and therefore the majority of colitis-associated tumors were microsatellite stable (MSS).

Surgery (proctocolectomy) is the most effective method for minimizing CRC in UC patients as this virtually eliminates the risk (note that rectal cancer can still occur in those with an ileal pouch due to remaining rectal mucosa). However, surgical prophylaxis in asymptomatic patients with longstanding colitis is now viewed with a large amount of skepticism by both patients and physicians.

Surveillance colonoscopy

Periodic colonoscopy with biopsy for dysplasia has become routine in the clinical management of longstanding UC. There is limited evidence that this surveillance actually results in a cancer-specific mortality benefit. In the absence of any prospective trials on this issue, a well-designed population-based case–control study showed that when patients with CRC deaths were compared with live controls matched for age, gender, disease distribution and disease extent, previous colonoscopy appeared to decrease the risk of CRC significantly [131]. If a patient had had one or two previous colonoscopies, the risk decreased three-fold. Decision analysis models have also demonstrated effectiveness of surveillance versus no surveillance. When surveillance is compared with prophylactic colectomy, the value of surveillance ranks between that of prophylactic colectomy (most life-years saved) and no surveillance (baseline state) [132].

If high-grade dysplasia is found, there is widespread agreement that the patient should undergo colectomy because of the substantial rate of concurrent adenocarcinoma. Considerable controversy surrounds the management of low-grade dysplasia (LGD). In a landmark study from St Marks Hospital, the rate of progression to advanced dysplasia from LGD was 54% at 5 years [133]. The Mayo Clinic found that the risk of progression was 50% at 40 months. [134]. Their recommendation was to perform total proctocolectomy in patients with LGD. In 1994, a review of 10 previously published surveillance trials found that 16% (3/19) of patients who underwent colectomy for LGD had a synchronous colorectal cancer [135], while a recent meta-analysis concluded that the risk of developing cancer in patients with LGD is high [136]. Variable rates of progression make it difficult to draw strong conclusions; however, early colectomy for LGD that has been confirmed by two pathologists should be strongly considered. If patients refuse colectomy, it is important that colonoscopic surveillance continues.

Mortality from colorectal cancer

Multiple studies in the 1990s showed an increased risk of mortality from CRC in UC with ratios between 2.0 and 4.4. [81,83,85]. A more recent study by Winther *et al.* [86], however, showed no increased mortality from colorectal cancer. The patients who died from CRC did not differ from patients who died from other causes with regard to disease extent at diagnosis or age at diagnosis. The EC-IBD 10 year follow-up of 781 patients showed only one death from CRC [137]. A 10 year follow-up period is, however, too short to conclude whether or not CRC has an impact on UC-related mortality, as this cancer is known to have

a long latency period. However, it is certainly concordant with the data from Winther *et al.* [86]. They proposed that the impact of surveillance colonoscopy, colectomy and 5-ASA agents has led to the apparent decline of CRC in UC. Future studies are needed to clarify these connections.

Does health education change the natural history of UC?

It could be hypothesized that by increasing patients' health education it may add significant and sustained benefits to patients' well-being and thus change the natural history of the disease. There is evidence to suggest that health education in patients with chronic arthritis has sustained health benefits while reducing health costs [138]. Chronic arthritis is a prototypic chronic disease which requires protracted management and thus has a parallel to UC. Lorig *et al.* evaluated patients who participated in the Arthritis Self Management Program over a 4 year period [138]. Although there was no formal control group, the results were very positive. Participants were taught in 6 weekly, 2 h sessions by pairs of trained lay-leaders. Each course was attended by 10–15 participants. Content included pathophysiology, design of individualized exercise and relaxation programs, appropriate use of injured joints, an overview of medications, aspects of patient–physician communications and methods for solving problems that arise from illness. The course was taught from a structured protocol in an interactive manner. The data indicate that this education had significant, long-lasting benefits in reducing pain and use of medical services.

By increasing education it could be proposed that the following components could be increased in UC: compliance with medication, reinforcement of early alarm symptoms that require treatment and education regarding colonoscopy surveillance. These factors could potentially impact on the natural history of UC. There are limited studies similar to this in IBD [139–142], including a randomized trial of self-management versus the standard "physician-led" approach in UC patients [136]. This study indicated that many patients are keen to manage their disease more independently, leading to potentially better outcomes, including fewer relapses and hence reductions in both admissions to hospital and unnecessary attendance at outpatient clinics. However, there was no difference in quality of life scores between groups.

Conclusion

The introduction of 5-ASA agents and corticosteroid therapy has revolutionized the medical management of UC over the past 50 years, and pouch surgery has offered patients a realistic alternative to a long term stoma. Many recent population-based studies support a better long term prognosis for this disease, with mortality rates similar to or better than those in the general population. However, with an increasing knowledge of the genetics of UC and a greater awareness of both phenotypic and genetic heterogeneity, it may be an appropriate time to review the current criteria commonly used to diagnose UC. The reassuring data on mortality need to be balanced against the variability in treatment paradigms utilized in different countries, the increasingly aggressive medical management of the disease and the need for far more outcome-based studies in a greater range of populations.

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Chapter 16
Crohn's Disease: Clinical Course and Complications

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Summary

- The clinical features of Crohn's disease depend to some extent, but not exclusively, upon the anatomic location of the disease, the severity of the inflammation and the age of the patient.
- Crohn's disease is best understood to be a disease of the gastrointestinal tract with protean extraintestinal manifestations involving diverse organ systems, including the musculoskeletal system, skin, eyes, urogenital tract, cardiovascular system and neurologic system.
- The intestinal complications of Crohn's disease are primarily categorized as perforating complications, which include fistulas, abscesses and free perforation and cicatrizing complications (stricture). Adenocarcinoma of large and small bowel is a rare complication of longstanding disease.
- Crohn's disease should be considered progressive and over long periods of observation, complicated disease behaviors occur in the majority of patients. However, the rate of progression of the disease is highly variable and difficult to predict for a specific individual.
- Clinical and subclinical features associated with complicated disease behavior include early age of onset, need for treatment with corticosteroids, presence of serologic markers against various microbial agents and genetic polymorphisms, including NOD2/CARD15 disease susceptibility polymorphisms.

Introduction

Crohn's disease is a clinically heterogeneous condition. The clinical presentations of Crohn's disease differ among individuals with respect to symptoms and signs, anatomic location, age of onset and complications arising within and outside the bowel (see Table 16.1). This marked variability in disease expression extends to large differences in natural history and prognosis. Recent findings of serologic and genetic heterogeneity among patients with Crohn's disease suggest that divergent pathophysiology is the basis of this clinical heterogeneity. However, despite the growing understanding of the pathophysiological basis of Crohn's disease, the ability to predict the course of disease remains elusive. As no single test establishes a diagnosis of Crohn's disease, it is important for the clinician to consider all relevant information, including points of the history and physical examination, laboratory data, endoscopy and imaging. Understanding the variable presentation of Crohn's disease and the ability to integrate diverse sources of clinical data are essential for expert management of this complex and challenging condition.

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Clinical presentations

Presenting complaints

The presenting symptoms and signs of Crohn's disease occasionally may be abrupt and dramatic in onset. Most often, however, the initial symptoms are not specific for the disease and are mild and insidious in onset. These factors, along with the relative rarity of the disease by comparison with the far more prevalent diagnosis of irritable bowel syndrome, may contribute to the historically long delay in time to definitive diagnosis. A population-based study reported from Manitoba, Canada, suggested that 41% of patients had a 3 year or greater delay in diagnosis from the onset of symptoms [1]. In another study, the time to diagnosis was, on average, 7 years in patients with Crohn's disease, even when those individuals meeting Rome criteria for irritable bowel syndrome were excluded from the analysis, as compared with less than 1 year to diagnosis for patients with ulcerative colitis [2]. A longer delay in diagnosis may be seen in older patients, as compared with those who present in childhood. As awareness of the disease increases and endoscopic and imaging techniques improve, it is possible that the delay in diagnosis of Crohn's disease will diminish over time. However, this will require clinicians to maintain a high index of suspicion

Table 16.1 Clinical features of Crohn's disease.

Common symptoms
Abdominal pain
Diarrhea
Fever
Fatigue
Rectal bleeding
Weight loss
Anorexia
Nausea
Common clinical signs:
Abdominal tenderness
Palpable mass in the right lower quadrant
Guaiac-positive stool
Common laboratory and radiographic findings
Mild anemia
Mild leukocytosis
Elevated erythrocyte sedimentation rate or C-reactive protein
Small bowel involvement
Fistulas
Strictures
Extra-intestinal manifestations
Joint manifestations (25%)
Arthralgia
Arthritis
Mucocutaneous manifestations (15%)
Erythema nodosum
Pyoderma gangrenosum
Aphthous ulcers of the mouth
Ocular manifestations (5%)
Episcleritis
Uveitis
Recurrent iritis

for the diagnosis as the predominant symptoms may be mild and extremely variable among patients.

The most common presenting symptoms of Crohn's disease are diarrhea and abdominal pain. It is possible, however, for affected individuals to present with neither symptom; rather, a host of atypical presentations may occur, particularly in children and the aged.

Diarrhea is the most common symptom and presentation of Crohn's disease, affecting approximately 85% of patients at diagnosis. Decreased stool consistency and increased stool frequency are consequences of mucosal inflammation. In severe cases, an individual may have in excess of 20 stools daily. Nocturnal diarrhea, when present, is a strong indicator of the organic nature of the illness and is a manifestation of more severe disease. Stool consistency may range from formed to watery. Elaboration of pro-inflammatory cytokines, such as interferon-γ and tumor necrosis factor, promotes electrolyte and water secretion by the intestinal epithelium and also contributes to relaxation of the epithelial tight junctions. Inflammation further contributes to disturbed intestinal motility, through the elaboration of cytokines, biogenic amines, prostaglandins and neuropeptides. Inflammation also results in altered function of the enteric neurons in circular and longitudinal muscle. As Crohn's disease often involves the terminal ileum, bile salt-induced diarrhea may occur. In some patients, the presence of stricture (see below) and bacterial overgrowth may contribute to diarrhea. The stool most often does not contain obvious red blood. Rarely, however, frank lower gastrointestinal hemorrhage may complicate active disease when ulceration is marked and penetrates deep into the mucosa and submucosa.

Abdominal pain is the second most common symptom of Crohn's disease. As with diarrhea, the causes of abdominal pain in Crohn's disease are likely to be multifactorial [3]. Descriptive studies have noted a range of abnormalities suggesting the contribution of the enteric nervous system to Crohn's disease. Neural dysfunction is suggested by the presence of hypertrophic ganglia in the myenteric plexus. Substance P, a key neuropeptide involved in the stimulation of pain fibers, is present in abundance in the inflamed gut in Crohn's disease, along with increased expression of receptors for substance P on the enteric neurons and lymphoid follicles surrounding the micro-vasculature in the gut [3].

The abdominal pain of Crohn's disease may be diffuse, but is most often localized in the right lower quadrant, in keeping with a predilection for terminal ileal and right colonic involvement. Abdominal pain may be continuous or intermittent, most often exacerbated by eating. Severe bloating and cramping may occur in patients who develop intestinal stricture as a complication of the disease. Visceral hyperalgesia may contribute to chronic abdominal pain, particularly in patients who have had multiple abdominal surgeries. Chronic narcotic dependence affects a small minority of Crohn's disease patients with chronic abdominal pain and has been identified as an independent risk factor for mortality in the disease [4]. As much as possible, chronic use of narcotics should be avoided by adequately treating the underlying condition and proactively avoiding the complications that may lead to repeated surgery.

Weight loss completes the triad of symptoms most commonly noted at presentation. As with diarrhea and abdominal pain, the factors contributing to weight loss are variable. Contributing factors may include accelerated transit through the gut, disruption of the absorptive surface of the bowel by ulceration, protein losses from the inflamed gut and increased caloric requirements from a catabolic state induced by inflammation. Anorexia may occur in the setting of active inflammation, while nausea and vomiting may occur as a result of stricture or, particularly in children, delayed gastric emptying related to gastroduodenal Crohn's disease. Rarely, weight loss and malnutrition may occur as a result of internal enteric fistulas and consequent bypassing of bowel. By far the most common cause of weight loss, however, is voluntary restriction of oral intake to minimize pain and diarrhea related to eating. It is

important to note that specific nutrients may be deficient, even while weight and overall nutrition are maintained and the disease is clinically quiescent. The most common specific deficiencies include vitamin B_{12}, iron, calcium and fat-soluble vitamins.

Fever may be the sole presenting complaint in Crohn's disease and it has been estimated that perhaps 2% of cases of fever of unknown origin may be attributed to this diagnosis [5]. Most often fever is not an isolated symptom, however, and is low grade. Higher spiking fever should raise the suspicion for perforating or pyogenic complications of the disease or superimposed infection. Clinical suspicion of infection should be especially high in patients on immune suppressing therapies, such as corticosteroids, antimetabolite therapy or anti-TNF biologic agents. Occasionally fever occurs in Crohn's disease in the absence of an identifiable abscess or infection.

The manifestations of Crohn's disease extend beyond the gastrointestinal tract to include extraintestinal complaints in addition to constitutional symptoms. Children in particular may have prominent constitutional signs and symptoms, as only 25% of children and adolescents present with the classic triad of abdominal pain, weight loss and diarrhea [6]. In children, growth failure or retarded development of secondary sex characteristics may also occur. Typical complications associated with Crohn's disease arising outside of the gut may include manifestations in the eyes, skin, joints and liver. There has been an increased awareness that Crohn's disease of long duration can be accompanied by adenocarcinomas of the small or large bowel and rarely lymphoma.

Common presentations

The symptomatic presentation of Crohn's disease usually depends on the site, extent and severity of the disease at the time of presentation. Patients with small bowel disease will often present with abdominal pain, whereas those with colonic disease suffer from diarrhea, hematochezia and a dull, aching, abdominal pain. Classifying patients according to disease location does not help to determine prognosis or disease severity; however, it can be useful in understanding disease manifestations, indications for surgery, risk of postoperative recurrence and for selecting the best available treatment options.

Ileocolonic disease

The majority of patients (50%; see Table 16.2) will present with disease involving the distal ileum and right colon (ileocolonic). Most patients (two-thirds) will experience abdominal pain in the right lower quadrant. If the patient has partial bowel obstruction from stenosis, the pain may be exacerbated within 30–60 min of a meal. Additional symptoms of ileocolonic Crohn's disease include blood in stool (22%) and malnutrition with weight loss and muscle wasting (12%). About 20% of patients with ileocolonic disease will suffer from perianal disease with fistulas [7–9]. Intestinal complications include obstruction, inflammatory mass or abscess. Extraintestinal manifestations, including pyoderma gangrenosum, colitic arthritis and spondylitis, occur less often in patients with ileocolonic disease when compared to patients with colonic disease alone.

Table 16.2 Anatomic disease location.

	Farmer *et al.* [7]		Mekhjian *et al.* [8]	
Disease location	Adult (%)	Child (%)	Adult (%)	Child (%)
Ileocolonic	41	50	55	60
Ileal	29	30	41	35
Colonic	14	10	27	15
Perianal	22	49	47	50
Jejunal	4		10	
Gastroduodenal	5	30	5	30
Esophageal	Rare	Rare	Rare	Rare
Oral	Rare	Rare	Rare	Rare

Adapted from data in [6] and [7].

Patients with ileocolonic involvement require surgery more often than those with small intestinal disease, colonic disease or anorectal disease. A cohort study from the Cleveland Clinic demonstrated that 91.5% of those with ileocolonic disease required at least one surgical resection of the distal ileum, the right colon or both [7]. The most common indications for resection include intestinal obstruction, perianal disease, intestinal perforation with abscess and toxic megacolon. The long-term survival rate was 94% after 10 years, which is similar to those of other presentations [7].

Ileal disease

Ileal disease is the second most common form of Crohn's disease, affecting 30% of patients. The onset of ileal disease can be sudden, with severe symptoms, or may be mild, with subtle symptoms. Patients with active ileal inflammation often present with anorexia, looser, more frequent stools and weight loss. During a physical examination, these patients may have low-grade fever or evidence of malnutrition. Occasionally, patients may present with acute right lower quadrant pain mimicking appendicitis. The most frequent complaint associated with ileal disease is diarrhea. The diarrhea of ileal Crohn's disease may be mild or severe, occur any time during the day or nocturnally and may be intermittent or postprandial in nature. Another common symptom is crampy lower abdominal pain localizing to the right lower quadrant. Postprandial exacerbation of pain is most typical and larger meals or those containing greater amounts of residue may heighten the pain. Signs and symptoms of malnutrition may be seen frequently in patients with ileal disease due to reduced caloric intake or extensive disease. Patients with ileal disease tend to have perianal fistulas less commonly than patients with colonic or ileocolonic disease. In addition,

rectal bleeding is a less prominent feature than in patients with colonic disease.

Colonic disease

Crohn's disease involving the colon can be difficult to distinguish from ulcerative colitis. Approximately 20% of patients with Crohn's disease present with disease involving the large bowel alone. Colonic disease may affect the right colon or it may extend distally to involve most or all of the colon. Half of patients with more extensive colitis will have relative or complete sparing of the rectum. Patients with Crohn's disease limited to the colon commonly present with rectal bleeding, perianal complications and extraintestinal manifestations involving the skin or joints. The majority of patients present with diarrhea, the severity of which tends to correlate with both the extent of colitis and the severity of inflammation. Abdominal pain is also common. Although most patients with Crohn's colitis have relative or complete sparing of the rectum, proctitis may be the initial presentation in some cases. One study with long-term follow-up found that 24% of patients with colonic disease at initial presentation may ultimately develop small intestinal involvement [10]. Approximately 50% of patients with colonic Crohn's disease require surgical resection within 10 years of diagnosis and half of those require ileostomy [10].

Perianal disease

Estimates for the cumulative incidence of perianal disease range from 22 to 45% [11,12]. In as many as 24% patients with Crohn's disease, perianal disease precedes intestinal manifestations with a mean lead time of 4 years [13]. More often, however, perianal disease occurs subsequent to the onset of symptoms of luminal disease. It is more common to see perianal fistulas complicating colonic or ileocolonic Crohn's disease. In patients with colonic disease, perianal manifestations are more common when there is left colonic involvement than when there is only proximal colonic disease. Although perianal disease activity does not always parallel activity in the intestine or at other extraintestinal sites, patients whose intestinal disease is quiescent are likely to have better outcomes after surgical treatment. Patients who develop perianal complications at an early age, have a fistula as the first manifestation of perianal disease or have rectal involvement may be at increased risk for ultimately requiring abdominoperineal resection [10].

The major perianal complications include fissures, ulceration, fistulas, abscesses and strictures. Symptoms can vary from anal pain and purulent drainage to bleeding and incontinence and can be associated with significant morbidity and impaired quality of life. Perianal complications can be subclassified into skin lesions (anal skin tags, hemorrhoids), anal canal lesions (anal fissures, anal ulcers and anorectal strictures) and perianal fistulas (see Table 16.3). The anal fissures in Crohn's disease tend to be eccentrically located rather than occurring in the midline as is more common with idiopathic fissures. In most cases anal strictures are asymptomatic, but occasionally obstructive symptoms and a sense of incomplete evacuation may occur, particularly if stool consistency improves in the course of treatment. Deeper perirectal abscesses may arise secondary to fistulas especially when the internal os of the fistula is located high in the rectum.

Three population-based studies have reported various estimates for the cumulative incidence of perianal fistulas. Hellers *et al.* reported that perianal fistulas occurred in 23% of patients in Stockholm County, Sweden [14]. The occurrence of perianal fistulas was strongly associated with the site of disease. Perianal fistulas occurred in 12% of patients with ileal Crohn's disease, 15% with ileocolonic disease, 41% with colonic disease with rectal sparing and 92% with colonic disease with rectal involvement [14]. Similarly, Schwartz *et al.* reported a cumulative incidence of perianal fistulas in 21% of Crohn's disease patients in the population-based cohort from Olmsted County, Minnesota, USA [15]. Most recently, Tang *et al.* reported only 12.6% of patients with Crohn's disease in Manitoba, Canada, developed perianal fistulas [12].

Less common presentations

Upper gastrointestinal involvement

Crohn's disease involving the gastrointestinal tract proximal to the ileum is relatively uncommon. In such cases, it is distinctly rare for the disease to be confined solely to the esophagus, stomach, duodenum or jejunum. Wagtmans *et al.* found 72 of 940 patients (8%) had disease of the mouth, esophagus, stomach, duodenum or jejunum [16]. All had gastrointestinal symptoms. Upper gastrointestinal involvement with Crohn's disease is most frequently found in the gastric antrum, the duodenal bulb and the duodenal loop. Involvement of the esophagus, gastric corpus and gastric fundus is less frequent, as is localization in the jejunum. The age of onset of affected individuals is often younger than those who did not have upper tract involvement. Nugent and Roy suggested specific criteria for diagnosing upper gastrointestinal Crohn's disease: (1) histologic presence of non-caseating granulomatous inflammation of the duodenum with or without obvious Crohn's disease elsewhere in the gastrointestinal intestinal tract and without evidence of systemic granulomatous disorder or (2) documented Crohn's disease elsewhere in the gastrointestinal tract and radiologic and/or endoscopic findings of diffuse inflammatory change in the duodenum consist with Crohn's disease. The same criteria may be applied to the esophagus, stomach and jejunum. Approximately one-third of patients with proximal Crohn's disease do not have evidence of distal Crohn's disease at the time of diagnosis. However, virtually all developed distal disease, over time.

Patients often present with non-radiating epigastric abdominal pain and malaise. Patients may also experience

Table 16.3 Classification of perianal disease.

Skin tag	Two types: Large, edematous, hard, cyanotic skin tags. Typically arising from a healed anal fissure or ulcer. Excision contraindicated due to problems with wound healing "Elephant ear" tags that are flat and broad or narrow, soft painless skin tags. May cause perianal hygiene problems and can be safely excised
Hemorrhoids	Prolapsing internal hemorrhoids. Uncommon in Crohn's disease. Often present as large external skin tags
Fissure	Anal fissures are broad based and deep with undermining of the edges. There may be associated large skin tags and a cyanotic hue to the surrounding skin. They tend to be multiple and may be placed either eccentrically around the anal canal or in the midline, in contrast to idiopathic fissure *in ano*, which tend to lie in the midline. Typically painless (pain should raise suspicion for perianal abscess or acute/chronic conventional anal fissure). Conventional anal fissures occasionally are treated by conventional fissure treatment including lateral sphinceterotomy
Anal ulcer	Anal ulcers are usually associated with rectal inflammation and may lead to destruction of the anorectum, anorectal strictures, complex anorectal fistulas and perianal abscess
Low fistula	Superficial, low intersphincteric or low transsphincetric fistulas. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum
High fistula	High intersphincteric, high transsphincteric, suprasphincteric, extrasphincteric fistulas. Arise from penetrating ulceration of the anal canal or rectum
Rectovaginal fistula	Superficial, intersphincetric, transsphinicteric, suprasphincteric, extrasphincteric fistulas. Arise from penetrating ulceration of the anal canal or rectum into the vagina
Perianal abscess	Potential anorectal spaces may become infected with an abscess, including perianal, ishiorectal, deep postnatal, intersphincteric and supralevator
Anorectal stricture	May be short annular diaphragm-like strictures <2 cm in length or longer tubular strictures arising from rectal inflammation. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum
Cancer	Squamous cell carcinoma, basal cell carcinoma or adenocarcinoma arising from malignant degeneration of non healing perianal fistulas or sinus tracts

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early satiety, nausea, bloating and weight loss. Diarrhea is not common with upper gastrointestinal involvement alone. Symptoms may mimic the presentation of peptic ulcer disease, gastric cancer or pancreatitis. As with other locations, upper gastrointestinal involvement may be complicated by stenosis, fistulization or abscess formation. Patients with upper tract disease do not undergo surgery more often than do patients with lower tract disease alone but when resection is needed, the length of bowel that is resected tends to be longer [16].

Esophageal Crohn's disease is rare, occurring in less than 2% of patients [7]. The presenting symptoms may include dysphagia, odynophagia, substernal chest pain and heartburn. Collectively, these symptoms are found in 80–100% of patients with esophageal disease [16]. Symptoms may be progressive and lead to profound weight loss. Aphthous ulcers may sometimes be found in the mouth and posterior pharynx. Esophageal stricture and, very rarely, esophagobronchial fistula may complicate the course. Endoscopic findings demonstrate ulcers in 85% of patients and strictures in as many as 25% [17].

Gastric Crohn's disease is also uncommon. A series by Wagtmans *et al.* reported gastric involvement in 0.5% of patients [16]. Gastric Crohn's disease often presents as *Helicobacter pylori* negative peptic ulcer disease with dyspepsia or epigastric pain as the primary symptoms. When outflow obstruction occurs because of stricture formation or edema, early satiety, nausea, vomiting or weight loss may predominate. Fistulas to the colon and spleen have also been reported. The radiographic findings typically include a rigid antrum tapering to a stenotic pyloric channel.

Duodenal involvement occurs in 1–7% of patients diagnosed with Crohn's disease [16]. The segments most often affected include the bulb and second portion and there is often concomitant involvement of the pre-pyloric antrum. Duodenal involvement usually occurs in conjunction with ileal or colonic disease. Symptoms may include abdominal pain, diarrhea, weight loss, anorexia and malaise. Early satiety, nausea and vomiting can occur with or without duodenal obstruction, a frequent complication.

In older series, jejunal involvement was found in 4–7% of patients [8,9]. This estimate is likely to increase as newer endoscopic modalities such as wireless capsule endoscopy [18] and double balloon push enteroscopy [19] increase the sensitivity for ascertaining small bowel abnormalities. One study compared findings of wireless capsule endoscopy with computed tomography (CT) enteroclysis in 56 consecutive patients. After excluding 15 (27%) of patients for stricture seen on CT enteroclysis, wireless capsule endoscopy found ileal or jejunal lesions in more than

twice as many patients than was found on CT enterography (25 vs 12, $p = 0.004$) [18]. Findings included aphthous ulcers, erosions and villus denudation. Jejunal Crohn's disease may involve single or multiple areas of jejunum and may occur in association with ileal or colonic disease. Many patients with overt radiographic findings of jejunal disease on barium studies experience abdominal pain and cramping. Weight loss and diarrhea may be prominent symptoms in more extensive disease and may be severe enough to include frank malabsorption and steatorrhea. Strictures may also complicate jejunal disease over time, necessitating intestinal resection or stricturoplasty. One study demonstrated a higher rate of re-operation in patients who required jejunal resection, compared with patients with ileocecal disease [20].

Diffuse jejunoileitis is a rare condition, most often occurring in children and young adults, and is characterized by multifocal ulceration and stenoses in the small bowel. The condition is often complicated by bacterial overgrowth and protein-losing enteropathy and may prove highly refractory to medical therapies usually effective for Crohn's disease. There is an increased mortality associated with ulcerative jejunoileitis. It is uncertain if this is a rare variant of Crohn's disease or a distinct pathophysiologic entity. In some cases, ulcerative jejunoileitis appears to be a rare variant of celiac sprue, as some patients respond well to a gluten-free diet.

Oral manifestations

Crohn's disease is a truly panenteric inflammatory condition and may also affect the oral cavity. Older studies have found the incidence of oral manifestations of Crohn's disease to range from 4 to 12% [21]. A more recent series suggest that oral disease may be particularly common in children. In one case series of 48 consecutive children with Crohn's disease examined by a dentist, more than one-third were found to have oral findings that included mucogingivitis, mucosal tags, deep ulceration, cobblestoning, lip swelling and pyostomatitis vegetans [22]. Granulomas were often found in biopsies of the oral lesions and, interestingly, oral disease was associated with the presence of perianal disease. By far the most common oral manifestation of Crohn's disease is aphthous stomatitis. It is not unusual for patients to note recrudescence of painful aphthous ulcers in the mouth prior to or simultaneous with a flare. However, aphthous ulcers are very common in the general population and may not be a specific sign of Crohn's disease. Orofacial granulomatosis is a condition of children and young adults characterized by aphthous oral ulcers with non-caseating granulomas, swelling of the face or lips, cobblestoning of the oral mucosa and gingival hyperplasia. When these findings are accompanied by recurrent facial paralysis, the condition is called Melkersson–Rosenthal syndrome. Both conditions bear a striking resemblance to Crohn's disease, including a striking Th1 cytokine profile in the lesions [23], but in neither condition are intestinal findings of Crohn's disease noted. At present, it is unclear if these conditions are distinct from typical Crohn's disease or simply represent an unusual variant of the disease. Topically applied corticosteroids are a mainstay in the treatment of oral Crohn's disease; however, the oral manifestations of the disease will most often respond to the same systemic treatments administered to treat disease occurring in more distal and usual locations.

Appendiceal disease

Reports from older literature have described varied presentations of Crohn's disease involving the appendix. Ileal or ileocolonic Crohn's disease occasional presents as a syndrome mimicking acute appendicitis, which may lead to the correct diagnosis at laparotomy. Other presentations have included appendiceal abscess and hemorrhage. A classic, although rare, presentation of Crohn's disease is fistula occurring after appendectomy. Such patients are likely to have had disease affecting the terminal ileum or cecum, as more recent studies suggest that isolated Crohn's disease of the appendix is distinctly rare, if it occurs at all. A study in Copenhagen County, Denmark, found that among more than 5000 patients who underwent appendectomy over a 5 year period, only 0.1% had epithelioid granulomas in the appendix [24]. None of the patients with granulomas in the appendix had recurrent gastrointestinal difficulties over a mean follow-up of 10 years. Conversely, less than 1% of patients diagnosed with Crohn's disease over a 25 year period had disease confined to the appendix at presentation. Notably, none of these patients experienced recurrent disease after appendectomy over a 6 year mean follow-up [24].

Presentation in the aging patient

Some, although not all, population-based studies of incidence suggest a second peak in incidence in the sixth decade of life. In the Olmsted County, Minnesota, cohort, approximately 7% of prevalent cases are 60 years of age and older, while the incidence rate in this age group is approximately 5–6 per 100,000, nearly half the rate observed for the age range of 20–29 years [25]. As the genetic basis of Crohn's disease is increasingly well understood, it has become apparent that identifiable genetic susceptibility may be more prevalent in younger patients, with as yet undefined environmental factors presumed to play a great role in older patients. This is substantiated by the observation that patients diagnosed at an older age are found less often to have affected relatives [26]. Accordingly, the clinical features and prognosis in older patients may also be distinct from that of patients who are diagnosed at younger ages. For example, most studies have noted a greater tendency for colonic disease in older populations. One study compared the clinical features in patients diagnosed after the age of 40 years with those diagnosed between 16 and 40 years of age [26]. Presenting symptoms were similar in

both age groups, but older patients reported abdominal pain and cramping less frequently. Given the heightened concern for neoplastic, ischemic and diverticular disease in older populations, it is not surprising that there was a shorter time to correct diagnosis in the older patients, with a lower threshold for radiographic and endoscopic investigation. The same study observed similar rates of surgery, but the diagnosis of Crohn's disease was more often established at the time of surgery in older patients [26]. Most studies report similar disease outcomes in older patients and a similar response to therapies. However, there is greater concern for adverse consequences of medical and surgical interventions in older patients. For example, patients older than 50 years have an increased risk of hypertension, hypokalemia and change of mental status during treatment with corticosteroids as compared with younger patients [27]. In addition, older patients appear to have an increased risk of serious infection and congestive heart failure during treatment with infliximab [28]. One study examined the complications of abdominal surgery for Crohn's disease comparing patients aged 55 years and older with younger patients. Whereas the rate of anastomotic leak and mortality was the same in both groups, a significantly higher rate of cardiac and respiratory complications was noted in the older patients [29].

Complications

Historically, clinicians have attempted to classify Crohn's disease on the basis of typical intestinal complications or what has been called "disease behavior". Such complications have generally been divided into penetrating (fistulizing) and cicatrizing (stricturing) behaviors. It has been suggested by some authors that the individual's tendency for a particular behavior is a consistent feature over time, with some patients tending to have consistent disease complications over time and others expressing only inflammatory disease without penetrating or cicatrizing behavior. It has been observed that among patients who require an intestinal resection for stricture, a second resection is very likely to be for stricture [30]. Similarly, patients who undergo intestinal resection for fistula are likely to have the same indication should they require a second resection [30]. In addition, patients with a perforating indication for intestinal resection were observed to require their next resection twice as quickly as patients who had surgery for stricture [30]. These findings held true when analyzed by anatomic location of disease, suggesting that disease behavior is not merely a feature of ileal or colonic disease. Furthermore, studies of cytokine expression in the intestinal tissue of patients with a non-perforating indication for intestinal resection demonstrated significantly increased levels interleukin-1β and interleukin-1 receptor antagonist when compared with the intestinal mucosa of patients with intestinal resection for a perforating complication [31]. Together, these findings suggest that the tendency to form fistulas or strictures is an intrinsic feature of each patient's disease expression.

It is worth noting, however, that although the type complicated disease behavior expressed by an individual patient tends to be consistent over time, there are important exceptions. Internal fistulas are frequently found to arise proximal to intestinal strictures [32]. This may occur, in part, due to mechanical factors such as back-pressure behind an obstruction, as in these circumstances fistulas most commonly track along perforating blood vessels, a point of low mechanical resistance [32]. In addition, the occurrence of perianal fistulas appears to be somewhat distinct from the tendency to form internal fistulas. Perianal fistulas are not associated with consistently increased risk of internal fistulas in patients with ileal disease [33]. By contrast, a more consistent association is seen between perianal and internal fistulas in patients with Crohn's colitis [relative risk 3.4, 95% confidence interval (CI) 2.6–4.6] [33].

The factors contributing to an individual patient's disease behavior are gradually being elucidated. Evidence of a genetic contribution to disease behavior has grown. The presence of CARD15/NOD2 mutations has been associated with a Crohn's disease phenotype notable for younger age of onset, ileal involvement and complicated disease behavior, including both strictures and fistulas [34–37]. As strictures generally occur more frequently in ileal disease, not all studies have concluded that the risk of stricture with CARD15/NOD2 mutations is independent of the propensity for ileal localization [38]. However, one study found that children with the 1007fs mutation had a 6.6-fold increased risk of stricture requiring surgery [39]. In addition, environmental factors may play a role. In one study, smoking was associated with a strong tendency for fistula or stricture, as opposed to purely inflammatory disease, in the first 8 years of disease [40].

A key advance in understanding, however, is that disease behavior is not static; rather, disease behavior evolves over time. Numerous independent cohort studies have observed that Crohn's disease is most often inflammatory in its behavior at the time of onset and that clinical behavior changes over time. In one study, the cumulative probability of change in behavior from inflammatory to complicated behaviors was 22, 38 and 63% at 3, 6 and 12 years, respectively [41]. The same study noted cumulative probabilities of penetrating complications of 22, 33 and 55% at 3, 6 and 12 years, respectively [41]. A separate cohort from Paris, France, demonstrated 20-year actuarial rates of inflammatory, stricturing and penetrating disease of 12, 18 and 70%, respectively [42]. A third study in Belgium noted a change in disease behavior in 45.9% of patients observed over 10 years, most often from non-stricturing, non-penetrating disease to either stricturing (27.1%) or penetrating (29.4%) disease [43]. These cohorts are derived from different populations, perhaps accounting for the wide variation in estimates for change in

behavior. Taken together, however, these data suggest that disease behavior evolves over time. The majority of patients observed for long periods of time will demonstrate complicated behaviors; however, the rate of progression to complicated behavior is highly variable among patients, most likely the result of genetic and environmental influences that vary between individuals.

Strictures

Strictures occur in Crohn's disease as a consequence of chronic transmural inflammation. Fibroblasts are a key cellular component in fibrostenotic disease. Narrowing of the lumen results from wound healing and deposition of extracellular matrix, causing distortion and contraction of the lumen. The muscularis propria is typically thickened and the muscularis mucosa may be disrupted. Over time, this often leads to intestinal obstruction. Strictures may occur at any location within the gastrointestinal tract, localizing to any location where inflammation has been active. Not all presentations of luminal narrowing represent fibrotic stricture, however. The classic "string sign" – a long segment of bowel with markedly narrowed lumen with wide separation from other loops of small bowel seen on barium studies as a typical finding of Crohn's ileitis – is thought to occur primarily as a consequence of spasm. This finding typically resolves in response to glucagon, which temporarily alleviates spasm of the smooth muscle. Another classic radiographic finding suggesting stricturing in Crohn's disease is pseudosacculation: one or more areas of dilation of bowel on the anti-mesenteric side, corresponding to areas of ulceration and contraction on the mesenteric side of the lumen. Strictures are usually asymptomatic until the lumen is sufficiently narrowed to cause relative obstruction. Classic symptoms of colicky postprandial pain and bloating, progressing to episodes of frank obstruction, indicate higher grade stenosis. In such cases, radiographic studies are likely to demonstrate prestenotic dilatation. It may be difficult in practice to differentiate between inflammatory and fibrotic stenosis and, in fact, most stenoses have both elements. Strictures also have the potential to be a manifestation of cancer, which may complicate Crohn's disease in any location. Anastomotic strictures often occur after ileal or ileocolonic resection for Crohn's disease.

Fistulas

Fistulas occur in Crohn's disease when inflammation penetrates through to the serosa. This occurs through the elaboration of numerous proteases and metalloproteinases which contribute to degradation of the extracellular matrix. Serosal inflammation results in the creation of adhesions between the inflamed segment of bowel and the surface of the adjacent organ. Further penetration of inflammation occurs resulting in communication from the lumen of the inflamed bowel to the affected organ. Fistulas are considered to be internal when the fistula terminates in another organ within the body or external when the fistula communicates with the body surface. A wide range of organs may be affected. The most common internal fistulas are enteroenteric or enterocolonic, with enterovesicular, colovesicular and rectovaginal fistulas also frequently occurring. Internal fistulas are estimated to occur in 5–10% of patients with Crohn's disease. More unusual fistulas such as esophagobronchial fistulas, cologastric fistulas and gastrosplenic fistulas are described in the literature, but very rarely seen. Most internal fistulas bypass relatively short segments of bowel and are therefore clinically silent. These minor internal fistulas may be seen on radiographic study or noted at the time of surgery. Rarely, an internal fistula will bypass a long segment of the gastrointestinal tract, resulting in clinically apparent nutritional deficiencies or malabsorption. The predominant symptoms will otherwise depend upon the organ receiving the fistula.

Fistulas to the bladder typically present with symptoms of dysuria, pneumaturia and fecaluria. Recurrent urinary tract infections may be the sole presentation of enterovesicular or colovesicular fistulas. As a rule, enterovesicular fistula rather than colovesicular fistula should be suspected in women unless previous hysterectomy has been performed. Enterovesicular fistulas occur in 2–8% of patients [44]. The diagnosis will be strongly suspected from the history, but may be extremely difficult to diagnose. The preferred diagnostic tests are cystourethrogram or cystoscopy, where the os of the fistula within the bladder can be visualized as a heaped up area of mucosa surrounded by inflammatory changes. Colonoscopy is rarely useful even when the rectum is the source of the fistula, as the fistula orifice may be extremely small and difficult to visualize. Barium enema may be useful if a rectovaginal fistula is suspected. Even if the fistula is not directly visualized, an X-ray of centrifuged urine voided after barium enema will be radio-opaque if even small amounts of barium are present. Another useful method is to administer charcoal by mouth and later to examine the urinary sediment for its presence.

Rectovaginal fistulas present a uniquely challenging problem, with a potentially large impact on sexual function and quality of life. It is estimated that as many as 3–5% of women with Crohn's disease may develop a rectovaginal fistula. The spectrum of rectovaginal fistulas may include fistulas to any part of the vagina, including to the labia or fornix, and will present with pain, persistent or intermittent drainage, passage of flatus per vagina and dyspareunia. The literature suggests that rectovaginal fistulas are very difficult to close with medical therapy and highly variable outcomes are reported with surgical therapy.

The most common external fistulas are perianal fistulas, with enterocutaneous and colocutaneous fistulas to the abdomen being far less common. External fistulas typically present with local pain and tenderness, often with fluctuance and abscess formation prior to rupture onto the skin surface. Drainage may be persistent or there may be

intermittent cessation of drainage, sometimes followed by abscess formation as superficial healing occurs and a closed space infection ensues. As noted previously, perianal fistulas are strongly associated with colonic disease and particularly with rectal involvement. Enterocutaneous fistulas occasionally present after intra-abdominal surgery, often with the external os of the fistula arising in the surgical scar. Fistulas occurring shortly after surgery should always raise a suspicion of inadvertent surgical trauma to the intestinal serosa as the underlying cause, rather than residual active Crohn's disease. Enterocutaneous fistulas are somewhat more difficult to heal by medical means than perianal fistulas [45]. At a minimum, these fistulas present a difficult management problem, with the potential for damage to the surround skin and major disruption of daily life. High output fistulas may also lead to nutritional and water/electrolyte deficiencies, particularly if these arise from a very proximal location.

Abscesses

As many as one-quarter of all patients with Crohn's disease will present with abscess at some point in their disease course [46]. This penetrating complication is the result of fistulization through the intestinal serosa to form a purulent collection. Typically this process becomes walled off; however, free perforation and peritonitis may also occur. The classic symptoms of tender abdominal mass and spiking fevers may be absent in patients being treated with corticosteroids. This necessitates maintaining a low threshold for cross-sectional imaging in these patients. A classic, though uncommon, presentation is psoas abscess, a complication of ileal or ileocecal disease penetrating through the retroperitoneum to the psoas muscle. This complication should be suspected in patients with knee, thigh or hip pain, inability to straighten the leg on the affected side and a limping gait. The ureter may also be affected in this situation, leading to unilateral ureteral obstruction. Perianal or perirectal abscesses frequently complicate perianal fistulas. Increasingly, radiographic drainage of intraabdominal and perianal abscess along with judicious medical therapy can lead to successful outcomes without surgery [47].

Systems of disease classification

Disease classification systems are intended to meet a number of goals, the most important of which are to create more homogeneous subgroups of patients for clinical trials and for genetic research. The underlying hypothesis is that differentiating these subgroups will allow heterogeneous responses to medical therapy to be identified prospectively and will permit precise genotype–phenotype correlations. An ideal system of disease classification would incorporate a complete understanding of the genetic and environmental factors that cause Crohn's disease and modify its natural history.

Table 16.4 Montreal Classification

Age at diagnosis	A1 below 16 years
	A2 between 17 and 40 years
	A3 above 40 years
Location	L1 ileal
	L2 colonic
	L3 ileocolonic
	L4 isolated upper disease*
Behavior	B1 non-stricturing, non-penetrating
	B2 stricturing
	B3 penetrating
	p perianal disease modifier†

*L4 is a modifier that can be added to L1–L3 when concomitant upper gastrointestinal disease is present.
† "p" is added to B1–B3 when concomitant perianal disease is present.
Reprinted from Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus and implications. *Gut* 2006; **55**:749–53, with permission from the BMJ Publishing Group Ltd [48].

At present, the promise of disease classification systems has been met only partially. Considerable progress has been made in identifying genetic variations associated with susceptibility to Crohn's disease and, to some extent, clinical phenotypes have also been associated with disease-related polymorphisms. However, the predictive values of the genetic and environmental factors thus far elucidated have been insufficient for use in the management of individual patients. For this reason, classification on the basis of anatomy, disease behavior and age of onset continue to be useful, albeit imperfect, descriptive systems primarily as a research tool. The most current system of disease classification is the Montreal Classification (see Table 16.4) [48].

Serologic profiles

Distinct profiles of antibodies expressed against a variety of microbial antigens are a feature of Crohn's disease that distinguishes it from other forms of intestinal inflammation. The growing list of antimicrobial antibodies associated with Crohn's disease includes anti-*Saccharomyces cerevisiae* antibodies (ASCA) IgA and IgG, IgA antibody against outer membrane porin C of *Escherichia coli* (anti-OmpC), IgA antibody against *Pseudomonas fluorescens* (anti-I2) and anti-flagellin antibody (anti-cBir1 flagellin) [49–52]. A distinct subset of patients manifesting left-sided Crohn's colitis may be positive for perinuclear antinuclear cytoplasmic antibody (pANCA) [53]. Phenotypic associations have been reported for each of the serologic tests. These include small bowel disease for anti-cBir1 and ASCA; fibrostenosis for anti-cBir1, ASCA and anti-I2; internal perforating disease for anti-cBir1, anti-OmpC and

ASCA; and the need for small bowel surgery with ASCA and anti-I2 [49]. Moreover, retrospective studies have suggested that there is an increasing tendency for more complicated disease behavior and surgery for individuals with an increasing number of positive serologies against microbial antigens [54,55]. This observation has been confirmed in a prospective study of children with Crohn's disease, with a shorter time to need for surgery and a faster rate of progression to complicated disease behavior among patients with one or more positive antimicrobial serologies and a more benign course of disease for seronegative patients [56].

Endoscopic and radiographic features

As the clinical and laboratory features of Crohn's disease may be relatively non-specific, endoscopic and radiographic imaging – complemented by histopathologic findings – remain mainstays of evaluation and management. Both endoscopy and radiography will demonstrate the anatomic location of the disease. However, conventional endoscopy does not adequately assess the presence of disease in the small bowel, except for obvious disease in the distal terminal ileum or the duodenum. With the growing use of wireless capsule endoscopy has come increased recognition of small bowel disease not detectable on conventional endoscopy or imaging [57]. The significance of small bowel lesions in patients with disease documented elsewhere may be clear, but less certain is the significance of small bowel lesions when no disease is detected in the more usual locations or in cases previously thought to be ulcerative colitis [58]. Findings seen on capsule endoscopy suggestive of Crohn's disease include erosions, denuded villi and aphthous ulcers; however, these lesions may not be sufficiently specific for Crohn's disease [18].

Typically, the patchy nature of Crohn's disease will be evident. Normal bowel will be interspersed with areas of obvious disease. On endoscopy, discrete ulcers interspersed with normal mucosa is classic. Ulcers may take many configurations, including aphthous ulcers, linear or serpigious ulcers, stellate, "rake" or "bear claw" ulcers and map-like ulcers, which are broad, superficial and irregular. Mucosal edema with intervening circular and longitudinal ulcers creates the appearance of "cobblestoning" seen in severe and chronic disease. Strictures may be noted endoscopically and radiographically, whereas fistulas of all sorts are best demonstrated on cross-sectional or barium imaging. Abscesses are most easily demonstrated on cross-sectional imaging.

A puzzling feature of Crohn's disease is the tendency for fatty proliferation of the mesentery. This has long been recognized as the "creeping fat" seen at surgery: mesenteric fat encroaching from the mesenteric side of the bowel and wrapping around to the anti-mesenteric surface. On CT this may be seen as fibrofatty proliferation of the mesentery. This feature is not completely pathognomonic of Crohn's disease, but is highly typical. Mesenteric obesity may be observed from the time of disease onset and has been associated with increased expression of PPAR γ and TNF expressed in mesenteric adipocytes [59].

Renal and urologic complications

In addition to fistulas to the urinary tract and hydronephrosis from extrinsic compression of the right ureter, nephrolithiasis and intrinsic renal disease may complicate Crohn's disease. Nephrolithiasis is associated with ileal or ileocolonic disease rather than disease confined to the colon and occurs in approximately 8% of patients [60]. Kidney stones are most common in patients with extensive small bowel disease or intestinal resection. The majority of stones are composed of calcium oxalate. With relative fat malabsorption, luminal calcium binds to fat, leading to increased absorption of oxalate, which when excreted in urine binds calcium to form calculus. Uric acid stones also occur in the setting of bicarbonate and water losses in diarrhea. This, in turn, results in precipitation of uric acid in concentrated urine of low pH.

Intrinsic renal disease seldom occurs in Crohn's disease. IgA nephropathy is rare but may result in nephrotic-range proteinuria. Amyloidosis occurs in less than 1% of patients with Crohn's disease as an extra-intestinal complication. Proteinuria is a hallmark of the disease, which may progress to renal failure and tissue biopsy will demonstrate classic negative birefringence on polarizing microscopy. Interstitial nephritis has been reported in untreated Crohn's disease, as well as a rare idiosyncratic reaction to 5-aminosalicylates.

Vascular complications

Population-based studies have confirmed previous sporadic reports of a predisposition to thrombosis in inflammatory bowel disease (IBD). Compared with the normal population, patients with Crohn's disease have an age-adjusted incidence ratio of 2.9 for pulmonary embolus and 4.7 for venous thrombosis [61]. Mutations in Factor II, Factor V, tetrahydrofolate reductase and Factor XIII do not appear to account fully for this increased thrombotic tendency [62] and deficiencies in antithrombin III, protein S and protein C, although occasionally seen in patients with IBD, are found inconsistently in various case series and are not seen in most patients. Hyperhomocysteinemia may occur as a result of deficiencies of vitamin B_{12}, vitamin B_6 or folic acid (the latter possibly a result of treatment with sulfasalazine or methotrexate). This may be a risk factor for venous thrombosis in patients with IBD [63]. It is uncertain whether the tendency for thrombosis in IBD is an indirect result of inflammation or, as some have suggested, a primary characteristic of Crohn's disease [64,65]. A variety of vasculitidies have been reported in patients

with Crohn's disease, but the number of affected patients appears to be very small, likely indicating that these cases occur by chance.

Cardiac and pulmonary complications

There are very rare reports of endocarditis, myocarditis and pleuropericarditis in patients with Crohn's disease. Cardiomyopathy also occurs rarely on the basis of specific nutrient deficiencies related to severe malabsorption. It should be noted that treatment with anti-TNF antibody has been associated with increased mortality in patients with concurrent moderate to severe congestive heart failure [66]. Rarely, new onset of congestive heart failure has occurred during treatment with these agents. One report suggests that mitral valve prolapse and pericardial effusion are found with significantly greater frequency among patients with Crohn's disease compared with normal controls [67].

Pancreatic disease

Antibodies directed against the exocrine pancreas have been detected in patients with IBD. Some studies suggest that pancreatic autoantibodies (PAB) are specific to Crohn's disease, but the best available evidence suggests that PAB are found in both Crohn's disease (33%) and ulcerative colitis (23%), and also their healthy relatives (22%, as compared with 0% for healthy controls) [68]. An extracellular staining pattern was significantly associated with Crohn's disease, as opposed to ulcerative colitis, and higher titers were observed in Crohn's disease [68]. In addition, one study detected impaired pancreatic function more frequently among Crohn's disease patients with PAB than in patients who did not have PAB [69]. Asymptomatic elevation of lipase or amylase is seen in as many as 14% of patients, with no apparent relationship to disease activity [70]. Clinically apparent pancreatitis may complicate treatment with 6-mercaptopurine or azathioprine in approximately 4% of patients. More rarely, pancreatitis may occur as a consequence of duodenal inflammation, possibly as a result of pancreatic duct obstruction or in association with primary sclerosing cholangitis, which according to some reports occurs as frequently in Crohn's disease as in ulcerative colitis [71]. Autoimmune pancreatitis and idiopathic fibrosing pancreatitis have been reported in patients with Crohn's disease, although very infrequently [72,73]. Direct involvement of the pancreas with granulomatous inflammation has also been described. Despite these various pancreatic findings, clinically significant exocrine pancreatic insufficiency is rare in Crohn's disease.

Neurologic and psychiatric manifestations

Diverse neurologic complications have been described in association with Crohn's disease. Direct extension of fistulizing disease has been reported occasionally to cause pre-sacral or epidural abscess. Cerebrovascular thrombosis, including cavernous sinus thrombosis, is presumed to occur because of the prothrombotic tendency associated with Crohn's disease. Patients with Crohn's disease have been described to have a high frequency of focally intense white matter lesions on MRI [74]. The clinical significance of this finding is uncertain; however, Crohn's disease is associated with multiple sclerosis, demyelination and optic neuritis at an incidence rate ratio of approximately 2 [75]. This observation confounds interpretation of rare reports of new onset of demyelination with anti-TNF antibody therapy. Other medications used to treat Crohn's disease have important neurologic side effects, including peripheral neuropathy with metronidazole and thalidomide and progressive multifocal leukoencephalopathy with natalizumab, a humanized anti-α_4 integrin antibody [76]. Other unusual neurologic syndromes occasionally seen in patients with Crohn's disease include those resulting from deficiencies of fat-soluble vitamins in patients with short bowel syndrome, cerebral vasculitis and autoimmune polyneuropathy.

It is easy to understand how the life-disrupting symptoms of Crohn's disease may contribute to anxiety and depression. As a personality trait, anxiety does not contribute in significant ways to the pathogenesis of Crohn's disease [77]. However, both anxiety and depression are often seen in association with the disease as a reaction to illness [77,78] and contribute to impaired health-related quality of life independent of disease activity [79].

The following topics are covered in other chapters: dysplasia and cancer; hepatobiliary disease; ocular manifestations; articular manifestations; cutaneous manifestations; bone disease.

Natural History and Prognosis

Clinical course of disease activity

Patients faced with a new diagnosis of Crohn's disease will have many questions about their prognosis. The disease is generally characterized as chronic, with periods of relapse and remission. Perhaps less than 10% of patients will have a chronic unremitting course of disease. As demonstrated in the placebo arms of clinical trials, flares often subside without intervention more frequently than the clinician would suspect. Effective medical and surgical therapy may modify the natural history of the disease, but outcomes remain somewhat unpredictable. The goals of medical therapy are to treat active flares of relapsed disease and to prolong periods of remission. Additional goals are to prevent complications of the disease that lead to temporary or permanent impairment of quality of life.

As symptomatically active Crohn's disease rarely goes untreated, observational studies serve to record the treated natural history, whereas the true natural history is largely unrecorded. Older outcome data from referral centers provide a skewed perspective on disease outcomes, which

are more accurately represented in population-based incidence cohorts. An incidence cohort from Copenhagen County, Denmark, looked at the frequency of relapse over the first 8 years from diagnosis. Within 3 years, approximately 50% of patients achieved a full year in remission [80]. After the third year, 22% experienced remission for the next 5 years, whereas 25% had a relapse every year. The remaining 53% had alternating relapse and remission, with 22% having periods of active disease during 3 or more of the 5 years and 31% with active disease during less than 3 years of the 5 years [80]. Over the entire 8 year period, two-thirds of patients experienced years of relapse and of remission, whereas 20% had relapsed disease in every year and 13% had no relapses after the initial presentation [81]. Very few patients experience chronic, unremitting disease, which occurred in only 4% of patients over 5 years and 1% after 10 years [80].

Applying retrospective definitions of various disease states to population-based data from Olmsted County, Minnesota, Silverstein *et al.* used Markov chain analysis to model time spent in each disease state [82]. The data represented 174 patients diagnosed with Crohn's disease between 1970 and 1993, with a median follow-up of 10 years. Over a projected life expectancy of 46.4 years from a mean diagnosis age of 28.1 years, it was estimated that 23.9% of time would be spent in medical remission on no medications and 40.7% of time would be in post-surgical remission on no medications. Time with disease treated with 5-aminosalicylates and similar medications comprised 27% of remaining life expectancy, while time spent on corticosteroids or immunosuppressive medications spanned 6.9% [82]. This model does not reflect current practice, with increased usage of immunosuppressive medications. However, recent data suggest that with increasing use of immunosuppressive medications, the rate of surgery has not decreased over time [83]. This model also does not reflect the introduction of anti-TNF antibodies, the use of which has been associated with decreased rates of hospitalization and surgery in both fistulizing and non-fistulizing Crohn's disease [84,85].

Population-based studies have also assessed the natural history of disease after the first use of corticosteroids. In the Copenhagen County, Denmark, cohort, 56% of patients received at least one course of corticosteroids [86]. At 1 month, 48% of patients had achieved complete remission and 32% obtained partial remission, whereas 20% of patients had no response [86]. When followed to 1 year, 55% of the initial responders and remitters maintained response, but 45% relapsed or were steroid dependent. Analysis from the Olmsted County, Minnesota, cohort produced very similar findings. Overall, 43% of patients required treatment with corticosteroids [87]. Initial outcomes were complete remission in 58%, partial remission in 26% and steroid refractory in 16% [87]. At 1 year, 32% of initial responders and remitters continued to enjoy prolonged response, whereas 28% became steroid dependent and 38% required surgery [87]. Other studies have consistently demonstrated that the need for treatment with corticosteroids is associated with unfavorable prognosis, including development of strictures [88], surgery [89] and disabling disease [90].

Surgery

Surgery is indicated when a patient proves refractory to available medications or develops a complication such as abscess, free perforation, stricture, fistula or cancer. The threshold for surgery is in flux, as new drugs become available and non-invasive techniques to manage complications, such as percutaneous abscess drainage, become available. Historically, however, most patients with Crohn's disease have required at least one surgery over the course of their disease. A population-based study of incident cases from Stockholm County, Sweden, diagnosed between 1955 and 1989 recorded rates of intestinal resection of 44, 61 and 71% at 1, 5 and 10 years, respectively [91]. Patients with ileal or ileocolonic disease were more likely to undergo resection. Operative rates in the Copenhagen County, Denmark, population-based cohort (diagnosis made between 1962 and 1987) found a cumulative probability of surgery of 35, 61 and 82 at 1, 10 and 20 years, respectively [80] At 15 years from diagnosis, 70% had at least one surgery: 34% with one surgery and 36% with two or more surgeries [80]. A more recent study from Copenhagen County looked at patients diagnosed between 2003 and 2005 [92]. A significantly lower rate of surgery was observed in the first year of diagnosis (12%) compared with the earlier cohorts [92]. The median time to first resection, which consisted of colectomy in 1% of patients, was just 1 month (range 0–8 months) [92]. In the Olmsted County, Minnesota, cohort, 57% of patients required at least one surgery over a median follow-up of 10 years [82].

The rate of recurrence after surgical resection depends on how recurrence is defined. Bernell *et al.* noted clinical relapse rates after intestinal resection of 33%, 44% and 50% after 5, 10 and 15 years [91,93]. Symptomatic relapse occurs at a lower rate in patients with colonic disease undergoing resection. After colectomy and ileostomy, symptomatic recurrence was observed in 24% at 10 years [94]. It is well documented that symptoms of recurrence after surgery are delayed from the time of endoscopic recurrence. Rutgeerts *et al.* reported that 73% of patients have endoscopic recurrence 1 year after ileal resection, whereas only 20% experience symptoms of recurrent disease at that time [95]. By 3 years after resection, endoscopic recurrence is observed in 85%, but only one-third of patients experience clinical relapse [95]. Olaison *et al.* observed similar findings, with first onset of symptoms with diagnostic confirmation of recurrent disease noted in 33 and 44% at 5 and 10 years, respectively, after resection [96].

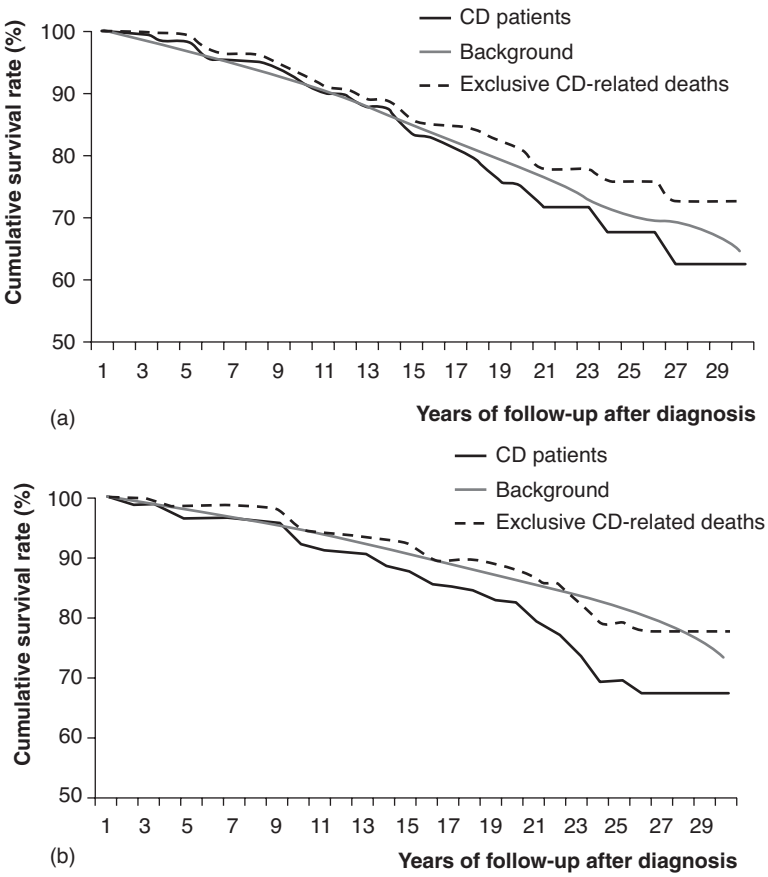

Figure 16.1 Cumulative survival among 157 men and 217 women diagnosed in Copenhagen County, Denmark, between 1962 and 1987 and followed up to 35 years (median 17 years). "Exclusive CD-related deaths" indicates all deaths except for those thought to have certain or possible relationship to Crohn's disease. Reprinted with permission from Jess T, Winther KV, Munkholm P *et al*. Mortality and causes of death in Crohn's disease: follow-up of a population-based cohort in Copenhagen County, Denmark. *Gastroenterology* 2002; **122**:1808–14. Copyright American Gastroenterological Association (2002).

Quality of life

All patients with Crohn's disease experience periods when their illness has detrimental effects on their quality of life. However, quality of life is highly variable from patient to patient and depends not only on disease activity, but also on the untoward effects of treatment, psychological constitution and social supports. One study from Denmark found that despite periods of illness, the majority of patients enjoy lives similar to healthy controls, with similar percentages being married and having children [97]. Employment and unemployment rates were similar in the two groups and socioeconomic status was, in fact, higher among the Crohn's disease patients [97]. Patients and controls had similar numbers of sick days. Despite these findings, 23% reported not being able to work at capacity and 21% reported that the disease affected their leisure activities [97].

A prospective study of generic quality of life demonstrated a significant impact of active disease on well-being [98]. Physical and mental components of the Short Form-36 (SF-36), a generic measure of quality of life, were affected. Even as active disease subsided and quality of life improved, patients continued to express concerns over the potential need for a stoma or surgery, the uncertain nature of the disease and diminished energy [98].

Mortality

Population-based studies reveal a small but demonstrable increase in mortality associated with Crohn's disease. Studies indicate that after 20 years from onset, the survival is approximately 95% of the expected rate for the general population [99]. In the Olmsted County, Minnesota, cohort, patients followed between 1940 and 2004 were found to have a standardized mortality ratio (SMR) of 1.2 (95% CI 0.9–1.6) [100]. In addition, 32% of patients died from disease-related complications, including non-malignant gastrointestinal causes (SMR 6.4, 95% CI 3.2–11.5), gastrointestinal cancers (SMR 4.7, 95% CI 1.7–10.2) and chronic obstructive pulmonary disease (SMR 3.5, 95% CI 1.3–7.5) [100]. Very similar findings were reported from Copenhagen County, Denmark, in patients diagnosed between 1962 and 1987 and followed at least until 1997 [101]. In this region, the SMR was 1.3 (95% CI 1.01–1.56). Of note, there was an excess mortality in women diagnosed at less than 50 years of age (SMR 3.42, 95% CI 2.21–5.04) accounting for the excess in mortality (see Figure 16.1) [101]. As in Olmsted County, complications related to Crohn's disease were the cause of death in approximately one-third of patients [101]. A third population-based study from Stockholm County, Sweden, likewise demonstrated increased mortality, with SMR 1.5

(95% CI 1.29–1.75) [102]. The general trends appear to show that slight increases in mortality occur as the disease duration increases.

Conclusion

Crohn's disease continues to challenge clinicians with its varied presentation and features. The mysteries of this clinical heterogeneity are gradually being unraveled as the genetic and environmental causes of the disease become better understood. Clear representations of the clinical features of Crohn's disease will continue to facilitate its management, and also the explication of the molecular basis of these features.

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Chapter 17
Practical Inflammatory Bowel Disease Pathology in Patient Management

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Summary

- The correct interpretation of biopsy material in cases of IBD requires detailed knowledge of the clinical information and endoscopy findings, along with carefully sampled normal and abnormal regions of the affected bowel.
- The biopsy appearances of CIBD change with time and treatment. Biopsies taken within 6 weeks of onset may closely mimic infective colitis whereas post-treatment material from ulcerative colitis patients may closely resemble Crohn's disease.
- While tempting to biopsy, focal lesions such as polyps and ulcers can only be interpreted properly and in context when biopsies from surrounding normal and abnormal mucosa are also submitted.
- "Indeterminate colitis" must only be used in the context of resection specimens when careful macroscopic and microscopic examination fails to distinguish definitively between ulcerative colitis and Crohn's disease. The term "IBD unclassified" is used when a diagnosis of CIBD can be made on biopsy material but a confident diagnosis of ulcerative colitis or Crohn's cannot.
- In cases where the patient's clinical course and response to treatment remains inconsistent with an original CIBD diagnosis, repeat biopsies can be very useful. Review of all previously submitted material, in conjunction with review of the radiologic and endoscopic findings, may also be necessary.

Introduction

Histopathologic examination of biopsies and resection specimens can guide patient management in inflammatory bowel disease (IBD) if used properly in context and in conjunction with history, examination, endoscopic findings and operative/radiologic findings [1]. It is often of limited value in isolation. In particular, history of drug therapy or surgical procedure is crucial to the interpretation of the biopsy findings in context and is necessary if important pitfalls are to be avoided. When interpreted properly, such findings will guide patient management. Patients who bear an incorrect label of chronic IBD due to over-interpretation of minor and normal histologic features are disadvantaged in life.

This chapter outlines practicalities and pitfalls in biopsy interpretation and examines the events in the history of a patient with IBD where pathology is crucial to patient management. In particular, it considers the initial biopsy differential diagnosis, the diagnosis of fulminant colitis, the diagnosis after medical and surgical treatment and the diagnosis of neoplasia.

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Abnormal biopsies, and indeed normal biopsies, may be misinterpreted out of context. The history, endoscopic appearances and site of the biopsy are very important for correct interpretation. This may be achieved by placing biopsies in separately labeled pots of formalin. However, this is labor intensive and a better alternative is to use one pot of formalin and to place the biopsies in order, either in a multiwell cassette or on acetate. What is normal in the cecal mucosa would be distinctly abnormal in the descending colon. A wonderfully skilled colonoscopic examination with accurate description of endoscopic mucosal appearances is wasted if the biopsies from eight carefully described sites are floating around in the same container of formalin. Accurate diagnosis of the presence or absence of IBD and differentiation of Crohn's disease from ulcerative colitis are crucially dependent upon accurate biopsy site labeling. Carryover of part of one patient's biopsy set to another patient's biopsy set is a disaster and requires simple processes to avoid it. It is easy to see that dysplasia in one patient's colon is not managed properly if it has been thought to originate from a different patient's colon. Indeed, the management of the other patient will be potentially damaging. Regular communication between the endoscopy nurses, endoscopists and pathologists is essential for good biopsy management. Endoscopy rooms

are semi-dark and labeling and biopsy handling may be difficult. It is important to have a good light on a dedicated area of bench for biopsy handling. Small forceps and/or hypodermic needles are useful for removing the biopsies from the jaws of the endoscopic biopsy forceps. The biopsy may then be placed on a small piece of thin card to maintain orientation. If the biopsy is placed muscularis mucosae side down, it will adhere successfully to the card. However, if it is placed the other way up, as the muscularis mucosae contracts the biopsy will fall off the card. We find that the simplest, most cost-effective way of handling biopsies and keeping carryover to the minimum is to use multiwell cassettes [2]. These have six numbered compartments to take a colonoscopic biopsy series in one or two cassettes depending on the number of sites biopsied. If fewer than two sites are biopsied, cassettes with single compartments are available and cheaper than multiwell cassettes. Tissue is processed in these cassettes in the laboratory; the biopsies are embedded in wax on the reverse side of the cassette and sections are cut from this wax block. To present the biopsies in a pot of formalin without a cassette entails someone removing the biopsies from the pot. This extra handling step, which introduces the potential for carryover, is removed if cassettes are used. It eliminates a huge amount of labor time in the laboratory and allows more biopsies to be processed by the same staff. It also has implications for storage space of both blocks and slides and represents both space and cost savings.

Ulcers, polyps and other lesions are tempting things to sample, but it must be remembered that to put them into context histologically, biopsies of surrounding flat mucosa and more distant flat mucosa or other lesions in the colon are equally important. A detailed description of the colonoscopic distribution of the colitis is important for differential diagnoses such as the presence of diverticular sigmoid and unusual patterns of ulcerative colitis, including the skip lesion in the appendix and the cecal patch lesion.

Chronic IBD biopsies taken post-surgery generate their own problems. Ileostomy ends always have chronic inflammation and non-specific changes rarely diagnostic of anything and ileoscopic biopsy from further along the ileum will usually be more rewarding. Biopsies from the proximal side of an anastomosis in Crohn's disease show recurrent disease within months of the anastomosis. Gastric and duodenal biopsies in Crohn's disease may reveal focal active gastritis and duodenitis, sometimes with granulomas, but similar changes may occasionally be seen in patients without Crohn's disease, including those with ulcerative colitis [3]. The differences between the two diseases in the upper gastrointestinal tract probably needs further research. Diversion of the fecal stream from the colon or rectum in chronic IBD produces different effects depending on the original diagnosis. Diversion of pre-existing normal large bowel produces diversion proctocolitis. Diversion of pre-existing ulcerative colitis produces more severe inflammation than previously present [4]. Diversion in Crohn's disease often results in the inflammatory changes becoming less pronounced; the mucosa may appear fibrosed with little inflammation and any granulomas may become degenerate and hyalinized. These appearances may resemble ulcerative colitis [5,6]. Biopsies from the pelvic ileal reservoir (pouch) mucosa are useful when coupled with the clinical history and endoscopic appearances to aid the diagnosis of pouchitis [7]. It is important histologically to exclude other causes of inflammation within the pouch and to assess adaptive changes within the pouch mucosa [6]. When the patient has symptoms of pouchitis but clearly does not have pouchitis, biopsies of the pre-pouch ileum and anal columnar cuff may provide the answer. Biopsies in dysplasia, particularly localized versus widespread lesions of dysplasia and their management, will be discussed later.

The initial biopsy differential diagnosis of IBD

Colonic mucosal biopsies within 6 weeks of onset of symptoms in IBD will rarely show much crypt architecture distortion and it may be difficult to distinguish histologic changes of early chronic IBD from an acute self-limiting colitis, i.e. infective colitis [1]. Surawicz and co-workers [8,9] helped with their studies some years ago by identifying some of the changes which may indicate the very early stages of chronic IBD. These include basal plasmacytosis and basal lymphoid aggregates or follicles. Edema in the lamina propria and neutrophils would be more in keeping with infective colitis [10], but this distinction is not always easy and infections such as *Campylobacter* may produce a colitis which closely mimics IBD. It also has to be noted that in severe UC, superficial edema and neutrophil clusters in the superficial part of the lamina propria are not uncommon. Crypt abscesses in infective colitis are usually eccentric and sometimes part of the pattern of crypt beading described by Day *et al.* [10]. The classical histologic features of infective colitis are not always present and cases of infective colitis sometimes mimic early Crohn's disease. *Campylobacter* colitis often provides close mimicry of chronic IBD histologically, particularly if there is chronic infection [11]. Amebic colitis is often difficult to diagnose, but characteristic hematoxyphilic necrotic slough on the surface of an ulcer can be a really good aid to diagnosis. However, a careful search is still required to find definite amebae. They are identified by means of a prominent nuclear karyosome and the presence of red blood cells within their cytoplasm [12]. Amebae may be difficult to distinguish from macrophages containing red blood cells, but close examination of the nuclear detail and the size of the cells is useful, as is extra staining with a periodic acid–Schiff (PAS)

stain and an immunohistochemical stain for macrophages (such as KP1/CD68). Most infective colitides resolve without leaving residual histologic stigmata. Pseudomembranous colitis due to *Clostridium difficile* toxin [13] will, in some cases, after resolution, retain a degree of crypt architectural distortion and hyperplastic change with serrated crypts. Chronic architectural changes in chronic shigellosis may occasionally provide close mimicry of chronic ulcerative colitis. In view of this, when considering biopsies from colitis in the tropics, even gross crypt architectural distortion should not be regarded as definitive histologic evidence of chronic ulcerative colitis.

Normal microscopic appearances which may cause diagnostic difficulty

There are several aspects of the normal large bowel mucosa which may be misinterpreted as abnormal. Within the normal large bowel, approximately one in seven crypts is racemosely branched. The histologic appearance of the normal density of lamina propria mononuclear cells in the right side of the colon is also important. There are always more chronic inflammatory cells in the cecum than in the rest of the large bowel. In the cecum, the lamina propria is filled out to its full thickness by a mixture of chronic inflammatory cells. This would be abnormal at any other site within the colon. If histopathologists do not appreciate this feature, there will be gross over-diagnosis of inflammation in the cecum. This may lead to an incorrect label of Crohn's disease. In some patients with left-sided ulcerative colitis, it may lead to an erroneous label of total colitis or a cecal patch lesion. In the cecum, focal active colitis is another important finding [14]. This is not normal, but is common at colonoscopy. It may be related to bowel preparation artifacts, recovering infection or drugs such as non-steroidal anti-inflammatory drugs (NSAIDs). In this lesion, focal collections of neutrophils are seen to cause focal destruction of part of a crypt wall. In the original study by Greenson *et al.*, very few adults with this condition were proven to develop Crohn's disease whereas in the pediatric series a small number of children did develop Crohn's disease [14].

Normal colonoscopic findings in patients with diarrhea

Normal colonoscopic findings in patients with diarrhea may have several explanations. Minimal change colitis [15] is a term used to denote microscopic evidence of ulcerative colitis with a normal or near normal colonoscopy. This is probably becoming less common with more magnification being used at colonoscopy. The histologic features are those of ulcerative colitis and the treatment is the same as for ulcerative colitis. Other important disorders in this category are the microscopic colitides. This is an umbrella term for several different microscopic forms of colitis, with little endoscopic evidence of their presence. These include lymphocytic colitis, collagenous colitis and granulomatous, giant cell and pseudomembranous forms of microscopic colitis [16]. The diagnosis of microscopic colitis depends on presentation with persistent watery and bloodless diarrhea, no obvious endoscopic findings and typical histologic findings of collagenous colitis or one of the newer variants.

Patients with small bowel Crohn's disease causing diarrhea may have normal colonoscopic findings, but examination of a random biopsy may reveal an isolated granuloma in the absence of other colonic inflammation.

Biopsies of ulcers

Ulcers may represent the most severe activity in chronic IBD [17] or they may be part of a superimposed or primary infection such as cytomegalovirus, amebae or other unusual infection or neoplasia. Patients with IBD may occasionally have solitary ulcer/mucosal prolapse which may be misdiagnosed as IBD due to the presence of inflammation in the biopsies taken from the ulcerated region in the rectum [18]. This shows the importance of taking biopsies from the ulcer and the mucosa away from the ulcer.

Biopsies of polyps

In longstanding IBD, one commonly expects to find a benign inflammatory polyp. Giant inflammatory polyps can cause confusion. They are usually found in the distal transverse colon in longstanding ulcerative colitis. Colonoscopic biopsies may reveal unremarkable large bowel mucosa or inactive ulcerative colitis, yet the polyp may be large enough to cause colonic obstruction [19]. Histology of the resection specimen may cause even more confusion, since in the region of the giant inflammatory polyp there may be transmural inflammation which will raise an erroneous suspicion of Crohn's disease. Cytomegalovirus has previously been mentioned as a cause of ulcers, although it may also result in polyp formation, as may amebic infection. In longstanding chronic IBD, amyloid may deposit as a polyp. As patients with IBD get older, they may develop adenomas as they enter the adenoma age group. These may be difficult to distinguish from DALMS (dysplasia-associated lesions or masses) [6] or ALMs (adenoma-like lesions or masses) [20], and both may give rise to adenocarcinoma. Finally, lipomas may also be found in the ascending colon.

Colonoscopic finding of colitis limited to the sigmoid colon

In cases of endoscopic colitis limited to the sigmoid, biopsy of the sigmoid mucosa alone or mixing the rectal and sigmoid biopsies together in the same pot causes considerable diagnostic difficulty. The usual cause of isolated sigmoid colitis is diverticular disease [21]. A small proportion of patients with diverticular disease-related colitis may develop ulcerative colitis [22]. If the sigmoid colon alone is biopsied or resected and examined, the histologic appearances may resemble Crohn's disease, ulcerative colitis or mucosal prolapse. It is crucial to take a separately labeled biopsy from the rectal mucosa and sigmoid mucosa when investigating a sigmoid colitis, since diverticula, and consequently diverticular colitis, do not occur within the rectum. It is rare for ulcerative colitis not to involve the rectum, although this may be seen rarely even in the absence of treatment [23].

Variation in microscopic appearances in ulcerative colitis

The biopsy in ulcerative colitis may have varying degrees of severity according to the quantity of neutrophils and the amount of epithelial damage (ulceration, erosion and crypt destruction) that they have caused (Figure 17.1). Several scoring systems have been devised to try to quantify the damage and activity in ulcerative colitis [9,17,24], although these are not in routine clinical use. They are useful in human drug trials and in animal studies where one wishes to quantify levels of improvement in activity in ulcerative colitis histology. The only time when a scoring system is used in routine clinical practice is in the evaluation of acute inflammatory changes in the ileoanal pouch mucosa to diagnose pouchitis. This may involve using the histologic scoring system devised at St Marks Hospital, London, or the combined clinical and endoscopic scoring system devised at the Mayo Clinic [25]. As previously stated, the biopsy pathology in ulcerative colitis varies with time and treatment. In the first 6 weeks following onset of symptoms there may be little crypt architectural distortion, making diagnosis difficult. Later in the course of the disease, after treatment, ulcerative colitis may become patchy and resemble Crohn's disease [26]. These microscopic areas of patchiness and occasionally skip lesions may become more apparent in healing ulcerative colitis. Since this is part of a healing process, it would be very unusual to find coexisting evidence of severe activity such as erosions or ulcers. The finding of such evidence with patchiness would raise the possibility of Crohn's disease rather than treated ulcerative colitis. Skip lesions are the hallmark of Crohn's disease. There are only two recognized skip lesions in ulcerative colitis: one in the appendix, described by Davison and Dixon [27], and the other in the form of the cecal patch lesion described by D'Haens *et al.* [28]. These two lesions are part of the spectrum of ulcerative colitis and are not contraindications to pouch surgery [6].

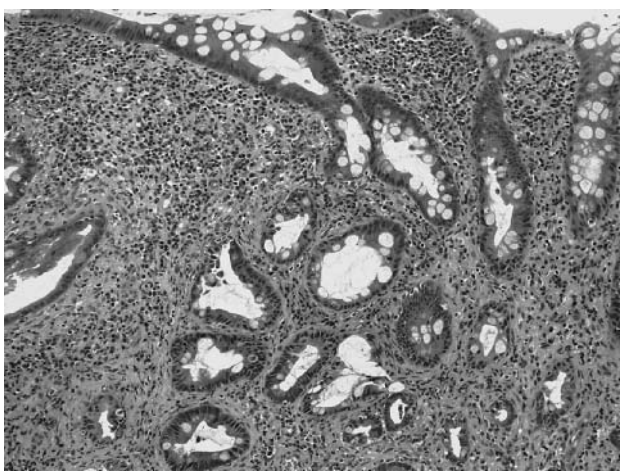

Figure 17.1 Biopsy appearance of ulcerative colitis. There is chronic inflammation in the lamina propria with marked crypt architectural disturbance.

Mucosal biopsies in Crohn's disease

In cases of small bowel Crohn's disease, the large bowel mucosal biopsy may be normal or may contain isolated well-formed mucosal granulomas which are not related to crypt rupture. Those granulomas which are related to crypt rupture contain mucin, neutrophils or epithelial cells. If there is inflammation with granulomas in relation to crypt damage, this may confuse and may lead to an erroneous diagnosis of Crohn's disease [29]. Granulomas may form in relation to crypt damage in many different forms of colitis. The cryptolytic granulomas as described by Lee *et al.* [30] were suggested to be specific for Crohn's disease. However, it soon became apparent that they are not specific and they may be seen in diverticular disease and many other forms of colitis [29]. Inter-observer variation is large in the biopsy diagnosis of chronic IBD, but the only feature which is usually agreed upon in inter-observer studies is the patchiness and acute and chronic inflammatory changes in Crohn's disease [31]. The classical features of Crohn's disease in the mucosa are focal erosion, focal inflammation and focal architectural change, with relatively little mucin depletion (Figures 17.2 and 17.3). Aphthoid ulcers are seen very early in the course of the disease. Resected specimens are easier to interpret since fat wrapping [32], transmural inflammation in the form of lymphoid aggregates and transmural connective tissue changes will be appreciated. Fat in the submucosa has been recognized for a long time radiologically as the fat halo sign, but has been

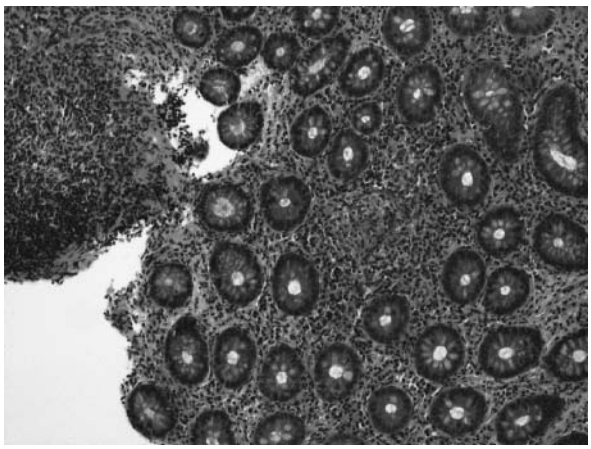

Figure 17.2 Biopsy appearance of Crohn's disease. There is chronic inflammation in the lamina propria with modest crypt architectural disturbance. There is focal crypt damage due to focal acute and chronic inflammation.

appreciated histologically only recently. One may also see perineural chronic inflammation and transmural granulomas. Transmural inflammation and lymphoid aggregates may also be seen in diverticular disease, but in this context the lymphoid aggregates are associated with, and seen to radiate around, individual diverticula rather than being diffusely distributed throughout the bowel wall.

The appendix in IBD

The appendix is involved in ulcerative colitis in 75% of colectomy specimens either by direct extension or as a skip lesion [27]. The histologic changes in ulcerative "colitis" affecting the appendix are mucosal and diffuse and have appearances similar to those seen in the large bowel. Histologic changes in Crohn's disease in the appendix are rather different and involve transmural inflammation in the form of lymphoid aggregates and connective tissue changes throughout the wall. Unfortunately, the diagnosis of Crohn's disease in the isolated appendicectomy specimen is not that straightforward. Many of the histologic changes of Crohn's disease may be seen in association with an appendix abscess or an appendix mass, including granulomas. Granulomatous vasculitis would offer most concern for a diagnosis of Crohn's disease, but since that has been seen more recently in other forms of proctocolitis [33,34], it is now also in question as an absolute marker for Crohn's disease.

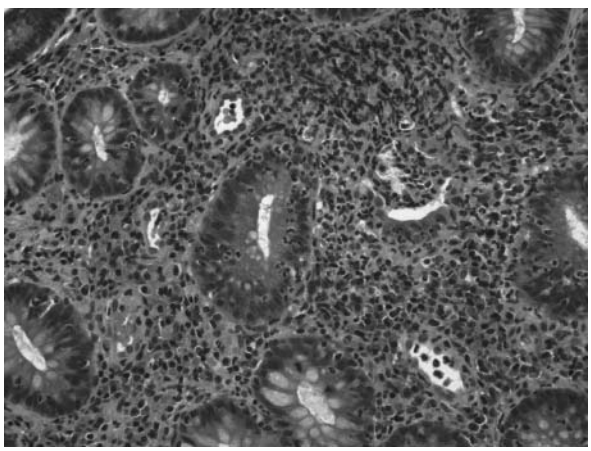

Figure 17.3 Biopsy appearance of Crohn's disease. Similar changes to Figure 17.2 are seen, but including a microgranuloma.

Mimicry of Crohn's disease by ulcerative colitis

Ulcerative colitis may closely mimic Crohn's colitis when there are granulomas in response to crypt damage [30]. Ulcerative colitis may also mimic Crohn's disease when there is patchiness of disease after treatment, resolution of histologic changes after treatment or when there is severe or fulminant colitis [26]. Diversion proctitis in the three-stage pouch procedure in ulcerative colitis develops many of the changes of Crohn's disease [4], and again this is not a contraindication to pouch surgery. The skip lesion mentioned above is also a mimic of Crohn's disease.

It may be particularly difficult to differentiate Crohn's disease from ulcerative colitis in the case of severe colitis, after treatment or where rare variants of ulcerative colitis are not recognized. In cases of follow-up biopsies of post-treatment IBD, the pathologist has to establish whether ulcerative colitis or Crohn's disease is still the diagnosis (often by referral to the pre-treatment biopsies), whether it has improved and if it is now complicated by superimposed infection. As stated above, it is important to go back to the whole patient and to the original pre-treatment biopsy series. The important forms of superimposed infection are pseudomembranous colitis and cytomegalovirus, both of which may induce an attack of severe ulcerative colitis.

Quiescent ulcerative colitis may contain post-inflammatory polyps. It is important to biopsy the polyps at the flat mucosa. Biopsies after surgical treatment and biopsies from the ileostomy may show more specific changes than in Crohn's disease and anastomotic biopsies in Crohn's disease will show recurrence which may or may not be symptomatic on the proximal side of the anastomosis.

Diversion in ulcerative colitis will mimic Crohn's disease. Diversion in Crohn's disease may mimic ulcerative colitis or show complete resolution [6].

Upper gastrointestinal tract biopsies

There has been a lot of excitement about the specificity of focal inflammatory changes in the upper gastrointestinal

tract to differentiate Crohn's disease from ulcerative colitis when the endoscopy is normal [3]. The value of this finding is not absolute, in that some cases of focal inflammatory changes have also been reported in ulcerative colitis [35].

Chronic IBD unclassified and indeterminate colitis

Indeterminate colitis was defined by Price as the term to describe colectomy specimens in which the macroscopic and microscopic features resembled both Crohn's disease and ulcerative colitis and in which a firm diagnosis of neither was possible [36]. Price later published a further paper describing the added value of radiology and clinical follow-up in achieving a diagnosis. Even with extra information, a number of cases remained indeterminate. The rectum is usually spared and the colitis is usually very severe. This contributes to the difficulty in subclassification. Since 1990, the term started to be used by some to describe the histologic changes of chronic IBD (CIBD) seen on colonoscopic biopsy series which are difficult to classify either as Crohn's disease or as ulcerative colitis. This occurs early in the disease history, after treatment or when very severe. The term "IBD unclassified" should be used for biopsy histopathology in which a diagnosis of CIBD can be confidently made but the features are equivocal for ulcerative colitis and Crohn's disease. It is fortunate that the World Congress of Gastroenterology Guidelines on IBD have clarified this issue [37]. Price's original description of the concept of indeterminate colitis, applied only to resection specimens, is a useful and specific one which is valuable in the management of CIBD. The term should not now be applied to biopsy material. Cases equivocal for ulcerative colitis and Crohn's disease on biopsy material should be designated as "CIBD unclassified" according to the World Congress of Gastroenterology Guidelines. Resections of such cases usually provide definitive evidence of one or other of the two major subtypes of CIBD and do not often show changes characteristic of indeterminate colitis.

The main reason for uncertainty in IBD differential diagnosis is that neither disease displays diagnostic histologic features that are universally present in one disease and invariably absent in the other. The most difficult large bowel resection specimens are those cases of acute severe colitis in which the inflammatory changes are so severe that distinction between the two major subtypes of CIBD becomes very difficult. A diagnosis of indeterminate colitis is made in 10–15% of colectomy specimens performed for severe colitis [36]. It is useful to state that most cases of indeterminate colitis diagnosed at the time of colectomy behave like ulcerative colitis and restorative proctocolectomy with ileoanal pouch formation is usually successful. Indeterminate colitis patients with pouches have a higher incidence of pelvic sepsis but not necessarily of pouchitis [38]. The prevalence of fistulae may be higher in pouch patients who had indeterminate colitis compared with those with ulcerative colitis, but the long-term function may be as good [39]. In a small proportion, the eventual diagnosis is realized to be Crohn's disease and these patients have a poorer outcome and have a pouch failure rate of up to 40% [40].

It should be emphasized, however, that the pathologic opinion must never be considered in isolation. Further clinical, radiologic or endoscopic information will often clarify the diagnosis of either Crohn's disease or ulcerative colitis in the absence of definitive differentiating histopathologic features on biopsy and sometimes in the resected colon.

Backwash ileitis in ulcerative colitis

There are varying degrees of severity and extent of inflammation described as part of backwash ileitis. The literature on systematic review of the histopathology of backwash ileitis is scant, as indicated in a recent review [41].

Pouchitis

Pouchitis is a diagnosis dependent upon three main features: clinical symptoms, endoscopic diffuse inflammation and severe acute histologic changes. The clinical features are of diarrhea, pouch discharge and systemic upset. The endoscopic features are of diffuse and severe ulceration. The histologic features are of ulceration and severe/acute inflammation [7] (Figure 17.4). The acute histologic changes in the pouch mucosa must include neutrophilic infiltration and damage which is severe enough to result

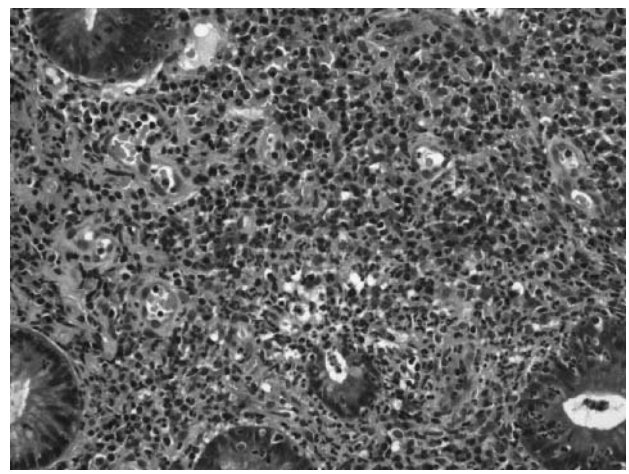

Figure 17.4 Pouchitis. There is severe acute inflammation. Ulceration was also present.

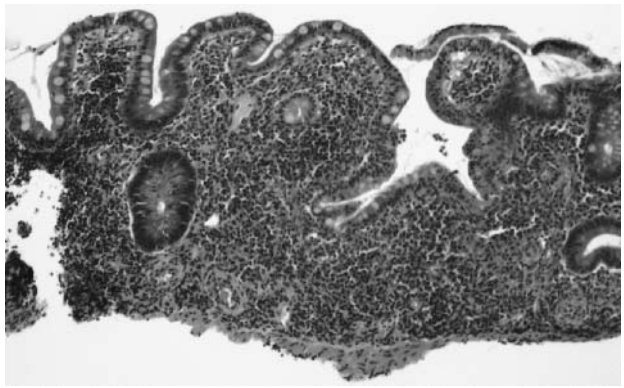

Figure 17.5 Adaptive changes in the pouch mucosa. There is villous atrophy in the absence of intraepithelial lymphocytosis. There is severe diffuse chronic inflammation and moderate focal acute inflammation insufficient to correlate with an endoscopic and clinical diagnosis of pouchitis.

in erosion or ulceration. Neutrophils in the pouch mucosa are often over-interpreted as representing pouchitis. Neutrophils are common in pouch mucosa as part of the adaptive or colonic phenotypic changes within the pouch mucosa. The amount of acute and chronic histologic change in the pouch mucosa is scored numerically using either the St Marks Hospital score or the Mayo Clinic score [25]. The Mayo Clinic score also provides a numerical score for the clinical findings, whereas the St Marks Hospital score is concerned only with the histologic features. Most pouch biopsies show merely adaptive changes in the pouch mucosa, which may include some acute inflammatory cells [6] (Figure 17.5). Pathologists must assess these adaptive changes accurately, especially when it comes to numerical scoring of the amount of inflammation present. They have to exclude other causes of acute and chronic inflammation in the pouch mucosa which may mimic pouchitis. The pouch is a neo-rectum and consequently may develop many of the causes of acute and chronic inflammation and connective tissue changes, such as those of mucosal prolapse, which may be seen within the rectum. Prolapse may be represented as a red patch in the mucosa, an ulcer or a polyp [42]. A focal area of inflammation may occur within the pouch mucosa as a result of secondary pouchitis, which may develop in response to localized inflammation outside the pouch such as an abscess or tumor. Mucosal prolapse may occur within the pouch anteriorly or at other points around the circumference. The granulomas that one sees in pouch mucosa may be seen in relation to crypt rupture or within lymphoid follicles. They do not appear to be related to pouchitis and are asymptomatic.

Cytomegalovirus has been mentioned as a complication of IBD. It may be seen in irregular Crohn's-like ulcers in the pouch but this is not Crohn's disease. Crohn's disease may occur within the pouch but it is a difficult place to make a diagnosis since many of the histologic changes of Crohn's disease may be seen in patients with pouchitis who have had ulcerative colitis. A diagnosis of Crohn's disease should be made only after consideration of all available pathologic material and of the whole patient.

Ulcer-associated cell lineage may be seen in ileoanal pouch mucosa soon after episodes of ulceration and may persist for a long time afterwards [6]. This is important in the patient who gets better after pouchitis, before they come to clinic. At that point, if a biopsy shows ulcer-associated cell lineage, one can be certain that there was ulceration previously but cannot be certain if it was due to pouchitis. However, it is useful when associated with the appropriate symptoms. In the patient with symptoms of pouchitis in whom one does not identify pouchitis, one most consider the columnar cuff of the anal canal below the pouch which may become inflamed and dysplastic. The ileum above the pouch may also become inflamed and should be inspected carefully and biopsied [6].

Colitis-associated neoplasia

The microscopic diagnosis and treatment of dysplasia in ulcerative colitis remains a confusing area [20]. The diagnosis should be accurate and in context. It is important not to over-diagnose dysplasia. Colitis-associated neoplasia (CAN) is classified as indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia or adenocarcinoma. The microscopic diagnosis of dysplasia depends upon having a well-orientated histologic section with areas of intact epithelium. Dysplasia is diagnosed when cells with a high nuclear-to-cytoplasm ratio are seen not only in the regenerative zone of the crypt, where they are normal, but also ascending from the regenerative zone at the base of the crypts to the surface. Similar cellular changes may be seen in association with severe epithelial regenerative changes. In this case, when seen in a similarly well-orientated crypt with areas of intact epithelium, the atypical epithelial cells will be seen to mature towards the surface with a decreasing nuclear-to-cytoplasm ratio. The term indefinite for dysplasia should be used when a clear-cut diagnosis of dysplasia cannot be made for one of the following three reasons. First, in severe acute inflammation with neutrophils in the epithelium, there may be such severe epithelial regenerative activity that it is uncertain if the nuclear changes seen represent dysplasia or not [20]. Second, the malorientation of sections may result in a transverse section of the crypt base being seen. Consequently, one cannot assess maturation of nuclear detail towards the top of the crypts at the surface of the mucosa. Third, if dysplasia is present in less than four crypts, it is considered as indefinite for dysplasia. Repeatedly, inter-observer studies have shown pathologists to be good at agreeing on the

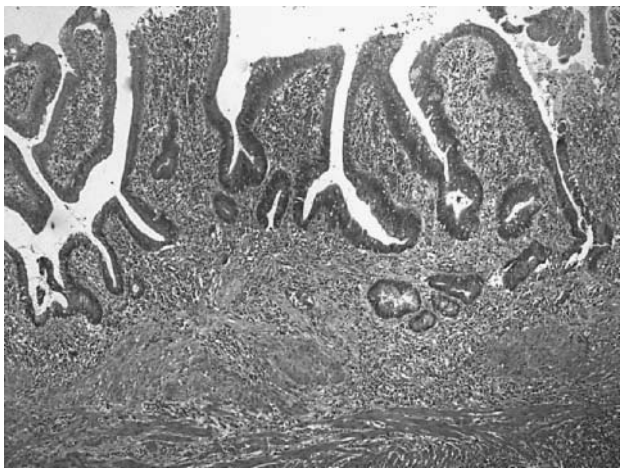

Figure 17.6 UC colectomy with severe villiform regenerative change due to cyclosporin and mimicking dysplasia.

diagnosis of high-grade dysplasia, but there is generally poor agreement for low-grade dysplasia. It has now become recognized that locally dysplastic lesions resembling adenomas (ALMs) may respond very well to local excision provided that there is careful colonoscopy to exclude other lesions and careful colonoscopic follow-up [43,44]. This may also apply to some dysplasia-associated lesions or masses (DALMs).

The histologic changes of villiform architecture and nuclear enlargement due to severe epithelial regeneration which are seen in ulcerative colitis after cyclosporin therapy may closely mimic dysplasia [45] (Figure 17.6). The main difference is that the pseudodysplasia will be seen in every histologic section taken from the affected colon. A diffuse distribution of this nature is never seen in true dysplasia. The duration of disease is also helpful in some cases, in that it would be very unusual to see dysplasia in a diffuse distribution and in a patient who had extensive ulcerative colitis for less than 10 years.

Conclusion

All biopsies in chronic IBD must be viewed in context and must be from known biopsy sites. One should consider iatrogenic disease and normal or unusual variants of disease. It is also very important to consider what a biopsy from a particular site at a particular time will tell you with regard to the patient's prognosis and treatment. It is also important to think about which site you need to biopsy to answer your current question. It is crucial when a lesion is visualized at colonoscopy to biopsy the lesion and apparent normal mucosa to put it into context with its background. If your patient with IBD is failing to get better, it is important not only to reconsider the diagnosis of IBD, but also to exclude a superimposed infection. Often, repeat biopsies are forgotten as an important way of excluding infections complicating ulcerative colitis in patients receiving more and more immunosuppressive therapy but failing to get better. It is crucial for the pathologist to work closely with clinicians managing IBD patients and to have regular meetings to review biopsy and resection material for optimal management of these complex cases.

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Chapter 18
The Role of Endoscopy in Diagnosis and Treatment of Inflammatory Bowel Disease

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Summary
- Endoscopic mucosal examination is an important modality in the evaluation of a suspected or known case of inflammatory bowel disease.
- Capsule endoscopy allows surveillance of the entire small intestine, one of the most important organs afflicted by Crohn's disease.
- The introduction of chromoendoscopy and confocal laser endomicroscopy may help identify dysplastic lesions for precise tissue sampling and ablation during endoscopic surveillance.

Introduction

Diagnosing and monitoring inflammatory bowel disease may be a challenging process and in the initial stages requires a thorough history and physical examination along with an understanding of how laboratory values and imaging modalities may be utilized. However, these tools may only provide clues for the physician towards a diagnosis and ultimately endoscopy of the upper, lower or both tracts of the gastrointestinal tract with tissue biopsy is necessary. It should be noted that in some cases, even endoscopy and tissue samples are insufficient in providing a conclusive answer. Nevertheless, it is important for the astute physician to recognize endoscopic and histologic features of inflammatory bowel disease that may determine the strategies employed in the care of the patient.

In this chapter, we focus on the latest role of endoscopy in diagnosing and differentiating inflammatory bowel disease and cancer surveillance. We discuss features of inflammatory bowel disease that may manifest in the foregut, midgut, hindgut and biliary tract. We also cover all the currently available endoscopic instruments and procedures and how each one may be most effectively used in different areas along the gastrointestinal tract. We hope to provide a complete understanding of the benefits that endoscopy provides when inflammatory bowel disease is suspected.

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Foregut: overview

Discussion about inflammatory bowel disease in the upper gastrointestinal tract revolves around Crohn's disease, although there are oral conditions that can also be associated with ulcerative colitis. Symptoms of inflammatory bowel disease involving the upper gastrointestinal tract are generally non-specific and include bloating, nausea, vomiting, midepigastric pain and, less commonly, hemorrhage. When strictures develop, weight loss may also occur. The differential diagnosis of inflammatory bowel disease of the upper gastrointestinal tract should include sarcoidosis, peptic ulcer disease, eosinophilic gastroenteritis, celiac disease, tuberculosis, malignancy, disseminated fungal disease and Brunner's gland hyperplasia. Studies available for the evaluation and diagnosis of disease in the upper gastrointestinal tract include esophagogastroduodenoscopy (EGD), upper gastrointestinal radiologic series and cross-sectional computed tomography (CT) studies. Upper gastrointestinal series can demonstrate strictures, ulcers, cobblestoning, other abnormalities and fistulae seen in Crohn's disease. EGD is particularly helpful because of the ability to detect subtle mucosal abnormalities and take biopsy specimens in the upper gastrointestinal tract. Granulomas are generally considered diagnostic of Crohn's disease but are not typically found in histopathology. Nonetheless, granulomas have been reported in 25–40% of upper endoscopic biopsies in the pediatric population [1]. The more common findings include non-specific chronic inflammation with

architectural mucosal distortion, mucosal atrophy and intraepithelial lymphocytosis.

Foregut: oral cavity

Oral mucosal manifestations of inflammatory bowel disease may be seen in both Crohn's disease and ulcerative colitis and may be a reflection of severity of disease. Aphthous ulcers are seen in up to 5% of Crohn's disease patients and differentiate themselves from herpes simplex virus lesions by having unkeratinized mucosa. They can be found on the lateral edges of the tongue, floor of the mouth, pharynx, soft palate, labial and buccal mucosa and generally spare the hard palate. Other oral manifestations of Crohn's disease include labial fissures, mucosal tags and cheilitis. Granulomatous cheilitis may cause firmness and enlargement of the lips and can produce non-caseating granulomas upon biopsy. Both Crohn's disease and ulcerative colitis can cause pyostomatitis vegetans, a condition described as miliary pustules found in the buccal and labial mucosa with sparing of the tongue and floor of the mouth. Although rare, this condition is found almost exclusively in inflammatory bowel disease and its activity typically parallels the severity of bowel pathology [2]. However, there are instances where pyostomatitis vegetans appear when inflammatory bowel disease is otherwise not evident. Its appearance in a healthy-appearing adult or a patient in remission may be an indicator of early disease activity [3,4]. As a result, patients with pyostomatitis vegetans should be investigated for inflammatory bowel disease regardless of any lack of gastrointestinal symptoms. These lesions typically regress when the underlying inflammatory bowel disease is properly treated.

Foregut: esophagus

Consistent with the rest of the upper gastrointestinal tract, specific inflammatory bowel disease involvement in the esophagus is rare. Its prevalence in adults with Crohn's disease has been shown to be as low as 1.8% [5], although the actual rate may be higher for several reasons; diagnosis is difficult as symptoms, macroendoscopic findings and histopathologic results are not specific. Furthermore, milder symptoms may be relieved by H2 blockers and proton pump inhibitors, leading many physicians to confuse this process with reflux esophagitis. Disease of the esophagus alone is also rare, as esophageal Crohn's disease is typically associated with disease elsewhere in the gastrointestinal tract [6]. Usually the presenting symptom in esophageal Crohn's disease is progressive dysphagia [7], possibly a reflection of the difficulty in diagnosing Crohn's early in its course of esophageal involvement. Common findings during EGD, none pathognomonic for Crohn's disease, include ulcers (typically of the superficial, aphthous variety), erosions and in more severe cases strictures and cobblestoning [8]. These findings may also be visualized during radiological studies with contrast. A few cases have also been reported involving esophageal fistulas, forming either within the stomach or tracheobronchial tree [9,10].

Histopathologic features of esophageal Crohn's disease are also non-specific, often revealing lymphocytic infiltrates that may be seen in other causes of chronic inflammation. Granulomas, as mentioned above, are also infrequent. Nevertheless, biopsy remains important because, particularly in the case of strictures and ulcerations, it may rule out malignancies. Given the overall difficulty in diagnosing Crohn's disease in the esophagus, a high index of suspicion is necessary. Although Crohn's patients presenting with esophageal symptoms may warrant further investigation with EGD and biopsies, more common causes of esophagitis may need to be excluded beforehand.

Due to the sporadic prevalence of esophageal Crohn's disease limiting subject size in trials, and varied opinions regarding prognosis of this process, there are no established guidelines regarding treatment. Treatment options include medical, endoscopic and surgical therapy, depending on the severity and presentation of disease. H2 blockers and proton pump inhibitors have been shown to be effective for mild symptoms, but there are conflicting reports as to whether they result in endoscopic healing [6] and prevent progression to strictures, fistulas and other serious disease complications. Steroids have been shown to resolve superficial mucosal lesions, but recurrence is frequent [11]. 5-Aminosalicylic acid (5-ASA) products are not believed to be of benefit in the proximal gut, as they are activated distally. Moderate disease has been treated with anti-secretory therapy, a longer course of steroids and immunomodulators. To date, the largest study evaluating immunomodulator therapy in esophageal Crohn's disease includes 11 subjects, in which six demonstrated response to cyclosporin, azathioprine or 6-mercaptopurine, although there is no mention of any follow-up for possible recurrence [12].

Attempts to treat esophageal strictures in Crohn's disease have been made with endoscopic balloon dilation with intralesion steroid injections, with varying success rates. There is a risk of perforation when dilating an inflamed esophageal wall and narrow fistulous tracts may not be readily accessible for needle injections. Thus far the role of balloon dilation or temporary esophageal stenting for Crohn's esophageal stricture or fistula has not been firmly established and refractory advance disease warrants consideration for surgical resection. Knowledge of surgical resection is confined mainly to case reports. Of note, one report describes a case of Crohn's disease

isolated to the esophagus requiring esophageal resection due to stricture refractory to balloon dilation, without any recurrence of symptoms or disease elsewhere after 36 months of follow-up [13]. However, post-operative mortality rates of patients undergoing resection due to esophageal Crohn's disease for strictures and fistulas have historically been considered high and it is generally recommended to avoid resection unless symptoms are refractory to other modalities of treatment.

In cases of fistulizing disease, biological therapy such as infliximab may be considered. The effectiveness of infliximab in fistulas arising from the esophagus is not as well documented as in fistulas of the lower gastrointestinal tract, but there have been a few case reports in which esophagogastric and esophagobronchial fistulas were able to resolve clinically with this treatment [13,14]. Other non-surgical alternatives have also been investigated, such as fibrin and polymer sealants, but there is little information on their long-term effectiveness. Ultimately, surgical resection may be needed, but as indicated above, mortality rates are high in this population.

Foregut: stomach

When the stomach is viewed during EGD, the most frequent lesions are non-specific for Crohn's disease, such as acute or chronic erosions, mucosa erythema and mucosa edema. To a lesser degree, ulcers varying from aphthoid lesions to extensive serpiginous lesions may be seen. Multiple endoscopic studies reveal the antrum to be the most common area in the stomach to detect Crohn's disease, while the proximal stomach often appears normal macroscopically [15]. Fistulas may develop from the stomach in Crohn's disease, although like those evolving from the duodenum they are infrequent.

The role of biopsy in EGD is particularly important in the stomach, because focal active gastritis with histopathologic characteristics of Crohn's disease can have high positive predictive value during diagnosis (estimated to be as high as 94% in one retrospective study [16]). These focal inflammatory infiltrations will reveal lymphocytes, histiocytes and granulocytes without concurrent *Helicobacter pylori* infection and are distinct from other causes of gastritis. They are also refractory to antibiotic regimens for *H. pylori*, so monitoring response to *H. pylori* treatment may help in diagnosis. One study recommends taking multiple biopsies in the antrum, corpus and angulus, regardless of whether the mucosa appears normal macroscopically, with tissue from the angulus shown to have the highest diagnostic potential [24]. Focal inflammatory infiltrations may also reveal granulomas. Non-caseating granulomas are pathognomonic for Crohn's disease and most often found in the stomach [23], but their occurrence is infrequent (in one study upper endoscopy found granulomas in the entire upper gastrointestinal tract in only 19.5% of cases [17]). Due to the rarity of Crohn's disease isolated to the stomach, there is little knowledge of treatment options specific to this condition. The role of 5-ASA products, steroids and biological therapies for gastric involvement is unclear, although symptomatic relief may be achieved with H2 blockers and proton pump inhibitors. Surgery may be an option for severe gastroduodenal obstruction.

Foregut: duodenum

Crohn's disease of the duodenum is uncommon, but when present manifests as duodenitis, stenosesor very rarely, fistulas arising from the duodenum. In cases of stenosis, EGD may allow for dilation. Duodenal Crohn's disease has also been infrequently associated with pancreatitis, presumably due to disease spreading to the ampulla of Vater or reflux of duodenal contents into the pancreatic duct. Duodenal strictures distal to the ampulla may add further to the possibility of duodenal pancreatic reflux. Common abnormalities of the duodenum in Crohn's disease include focal ulcerations, polypoid lesions and thickened duodenal folds. When seen, notching in the valves of Kerckring may be pathognomonic [18].

The diagnosis of duodenal Crohn's disease is generally dependent on finding either granulomas on biopsy or inflammatory changes consistent with Crohn's disease in the setting of documented disease elsewhere. Non-caseating granulomas are considered pathognomonic for Crohn's disease but their incidence in the upper gastrointestinal tract as a whole is low. However, duodenal biopsy remains important, particularly to rule out inflammation from celiac disease. Patients with small-bowel Crohn's disease are at increased risk for developing adenocarcinoma though duodenal adenocarcinoma is rare [19]. Nevertheless biopsy is also important when there is duodenal obstruction.

There has been speculation that ulcerative colitis may manifest as duodenitis, contrary to the traditional concept of this disease being confined to the lower gastrointestinal tract. One recent study involving EGD of 250 patients with ulcerative colitis detected gastroduodenal involvement as granular mucosa, friability and/or multiple aphthae in 7.6% of subjects, with pancolitis and lower steroid dosage as risk factors [20]. The entity of upper gastrointestinal ulcerative colitis remains unclear and further investigation may be necessary.

Foregut endoscopy: esophagoduodenoscopy

The most common finding during esophagogastroduodenoscopy in Crohn's disease is normal upper

gastrointestinal mucosa, as involvement of Crohn's disease in the upper gastrointestinal tract usually occurs when there is already distal disease. Thus, the American Society for Gastrointestinal Endoscopy's guidelines for endoscopy in the diagnosis of inflammatory bowel disease in 2006 do not recommend EGD to be routinely used in patients suspected of having Crohn's disease. However, EGD does have some utility in diagnosis in cases of indeterminate colitis [21]. Furthermore, there are pediatric studies that support EGD as a first-line investigatory study when diagnosing inflammatory bowel disease [22].

In the upper gastrointestinal tract, Crohn's lesions are most often found in the stomach and are least likely to be found in the esophagus. In an early study of patients with known Crohn's disease of the lower gastrointestinal tract, upper endoscopy revealed stomach lesions in 49% of the study group, duodenal lesions in 34% and esophageal lesions in 15% [23]. The non-specific nature of endoscopic lesions and the overwhelming frequency of normal macroscopic findings suggest why EGD without any histopathologic results has not been shown to differentiate patients from controls [24]. The exception is when aphthous lesions are seen, as they may raise suspicion for Crohn's disease. However, their occurrence is infrequent, with the lesions found only in 11.1% of patients with known disease undergoing EGD in one study [24].

Midgut: overview

Small bowel Crohn's disease is usually associated with colonic lesions. However, up to one-third of Crohn's may occur in the small intestine alone [25]. The overwhelming location of intestinal involvement is in the terminal ileum, typically accessible with a colonoscope. Jejunal Crohn's disease is considered rare and has a more aggressive early course [26]. The reported small bowel distribution of Crohn's lesions is likely biased by the surgical literature and difficult access of the jejunum and proximal ileum. Nonetheless, capsule endoscopy has confirmed the distal dominance of small bowel Crohn's disease [27]. On the other hand, we have identified isolated mid-small bowel disease in roughly 10% of patients, suggesting that thorough small bowel examination using capsule endoscopy may discover Crohn's-like lesions in 16% of symptomatic patients with a prior diagnosis of indeterminate or ulcerative colitis [27].

The vast length of the small intestine poses a difficult issue in determining disease activity and extent of involvement of Crohn's disease. Having a disease activity index that parallels what is available for the colon may prove to be an important advance in disease management and scientific research. A capsule endoscopy Crohn's disease index has been proposed for this purposed but it has not yet been validated [28]. It is often difficult to differentiate disease exacerbation and development of small bowel cancer arisen from inflamed tissue. Performing tissue sampling would minimize confusion and expedite proper disease management. Intestinal strictures and fistula, often the reasons for repeated surgery, may now be treated with balloon dilation or stenting.

Midgut endoscopy: push enteroscopy, capsule endoscopy, single and double balloon enteroscopy

The first form of small bowel endoscopy, push enteroscopy may visualize the distal duodenum and the proximal jejunum. In spite of the ability to perform biopsy and stricture dilation, the value of push enteroscopy is limited by its short length of passage and technical difficulty. With the rapid expansion of other competitive endoscopic technologies, dedicated push enteroscopes are rarely used these days. Rather, many endoscopists choose to use a pediatric colonoscope to carry out a limited upper small bowel examination.

Today, capsule endoscopy is widely considered the best endoscopy method to diagnose suspected Crohn's disease. Before 2000, the only way to visualize the mucosal lining of the entire small intestine was with a sonde enteroscope or a push enteroscope through a small bowel opening at laparotomy. Sonde enteroscopy is a time-consuming and technically complicated procedure and is not suited for evaluation of inflammatory bowel disease. Intraoperative enteroscopy is even more invasive and should not be used for diagnosis or evaluation of known inflammatory bowel disease. Multiple studies have shown that capsule endoscopy has a significant diagnostic yield when other conventional studies fail to confirm suspected cases of Crohn's disease in the small intestine ([29,30].). It is principally used to diagnose Crohn's disease when small bowel follow-through (SBFT) and ileoscopy are negative. A meta-analysis has also shown statistically significant advantage of capsule endoscopy over barium radiography, colonoscopy, CT enterography and push enteroscopy for suspected Crohn's recurrence [31]. The single most important contraindication to capsule endoscopy is the presence of significant inflammatory or fibrotic strictures because of capsule retention. In spite of pre-procedure screening with barium small bowel studies, roughly 3% of capsules are retained as a result of occult Crohn's strictures. Therefore, the decision to employ capsule endoscopy in a patient with known Crohn's disease should be supported by good reason.

Double balloon enteroscopy was introduced shortly after capsule endoscopy became available. The two balloons are designed to pleat the small intestine on to its overtube, allowing the overtube balloon to serve as an anchor and facilitate forward passage of the thin endoscope. In spite

of the ability to insert a double balloon enteroscope deeply inside the small intestine, it is not possible reach the cecum in most cases. Nonetheless, it is realistic to achieve a total enteroscopy in roughly half of the patients if the small bowel is approached from both the oral and rectal routes. The value of double balloon enteroscopy has not been fully assessed. It is typically used in the setting of Crohn's stricture for removal of retained endoscopic capsules [32], and to confirm the disease when other studies are only able to suggest some intestinal abnormalities [33]. Although double balloon enteroscopy may have valuable diagnostic and therapeutic contributions to make to inflammatory bowel disease, caution must be exercised to avoid inadvertent perforation of ulcerated or fixed small bowel lesions [34]. Single balloon enteroscopy, modified from double balloon enteroscopy, was introduced about 2–3 years ago. Its only significant difference from the double balloon enteroscopy is the absence of a balloon at the distal tip of the scope. Published clinical experience of single balloon enteroscopy is too limited to address its use in the assessment and management of small bowel Crohn's disease at this time.

Biliary tract: overview

The biliary tract is not typically the initial site of evaluation in the setting of suspected inflammatory bowel disease. However, some hepatobiliary disorders can provide clues to the astute clinician, most commonly primary sclerosing cholangitis (PSC). Multiple studies have been carried out in which inflammatory bowel disease patients without any known hepatobiliary disease or symptoms received evaluation for other disorders via liver function tests and ultrasound; results have ranged from 12 to 55.9% of subjects having some sort of hepatobiliary abnormality [34,35]. Abnormalities found varied from cholelithiasis, hepatitis, hepatomegaly to hepatic steatosis, although there has been little comment on the clinical significance of these abnormalities or how frequently they are secondary to medications used for inflammatory bowel disease. Regardless, given the risk of PSC and its association with malignancy, hepatobiliary abnormalities should warrant further investigation.

PSC is a disease characterized by inflammatory and fibrotic changes of the intrahepatic and extrahepatic bile ducts that ultimately lead to cholestatic liver disease. Its pathophysiology is not well known and there is speculation about immunologic involvement because it frequently occurs with inflammatory bowel disease. In North America and Europe, it has been estimated that 80% of primary sclerosing cholangitis patients have concomitant inflammatory bowel disease [36] and its occurrence is more frequent in ulcerative colitis than Crohn's disease. In contrast, the incidence of PSC in inflammatory bowel disease is smaller (2–4% of ulcerative colitis cases, 1.4–3.4% of Crohn's disease cases) [24]. In addition to progression to liver failure, complications include increased risk of colorectal malignancy, cholangitis and a 10–15% lifetime risk of developing cholangiocarcinoma [37]. There is speculation that PSC is an additional risk factor for colorectal cancer compared with cases of inflammatory bowel disease alone and meta-analytic studies have revealed as high as a four-fold increase in rates of colorectal cancer in ulcerative colitis patients with PSC compared with those with ulcerative colitis only. In the light of this speculation, since 2002 British guidelines have recommended annual colonoscopy in patients with PSC and inflammatory bowel disease [38].

Diagnosing PSC in the inflammatory bowel disease patient does not differ from diagnosing it in an otherwise healthy individual. Common presenting symptoms include fatigue, weight loss, prurutis and jaundice and cohort studies have shown that 15–40% of patients are asymptomatic, with investigation prompted by abnormal liver function tests. Typical biochemical features will present an obstructive picture, with alkaline phosphatase 3–10 times normal and alanine/aspartate aminotransferase 2–3 times normal. Bilirubin levels may be elevated or normal, depending on how extensive the disease is [24]. Cholangiography and magnetic resonance cholangiopancreatography (MRCP) are the typical radiologic studies employed for diagnosing PSC, with the former being considered the "gold standard". The "beads-on-a-string" description reflects multiple focal strictures along the bile ducts with saccular dilation of unaffected areas. However, given the risks from direct cholangiography, MRCP has been increasingly used and is believed to have a comparable accuracy rate. Images from MRCP may also include bile ducts proximal to obstructed sections and evaluate the liver for features of cirrhosis and portal hypertension [24]. The role of liver biopsy is vital in staging but not in establishing the diagnosis because its findings may not be representative of the involved tissue or specific to PSC.

The treatment for PSC revolves around ursodeoxycholic acid and endoscopic dilatation. If medical and endoscopic therapies are unsuccessful, surgical resection may occasionally be considered in highly selected patients. Liver transplantation is the only treatment that may provide absolute cure and one center's 12 year experience with liver transplantation for PSC had a 5 year survival rate of 85%, although this study was not focused exclusively on PSC with inflammatory bowel disease [39]. Much has been debated about the risk of exacerbating inflammatory bowel disease after liver transplantation (and also other solid organ transplantation). Various studies have shown development of *de novo* disease or recurrence, even worsening disease and acceleration of colorectal dysplasia after liver transplantation, and

other studies have shown the contrary, that transplantation in the setting of inflammatory bowel disease will reduce symptoms. Due to the small subject size in these studies and varying exclusion criteria, it is difficult to conclude whether transplantation is indeed a risk factor for inflammatory bowel disease or improves symptoms in preexisting disease. Post-graft patients frequently experience diarrhea and other gastrointestinal symptoms that can be attributed to infections and drug side effects. However, if these causes are ruled out and symptoms are persistent, endoscopy to examine for inflammatory bowel disease may be necessary in the light of the studies mentioned above.

Surveillance guidelines of the biliary tract in the setting of inflammatory bowel disease are not well defined. However, a patient presenting with symptoms suggestive of obstructive jaundice or as abnormal liver biochemical levels should prompt investigation of the biliary tree. The role of surveillance colonoscopy in recipients of liver grafts due to PSC is also not well defined; it is thought that the progression from inflammatory bowel disease to colorectal neoplasia is accelerated after liver transplant, although the overall incidence is not believed to be higher compared with other individuals with inflammatory bowel disease [24,40]. For the time being, there are no formal recommendations regarding whether this subgroup of patients should undergo colonoscopy sooner after their transplant or more frequently than other inflammatory bowel disease patients. Small-duct PSC has also been described in inflammatory bowel disease patients. This is considered a variant of PSC and is characterized by normal findings on cholangiography despite biochemical features of chronic cholestasis. Due to the lack of findings on cholangiography, liver biopsy is required to establish the diagnosis where features of classic PSC appear. Studies thus far on small-duct PSC are limited by small subject sizes, but it is thought there is progressive potential where a small proportion of these patients will develop large-duct involvement and hence classic PSC, and in the absence of such progression of disease there is no association with cholangiocarcinoma [41–44]. Using liver failure or transplantation as endpoints with an average follow-up period of just over 8 years, one study showed 9% of small-duct patients reaching this stage whereas 47% of those with large-duct disease required transplant or died [31]. Hence the general consensus in the studies available appears to be that long-term prognosis is favorable compared with classic PSC.

Autoimmune hepatitis can also be seen with PSC and in this case the term AIH-PSC overlap syndrome is often used. Estimates of PSC patients with concurrent autoimmune hepatitis have varied due to difficulty with classification and range from 7.6 to 53.8% [25]. Furthermore, this overlap is more commonly seen in the pediatric and adolescent population [25].

Biliary tract endoscopy: endoscopic retrograde cholangiopancreatography, choledochoscopy, intraductal ultrasound and conventional endoscopic ultrasound

Direct cholangiography is traditionally the only trusted method to diagnose PSC, which can involve a combination of the extra- and intra-hepatic ducts. Percutaneous or endoscopic retrograde cholangiography are considered equivalent, although the latter method has been mostly employed in recent years. Improvements in magnetic resonance cholangiography (MRCP) have given this technology equal ability to ERCP in diagnosing the condition [45]. However, ERCP provides superior quality cholangiograms to MRCP and in selected cases is still needed to establish the diagnosis.

Perhaps the most important role of ERCP in PSC is in its ability to deliver endoscopic therapy, which consists of dilation and stenting. Repeatedly treating a dominant stricture to maintain biliary patency has been shown to improve 5 year survival in PSC patients [46]. Balloon dilation, followed by stenting for 3 months, was previously regarded as the standard therapy during ERCP. However, newer studies have identified more complications and no additional benefit from stenting of PSC [47]. If stenting is preferred, it is usually done for 1–2 weeks these days. Surveillance and diagnosis of malignancy in the setting of PSC continue to be a difficult task to carry out. Brushing, biopsy, intraductal ultrasound, conventional endoscopic ultrasound and choledochoscopy have all been tried but with limited success. Repeat examinations and tissue sampling may be necessary to ensure the benign nature of a dominant PSC stricture.

Hindgut: overview

Endoscopy is important in differentiating ulcerative colitis from Crohn's disease as it may provide several macroscopic clues. In ulcerative colitis, inflammation is contiguous as opposed to patchy. Endoscopic examination should show continuous disease involvement along the colon, starting in the rectum and extending proximally. All sides of the bowel wall are involved due to the circumferential behavior of the colitis. Unless the patient has already received medications per rectum, inflammation is worse at the distal end of the bowel. Ulcerative colitis may first appear as merely loss of fine vascular markings. As the disease progresses and there is increased re-vascularization and submucosal edema, hyperemia of the surface will develop and eventually proceed to severe cases of granular, friable mucosa that bleeds with minimal trauma. The granular appearance of the lumen, caused by multiple

small points of light reflection, is often described as like "wet sandpaper". Ulcers may appear in as early as moderate disease, but are typically surrounded by inflamed mucosa and may proceed to form large, continuous ulcers. Pseudopolyps, raised areas of inflammation that resemble polyps, are the result of chronic repetitive cycles of inflammation and ulceration followed by deposition of granulation tissue during healing. Like cobblestoning, they may be seen in both Crohn's disease and ulcerative colitis, although they are more often associated with the latter.

Features favoring Crohn's disease include sparing of the rectum and discontinuous or patchy disease involvement, also referred to as skip areas, sections of normal mucosa separating diseased parts along the bowel. Rectal sparing and skip areas are particularly important in cases of moderate to severe disease where other endoscopic findings of Crohn's disease may be indistinguishable from ulcerative colitis, thus making differentiation between the two diseases especially challenging. Also, Crohn's disease is usually most severe in the right colon and cecum, tends to affect the side of the colon opposite the mesentery more and may demonstrate fistulization (although rare rectovaginal fistulas may also develop in ulcerative colitis) along with perianal disease. Aphthous ulcers are classically identified with Crohn's disease, although they are not exclusive to this disease. In early disease they may appear punched out in otherwise normal-appearing mucosa, but as the disease progresses and lymphoid follicles accumulate and expand, these ulcers will aggregate into star-shaped ulcers called stellate ulcers. In severe disease, ulcers may be identified as deep and serpiginous. Parallel rows of linear ulcers can also be seen and are referred to as "bear-claw" ulcers. Finally, the effects of chronic injury and submucosal edema from severe disease may cause cobblestoning of the lumen. But perhaps the most important evidence of Crohn's colitis occurs in the terminal ileum. The presence of discrete ulcers or strictures of the terminal ileum or ileocecal valve should distinguish Crohn's disease from backwash ileitis of ulcerative colitis [48].

When endoscopic and radiographic appearances fail to separate Crohn's disease from ulcerative colitis, mucosal histology becomes an important part of the evaluation. Tissue sampling in diseased and normal mucosa also applies when differentiating Crohn's disease from ulcerative colitis. In one prospective study of 357 inflammatory bowel disease patients with an average follow-up of 22 months, endoscopic biopsy has been shown to have diagnostic accuracy of Crohn's disease or ulcerative colitis in 89% of cases and to err in 4% [49]. Histopathologic features of Crohn's disease include focal inflammation in a background of normal mucosa and granulomas (Table 18.1). Granulomas are neither pathognomonic for Crohn's disease nor indicative of disease severity and they may appear in a variety of other diseases, including tuberculosis, sarcoidosis and fungal and bacterial infections. Their occurrence during endoscopic biopsy of inflammatory bowel disease has been shown to range from 15 to 36% [50] and even higher in surgical specimens. Biopsies taken from the edge of ulcers are reputed to have the highest yield when finding granulomas [51]. In ulcerative colitis, typical histopathologic traits include ulcers in a background of inflammation. Granulomas are not expected; rather, ulcers and crypt abscesses are considered the defining but not pathognomonic lesions as they may appear in Crohn's disease at a lesser frequency.

Table 18.1 Crohn's disease versus ulcerative colitis: typical histopathologic findings.

	Crohn's disease	Ulcerative colitis
General features	Focal areas of inflammation in a background of normal mucosa	Ulcers in a background of inflamed mucosa
Granulomas	Frequent though not pathognomonic	Infrequent, ruptured crypt abscesses may appear as small granulomas
Crypt abscesses	Not common but possible	Defining lesion, though not pathognomonic

Biopsy of the terminal ileum is of particular importance in distinguishing Crohn's disease from ulcerative colitis and should be pursued in most cases. Backwash ileitis appears only as inflammation without ulcerations macroscopically. Non-caseating granulomas have been shown to occur in less than 10% of terminal ileal biopsies, but their presence in this setting is regarded as pathognomonic for Crohn's disease [47]. Along with diagnosis, endoscopic biopsy may assist in assessing the distribution and severity of inflammatory bowel disease. Determination of whether disease is confined to the rectum or left-sided colon or is rampant throughout the entire colon (pancolitis) may guide the physician towards proper medical or surgical therapy. Biopsy is integral in distinguishing inflammatory bowel disease from other etiologies of colitis such as ischemia, use of NSAIDs and infection, as they may all mimic inflammatory bowel disease on a macroscopic level. Infection has been documented to be the cause of colitis in as much as one-third of bloody diarrhea cases suspicious for inflammatory bowel disease [47]. As a result, biopsies should be taken in diseased and normal-appearing bowel. Features of chronic disease suggestive of inflammatory bowel disease include destruction of cell architecture, accumulation of plasma cells near the mucosal base, increased cellularity of the lamina propria and Paneth cell metaplasia.

Hindgut endoscopy: colonoscopy and ileoscopy

The gold standard for diagnosing inflammatory bowel disease in the large bowel is colonoscopy with biopsies. This is also true when encountered with the challenge of differentiating ulcerative colitis from Crohn's disease that otherwise has not manifest itself in other parts of the digestive tract (see Table 18.2). There is some value with imaging studies, particularly in cases where immediate endoscopy may be contraindicated or areas of the bowel are otherwise inaccessible. However, diagnosis of inflammatory bowel disease ultimately still requires endoscopy and biopsy. Symptoms prompting investigation for inflammatory bowel disease include abdominal pain, diarrhea, tenesmus, rectal bleeding and bloating. Patients may also present with associated weight loss or anemia.

Colonoscopy plays many vital roles in a patient with known or suspected inflammatory bowel disease; it has value in diagnosis, differentiation and monitoring disease, along with cancer surveillance. In cases where strictures develop, endoscopy also allows for dilation. It is advantageous to imaging studies because along with allowing biopsies to be taken, it provides direct visualization within the lumen to find early disease changes that may otherwise not show up on MRI or CT. Thus colonoscopy with biopsy is recommended in the initial evaluation of any patient with symptoms suspicious of inflammatory bowel disease and is considered the gold standard in diagnosis. However, there are contraindications to endoscopy, mainly toxic megacolon or severe colitis, conditions where bowel perforation is at an increased risk. One would also be careful to pursue colonoscopy in patients with other comorbidities who may be neutropenic or coagulopathic, given the relative increased risk of infection and bleeding. Finally, it should be noted that NSAIDs and sodium phosphate-based bowel preparations may cause mucosal changes that can be mistaken for inflammatory bowel disease [52,53]. Colonoscopic identification of a true inflammatory bowel disease may be easier than differentiating Crohn's disease from ulcerative colitis [54].

Table 18.2 Crohn's disease versus ulcerative colitis: typical macroscopic findings.

	Crohn's disease	Ulcerative colitis
Lesions (early to severe disease)	Aphthous ulcers Stellate ulcers Serpiginous "bear-claw" ulcers Cobblestoning	Loss of fine vascular markings Hyperemia Friable, granular mucosa, occasional large ulcers surrounded by inflamed mucosa Pseudopolyps
Distribution along colon	Patchy, skip lesions with intervening areas of normal mucosa	Continuous involvement throughout affected segments, cecal patch also possible
Distribution along luminal wall	Predilection for wall opposite mesentery	Circumferential, affecting all walls equally
Rectal involvement	Usually spared	Usually involved with disease spread extending proximally
Perianal involvement	Anal skin tags, fissures, complicated fistulas, abscesses	Rare, uncomplicated fissures and fistulas may be present
Ileal involvement	Involvement in most cases	Only backwash ileitis in pancolitis
Fistulization	Multiple types possible; enterocutaneous, perianal, rectovaginal, enterovesicular	Only rare rectovaginal fistulas

Hindgut endoscopy: colonoscopic disease activity monitoring

There has been much investigation regarding the role of endoscopy when monitoring inflammatory bowel disease, particularly in Crohn's disease. Scoring systems have been developed based on ulcerations, areas of inflammation, stenoses and other disease findings in an attempt to use endoscopic findings to foresee prognosis and response to therapy. Notable scoring systems include the Crohn's disease endoscopic index of severity (CDEIS), the simple endoscopic score for Crohn's disease (SES-CD) and the Rutgeerts endoscopic grading scale. With the exception being Rutgeerts score, the clinical value of such scoring systems is limited because disease activity appears to be independent of endoscopic findings. When compared with the Crohn's disease activity index (CDAI), an index based on clinical symptoms, no correlation is found between endoscopic findings and symptoms [55,56]. Endoscopic findings are also shown to have no predictive value in determining response to steroids in the setting of an acute flare [51], nor prognostic indicators once disease remission is achieved [57]. Studies geared towards correlating histopathologic findings with symptomatic disease in both Crohn's disease and ulcerative colitis have generated only mixed results [58], although in the setting of an acute exacerbation biopsy properly rules out infection. Thus, repeat colonoscopies to monitor Crohn's disease are generally not recommended and determining disease activity relies more towards assessment of patient symptoms.

The Rutgeerts endoscopic grading scale (Table 18.3), on the other hand, assesses disease recurrence and severity in Crohn's disease patients who have had ileocolonic resection. Grading is based on ulcers, inflammation and lumen

Table 18.3 Rutgeerts endoscopic grading scale [60].

Grade	Endoscopic findings
0	No lesions
1	<5 aphthous ulcers
2	>5 aphthous ulcers with normal mucosa between lesions or lesions confined to ileocolonic mucosa
3	Diffuse aphthous ileitis with diffusely inflamed mucosa
4	Diffuse inflammation with larger ulcers, nodules and/or narrowing

narrowing that may be seen in the area of and before the anastomosis. Routine colonoscopy to monitor disease activity is important in this situation because studies have shown up to 30% of ileocolonic resection patients to have symptomatic disease recurrence and 85% endoscopic recurrence in this area within the first postoperative year [59]. Recurrence rates generally do not increase significantly beyond the first year. Furthermore, the severity of early post-operative endoscopic findings alone have been shown to be a prognostic factor for symptomatic recurrence [54]. Current strategies of postoperative maintenance therapy are not well defined, although some medications, notably immunomodulators, have at times shown potential. Risk assessment of postoperative patients for disease recurrence at large is still inadequate. Nevertheless, given the significant rates of both symptomatic and endoscopic recurrence with in the first postoperative year and the prognostic value in evaluating severity of endoscopic findings, routine colonoscopy at 6 and 12 months after resection is helpful for monitoring disease activity and is generally recommended [60].

Hindgut endoscopy: colonoscopic cancer surveillance

Endoscopy for cancer surveillance plays an integral role in the long-term care of the inflammatory bowel disease patient. In a cohort study comparing patients with either Crohn's disease or ulcerative colitis with random age-matched subjects in the same region without inflammatory bowel disease, both Crohn's disease and ulcerative colitis patients were shown to have an increased risk of developing colon carcinoma. Ulcerative colitis also carries an increased risk of rectal carcinoma [61]. In patients with severe inflammatory bowel disease, surveillance colonoscopies have been shown to detect colorectal cancer at earlier stages with subsequent better prognosis, although this may be influenced by lead-time bias. There is also indirect evidence that surveillance reduces death from colorectal cancer and is cost-effective in inflammatory bowel disease. However, it should be noted that there is no clear evidence that surveillance prolongs survival [62]. Regardless, guidelines for screening and surveillance colonoscopies are recommended and commonly practiced.

Table 18.4 Factors increasing risk of colorectal cancer in inflammatory bowel disease patients.

<15 years of age at onset of disease
Long duration of disease
Ulcerative colitis extending proximally to at least the left-sided colon
Crohn's disease with extensive disease involvement of at least one-third of the colon
Family history of colorectal cancer
Prior dysplasia
History of PSC
Backwash ileitis

There are several risk factors thought to carry a greater likelihood of developing colorectal cancer (Table 18.4), but regardless, patients should undergo a screening colonoscopy 8–10 years after initial onset of symptoms. If their screening colonoscopy is negative for dysplasia but demonstrates left-side colitis or Crohn's disease with involvement in at least one-third of the colon, further surveillance colonoscopies are recommended every 1–2 years [63] and annually in patients with PSC. Four-quadrant biopsies should be taken from the proximal area of disease then every 10 cm, with a minimum of 33 tissue samples. In ulcerative colitis, where the likelihood of cancer in the lower sigmoid and rectum is greater, biopsies should be taken every 5 cm these areas [64]. Macroscopic abnormalities including strictures and mass lesions should all be biopsied. In Crohn's disease, where colonic involvement of is not as extensive as in ulcerative colitis, the guidelines for surveillance are debatable. Following general population guidelines for colorectal cancer screening may be appropriate, although some advocate more frequent colonoscopy in those with risk factors for colorectal cancer.

Abnormal histopathologic findings taken from colonoscopy include those that are "indefinite for dysplasia", "low-grade dysplasia" or "high-grade dysplasia". Biopsies read as "indefinite for dysplasia" require a second evaluation by an experienced gastrointestinal pathologist, with follow-up surveillance every 3–6 months if this read is verified [58]. It is also important to characterize abnormal biopsies as arising from flat or raised lesions, since management differs.

In flat mucosa, management strategy for single low-grade dysplasia is undefined because of ambiguity regarding the risk of cancer these lesions entail. Five-year progression rates of low-grade dysplasia to high-grade lesions or colorectal cancer have been shown to vary from virtually nil to 50–55% [64]. Furthermore, in cases of colon resection where a single low-grade dysplastic lesion was the worst finding, studies have shown a 20% rate of

Table 18.5 Surveillance colonoscopy findings requiring colectomy [64].

Multifocal low-grade dysplastic flat lesions
Any high-grade dysplastic flat lesion(s)
Dysplasia in the surrounding mucosa of any raised lesion
Dysplasia-associated lesions or masses (DALMs) not removable by polypectomy

concurrent colorectal cancer in surgical specimens that were likely missed during colonoscopy [64]. Currently, prophylactic colectomy for a single low-grade dysplasia is controversial, but in the light of possible concurrent colorectal cancer this option should be discussed with patients. Multifocal low-grade dysplastic lesions, on the other hand, require colectomy, as do high-grade dysplastic lesions. Single low-grade dysplastic lesions during repetitive colonoscopies may also warrant prophylactic colectomy, since the 5 year progression rate of a single low-grade lesion to high grade or malignancy may approach the rate in cases of multifocal low-grade dysplastic lesions [59].

Raised lesions may resemble typical sporadic adenomas or dysplasia-associated lesions or masses, also known as DALMs. Biopsies of the surrounding mucosa are required to help determine management. Simple polypectomy may be sufficient for removal of adenomas [65]. If adjacent mucosal biopsies and the rest of the colon are negative for dysplasia, surveillance colonoscopy 6 months later is recommended with regular follow-up afterwards if no dysplasia is found subsequently as well [64] (see Tables 18.5 and 18.6). The management of DALMs and whether polypectomy alone is adequate is more complex, especially if the patient has other risk factors for developing colorectal cancer. Generally, dysplasia of the surrounding mucosa regardless of lesion and DALMs that are sessile or otherwise not removable by polypectomy [64] require colectomy. However, in one recent study with nine patients with high-grade DALMs, the patients were shown not to have colorectal cancer in subsequent surveillance or from surgical resection specimens over a mean of approximately 4 years of follow-up, implying that immediate colectomy for these lesions may not be necessary [66]. In cases of uncertainty, referral to a tertiary center with possible tattooing of the lesion in question may be necessary.

Chromoendoscopy is a new technology that is currently being investigated in cancer surveillance of inflammatory bowel disease patients. In this procedure, segments of colon are stained with either a methylene blue or indigo carmine dye to enhance visualization of mucosal abnormalities and guide biopsies. Studies regarding chromoendoscopy are promising; a recent prospective trial of note involving 115 inflammatory bowel disease patients undergoing both colonoscopy with four biopsies taken every 10 cm of colon and chromoendoscopy with target-guided biopsy was able to show increased detection of both low- and high-grade dysplasia by chromoendoscopy as compared with regular colonoscopy (17 patients with dysplasia in chromoendoscopy, three in colonoscopy) [67]. Chromoendoscopy is not commonly performed and there are no defined guidelines for its practice. However, this may change in the near future given its potential thus far.

Confocal laser endomicroscopy is another technique that aims to identify suspicious lesions to guide endoscopic biopsy at the time of endoscopy. With resolution down to the cellular level, confocal microscopy and chromoendoscopy have been shown to detect significantly more intraepithelial neoplasia than randomly obtained biopsies [68].

Table 18.6 Surveillance colonoscopy findings that warrant consideration for colectomy [64].

Unifocal low-grade dysplastic flat lesion, especially if risk factors for colorectal cancer are present
Unifocal low-grade dysplastic flat lesion on repeat colonoscopies

Hindgut endoscopy: flexible sigmoidoscopy

In cases suspicious for inflammatory bowel disease, flexible sigmoidoscopy may be utilized in situations such as severe colitis where the risk of bowel perforation is high with colonoscopy. Flexible sigmoidoscopy may be adequate in diagnosis, but a future colonoscopy at a later time may still be needed to exclude skipped lesions and to assess the extent of colitis. In patients with a prior diagnosis of ulcerative colitis presenting with an acute flare, flexible sigmoidoscopy may be sufficient in confirming disease recurrence and ruling out infection, ischemia and other causes of colitis.

Conclusion

Inflammatory bowel disease is a condition that is diagnosed on the basis of multiple factors including clinical history and results of diagnostic studies such as radiology, endoscopy and histology. It is our opinion that endoscopic mucosal examination is the most important modality in the evaluation of a suspected or known case of inflammatory bowel disease. The additional benefit of endoscopy is its ability to obtain tissue for histologic examination and to perform therapy. Advances in technology finally allow us to investigate the entire small intestine, one of the most important organs afflicted by Crohn's disease. There are also clinical data showing that endoscopic treatment of sclerosing cholangitis may improve patient survival from liver failure, although the value of ERCP diagnosis of this

condition has diminished with the advances in MRCP. The introduction of chromoendoscopy and confocal laser endomicroscopy may help identify dysplastic lesions for precise tissue sampling and ablation during endoscopic surveillance. We now have a fantastic array of tools and techniques that should make the diagnosis and management of inflammatory bowel disease easier than ever.

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Chapter 19
Imaging in Inflammatory Bowel Disease: Computed Tomography and Magnetic Resonance Enterography, Ultrasound and Enteroscopy

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Summary

- Transabdominal ultrasound may be an effective non-invasive method of assessing disease activity and extent in Crohn's disease. The addition of power Doppler and sonographic signal-enhancing agents may increase the accuracy of diagnosing intestinal complications of Crohn's disease.
- Endoscopic ultrasound (EUS) is an effective way to evaluate perianal disease and an EUS-directed combined medical–surgical approach may improve long-term healing rates.
- Computed tomography enterography (CTE)/enteroclysis is highly sensitive and specific for diagnosing small bowel Crohn's disease and has the added advantage of detecting extraluminal complications in 20% of patients. Concerns about exposure to ionizing radiation are leading to dose reduction and increased awareness about judicious use of this technique.
- The operating characteristics of magnetic resonance enterography/enteroclysis are improving to the point where it is a reasonable radiation-free alternative to CTE.
- These advanced diagnostic techniques not only improve diagnostic accuracy but also allow us to assess disease activity and diagnose intestinal complications such as stricture or penetrating disease.

Introduction

Crohn's disease, one of the major subtypes of the idiopathic inflammatory bowel diseases (IBDs), is characterized by chronic, transmural, often granulomatous, intestinal inflammation. There are over 600,000 persons in North America and nearly one million persons in Europe suffering from this condition [1]. Although involvement can occur anywhere in the gastrointestinal tract from mouth to anus, predilection for the small bowel is often observed. Non-specific symptoms such as abdominal pain, diarrhea or fatigue and variable disease behavior (inflammatory, penetrating or stricturing) add to diagnostic uncertainties. As no single test result is pathognomonic for Crohn's disease, clinicians frequently must utilize multiple modalities to secure a clinical diagnosis before the appropriate therapy can be initiated [2].

Some of the most important diagnostic tools from the clinician's viewpoint have included ileocolonoscopy and some form of small bowel imaging. Until recently, the latter largely consisted of small bowel follow-through (SBFT), but the past few years have seen increasing use of computed tomography enterography (CTE) or CT enteroclysis at certain centers. This imaging modality appears to be both sensitive and specific for detecting active small bowel inflammation, extraluminal complications and even select extraintestinal manifestations. These superior operating characteristics may result in changes in clinicians' decision-making and diagnostic algorithms. Universal adoption of CTE, however, may be constrained by concerns over radiation exposure and costs. In some countries, gastroenterologists perform abdominal ultrasounds to assess disease extent non-invasively and exclude complications of Crohn's disease. Recent years have seen advances in magnetic resonance (MR) technology such that MR enterography (MRE) or enteroclysis may be a viable radiation-free imaging option.

An equally important function of diagnostic imaging is the assessment of disease activity. Although historically the activity of Crohn's disease has been gauged by the

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Crohn's Disease Activity Index (CDAI), which is primarily symptom based (abdominal pain, diarrhea and overall sense of well-being account for most of the score), we are beginning to appreciate that this widely used clinical trial measure may not necessarily track well with more objective markers of disease activity, such as serum C-reactive protein, fecal lactoferrin or calprotectin, endoscopic appearance or radiologic appearance [3]. Furthermore, a growing body of evidence suggests that earlier and more aggressive medical therapy will be needed to alter truly the natural history of Crohn's disease. In this setting, it becomes all the more imperative to assess accurately biological evidence of disease activity before making decisions about initiating or altering therapy.

Ultrasound

Although grossly underutilized in the United States and Canada, transabdominal ultrasound (US) has been shown in numerous studies to be an effective non-invasive method of assessing disease activity and extent and of detecting intestinal complications in Crohn's disease [4]. It is low cost, non-invasive and radiation free. Bowel wall thickness is one of the primary findings to determine disease location. A prospective blinded study in children with known or suspected IBD showed that bowel thickness of >2.9 mm was relatively specific (but not sensitive) for moderate to severe IBD [5]. Doppler flow analysis of superior mesenteric arterial (SMA) blood flow may aid in the assessment of disease activity [6–9]. It may also be useful to predict risk of relapse [9]. The three best parameters on Doppler analysis of the SMA blood flow may be end diastolic velocity, time-averaged maximum velocity and maximum flow volume, but there was still overlap between actives and inactives [10]. Left colonic involvement was associated with increased Doppler blood flow in the internal mammary artery (IMA) [11]. Echo-signal enhanced US assesses bowel wall vasculature and correlates well with MR assessment of disease extent [12]. The addition of intravenous contrast-enhanced power Doppler after US may be useful in detecting inflammatory activity in the bowel wall [13] and be more reliable in diagnosing and assessing disease activity than color power Doppler alone [14]. The focal disappearance of intestinal wall stratification on US appears to correlate well with the presence of longitudinal ulcers in Crohn's disease [15].

Transabdominal US may be able, in experienced hands, to detect intestinal complications of Crohn's disease [16, 17]. Sonographic detection of mesenteric lymphadenopathy occurs more often in younger patients and in those with fistulae or abscesses, but this finding is neither sensitive nor specific enough to affect management decisions [18]. US may be particularly useful in detecting strictures in patients with acute obstructive symptoms – one study of US versus small bowel enteroclysis versus plain films showed it was only slightly less sensitive (52%) than small bowel enteroclysis (64%), but it was highly specific (100%) [19]. The combination of power Doppler with application of a sonographic signal-enhancing agent assesses intramural blood flow and may allow recognition of fibrostenotic strictures in Crohn's disease patients with obstructive symptoms [20], in addition to evaluating inflammatory masses [21]. The sensitivity of US to detect stricture ranges from 79 to 90% and the specificity ranges from 95 to 100% [22]. A prospective study of 17 children and young adults with IBD showed that changes in findings on abdominal US (bowel wall thickness, color and power Doppler) correlated many clinical parameters well [23].

Transabdominal US may not be as accurate at identifying internal fistulae as strictures, although data are conflicting. One study suggested that power Doppler analysis of internal fistulae could reveal vasculature both within and around the fistula walls and that blood flow characteristics such as resistance index correlated with clinical and biochemical parameters of inflammation [24]. An Italian study of 625 Crohn's disease patients suggested that internal fistulae were present in up to 44% and intra-abdominal abscesses in 20% and that transabdominal US had an accuracy in detecting fistulae comparable to that in radiographic studies [25].

The addition of oral administration of a non-absorbable anechoic solution appears to improve the sensitivity in detecting inflamed bowel and also strictures [26]. Indeed, even in the hands of a relatively inexperienced operator, the addition of an oral contrast agent is at least equivalent to standard transabdominal US performed by an experienced operator with respect to identifying small bowel abnormalities and it was superior to standard US for detecting strictures [27]. One group combined aspects of enteroclysis and US by infusing an oral contrast agent via a nasojejeunal tube followed by transabdominal US. This US–enteroclysis technique correctly identified active small bowel Crohn's disease in 94% of patients studied and intestinal complications of Crohn's disease in 90% [28]. A 2005 meta-analysis of all studies assessing the diagnostic accuracy of US to date estimated a sensitivity between 75 and 94% and a specificity between 67 and 100% – the point estimate varied on the cutoff value used for bowel wall thickness [29].

US may be useful in the perioperative period – one study suggested that preoperative sonographic appearance might predict risk of recurrence [30]. Ultrasonography may be useful in the postoperative setting to determine if there was been a recurrence of Crohn's disease [31]. Systematic US in the postoperative setting may identify patients with early bowel wall thickening who are at greater risk of Crohn's disease recurrence [32]. The finding of a bowel wall thickness of >5 mm in this setting is highly correlative of a severe endoscopic recurrence of Crohn's

disease [33]. The specificity of US in a 3 months postoperative setting for identifying those with endoscopic recurrence (90%) was higher than that of fecal calprotectin (75%), but calprotectin was more sensitive (63 versus 26%) [34]. In patients with a medically induced remission, the finding of increased vascularity in the bowel wall on color Doppler sonography presaged an unfavorable subsequent clinical course [35].

How does US compare with other diagnostic modalities? In one study of 73 Crohn's disease patients, US was compared with enteroclysis, CT and tagged white cell scanning. The sensitivity of US was 88% and the specificity was 93%. Although CT was numerically more sensitive than US, it was less specific [36]. Transabdominal US and MR abdomen tests were compared in 30 patients with known Crohn's disease and although both tests were sensitive, US was significantly more specific [37]. A German study of 48 patients with known Crohn's disease compared small bowel enteroclysis, transabdominal US and abdominal MR for assessing disease extent and intestinal complications and found that all three techniques were comparable [38]. Similarly, a study of 61 patients with IBD showed that there was a higher correlation between an endoscopic activity index and an ultrasonographic activity index ($r = 0.88$) than between the endoscopic index and an MR activity index ($r = 0.34$) [39].

Not all studies have demonstrated the utility of US, however. A study of 169 IBD patients, most of whom had Crohn's disease, could not demonstrate a significant correlation between the volume of inflamed bowel wall and biochemical markers of inflammation or between the volume of inflamed bowel wall and clinical disease activity [40].

Endoscopic US of the rectum and perianal area may be useful to assess disease activity and to locate and classify perianal disease. Although several studies have employed rectal EUS in ulcerative colitis [41,42], its primary indication is in patients with perianal Crohn's disease. Several studies have compared the diagnostic accuracy of rectal EUS with pelvic MR imaging and examination under anesthesia for perianal Crohn's disease and have suggested that all three modalities are useful [43,44]. In the study by Schwartz *et al.*, the accuracy for all three modalities was 85% or greater and the accuracy rose to 100% when any two tests were combined [44]. The use of endoanal US to assess rectovaginal fistulae ($n = 25$) was not found to be clinically useful in one study, but the authors found some clinical utility preoperatively with respect to identifying occult sphincter defects, allowing for concomitant anal sphincter reconstruction [45].

Ultrasonographic assessment after injection of hydrogen peroxide into fistula tracts appears to be as accurate as barium fistulogram in detecting fistula tracts and associated abscesses in patients with Crohn's disease and suspected enterocutaneous fistulae [46]. Compared with endoanal US alone, the addition of hydrogen peroxide injection may increase the accuracy of location and classification of anal fistulae [47,48]. Hydrogen peroxide-enhanced three-dimensional endoanal US and endoanal MR were equivalent to examination under anesthesia in locating and classifying perianal fistulae [49]. The endosonographic assessment of fistulae will often demonstrate persistence of the fistula tract despite clinical resolution of the fistula [50]. Only a minority of patients will demonstrate complete sonographic healing of perianal fistulae with 1 year of infliximab [51]. Sonographic healing of fistulae is associated with a lower fistula recurrence rate [52]. The results of serial EUS can guide a combined medical–surgical approach to perianal Crohn's disease and result in high long-term healing rates [53], and a small randomized trial of clinically directed medical–surgical therapy versus EUS-guided combined therapy suggested improved outcomes in the EUS-guided group [54]. Transperineal US may aid in the assessment of patients with severe perianal or rectovaginal complications of Crohn's disease [55].

The clinical utility of transabdominal US in ulcerative colitis is less clear. Some investigators have attempted to enhance their findings by administering water per rectum prior to sonographic investigation of the colon (so-called hydrocolonic sonography). In a study comparing hydrocolonic sonography with tagged leukocyte scanning among 68 IBD patients, the overall accuracy of the former was 87%, compared with 77% with scintigraphy [56]. Ultrasonographic assessment of colonic wall thickness may be useful in the assessment of disease activity in ulcerative colitis and correlates with radiographic extent and with biochemical and endoscopic activity of colitis [57]. Several studies indicate that mesenteric blood flow assessment may be useful to assess disease activity and predict relapse in ulcerative colitis (UC) [58,59]. Doppler analysis of the superior mesenteric artery may be a useful tool to assess for proximal extension of UC, since blood flow measurements were significantly increased in those with pancolitis but not in those with left-sided disease [60]. Increased rectal wall thickness may identify UC patients at higher risk of relapse [61]. Some have suggested that EUS might be useful not only to assess disease activity but also to predict response to medical therapy or determine the need for surgery in UC [62], but this remains to be confirmed.

CT enterography and enteroclysis

CTE combines high-resolution CT scanning with some of the concepts of barium radiography [63–67]. CTE allows for the evaluation of small intestinal regions inaccessible to conventional endoscopy and has replaced SBFT at many centers. Indications for CTE include suspected Crohn's disease, assessment of disease activity (either at baseline or in response to medical therapy), evaluation of

suspected stricture (inflammatory versus fibrostenotic) and assessment of degree of bowel obstruction (by measuring degree of prestenotic bowel dilation). The presence of intestinal fistulae can often be imputed from CTE findings, as fistula tracts typically enhance and are often filled with fluid and/or air. One of the great advantages of CTE over SBFT is the ability to detect penetrating disease or extraintestinal IBD manifestations [68]. Thus, a Crohn's disease patient with a suspected penetrating complication such as fistula or abscess would require only one examination for assessment of both bowel wall disease and extraenteric disease with CTE, instead of two examinations (i.e. SBFT plus CT of abdomen and pelvis).

In the 60–75 min prior to the examination, the patient ingests a large volume (between 1500 and 2000 ml) of a neutral enteric contrast agent either by mouth (CT enterography) or via a nasojejunal tube (CT enteroclysis). A small feasibility study comparing peroral ingestion of water with nasojejunal administration of methylcellulose suggested that both methods had similar overall accuracy [69]. The use of a neutral rather than a positive contrast agent maximizes the contrast between the bowel lumen and the bowel wall. Neutral agents include water, polyethylene glycol, methylcellulose or a highly diluted barium sulfate solution in sorbitol (VoLumen, EZ-EM, Inc., Lake Success, NY, USA). An intravenous iodinated contrast agent such as iohexol (Omnipaque 300, GE Healthcare, Princeton, NJ, USA) is administered (150 ml at 4 ml s^{-1}) and the scan is generally performed between 50 and 70 s after administration, in the portal venous and/or hepatic uptake phase. The examination is performed using thin slices (2.5 mm every 2.5 mm) on a helical CT scanner (four-slice or higher). The images can be viewed in either axial or coronal fashion. Viewing of coronal images may be preferred by some readers as the primary view [70].

Signs of active bowel inflammation on CTE include mural hyperenhancement, mural stratification (produced by differential uptake of contrast in the bowel wall layers), increased mural thickness, peri-enteric fibrofatty proliferation and engorgement of peri-enteric vessels, often called the "comb sign" [71] since the engorged vessels resemble the teeth of a comb [66] (Figures 19.1–19.3). The finding of asymmetric (as opposed to generalized) mural enhancement and thickening is thought to be highly specific for Crohn's disease. Up to 20% of patients with known Crohn's disease are found on CTE to have potentially significant extraluminal findings, including penetrating disease (Figure 19.4), pancreatitis, sacroiliitis, nephrolithiasis, cholelithiasis, primary sclerosing cholangitis or portal/mesenteric vein thrombosis [68].

Several studies have demonstrated that individual CTE findings such as mural hyperenhancement, increased mural thickness and increased mesenteric fat density are relatively sensitive markers for active small bowel inflammation (80–90%). These findings also track well with other

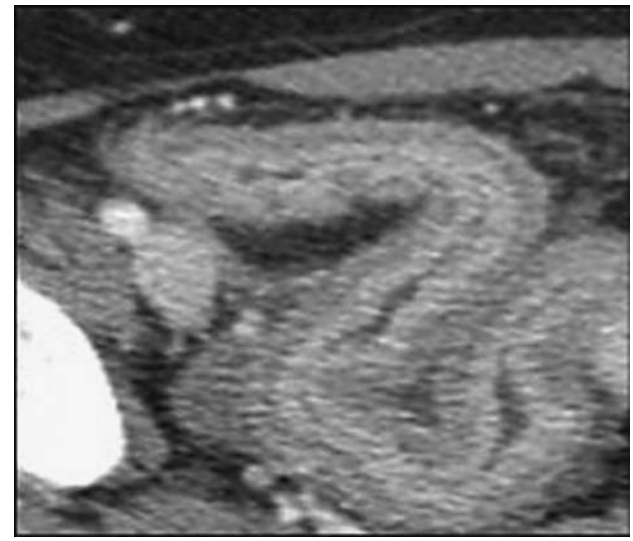

Figure 19.1 CT enterography demonstrating mural thickening, contrast hyperenhancement and mural stratification of the ileum in a patient with Crohn's disease. Courtesy of Joel G. Fletcher MD.

measurements of Crohn's disease activity, such as ileal erosions on endoscopy and elevated C-reactive protein [72–74]. A study correlating the findings on CT enteroclysis with surgical pathological findings noted high correlation with respect to inflammation and fibrostenosis [75], with the comb sign demonstrating the highest association with inflammatory activity and the presence of radiographic stenosis being most predictive of fibrostenosis. In another study using surgical findings as the reference standard, CTE correctly identified intestinal complications

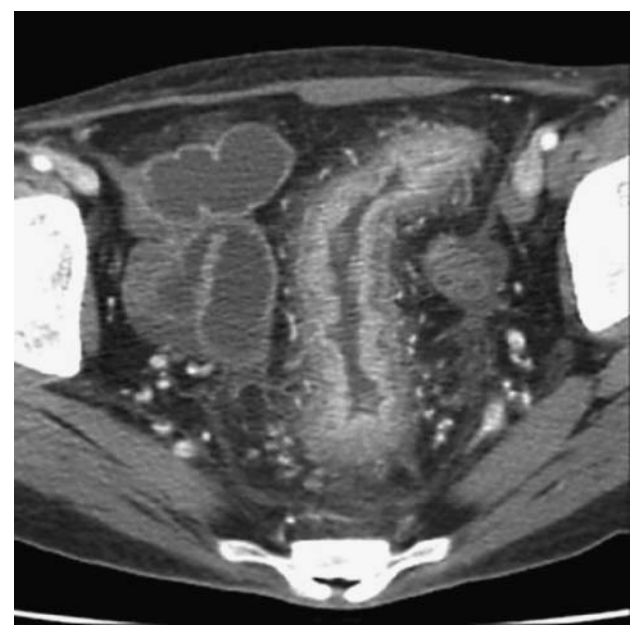

Figure 19.2 Peri-enteric fat stranding and inflammation of the rectosigmoid in a patient with Crohn's disease. Courtesy of Joel G. Fletcher MD.

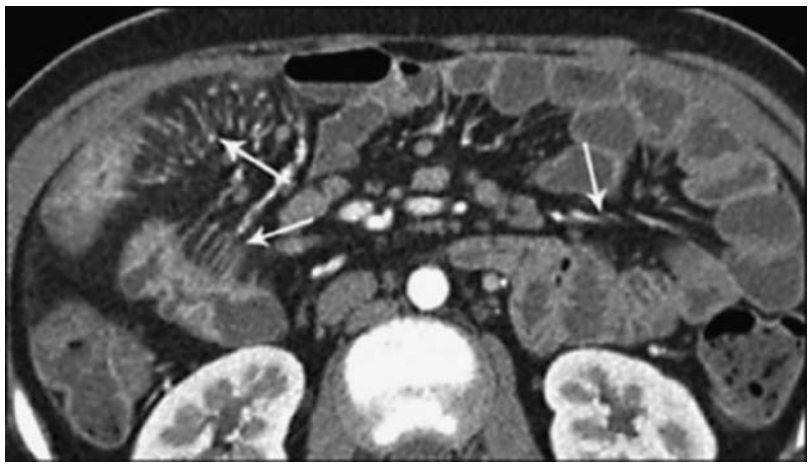

Figure 19.3 Engorgement of the vasa recta ("comb sign") of the ileum in a patient with Crohn's disease. Courtesy of Joel G. Fletcher MD.

(i.e. stricture, fistula, phlegmon, abscess) over 94% of the time [76]. Studies comparing the operating characteristics of CTE or CT enteroclysis with ileocolonoscopy have demonstrated high overall accuracy [77,78]. Compared with conventional barium enteroclysis, CT enteroclysis detects more prestenotic bowel dilation, fistulae, skip lesions and abscesses [79].

CTE, however, is not suitable for every patient. Either absence of adequate bowel distension or lack of intravenous contrast can severely limit its utility. This means that patients who cannot ingest the 1500–2000 ml of oral contrast agent or who cannot receive iodinated intravenous contrast (renal insufficiency or severe contrast dye allergy) should undergo alternative imaging.

Some have also expressed concerns that CTE is too expensive. Issues related to cost should be addressed by better quantifying how the information obtained at CTE translates into benefits with respect to managing patients. In other words, do the findings on CTE result in a change in patient management or a clinician's level of suspicion for active disease, fistula, abscess or stricture? Such studies are much needed and are currently under way. The earliest studies suggest that CTE indeed provides significant added value over the clinician's initial impression, because the findings appear to influence management plans [80,81]. In a study of IBD patients evaluated at the University of Michigan, the clinician's initial assessment (which included their perception of the patient's benefit from corticosteroids) was compared with their clinical assessment after revealing findings on CTE [80]. There was a poor correlation between the initial impression and that after CTE, suggesting that the CTE findings were resulting in a change in patient management. For example, in about half of patients with a clinical suspicion of structuring, CTE revealed no strictures. The findings on CTE changed the clinician's impression of the benefit from corticosteroids in 61% [80]. In a prospective study of 273 patients with known or suspected Crohn's disease evaluated at the Mayo Clinic, the findings on CTE resulted in a change in management in about 50% of patients [81]. Furthermore, CTE resulted in a significant change in the clinician's level of confidence for active Crohn's disease in 50% of the study group and for complications of Crohn's (stricture, fistula, abscess) in 34–47% [81]. Serial CTE to monitor disease activity in Crohn's disease has been shown in a proof-of-concept study to be feasible and can identify a subset of patients who are clinically unchanged but radiographically worsening [82].

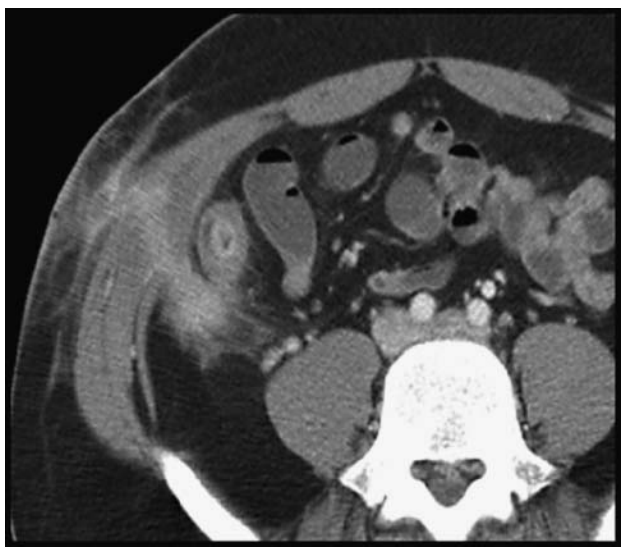

Figure 19.4 Enterocutaneous fistula arising from small bowel in a patient with Crohn's disease.

Another issue that comes to light when discussing widespread CTE implementation in the diagnosis and assessment of IBD patients is that of exposure to ionizing radiation. This concern has been prominently featured in major medical journals in the recent past [83,84]. We measured the mean cumulative dose of diagnostic ionizing radiation in a population-based inception cohort of 215 IBD patients from Olmsted County, Minnesota (diagnosed

between 1990 and 2001) [85]. A total of 115 Crohn's disease patients received a median cumulative dose of 26.6 millisievert (mSv) over a mean follow-up of 10.9 years, whereas 100 UC patients received a median cumulative dose of 10.5 mSv. Although the annualized median dose for Crohn's disease patients (3.1 mSv per year) was not much greater than the average annual background radiation exposure in the United States, the cumulative dose among patients in the upper quartile of exposure was significantly higher (48–279 mSv) [85]. Much of the difference in radiation dose between patients with Crohn's disease and UC could be explained by the higher rate of CT use among Crohn's disease patients. Other groups have documented the increased effective dose of radiation among Crohn's disease patients with the advent of CT-based imaging [86]. On the other hand, prolonged fluoroscopy with multiple films during SBFT may result in effective doses of radiation equivalent to that of CTE [87]. It is hoped that this issue will become less clinically relevant with the refinement of CT dose reduction techniques, particularly in patients requiring serial imaging. Our radiologists have been able to reduce the effective radiation dose by 30% and this may decrease further over time [88].

How does CTE compare with other diagnostic modalities? Both CTE and capsule endoscopy (CE) can demonstrate non-obstructive small bowel Crohn's disease when ileocolonoscopy and SBFT are negative [89]. CE has also been shown to be a sensitive tool to detect mucosal abnormalities in the small bowel. One meta-analysis of 11 studies compared the "diagnostic yield" (prevalence of abnormal findings) between CE and other modalities in patients with suspected or known Crohn's disease [90]. The diagnostic yields for CE and CTE were 69 and 30%, respectively ($p = 0.001$). When this analysis was restricted to those with suspected Crohn's disease only, the difference in diagnostic yield (40%) was no longer statistically significant [90]. However, the difference in yield among patients with known Crohn's disease remained significant. Diagnostic yield, however, is an imperfect marker of sensitivity at best, since it does not take into account specificity. Indeed, most studies examining the diagnostic utility of CE are flawed by the lack of a reference standard.

We performed a prospective four-way blinded comparison of ileocolonoscopy, SBFT, CTE and CT in 41 patients with known or suspected Crohn's disease using a clinical consensus gold standard [91]. Although both CE and CTE had high sensitivities (82–83%) for active small bowel inflammation, CTE was significantly more specific (89 vs 53% for CE, $p < 0.05$) (Figure 19.5). Furthermore, 17% of study patients had an asymptomatic partial small bowel obstruction on CTE, precluding ingestion of the endoscopy capsule. Using this algorithm, our capsule retention rate was 0%. We concluded that the lower specificity and the need for preceding small bowel imaging due to the high frequency of partial obstruction limited the utility of CE as a first-line test for Crohn's disease [91]. While at least one other study has suggested that CE was superior to CT enteroclysis, it is important to note that over one-quarter of patients approached for this study were excluded from undergoing CE because of the finding of small bowel strictures at CT enteroclysis [92]. Another important limitation to CE for visualizing the distal small bowel is its finite battery life. In up to 25% of patients, the small bowel is incompletely visualized because the examination ends while the capsule is still within the small bowel. In our practice, CE has been reserved for patients in whom the clinical suspicion for Crohn's disease remains high despite a negative evaluation with ileocolonoscopy and CTE.

MR enterography and enteroclysis

Magnetic resonance enterography (MRE) and MR enteroclysis may have intrinsic advantages over both CTE and CE, including avoidance of ionizing radiation exposure

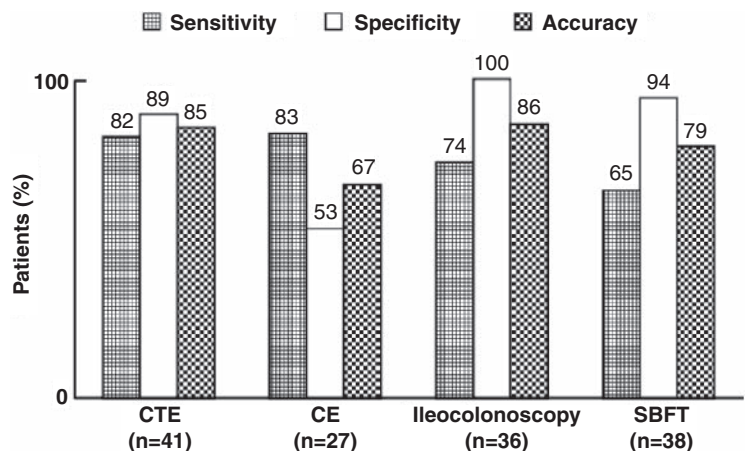

Figure 19.5 Sensitivity, specificity and diagnostic accuracy of CT enterography, capsule endoscopy, ileocolonoscopy and small bowel follow-through in a blinded four-way comparison study for detecting active small bowel Crohn's disease.

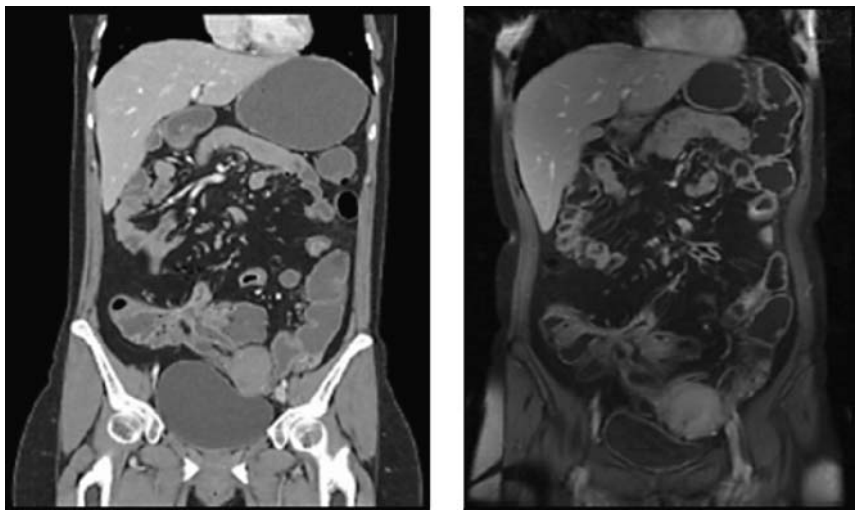

Figure 19.6 Comparison of spatial resolution of CT enterography (left) and MR enterography (right) in a patient with Crohn's disease and a complex internal fistula between the ileum, cecum and sigmoid colon.

and safety in pregnancy, while detecting luminal, extraluminal and perianal disease. Advances in the spatial resolution of MR imaging have largely overcome difficulties related to motion artifact (Figure 19.6).

MRE utilizes many of the same principles as CTE, in that a large volume of neutral enteric contrast agent is ingested to distend loops of small bowel adequately, and this is followed by intravenous administration of contrast agent, in this case gadolinium, to detect hyperenhancement in inflamed tissue [93–95]. Like CT, the enteric contrast can either be ingested by mouth (MRE) or administered via a nasojejunal tube (MR enteroclysis). Studies that compared both techniques suggested that both MRE and MR enteroclysis have high diagnostic accuracy compared with conventional barium-based imaging, although bowel distension and visualization of mucosal abnormalities may be superior with the MR enteroclysis technique [96,97]. However, patients prefer the oral ingestion of contrast agent with enterography over the discomfort of nasojejunal intubation with enteroclysis [98]. A study of MR enteroclysis with nasojejunal administration of 800 ml of iron oxide contrast medium found it to be safe and the accuracy of detecting active Crohn's disease improved significantly compared with images obtained prior to contrast ingestion [99]. In one study, T2-weighted images with oral contrast agent alone were equivalent in accuracy to T1-weighted gadolinium-enhanced images in patients with Crohn's disease [100].

MR imaging (MRI) findings include mural thickening, mural hyperenhancement and the comb sign (Figures 19.7 and 19.8) and have been shown to correlate with the Crohn's Disease Activity Index (CDAI) [101], the modified International Organization for the Study of Inflammatory Bowel Disease (IOIBD) index [101] and the Crohn's Disease Endoscopic Index of Severity (CDEIS) [102,103]. Internal fistulae can be detected by MRI and are often indicated by the "star sign" [104,105].

Bernstein *et al.* compared MRE and SBFT in 30 adult patients with recurrent Crohn's disease and found that MRE provided additional information in 27% ($n = 8$) [106]. MR-based techniques have been compared with CT-based techniques in several studies [107]. Limited data are available to compare the performances of MRE and CTE, with studies under way to address this key issue. Most findings have good or excellent interobserver agreement [108]. Inter-observer agreement for bowel wall thickening, mural hyperenhancement, may be higher for CT-based techniques [107]. When compared with the Van Hees

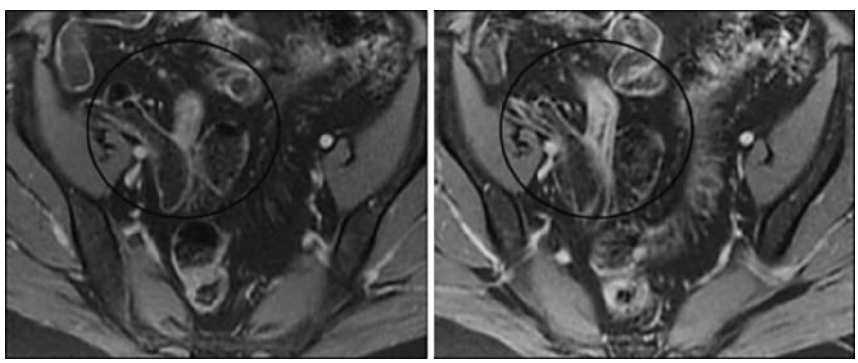

Figure 19.7 Mural thickening and contrast hyperenhancement of the neo-terminal ileum in a Crohn's disease patient with symptoms compatible with a flare who had a normal ileocolonoscopy and small-bowel follow-through. Courtesy of Jeff L. Fidler MD.

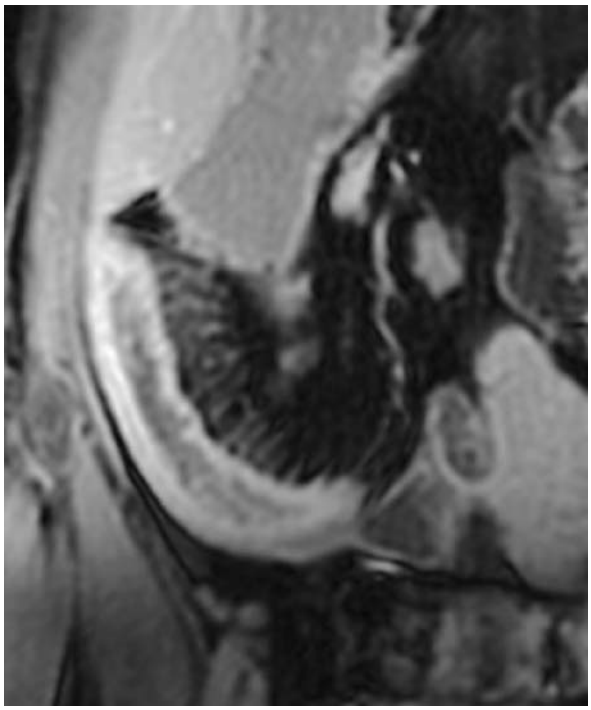

Figure 19.8 Comb sign of terminal ileum in a patient with Crohn's disease. Courtesy of Jeff L. Fidler MD.

activity index of Crohn's disease, the findings of bowel wall thickness and the enhancement ratio of bowel wall after intravenous gadolinium are weak to moderate indicators of severity [109].

Two studies comparing MR enteroclysis and conventional barium enteroclysis showed a strong correlation between the two modalities for evidence of inflammation, but MR provided additional extraluminal information [110,111].

The data from studies comparing MRE with CE are somewhat conflicting. A German study of 36 patients with suspected small bowel disease underwent both MR enteroclysis and CE and these modalities provided complementary information, in that CE detected more inflammatory lesions, especially in the proximal and mid-small bowel, while MR provided extraluminal information [112]. A German study comparing MR enteroclysis with CE in 19 patients with biopsy-proven Crohn's disease found that these two modalities were in agreement concerning the presence or absence of Crohn's disease for 85% of the bowel segments visualized; however, there was more variability between these modalities when the assessments of disease severity were compared [113]. It was concluded that the two modalities were complementary, in that CE seemed superior in the detection of superficial mucosal disease, whereas MR enteroclysis was better at assessing transmural and extraenteric involvement.

There are few data available to compare MR-based techniques with double-balloon enteroscopy, but in at least one preliminary study they were felt to be complementary [114], similar to the relationship between CE and cross-sectional imaging.

We currently consider utilizing MRE in young patients with established Crohn's disease who require frequent imaging or in patients with contraindications to CTE. If operating characteristics (sensitivity and specificity) are found to be favorable compared with CTE, access and cost issues will need to be examined. One must also be cognizant of the small but real risk of nephrogenic systemic fibrosis (NSF), a rare but potential serious and even fatal condition characterized by fibrosis of the skin and other organs, thought to be related to deposition of gadolinium derivatives [115–117]. Risk factors for NSF include renal failure and administration of gadolinium contrast agents.

A 2008 meta-analysis of 33 studies meeting methodologic quality criteria sought to determine which radiographic test has the best overall accuracy in the diagnosis of IBD [118]. All tests had high sensitivity (90% for US, 93% for MR, 88% for leukocyte scintigraphy, 84% for CT), but the specificity varied more (96% for US, 93% for MR, 85% for scintigraphy and 95% for CT). It was concluded that there were no significant differences in diagnostic accuracy and that for patients who would require frequent repeat imaging, modalities that did not involve the use of ionizing radiation were preferable [118].

Other radiographic measures of disease activity

Tagged white cell scanning has been shown in a number of pediatric studies to have reasonably good sensitivity and specificity for detecting gut inflammation in children with known or suspected IBD [119]. Technetium-99m-HMPAO (hexamethylpropylenamine oxime)-labeled leukocytes identified subclinical intestinal inflammation in approximately half of 27 HLA-B27-positive children and adolescents with spondyloarthropathy and no gastrointestinal symptoms [120]. A study of 28 children with known IBD underwent leukocyte-labeled scintigraphy and the sensitivity of 75% and specificity of 92% compared well with those of US and contrast radiography [121].

The exact role of positron emission tomography (PET) in the diagnosis and assessment of IBD patients has not been fully elucidated. One preliminary study suggested that fluorine-18-labeled fluorodeoxyglucose (FDG) PET could be useful in diagnosing subtle forms of colitis when other tests such as MR and US were inconclusive [122]. A retrospective analysis of 23 pediatric patients with suspected IBD who underwent PET scanning suggested excellent sensitivity but only moderate specificity [123]. Operating characteristics in the small bowel were improved compared with other sites. Like tagged leukocyte scanning, PET may be most attractive for use in pediatric patients

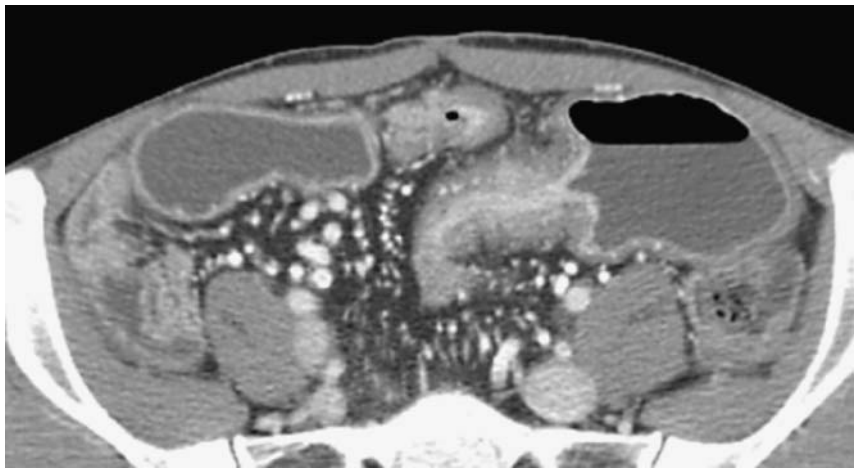

Figure 19.9 Tight intestinal stricture with significant proximal bowel dilation in a patient with Crohn's disease. Although an inflammatory component is present, the narrow diameter of the stricture and the degree of proximal dilation are suggestive of a significant fibrostenotic component that will not resolve with medical therapy alone.

due to its non-invasive nature. The combination of FDG-PET with CT has been studied in patients with Crohn's disease by several groups and this combination has excellent sensitivity in detecting moderate to severe mucosal activity with a high correlation with endoscopic findings [124,125]. One group combined PET–CT with enteroclysis, in that the PET–CT was repeated after nasojejunal administration of methylcellulose and showed promising preliminary results [126]. The high costs of PET scanning, however, may be prohibitive.

What information does the gastroenterologist seek from enterography?

The information obtained by radiologic assessments is utilized for two general purposes. The first is to aid in establishing a solid diagnosis of Crohn's disease. In this era of movement towards earlier and more intensive medical therapy, the gastroenterologist wishes to diagnose Crohn's disease accurately and seeks to avoid exposing patients without the condition to the potential morbidity associated with intensive treatment, including opportunistic infections and possibly malignancy. The second role of radiologic testing is to assess disease extent, activity and severity and to exclude penetrating disease. Extent (widespread small bowel, distal terminal ileum, right colon or pan-colonic) is an important feature, as it may influence both medication selection (e.g. oral delayed-release budesonide for isolated mild–moderate terminal ileum disease) and the type of surgery offered. The presence of peri-enteric inflammation, fistula(e) and partial small bowel obstruction with proximal bowel dilation (fibrostenotic versus inflammatory) (Figure 19.9) are significant findings, as they may alter management decisions. This includes determining the safety of initiating aggressive immunosuppression, the need for antibiotic therapy and a lower threshold for surgical intervention in patients with stricturing disease not responding to medical management. In patients with obstructive symptoms, the number of diseased segments and the length of each can be important for surgical planning (e.g. resection versus stricturoplasties). The wealth of information provided by radiologic imaging highlights the need for developing standardized reporting systems and a radiologic activity index.

Radiologic imaging such as CTE is generally used in patients with established small bowel disease when they experience a change in their clinical status such as new abdominal pain, unexplained weight loss or obstructive symptoms. Additional imaging applications may include assessment for reoccurrence after resection of a diseased segment of intestine. The interval between imaging is individualized, taking into account patient response to therapy, disease duration and extent of disease.

Conclusion

The diagnosis of Crohn's disease remains a vexing clinical challenge. It often requires a constellation of findings from multiple sources, including a key contribution from radiologic imaging. It is only after carefully interpreting this information that a diagnosis of Crohn's disease can be firmly established. There is no doubt that the advent of advanced diagnostic techniques such as US, CT enterography and MR enterography have advanced our ability to diagnose and manage inflammatory bowel disease and its complications.

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Chapter 20
New Diagnostic Approaches: Integrating Serologics, Endoscopy and Radiology and Genomics

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Summary
- Understand the role of biomarkers as non-invasive diagnostic tests.
- Identify biomarkers that aid in predictors of disease course.
- Expand on role of immune markers in IBD diagnosis and management.
- Explore non-invasive ways of predicting therapeutic responsiveness.
- Examine the possibilities of identifying markers of disease susceptibility.

Introduction

For certain diseases that can only be diagnosed clinically, physicians rely heavily on the presence of disease markers to support or even at times modify their clinical impression. Typically these markers play an important role in helping to establish a diagnosis and to evaluate the activity of a chronic disease over time. The diagnosis of inflammatory bowel disease (IBD), however, is not based solely on clinical grounds. Invasive endoscopic and radiologic and also histopathologic criteria need to be met in order to make a correct diagnosis and differentiate disease subtypes. The search for novel diagnostic approaches that accurately distinguish a group of patients with IBD from those unaffected by the disease has become an important focus in IBD research. Moreover, this search has taken a very exciting turn in the direction of finding biologic and genetic markers that can assess the natural history and perhaps predict the course of individual's disease including response to treatments over time.

This chapter highlights the recent advances in the area of diagnostic testing, focusing on serologic immune markers and genomics, and discusses the utility and feasibility of these novel diagnostic approaches.

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Biological markers of inflammation

Serologic immune markers

The search for the underlying trigger of the abnormal intestinal inflammatory reaction characteristic of IBD has led to the discovery of antibodies present specifically in the blood of patients with Crohn's disease and/or ulcerative colitis. Immune responses to resident intestinal flora in humans have been reported. Duchmann *et al.* demonstrated that patients with Crohn's disease (CD) boast reactivity to hundreds of bacterial antigens created from sonification of multiple bacterial specifies including enterobacteria, bacteroides and bifidobacterium [1].

Perinuclear anti-neutrophil antibody (pANCA) is noted for its association with ulcerative colitis (UC) or a UC-like phenotype. This IBD-specific ANCA displays a unique perinuclear highlighting (pANCA) on immunofluorescence staining and is DNAse sensitive [2]. Although it remains undefined, it has been suggested that the antigen to which pANCA is directed is a nuclear histone (H1) [3]. This antigen is clearly distinct from the proteinase 3 or the myeloperoxidase reactivity observed in those pANCA and cANCA patients with vasculitic disorders. pANCA is likely an autoantibody that is representative of a cross-reactivity with a luminal bacterial antigen [4–6]. pANCA has been shown repeatedly to be prevalent in the sera of approximately 60% and 20% of UC and CD patients, respectively [7–13]. ASCA (anti-*Saccharomyces cerevisiae* antibody) is another important antibody marker that is present in the blood of individuals with IBD.

Studies in both the adult and pediatric IBD population have demonstrated that ASCA is found in the blood of approximately 60% of CD, 10% of UC and <5% of non-IBD patients [7–9]. ASCA was the first CD-specific immune response thought to be targeted towards microbial antigens. IgA and IgG antibodies are directed against a specific oligomannosidic epitope present on the cell wall of the yeast *Saccharomyces* that shares homology with an intestinal bacteria [14]. To date it remains unclear as to the specific bacterial drive behind ASCA production. One interesting study published in *World Journal of Gastroenterology* looked at whether or not ASCA in the serum was correlated with *S. cerevisiae* present in the mucosa [15] The investigators found that the presence of *S. cerevisiae* in the colonic mucosal biopsy was very rare. This attempted to examine whether there was some cross-reactivity with yeast in the mucosa, but there did not appear to be an association. ASCA is part of the family of anti-glycan (carbohydrate) antibodies. Other anti-glycan antibodies, antibodies against laminaribioside (ALCA) and chitobioside (ACCA) have been studied in IBD [16]

In addition to the anti-glycan antibodies, three additional markers representative of microbial-driven immune responses have been identified; antibodies to the *Escherichia coli* outer-membrane porin C (OmpC), the *Pseudomonas fluorescens* CD related protein [anti-CD related bacterial sequence (I2)] and the CBir1 flagellin. Antibodies to OmpC, whose antigen is purified from commensal *E. coli* [6,17], have been reported in 37–55% of patients with CD and 2–11% of patients with UC, whereas no more than 5% of non-IBD individuals express anti-OmpC [18–20]. I2 was isolated from affected colonic mucosa in CD patients yet not in the unaffected segments [17]. Immune responses to this antigen are present in up to 55% of CD patients; it has also been detected in the serum of UC patients (10%) and in up to 20% of non-IBD patients, rendering this marker less specific for CD ([17], data on file Prometheus Laboratories). Serologic expression cloning was used to identify an immunodominant antigen, CBir1 flagellin, to which strong immune responses (B cell and CD4T cell) occurred in colitic mice [21]. Subsequent human studies reported 50% prevalence of seroreactivity to CBir1 in CD patients whereas UC, inflammatory and healthy controls exhibited little to no reactivity to this flagellin [22]. As seen with the genetic and clinical heterogeneity of CD, studies have shown immune response (immune phenotype) heterogeneity exists among CD patients. Landers *et al.* analyzed immune response heterogeneity in 330 patients and found that ASCA was detected in 56% of patients; 55% were seroreactive to OmpC C, 50% were seroreactive to I2 and 23% were pANCA [17]. About 85% responded to at least one antigen; only 4% responded to all four. Among microbial antigens (ASCA, OmpC, I2), 78% responded to at least one and 57% were double positive, but only 26% responded to all three. The level of response was stable over time and with change in disease activity. Among patients with the same qualitative antigen-response profiles, quantitative response differed. Moreover, this study demonstrated that CD patients could be clustered into four distinct groups depending on their immune response patterns to microbial or autoantigens. One cluster was ASCA, a second was antibodies to OmpC and I2, the third was pANCA and the fourth was low or no immune response to any tested antigens. Subsequent analyses incorporating CBir1demonstrated that antibodies to CBir1 are present in approximately 40% of CD patients negative for antibodies to specific microbial antigens (ASCA, OmpC and I2), which suggests a unique immune phenotype [21]. Immune reactivity to CBir1 may further define CD phenotypes in that anti-CBir1 expression is present in 40–44% of pANCA-positive CD patients versus only 4% in pANCA-positive UC patients. This difference may denote a unique etiopathogenic mechanism of disease that helps to stratify patients further based on immunogenetic phenotypes.

Given the CD- and UC-specific characteristics of ASCA and pANCA, these markers were initially introduced as markers used to differentiate CD from UC in indeterminate cases. However, as the number of identified antibody markers increased and also improved test sensitivity, consideration has been given to these tests are adjunctive diagnostic tools and as possible prognostic indicators given their association with disease phenotype.

Differentiating IBD from non-IBD

The recognition of IBD and subsequent diagnostic evaluation, in most cases, can be straightforward when the clinical presentation is unambiguous. However, a diagnostic challenge arises in patients who present with overlapping, non-specific and indolent symptoms that are characteristic of both organic and non-organic disorders. In the face of diagnostic uncertainly, clinicians are often obligated to exclude IBD using invasive diagnostic testing, in particular contrast radiography and colonoscopy with biopsies. Suspicion of IBD commonly results in extensive diagnostic investigations of patients who are ultimately found to have a functional bowel disorder. In contrast, the diagnosis of IBD, particularly CD, can be missed or delayed due to the non-specific nature of both the intestinal and extra-intestinal symptoms at presentation. Given these clinical challenges, the search has intensified for an accurate non-invasive diagnostic marker to aid clinicians in the prompt recognition of IBD and the differentiation of these disorders from mimickers.

The ideal non-invasive diagnostic test is both highly sensitive and specific. Moreover, it should be as good as the gold standard. To date, no such test has been developed, but advances in testing strategies and the addition of novel markers have helped the characteristics of available tests. Numerous studies have examined the diagnostic value of these markers, ASCA and pANCA in particular, in IBD and non-IBD patients. Peeters *et al.* found that positivity for both markers was significantly

lower in healthy and non-IBD controls [23]. The sensitivity, specificity, positive predicted value (PPV) and negative predicted value (NPV) for differentiating IBD from controls were as follows, respectively: ASCA+, 60% (243/407), 91% (345/378), 88% (243/276) and 68% (345/509); pANCA+, 50% (73/147), 95% (605/638), 69% (73/106) and 89% (605/679); ASCA+/pANCA–, 56% (229/407), 94% (355/378), 91% (229/252) and 67% (355/533); and pANCA+/ASCA–, 44% (65/147), 97% (620/638), 78% (65/83) and 88% (620/702). This study concluded that the specificity of serological markers for IBD is high, but with low sensitivity, making them less useful as diagnostic tests. The combination of these tests, however, is probably more powerful as a tool to differentiate IBD from non IBD. A similar study was performed in the pediatric age group [24]. Serum was collected from 120 children with new or established diagnoses of UC ($n = 25$) or CD ($n = 20$ and non-IBD patients ($n = 74$). This group also confirmed that the highest sensitivity for detecting inflammatory bowel disease, 71%, was achieved by using ANCA and ASCA together. A prospective study was then performed in children with non-alarm type-symptoms undergoing a complete diagnostic evaluation to rule in or out IBD [small bowel follow-through and esophagogastroduodenoscopy (EGD) and colonoscopy] at the same time as they underwent serologic testing [25]. Diagnosis of IBD versus non-IBD was made based on gold standard and blinded to serological analyses. The test characteristics of these markers were then examined using a sequential diagnostic testing strategy. In this study, the modified serodiagnostic assay was more sensitive (81 vs 69%), whereas the traditional assay had a higher specificity (96 vs 72%) for IBD ($p < 0.05$). The results of this study suggested that by sequencing from a sensitive test to a more specific test the false-positive diagnoses would have been reduced by 81%, yielding an overall sequential testing strategy accuracy of 84%. A decision analysis followed which reported that the sequential serodiagnostic strategies resulted in the largest cost savings ($550 per average patient) with an average cost per correct diagnosis of $1640 compared with $2188 for standard invasive testing. Cost savings were attributable to a 39% reduction in the use of invasive tests [26]. This sequential testing strategy is no longer commercially available and results from a similar study design using more updated diagnostic algorithms are needed to evaluate truly the accuracy of non-invasive sero-diagnostic markers in children with symptoms suggestive of, but not diagnostic of, IBD. Subsequent pediatric studies confirmed the specificity of these markers for IBD but continued to question the sensitivity of these tests as a screening tool to differentiate IBD from non-IBD [27].

The addition of CD specific CBir1 and OmpC to serodiagnostic panels has the potential to improve the sensitivity of these markers for CD versus UC and versus IBD. The same can be said perhaps for the anti-glycan antibodies. Dotan *et al.* found that in addition to ASCA, antibodies to laminaribioside (ALCA) and chitobioside (ACCA) had the highest discriminative capability between CD and ulcerative colitis ($p < 0.001$ and $p < 0.05$, respectively) [16]. Importantly, 44% (12/27) of ASCA-negative CD patients were positive for ALCA or ACCA. In patients with IBD positive for antibodies against either ALCA, ACCA or ASCA, the diagnosis of CD was suggested with a sensitivity of 77.4% and specificity of 90.6%. Having at least two of these antibodies increased the specificity to 99.1%. In CD, higher levels of antibodies against ALCA or ASCA were significantly associated with small intestinal disease ($p = 0.03$ and $p < 0.0001$, respectively).

Although conflicting, studies do support the use of these markers, particularly in children, to guide clinicians in cases of diagnostic uncertainty [9,23,25].The addition of new markers and the use of algorithmic testing based on pattern recognition rather than receiver operating characteristic (ROC) determined cut-offs should lead to increased accuracy of these tests in both children and adults. Further studies are needed in prospective cohorts where these tests are compared where gold standard diagnostic criteria in cases of diagnostic uncertainty.

Differentiating CD from UC

Although UC and CD share many epidemiologic, immunologic, therapeutic and clinical features, they are currently considered to be two distinct subtypes of IBD. Clinical, endoscopic, histopathologic and radiographic criteria have been put forth to help clinicians differentiate between these two diseases. However, despite published criteria, this discrimination may still prove to be difficult in patients with disease limited to the large bowel. This entity, referred to as indeterminate colitis (IC), occurs in approximately 10–15% of IBD patients. Classically, this term had applied to those patients whose diagnosis remained unknown even after careful examination of resected surgical specimens. However, the modern definition of IC refers to all patients pre- or post-colectomy whose categorization remains undefined. There still remains, however, a lot of inconsistency in the literature when defining IC since it is generally based on imprecise clinical definitions and very small retrospective studies. It must be emphasized that both surgical options and perhaps medical treatment rely on a correct diagnosis.

There is always a hesitation when offering pouch surgery to IC patients because of concerns regarding pouch failure, refractory pouchitis and a postoperative diagnosis of CD. Yu *et al.* compared the 10 year outcome of IC and chronic UC patients undergoing ileal pouch anal anastomosis (IPAA) [28]. Those patients going into surgery with a diagnosis of IC had significantly more episodes of pelvic sepsis (17% indeterminate colitis vs 7% chronic ulcerative colitis; $p < 0.001$), pouch fistula (31 vs 9%; $p < 0.001$) and pouch failure (27 vs 11%; $p < 0.001$). Moreover, 15% of patients with IC, but only 2% of patients

with chronic UC, had their original diagnosis changed to CD ($p < 0.001$).

Given the CD specificity of ASCA and the UC specificity of pANCA, these antibodies have been widely studied and have become with the addition of novel markers more widely accepted as useful discriminatory markers that help clinicians differentiate UC from CD. However, the discriminatory strength of these markers is amplified when they are evaluated in combination. A pANCA+/ASCA– serological profile was shown to be 19 times more likely to be present in the serum of a patient with UC than CD. Conversely, pANCA–/ASCA+ is 16 times more likely in CD than UC [29]. Quinton *et al.* obtained serum samples from 100 patients with CD, 101 patients with UC, 27 patients with other miscellaneous diarrheal illnesses and 163 healthy controls [30]. The combination of a positive pANCA test and a negative ASCA test yielded a sensitivity, specificity and positive predictive value of 57%, 97% and 92.5%, respectively, for UC. The combination of a positive ASCA test and a negative pANCA test yielded a sensitivity, specificity and positive predictive value of 49%, 97% and 96%, respectively, for CD. It should be noted that in patients with pure colonic CD, the prevalence of ASCA positivity is relatively low. Ruemmele *et al.* also studied ASCA and pANCA in cases of colitis among children with IBD [7]. IgA and IgG ASCA titers were significantly greater and highly specific for CD (95% for either, 100% if both positive). pANCA was 92% specific for UC and absent in all non-IBD controls. The majority of patients with CD positive for pANCA had a UC-like presentation. A meta-analysis was performed to examine the test characteristics of ASCA and pANCA [31]. Sensitivity, specificity and likelihood ratios (LR+, LR–) were calculated for different test combinations for CD, UC and IBD compared with controls. A total of 60 studies comprising 3841 UC and 4019 CD patients were included. The ASCA+ with pANCA– test offered the best sensitivity for CD (54.6%) with 92.8% specificity and an area under the ROC curve (AUC) of 0.85 (LR+ = 6.5, LR– = 0.5]. The sensitivity and specificity of pANCA+ tests for UC were 55.3 and 88.5%, respectively (AUC of 0.82; LR+ = 4.5, LR– = 0.5). The sensitivity and specificity were improved to 70.3 and 93.4% in a pediatric subgroup when combined with an ASCA negative test. Meta-regression analysis showed decreased diagnostic precision of ASCA for isolated colonic CD [relative diagnostic odds ratio (RDOR) = 0.3]. This study concluded that ASCA and pANCA testing are specific but not sensitive for CD and UC. It may be particularly useful for differentiating between CD and UC in the pediatric population. The first prospective study was conducted in IC patients and reported by Joosens *et al.* in 2001 [32]. They enrolled 97 predefined IC patients and followed them prospectively over time blinded to their ASCA and pANCA status. Over 6 years, 17 of 97 patients were diagnosed with CD, 66 remained indeterminate and 14 declared as UC. Thus a definitive diagnosis was reached for 31 of 97 patients (32%). Their initial serum antibody characterization demonstrated that 48% of the population were ASCA–/pANCA-, 27% were ASCA+/pANCA–, 21% were ASCA–/pANCA+ and 4% were ASCA+/pANCA+. ASCA+/pANCA– correlated with CD in 8 of 10 (80%) patients, whereas ASCA–/pANCA+ correlated with UC in 7 of 11 (63.6%) patients. The remaining four cases became CD, behaving clinically as UC-like CD. Thus, 100% of UC or UC-like CD patients were pANCA positive. At the time of last follow-up, almost half of the patients [47 of 97 (48.5%)] were negative for ASCA and pANCA. Only seven seronegative cases (14.9%) became CD or UC compared with 48% (24 of 50) of seropositive patients ($p < 0.001$). The conclusions from this study are that IC may represent a distinct form of IBD based on the lack of IBD-associated antibodies.

Four years later, the same group investigated whether anti-OmpC and anti-I2 were additive to ASCA and pANCA in their IC cohort and whether patients who remained unclassified over time also lacked response to these microbial antigens in addition to ASCA and pANCA [33]. The results of this study indicated that by adding anti-OmpC and anti-I2, the predictive capacity of serological tests increases only marginally and the specificity drops significantly. Despite another 1.5 years of follow-up, there still remained a large group of IC patients who remained negative for serological markers and may represent a separate phenotype. The entity of a UC-like Crohn's phenotype was first introduced by Vasiliauskas *et al.* in 1996 [34]. pANCA-positive patients with CD were reported to have endoscopically and/or histopathologically documented left-sided colitis and symptoms of left-sided colonic inflammation, clinically reflected by rectal bleeding and mucus discharge, urgency and treatment with topical agents; 100% of patients with CD expressing pANCA had "UC-like" features. The presence of pANCA in up to 25% of CD patients, however, limits its ability to distinguish UC form CD on its own. Novel antibodies such as anti-CBir1 may help to dissect the pANCA-positive IBD group. Targan *et al.* found CBir1 reactivity in 44% of pANCA-positive CD patients compared with only 4% of pANCA-positive UC patients [21]. This suggests that pANCA-positive/anti-CBir1-positive colonic CD patients may represent a unique UC-like phenotype. It is unclear as to whether the natural history of UC-like CD is different from chronic UC, especially when it comes to therapeutic responses and postoperative outcomes.

Phenotypic stratification

If indeed these immune responses represent the sum of a genetic and environmental predisposition to IBD, quantitative and qualitative expression of these immune responses may serve as an immunologic risk marker for IBD phenotypes. Vasiliauskas *et al.* introduced the notion of immune response stratification when they first reported that high ASCA levels were found to be associated with

fibrostenosing (FS) and internal-penetrating (IP) disease as well as the need for small bowel surgery [35]. Similar associations were then reported between NOD2/CARD15 and small bowel fibrostenosing CD [36–39]. These studies, however, did not take into account the immune responses as a confounding variable to all reported associations. Another cross-sectional study demonstrated that patients who were ASCA IgA or IgG positive were 8.5 and 5.5 times more likely to undergo early surgery (within 3 years of diagnosis) than ASCA IgA or IgG negative patients [40]. Mow *et al.* examined the association of multiple immune responses and disease phenotype [41]. Reactivity to OmpC was independently associated with IP disease, whereas reactivity to anti-I2 was independently associated with FS disease and the need for surgery. Both the presence and magnitude of the immune response was associated with more aggressive disease behaviors. A similar study in a Scottish CD cohort reported that the cumulative reactivity to ASCA, I2 and OmpC was associated with small bowel complications [42]. Antibodies to CBir1 were examined in a later study and were found to be independently associated with small bowel disease, IP and FS disease [21]. Xue *et al.* demonstrated that reactivity to ASCA, OmpC and CBir1 was associated with early disease onset, FS and IP disease and the need for surgery [43]. Cross-sectional studies confirmed that there is a significant association between the presence of microbial-driven immune responses and more aggressive disease phenotypes. More recent pediatric cohort studies suggest that these markers are present in patients before a complication occurs and thus predictive of disease progression from uncomplicated to complicated state. This could address the suggestion that a complication leads to an alteration in mucosal permeability and hence sero-reactivity to microbial antigens. Desir *et al.* demonstrated that baseline ASCA reactivity was associated with a more relapsing course in a pediatric CD cohort [IgA: odds ratio (OR) 2.9; 95% confidence interval (CI) 1.33–6.35] [44]. Serial antibody measurements did not predict the occurrence of clinical outcomes and there was limited variability in the antibodies over time. A multicenter study examined the association of ASCA, anti-I2, anti-OmpC and anti-CBir1 reactivity with disease course in 196 pediatric CD patients [45].The qualitative and quantitative reactivity to I2 and OmpC were each independently associated with the development of IP and FS disease behavior and the frequency of development of disease complication increased in parallel with reactivity to increasing numbers of antigens. The OR for the development of IP/FS disease was 5.3 and 11.0 for children with reactivity to three and four antigens, respectively. Furthermore, survival analysis demonstrated that reactivity to at least one microbial antigen was associated with the development of IP/FS disease faster than in patients negative for all markers, suggesting that these markers may predict more aggressive disease behaviors. This cohort has now been expanded to include close to 800 pediatric patients. The data confirm

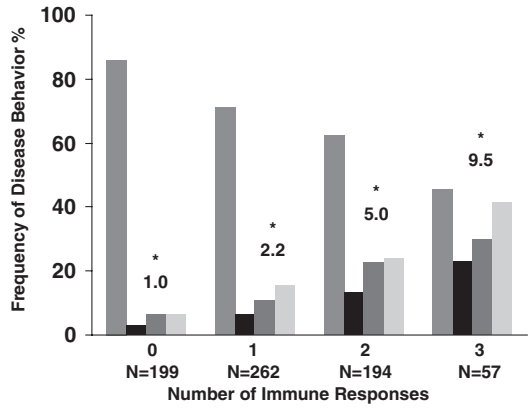

Figure 20.1 The frequency of disease behavior [non-penetrating, non-stricturing disease (NPNS), internal penetrating (IP), stricturing (S) and surgery] based on number of immune responses. The test for trend demonstrated a positive linear trend in the frequency of patients with IP disease as the number of positive immune responses towards OmpC, ASCA and CBir1 increased ($p < 0.0001$). The odds ratios (OR) reflect the odds of having internal penetrating when positive for any one, combination of two or all three immune responses, as compared with those patients negative for all immune responses (baseline group).

that an increasing number of immune responses and also the magnitude of the immune response are predictive of more rapid disease progression [46] (Figure 20.1).

Amre *et al.* also studied a cohort of pediatric CD patients who had sera drawn at diagnosis and were examined for the subsequent development of complications of their disease [47]. Survival analysis revealed that the time to first complication was more rapid for ASCA-positive patients than those who were ASCA negative. Moreover, the relative risk (RR) of a recurrent complication (RR = 3.68) and the need for an additional surgery (RR = 1.95) were significantly higher in ASCA-positive patients.

Data now exist that link sero-reactivity to microbial antigens to underlying genetically determined innate immune defects (*NOD2*) [39,48–50]. Devlin *et al.* demonstrated that a significant proportion of the cumulative sero-reactivity to microbial antigens was determined by the presence of variants in the *NOD2* gene in CD patients [48]. Ippoliti *et al.* also demonstrated an association between NOD2/CARD15 and magnitude of immune response in patients with fibrostenosing CD [51]. Those patients homozygote for NOD2/CARD15 had higher levels of immune reactivity as compared with those heterozygote and homozygote wild type, respectively. Thus, it is hypothesized that the more defective the innate immunity is (NOD2−/NOD2− vs NOD2+/NOD2+), the more intolerant/maladaptive the adaptive immune response is as expressed by higher immune responses. This in turn translates to a more aggressive clinical phenotype [48].

As compared with the positive association between ASCA, antibodies to OmpC, I2 and CBir1 and disease complication, pANCA has been shown to be associated with

a more benign, UC-like disease course and negatively associated with small bowel complicating disease [34,35]. High pre-colectomy levels of pANCA ($>100\,\text{EU}\,\text{ml}^{-1}$) have been prospectively shown to be associated with the development of chronic pouchitis in all IBD patients undergoing IPAA [52]. More recently, the same group reported that anti-CBir1 may accelerate the development of chronic pouchitis in the face of high pANCA levels [53].

Predictor of response to therapy

If indeed these B cell responses are a surrogate marker for antigen-driven specific T cell pathways, it is conceivable that individuals with certain immune response profiles will respond better to specific therapeutic targets. To date, most of the interest has been in the immune responses and infliximab. It is clear from both the clinical trials and clinical experience that not all patients respond to infliximab therapy. Taylor *et al.* were the first to report on the negative association between pANCA and infliximab response [54]. A subsequent study could not confirm that either ASCA or pANCA could predict response to treatment. However, lower response rates were observed for patients with refractory intestinal disease carrying the pANCA+/ASCA– combination ($p = 0.67$) [55].

The use of infliximab in UC has added another level of treatment for patients in whom colectomy may have been the only alternative. That being said, however, not all patients respond to infliximab and the *a priori* knowledge of which patients would be unlikely to respond to therapy may help clinicians and patients make the appropriate decision regarding medical versus surgical therapy. A total of 100 UC patients followed at a single center were enrolled to examine predictors of early clinical response to infliximab [56]. Of these 100 patients, 60 (60%) had pancolitis, 63% were on concomitant immunosuppressive therapy, 9% were active smokers, 64% had C-reactive protein $\geq 5\,\text{mg}\,\text{dl}^{-1}$ and 44% were pANCA+/ASCA–. Only five patients in this study received infliximab because of severe acute colitis refractory to intravenous corticosteroids. Early complete and partial clinical responses were observed in 41 and 24% of patients, respectively. Patients who were pANCA+/ASCA– had a significantly lower early clinical response [55 vs 76%; OR = 0.40 (95% CI 0.16–0.99); $p = 0.049$]. Concomitant immunosuppressive therapy and the use of an infliximab induction scheme did not influence early clinical response.

Predictor of disease susceptibility

The results from a very thought-provoking study from Israel suggested that immune reactivity to microbial antigens could occur in advance of clinical presentation in CD [57]. Sera were collected from members of the Israeli Defense Force (IDF), at the time of recruitment, and 32 individuals subsequently developed CD at a mean of 38 months post-recruitment. Over 30% of patients who subsequently developed CD were ASCA positive at the time of recruitment into the IDF.

Taking this one step further, there has been interest in evaluating whether immune responses are familial traits due to genetic factors. Sutton *et al.* demonstrated that the quantitative and qualitative expression of ASCA may be familial [58]. Studies in a twin population demonstrated an agreement in ASCA titers within concordant monozygotic twin pairs with Crohn's disease and suggested that the level of increase (magnitude) is genetically determined [59]. A recent study has shown that antibodies to OmpC have a strong familial aggregation pattern [60]. Preliminary studies in a pediatric cohort suggest that the quantitative but more so the qualitative expression of immune responses to microbial antigens is increased in the parents of pediatric CD patients, suggesting that the immune dysregulation observed in such patients is a familial trait [61]. Further research in a larger number of parents will increase our understanding of the role of familial expression of serological immune response. It will be important to evaluate further the subclinical or even preclinical nature of these markers of disease.

Genetic markers

The search for susceptibility genes continues to be a major focus among IBD researchers. The multifactorial etiology of IBD likely precludes the use of these genetic markers alone as confirmatory diagnostic tools in IBD. However, the presence of these candidate genes may identify at-risk populations. Genes may regulate distinct immune processes, which in turn are manifested as specific disease behaviors in patients with IBD. As work goes ahead in identifying these, it is likely that some of this will become part of a diagnostic or perhaps more applicable a prognostic panel. Association studies have identified important susceptibility genes for IBD (NOD2, ATG, IL23R, IBD5). These major genetic variants can be divided into defects involved in innate immune response: NOD2/CARD15 and ATG16L1 and those part of the adaptive immune response such as the IL-23R gene. To date, NOD2 has been shown to have the highest attributable risk of disease as compared with other novel genes identified. However, the susceptibility risk associated with individual genes is still less than the risk attributable to a positive family history. Moreover, the presence of NOD2 in both non-IBD and UC patients makes the use of genetic testing as a screening tool or to differentiate between IBD and non-IBD or between CD and UC inappropriate at this point. The impact of multiple susceptibility genes at predicting risk, diagnosis and differentiation merits further investigation. NOD2 has consistently been shown to be associated with small bowel CD [36–39]. Its association with disease progression and prediction of natural history is an evolving story and further work is being done to examine the contribution

of genetic predictors of natural history as compared with immune responses [41,45,46,48]. The association of the newly identified susceptibility genes and disease phenotype also merits further investigation. The future of IBD genetics perhaps lies in the use of candidate genes as predictors of response to therapeutics specifically targeted to immune pathways. Most of the original work was done examining the genetic markers associated with response to anti-tumor necrosis factor (TNF) alpha therapies. Pierik *et al.* studied the functional TNFR2T587G and the TNFR1A36G mutation in 344 CD and 152 UC patients and investigated the relationship with disease phenotypes [62]. An association with response to infliximab was evaluated in 166 CD patients. The TNFR2 587G allele was more frequent in UC. Both single nucleotide polymorphisms were negatively associated with smoking in CD. A relationship between TNFR1A36G and pancolitis was found in UC. There was no clear effect of the polymorphisms on infliximab response, although the TNFR1 minor was associated with a lower response to infliximab [62]. Another study assessed the association of the IgG Fc receptor FCGR3A-158 gene polymorphism with the biological and clinical response to infliximab in CD. A subset of 344 patients from the ACCENT I study were enrolled. No association could be observed between FCGR3A-158 gene polymorphism and the clinical response or remission to infliximab. However, they observed a trend towards a greater decrease in C-reactive protein after infliximab in V/V homozygotes as compared with V/F heterozygotes and F/F homozygotes (–79.4, –76.5 and –64.3%, respectively, at week 6; $p = 0.085$; one-tailed $p = 0.043$) [63]. Despite the fact that the C-reactive protein and FCGR3A genes are located on the same 1q23 locus, no association was found between C-reactive protein gene polymorphisms and decrease in C-reactive protein serum concentration after infliximab treatment in CD [64]. Understanding the immune pathways and their genetic determinants involved in the pathogenesis of IBD is critical for the future treatment of IBD patients. Targeting these pathways and developing antagonists and agonists will lead to an era of individualized medicine in the field of IBD.

Functional genomics and IBD

Functional genomics has the potential to expand significantly our understanding of the pathogenesis of IBD, UC and CD [65]. In addition, sophisticated statistical approaches may now be combined with existing gene expression platforms to begin to define clinically important subtypes of IBD. This type of analysis could potentially be used to predict disease course, including response to therapy and risk for complicated disease behavior including stricturing and the development of cancer [65]. Ideally, biomarkers might be identified from this analysis which could then be reduced to clinical practice. Support for this has come from the successful application of this approach in other fields, such as risk prediction and therapeutic stratification for lymphoma.

The two predominant platforms for determining the global pattern of gene expression in clinical samples currently are cDNA arrays and GeneChips [65]. Although the production methods differ for these two platforms, in both cases data regarding genome-wide gene expression are derived by determining the degree of hybridization of labeled cDNA or cRNA prepared from patient samples to gene-specific cDNAs or oligonucletodes immobilized on solid-phase matrices [65]. After normalization of the individual signals, data are then analyzed, typically to define fold differences relative to a predefined reference group. A number of factors may introduce significant variation into the experimental design and affect the reliability and ultimate reproducibility and comparability of a given study [66]. For IBD studies, these may include the intestinal location from which samples were obtained, the stage and severity of disease, medication exposure and whether full-thickness surgical specimens, endoscopic biopsies or isolated cell preparations are used as the source for mRNA [66]. Methods then used to normalize and analyze the resulting data also vary widely and will significantly affect the final interpretation. These issues have recently been reviewed in detail [65,66].

Most microarray studies in the IBD field to date have focused upon identifying novel aspects of pathogenesis and distinguishing CD from UC in this regard. The first such study compared colonic gene expression between eight subjects with UC and seven controls. The UC samples were obtained from colectomy specimens and so represented advanced disease [67]. The controls included three non-inflamed samples, one with non-specific inflammation and three with inflammation due to CD. The Affymetrix GeneChip (Hum 6000) used included ~6500 human genes and expressed sequence tags (ESTs). Seventy-four genes were found to be upregulated in the majority of UC specimens; most of these had not previously been implicated in the disease pathogenesis. Of particular interest was upregulation of members of the regenerating gene family, PSP, REG and PAP. A supervised cluster analysis [self-organizing maps (SOMs)] was then performed utilizing the 1087 genes which were either up- or down-regulated more than three-fold relative to the mean of the non-inflamed controls. The clinical specimens were scored relative to activity, chronicity and Paneth cell metaplasia and the histopathologic features were compared with the gene expression patterns [67]. Twenty nodes were found to represent this relationship adequately, with the expression of genes present within three clusters, 17,18 and 19, correlating well with disease activity scores [67]. These included pro-inflammatory cytokines, chemokines, adhesion molecules and a variety of neutrophil and

lymphocyte cell-surface markers. These results indicated that SOM analysis can be used to identify novel genes involved in specific biological processes. In this case, these related to inflammatory activity, but stricturing or penetrating disease or malignant transformation could be examined in a similar manner.

Three more recent studies have expanded upon this approach and in some cases have utilized endoscopic biopsies obtained at an earlier stage of disease. Lawrance *et al.* utilized a pool of RNA obtained from colectomy specimens from six subjects with UC, six with CD and six controls [68]. While this approach likely masked heterogeneity within the groups, it was felt to be more feasible from a financial point of view. The Affymetrix HuGene FL array containing 7070 genes/ESTs was used. Because of differences in subject selection, gene expression platforms and data analysis, most IBD microarray studies to date have not exhibited overlap for the majority of identified genes. In this case, 28% (21/74) of the genes found to be upregulated in the majority of the UC patients in the Dieckgraefe study were also up regulated in the UC group in the Lawrance study [67,68]. These included the *REG* family members, *MMPs*, *defensin 5* and the neutrophil-associated gene *S100*. Only 19% (33/170) of the genes differentially expressed relative to control were represented in both the CD and UC groups. However, in no case was a gene upregulated in CD and downregulated in UC or vice versa. While this suggests significant differences in disease pathogenesis, it may also reflect the relatively advanced stage of disease at which samples were collected. Genome-wide expression results may be used to advance efforts to identify susceptibility or modifier genes. In this case, a majority of the differentially expressed genes were located on chromosomes 4 and 17, potentially pointing to novel genetic regulators in these regions. Overall, the UC profile suggested activation of adaptive immunity combined with downregulation of epithelial metabolic and ion-transport functions and upregulation of epithelial proliferative and regenerative functions [68]. By comparison, the CD profile was notable for upregulation of antimicrobial defensins [68]. Taken together, these data were consistent with current hypotheses regarding the pathogenesis of UC and CD.

Studies by Costello *et al.* [69] and Wu *et al.* [70] utilized mRNA prepared from CD and UC biopsy samples and so may provide data regarding pathogenesis at an earlier stage of disease. In the study by Wu *et al.* [70], high-density cDNA microarrays representing ~23,000 unique transcripts were used, whereas Costello *et al.* [69] utilized the Affymetrix Human Genome U95Av2 array which contains 9662 unique transcripts. Costello *et al.* identified 500 and 272 transcripts differentially regulated in CD and UC, respectively. The greater number of transcripts identified for CD relative to the Lawrance study likely reflects the earlier stage of disease at which samples were obtained. Overall, the transcripts fell into functional groups involving the immune and inflammatory response, cell proliferation and growth and epithelial structure and permeability [69]. In addition, multiple genes differentially expressed within previously identified IBD susceptibility loci may guide future efforts to pinpoint candidate genes [69]. A primary goal of this study was to identify novel genes involved in disease pathogenesis, by including previously uncharacterized ESTs. In this regard, several unknown genes with putative functions including endocytosis, regulation of apoptosis, phospholipid metabolism, cell adhesion, intracellular signaling and gene transcription were identified, which will warrant further characterization [69]. Wu *et al.* also identified multiple differentially expressed genes which may point to differences in pathogenesis between CD and UC. For CD, a role for IFNγ-inducible TH1 processes and antigen presentation was suggested, with upregulation of *IFITM1*, *IFITM3*, *STAT1*, *STAT3* and *TAP1*, *PSME2* and *PSMB8*, respectively [70]. Consistent with the Lawrance study, reduced expression of genes likely regulating epithelial biosynthesis, metabolism and transport (*HNF4G*, *KLF5*, *AQP8*, *ATP2B1* and *SLC16A*) was a prominent feature of the UC transcriptome [70]. However, in interpreting results from inflamed UC specimens, it will be important to account for the relative reduction in epithelial cell number, compared with the increase in immune cell number, within the biopsy specimen. Unsupervised cluster analysis by multidimensional scaling confirmed that the majority of the CD and UC subjects had similar expression patterns, relative to the inflamed and healthy controls. Interestingly, however, two CD samples clustered with the UC group. These two patients had distal colonic disease similar to UC and were ANCA positive, potentially suggesting common pathogenic mechanisms for UC and this form of CD. With larger numbers of samples, this approach could be used to define subgroups of IBD patients who would potentially benefit from therapies targeting the specific pro-inflammatory pathways identified.

In approximately 10% of patients, standard approaches do not allow for classification of IBD as CD or UC. This has implications for the choice of medical therapies and surgical outcomes. Burczynski *et al.* recently utilized a genome-wide expression analysis in peripheral blood mononuclear cells (PBMCs) to define a limited set of genes which may distinguish CD from UC [71]. PBMC mRNA was prepared from 59 CD patients, 26 UC patients and 42 healthy individuals. Gene expression was measured using the Affymetrix HG-U133A array, which contains ~39,000 transcripts derived from ~33,000 well-substantiated human genes. Analysis of covariance methods were used to adjust for differences in PBMC cell type composition when testing for differences in gene expression between the groups. The percentage of eosinophils, monocytes and neutrophils were included as covariates in the analysis when defining genes differentially expressed between the IBD patients and controls. This identified 220 transcripts

for CD and 120 for UC, with 45 differentially expressed in both CD and UC [71]. Sixty-seven transcripts were uniquely identified for CD and 22 for UC. Interestingly, the CD genes included enzymes involved in prostaglandin metabolism, transcription regulators and transmembrane receptors, whereas the UC genes encoded primarily immunoglobulin constant regions [71]. A supervised class prediction approach was used to identify the smallest gene set which would discriminate between CD and UC. A set of 12 genes was found to provide 94% accuracy in discriminating between CD and UC with a test set containing 15 CD profiles and 6 UC profiles. The genes which favored CD included lipocalin 2, histone family members, CXCL5 and β3 integrin, whereas the genes which favored UC included granzyme K and several immunoglobulin constant regions. This exciting result now requires confirmation in a larger prospective population.

In this regard, two groups have recently designed microarray experiments to determine whether specific cytokine-dependent pro-inflammatory networks may drive mucosal inflammation in individual patients. Puleston *et al.* determined the pattern of gene expression in colon biopsies obtained at the time of diagnosis from patients with CD or UC and controls [72]. A microarray designed to detect the 41 known human chemokines and 21 chemokine receptors was used. Data suggested that a specific subset of chemokines including CXCL1–3, CXCL8 and CCL20, and their cognate receptors CXCR1, CXCR2 and CCR6, were upregulated in colonic IBD of the CD or UC type [72]. The CXCL chemokines would be expected to promote recruitment of neutrophils and monocytes to the inflamed gut, whereas CCL20 via CCR6 would be expected to promote recruitment primarily of lymphocytes and dendritic cells [72]. Tissue culture studies showed that IL-1β and TNFα could have induced expression of these chemokines in colonic epithelial cell lines, implicating these cytokine dependent chemokine networks in the mixed cellular infiltrate observed in active IBD [72]. Using a similar approach, Carey *et al.* sought to identify cytokine-dependent pro-inflammatory networks upregulated in pediatric colonic IBD at diagnosis and in treatment refractory disease [73]. In this case, samples were obtained from the affected colon of patients with pediatric onset CD or UC at diagnosis, treatment-refractory CD and controls [73]. The Affymetrix Human Genome U133 array which contains ~39,000 transcripts derived from ~33,000 well-substantiated human genes was used. Similarly to the Puleston study, analysis using Ingenuity systems software identified IL-6 and IL-1β-dependent biological networks regulating leukocyte recruitment, HLA expression and bacterial antigen pattern recognition which were upregulated in patients with both CD and UC. Data suggested that these networks remained activated, to a very similar degree, in patients with treatment-refractory CD [73]. Moreover, unsupervised cluster analysis demonstrated that the subset of CD patients with overall gene expression patterns for these networks similar to the known refractory patients were more likely to be steroid dependent during the first year following diagnosis. Taken together, these studies indicated that microarray data derived from diagnostic intestinal biopsies may be used to define subsets of patients with mucosal inflammation driven by specific cytokine-dependent pathways. These results may ultimately be used to guide more targeted biologic approaches.

Microarray experiments may also be used to identify novel biomarkers with respect to medication targets. Dooley *et al.* reported results from an interesting study in which data for colonic gene expression for CD and UC patients were compared with genes differentially regulated by IBD medications including azathioprine (AZA), metronidazole or 5-aminosalicylic acid (5-ASA) [74]. As expected, a number of genes involved in immune responses and epithelial metabolism and repair were differentially regulated in the IBD samples [74]. This included upregulation of metallothionein in CD. Interestingly, AZA was found to downregulate metallothionein in the CaCo2 intestinal epithelial cell line. This "anti-regulation" was felt to be consistent with the potential use for metallothionein as a biomarker of AZA response in individual CD patients [74]. A systematic comparison of genome-wide expression between affected intestinal samples and medication-treated cell lines is likely to yield additional insights into the mechanism of action of established and experimental therapies and to point to novel biomarkers of drug response worthy of further development. Similarly, longitudinal studies comparing the global pattern of gene expression within patients during clinical trials of new agents are also likely to afford novel insights into mechanisms of drug action and mucosal healing and potentially provide clues to the basis for individual variation in response.

Clinically useful parameters to predict risk for the development of colorectal cancer (CRC) in individual UC patients are lacking. Watanabe *et al.* recently used a microarray approach to define biomarkers for the development of CRC in longstanding UC [75]. Genome-wide gene expression was determined using the Affymetrix UG133 array and mRNA prepared from rectal biopsies obtained from 10 patients with UC-associated adenocarcinoma ($n = 8$) or dysplasia ($n = 2$) (UC-Ca) and 43 UC patients without neoplasia (UC-NonCa). All patients had pancolitis and disease duration $\geq$7 years. The neoplasia in all cases was in a segment of the colon proximal to the rectum. Forty genes were found to be differentially expressed between the UC-Ca and UC-NonCa groups [75]. As might be expected, these included genes which regulate cell proliferation, survival, cell cycle and signal transduction. Hierarchical cluster analysis using these genes correctly grouped the UC-Ca versus the UC-NonCa patients, except for three cases in the UC-NonCa group (Figure 20.2). Supervised class prediction showed that the pattern of gene

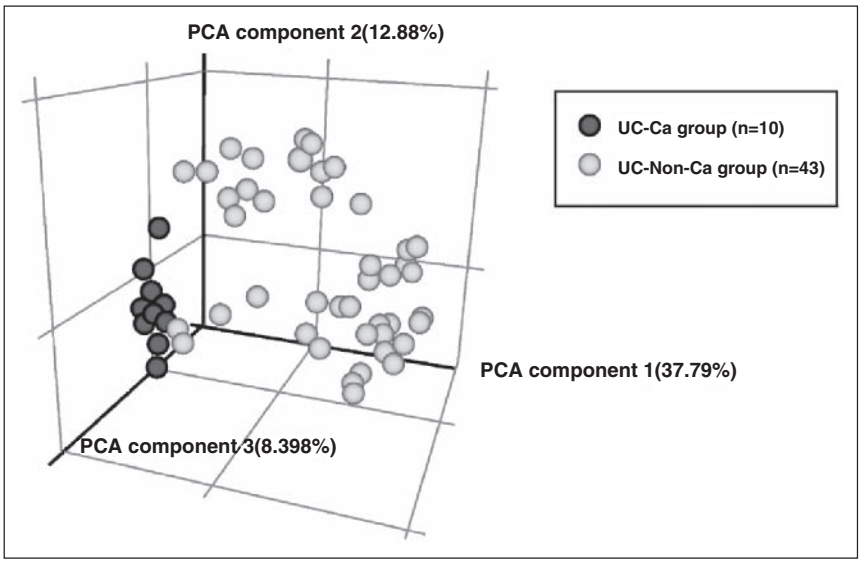

Figure 20.2 Principal component analysis (PCA). The global pattern of gene expression was determined by DNA microarray analysis in rectal biopsies obtained from UC patients with (UC-Ca) and without (UC-NonCa) colorectal cancer. Discriminating genes were used to generate a three-dimensional plot (from a 40-dimensional plot) of the data. PCA-based multidimensional scaling visualization separated samples in the UC-Ca (dark circles) and UC-NonCa (light circles) groups into linearly separable gene expression data space.

expression for this set could potentially predict the development of neoplasia with a sensitivity, specificity, PPV and NPV of 100%, 84%, 59% and 100%, respectively. Seven samples in the UC-NonCa group were mis-classified as UC-Ca using this approach. However, it should be noted that neoplasia may develop in these patients with longer follow-up. If confirmed in larger prospective studies, either the gene expression pattern as a whole or selected genes from the group may ultimately serve as useful biomarkers for UC patients at higher risk for CRC development and therefore more aggressive screening and/or therapy.

The preceding discussion has highlighted recent uses of functional genomic approaches to uncover novel aspects of IBD pathogenesis and to identify biological networks relevant to predicting clinical course and targeting therapy. Ideally, moving forward, studies will be designed with relatively uniform sample selection and processing, expression platforms and data analysis methods, in order to minimize variation and promote comparability. Perhaps most importantly, collaboration with industry will be required to validate these initial results on a scale which may in turn translate to clinically useful diagnostic assays.

Conclusion

The clinical utility and importance of IBD-specific immune and genetic markers have been reviewed in this chapter. Research and technological advancements have fostered a novel approach to understanding the intricate relationship between genetic and clinical expression of disease. Both genetic and serum antibody markers hold the most promise in helping researchers better comprehend disease heterogeneity and natural history. Although our current gold standard diagnostic tests do not possess this capability, exciting preliminary research suggests that IBD-specific genetic and antibody markers may serve as predictors of an individual's disease course (Figure 20.3). Thus, the foundation has been laid upon which the discovery of novel IBD-specific and IBD-sensitive markers will enable researchers to identify at-risk individuals, and also diagnose IBD and stratify patients into homogeneous subtypes with certainty. Clinicians can then create and implement individual treatment plans designed to improve the long-term prognosis of this chronic disease.

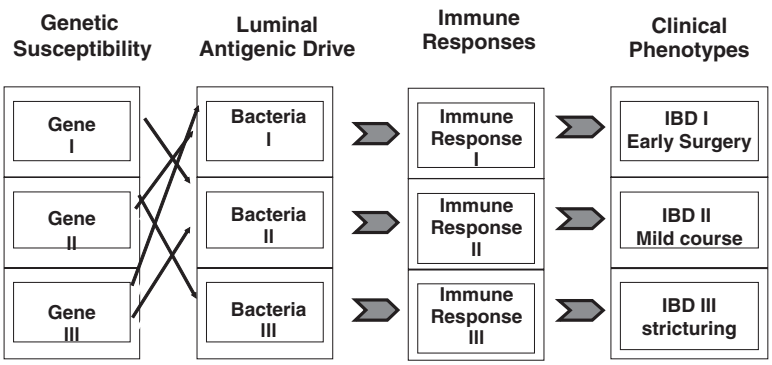

Figure 20.3 The classification of IBD: phenotype stratification based on immune response categories reflective of the genetic–bacterial interaction.

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Chapter 21
Considerations in the Differential Diagnosis of Colitis

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Summary
- Histopathology is an excellent diagnostic method to differentiate IBD and infectious colitis
- Patients with IBD can develop enteric infections, which can mimic IBD flares
- Patients with IBD are at increased risk of *C. difficile*, which may be more severe
- Other causes of colitis include ischemia, diverticular colitis, radiation, and medications including NSAIDS
- Microscopic (lymphocytic or collagenous) colitis presents with chronic watery diarrhea; colorectal biopsy is necessary for diagnosis

Introduction

The classic symptoms of colitis are diarrhea, watery or bloody, and abdominal pain, although symptoms may include nausea, vomiting, fever and malaise. When symptoms of colitis begin suddenly, the main diagnostic challenge is to differentiate an infectious colitis from new onset of idiopathic inflammatory bowel disease (IBD). However, there are many other causes of colitis that should be considered in the differentiatial (Table 21.1). Determining the cause of colitis can be challenging, as many of the clinical characteristics of the various colitides are nonspecific. A definitive diagnosis is essential, however, as misdirected treatment can lead to significant morbidity and mortality (e.g. the use of steroids in the setting of infection) [1,2]. Diagnostic clues can be obtained from a detailed history and physical examination, laboratory tests, stool cultures and endoscopy with biopsy . This chapter describes the differentiation of IBD from the other major colitides. Clinical, endoscopic and histologic features are reviewed, highlighting those features that mimic IBD.

Infectious colitis and proctitis

Gastrointestinal infections can mimic IBD and also aggravate it. Common causes of infectious colitis include *Salmonella*, *Shigella*, *Campylobacter*, enterohemorrhagic *Escherichia coli*, *Yersinia*, *Clostridium difficile* and *Entameba histolytica* (Table 21.2). Infectious causes of proctitis (inflammation confined to the rectum) that can mimic ulcerative proctitis include *Neisseria gonorrhea*, *Chlamydia trachomatis*, herpes simplex virus and, less commonly, *Treponema pallidum* [3].

History and physical examination

A detailed history may provide important clues as to the underlying cause of colitis. Bacterial colitis is most commonly acquired through ingestion of contaminated food and water. Historical clues that suggest an infectious etiology include recent travel and family members with similar symptoms. Recent or ongoing antibiotic use, prolonged hospitalization and advanced age are the main risk factors for *C. difficile* infection; however, sporadic cases can occur. Infectious proctitis more commonly occurs in individuals who engage in receptive anal intercourse. Immune status should also be assessed as immunosuppression from HIV/AIDS or organ transplantion places individuals at risk for opportunistic infections, including viral and protozoal causes of colitis.

Presenting symptoms are often nonspecific and include diarrhea, cramping abdominal pain, fevers and malaise. The character of the diarrhea can vary from watery to grossly bloody. Most infections are self-limited, with resolution of symptoms within 1 week. Symptoms can persist, however, for up to several weeks or even months (e.g. *Yersinia*, *Amebiasis*). Relapse (e.g. *C. difficile* and *Campylobacter* [4]) and chronic infection (*Yersinia* [5] and *Aeromonas* [6–8]) can also occur, mimicking the course typical of IBD. A lack of response to antibiotic therapy or relapse after antibiotics are stopped can be seen in

Inflammatory Bowel Disease. Edited by S. R. Targan, F. Shanahan and L. C. Karp.

Table 21.1 Differential diagnosis of colitis.

I. Idiopathic
  A. Inflammatory bowel disease
    1. Crohn's disease
    2. Ulcerative colitis
    3. Indeterminate colitis
  B. Diversion colitis
  C. Collagenous colitis
  D. Microscopic (lymphocytic) colitis
II. Infections
  A. Bacteria
  B. Parasites
  C. Viruses
  D. Fungi
III. Ischemia
  A. Mesenteric ischemia or thrombosis
  B. Drug induced (cocaine, oral contraceptives)
  C. Proximal to mechanical obstruction
IV. Physical agents
  A. Radiation
  B. Solitary rectal ulcer syndrome (prolapse)
  C. Glutaraldehyde hydrogen peroxide (endoscopic cleaning solutions)
  D. Drug induced
    1. Gold
    2. Isoretinoin
    3. Laxatives
    4. Allopurinol
    5. Non-*C. difficile* antibiotic-induced, i.e. ampicillin (usually right-sided colitis)
    6. Chemotherapeutics (5-fluorouracil)
    7. Non-steroidal anti-inflammatory drugs
V. Immunologic
  A. Allergic proctitis
  B. Eosinophilic colitis
  C. Graft-versus-host disease
  D. Immunodeficiency syndromes
VI. Associated with systemic disease
  A. Vasculitis
  B. Behçet's disease
  C. Sarcoidosis
VII. Miscellaneous
  A. Diverticular colitis
  B. Colon cancer

Adapted with permission from Surawicz CM. Diagnosing colitis. *Contemp Intern Med* 1991; **3**:17.

Table 21.2 Infectious colitis in immunocompetent individuals.

Bacteria
  Campylobacter spp.
  Salmonella spp.
  Shigella spp.
  C. difficile
  Shiga toxin *E. coli* (*E. coli* 0157:H7 +others)
  Non-*Cholera vibrio*s (*Vibrio parahaemolyticus* + *Vibrio vulnificans*)
  Yersinia enterocolitica
  Y. pseudotuberculosis
  Aeromonas hydrophilia
  Plesiomonas shigelloides
  Tuberculosis
Parasites
  E. histolytica
  Schistosomiasis
  Strongyloides stercoralis
  Dientamoeba fragilis
  B. hominis (possible pathogen)
Viruses
  Herpes simplex virus type II
  Cytomegalovirus

immunosuppressed patients with infectious colitis [9–11] and therefore does not distinguish between infectious or other causes in such patients. In a prospective study of first attacks of colitis, historical clues favoring infectious colitis included acute onset, fever and at least 10 bowel movements per day. In patients eventually diagnosed with IBD, the onset was more commonly insidious, without fever and with no more than six bowel movements per day [12]. Symptoms of infectious proctitis include anorectal pain, fecal urgency and mucopurulent rectal discharge with or without rectal bleeding. Diarrhea and abdominal pain are not typical features. In fact, constipation can be seen because of the pain associated with bowel movements

Physical examination findings are also nonspecific and include diffuse or focal abdominal tenderness. Extraintestinal symptoms including arthritis, erythema nodosum, pyoderma gangrenosum and perianal involvement (perianal fistulae, fissures and perirectal abscesses), when present, suggest IBD. However, certain infections can also cause extraintestinal manifestations, including arthritis, erythema nodosum and oral apthous ulcerations. Lymphogranuloma venereum (LGV) strains of *Chlamydia trachomatis* may cause a severe form of proctitis with perianal fistula formation, mimicking Crohn's disease [13].

Laboratory evaluation

Laboratory evaluation may be helpful in differentiating infectious colitis from IBD, but is often nonspecific. For example, an elevated white blood cell count, erythrocyte sedimentation rate and C-reactive protein are nonspecific markers of inflammation and can be elevated in infectious and idiopathic colitis. Anemia from acute blood loss can be seen in severe infectious colitis and IBD. However, iron deficiency anemia, indicating chronic blood loss or vitamin B_{12} deficiency, possibly due to malabsorption from terminal ileal involvement, would favor a diagnosis of IBD. A study of clinical and laboratory parameters in 239 adults who were ultimately diagnosed with infectious diarrhea or IBD revealed that features predictive of IBD were anemia, leukocytosis, thrombocytosis and a decrease in serum albumin [14]. The most helpful differentiating feature was the platelet count, which was greater than 450×10^9 per

liter in 59% of patients with IBD compared with only 1.6% of patients with infective diarrhea.

The presence of fecal leukocytes and red blood cells occurs in most patients with invasive bacterial colitis, but is nonspecific as a positive result can be found in any inflammatory condition of the bowel [15].

Stool cultures can provide a definitive diagnosis in patients with infectious colitis, although organisms are isolated in less than half of cases of presumed infectious colitis. Positive results are more frequently obtained in stool specimens tested within the first few days of symptoms [16]. Routine stool cultures for enteric pathogens identify *Shigella*, *Salmonella* and *Campylobacter*. *Escherichia coli* 0157:H7, *Yersinia*, *Aeromonas*, *Plesiomonas* and *Vibrio* can also be detected by culture; however, a request to the laboratory may be necessary as specific culturing techniques are required to isolate these organisms. Intraluminal fluid obtained during colonoscopy can also be sent for culture; however, biopsy cultures add little to the diagnosis of infectious colitis [17]. Stool microscopy can reveal the presence of ova and parasites. Serologic testing for elevated antibody titers is also available for certain enteric pathogens (*Yersinia*, *Entamoeba histolytica*). *C. difficile* can be diagnosed by isolation of the bacteria from culture, but rapid enzyme immunoassays for the detection of toxin A and B are more commonly used. Definitive diagnosis of infectious proctitis can be obtained by culture (gonorrhea, herpes simplex virus) or polymerase chain reaction (PCR) (*Chlamydia*) of mucopurulent material obtained from rectal swab.

Endoscopy

Endoscopic findings in infectious colitis usually do not allow differentiation between one infection and another and often do not exclude a diagnosis of IBD. The mucosal appearance in infectious colitis can vary from normal or mild erythema to friable colonic mucosa with ulceration and hemorrhage. Areas of involvement are often patchy, but can be diffuse. Typical signs at endoscopy for Crohn's disease (CD) include apthous ulcers, cobblestoning, noncontinuous disease with skip areas and rectal sparing. Apthous ulcers, generally thought to be classic for CD, occur in a variety of infections including *Salmonella*, *Shigella* and *Yersinia* [18] and cytomegalovirus (CMV) [19,20]. Isolated terminal ileal involvement, also commonly associated with CD, is also frequent in *Salmonella*, *Yersinia* and tuberculosis. Characteristic endoscopic changes of ulcerative colitis (UC) include diffuse erythema, granularity, friability and loss of vasculature. Diffuse mucosal disease almost always involves the rectum with variable proximal extension. The rectosigmoid is the most frequently and severely affected area of the colon in shigellosis, with variable proximal extension and occasionally pancolitis [21], similar to the distribution of UC. The terminal ileum can be the only site of involvement, however, with endoscopic findings mimicking CD [22]. Mucosal involvement in CMV colitis also varies from diffuse or segmental disease to ileocolitis similar to CD [20].

Table 21.3 Histology of infectious colitis.

Nonspecific features	
Preservation of normal architecture	
Acute inflammation	
Crypt abscesses	
Suggestive specific features	
Pseudomembranes	*C. difficile*
	E. coli 0157:H7
Viral inclusions	
Intranuclear and/or intracytoplasmic	Cytomegalovirus
Intranuclear	Herpes simplex virus type 11
Parasites	
Diagnostic organism on surface of biopsy	*E. histolytica*
	Cryptosporidium
Granuloma around organism	Shistosomiasis
Granulomas	*C. trachomatis*
	Syphilis
	Tuberculosis
Microgranulomas	Nonspecific can be seen with *Campylobacter* or *Yersinia* colitis, for example

Histology

Histologic findings can be helpful in distinguishing acute infectious colitis from IBD (Table 21.3). Crypt architectural distortion is the most important histologic feature in differentiating acute infectious colitis from IBD. When architecture is normal, infectious colitis is very likely and IBD is unlikely. Since Crohn's disease is focal, architecture can be normal. Although crypt distortion is rare in infectious colitis [23–25], it has been reported in *Shigella*, *Salmonella*, *Aeromonas* and *E. histolytica* [18]. The presence of crypt architectural distortion, therefore, does not completely exclude the possibility of an infectious etiology. The nature of the inflammatory infiltrate in the lamina propria may also be useful in differentiating infectious colitis from new onset IBD. Inflammation in IBD is characterized by an increase in both acute and chronic inflammatory cells (neutrophils, plasma cells and lymphocytes) (Plate 21.1), whereas a purely neutrophilic inflammatory infiltrate is typical of infectious coltis (Plate 21.2). A mixed inflammatory infiltrate can, however, be seen in some patients with acute infectious colitis [24]. Specific inflammatory changes at the base of crypts can also be helpful in distinguishing IBD from infectious causes. An increase in plasma cells and nodular collections of lymphocytes near the crypt bases, termed basal plasmacytosis and basal lymphoid aggregates, respectively, are seen commonly in patients with IBD and only rarely in those with infectious colitis [24]. Cryptitis (neutrophils in the crypt wall) and crypt abscesses (accumulation of neutrophils in the lumen

Table 21.4 Differentiation of ulcerative colitis from Crohn's disease.

	Ulcerative colitis	Crohn's disease
Site of disease distribution	Colon only Diffuse	Any part of GI tract Focal (segmental) skip areas
	Mucosal	Transmural
Colonoscopic appearance	Diffuse friability	Focal/aphthous ulcers Cobblestoning Linear ulcers with normal surrounding mucosa
Histopathology		
Crypt architecture	Distorted	Normal or focally distorted
Inflammation	Acute or chronic	Normal or acute/chronic
Epithelioid granulomas	Rare	Yes (20–30%)
Complications	Fistulae/abscess never or rarely occur	Fistulae/abscess can occur
Strictures	Uncommon	Common
Cancer risk after long-standing disease	++++	++

of destroyed crypts) are a nonspecific indication of inflammation and do not indicate a specific diagnosis. The presence of granulomas strongly suggests Crohn's disease (Plate 21.3), although they too can occur in rare cases of infectious colitis, including tuberculosis, *Yersinia*, ameba and syphilis and (LGV) strains of *Chlamydia trachomatis* [26,27] (Table 21.4).

Diagnostic histologic features are produced by certain infectious pathogens. Organisms may be visualized microscopically in amebiasis and strongylodiasis. Intranuclear inclusions can be seen in CMV infection (Plate 21.4) and HSV. Immunohistochemical staining of tissue can also identify the presence of CMV. The histologic findings of *C. difficile* pseudomembranous colitis are characteristic, with a pseudomembrane composed of fibrin, polymorphonuclear cells and debris often emanating from the surface epithelium in a "volcanic" fashion, called a "summit" lesion (Plate 21.5). Pseudomembranes, commonly thought to be diagnostic of *C difficile*, however, can occur in other infections, including CMV, *Shigella* [21], *Salmonella* [28], *Plesiomonas* and *E. coli* 0157:H7 (Plate 21.6).

Specific Infectious Agents

Bacteria

Several bacteria can cause a dysenteric syndrome that can mimic IBD: *Shigella*, *Campylobacter*, *Salmonella*, enterohemorrhagic *E. coli* and *Vibrio parahaemolyticus*, among others.

Shigellosis

Shigella spp. cause colitis by invading the colonic epithelium and by producing an enterotoxin.The colon is the major site of infection, with frequent rectal involvement causing a dysenteric syndrome. Stool cultures are usually diagnostic; bacteremia can occur.

Salmonlla

Non-typhodial *Salmonella* spp. infection causes gastroenteritis with diarrhea, due to small intestinal involvement. Less frequently, it causes colitis and a dysenteric syndrome. Symptoms can sometimes last for 2–3 months.

Campylobacter

Campylobacter spp. are often the most common pathogens isolated from diarrheal stool samples in the United States. Symptoms are watery diarrhea and a dysenteric syndrome. Fever is common.

Escherichia coli (enteroinvasive and enterohemorrhagic)

Enteroinvasive *E. coli* cause watery diarrhea as well as colitis with a dysenteric syndrome. Colitis due to enterohemorrhagic *E. coli* (Shiga toxin *E. coli,* including *E. coli* 1057:H7) causes watery diarrhea, which can become bloody. Fever may be absent. The organism has a predilection for the right colon and rectal sparing, hence clinically may present as ischemic colitis or CD (Plate 21.6). In children, this infection may present as suspected IBD or intussusception. Prompt recognition and appropriate cultures using special media will allow proper diagnosis. Although the organism is susceptible to most antibiotics, antibiotic therapy and antidiarrheal agents should be avoided as they may predispose to development of hemolytic uremic syndrome [29].

C. difficile

C. difficile is usually related to prior antibiotic therapy but sporadic cases can occur. The organism produces toxins, an enterotoxin A and a cytotoxin, toxin B. This illness can range from mild diarrhea to severe colitis with a toxic colon or even megacolon [30]. In addition to antibotics, other factors that predispose to severe disease include prolonged hospitalization, gastrointestinal tract surgery, elderly age and severity of illness. In North America and Europe, a more virulent strain emerged in 2000–2002 (NAP1 BI), which has led to epidemics with large numbers of cases and a marked increase in morbidity and mortality [31,32]. Associated with increased use of quinolones, the strain produces more toxins A and B *in vitro*, which may account for its virulence [33,34]. Early diagnosis and treatment are important, in addition to infection control measures and wise use of antibiotics to prevent disease.

Yersinia

Y. enterocolitica and *Y. pseudotuberculosis* are pathogens that can cause gastroenteritis and acute or chronic colitis, with terminal ileitis and mesenteric adenitis, that can be misdiagnosed as acute appendicitis or CD. From a large Dutch study of 261 patients with *Yersinia* enterocolitis 8.9% were complicated by arthritis and nearly 25% had chronic symptoms lasting from several months to 1 year [5]. The colonoscopic features include aphthoid ulcers in the left or right colon; terminal ileoscopy reveals edema, ulcers and round or oval elevations of the mucosa [35]. *Yersinia* culture requires a special cold-enrichment medium [36]. Granulomas in biopsies can suggest CD. Serology with elevated antibody titers in a typical clinical setting may be useful to make the diagnosis.

Aeromonas

Aeromonas spp. are pathogens ingested from fresh or brackish water. Symptoms include diarrhea with blood and mucus. *Aeromonas* associated colitis is rare, but there have been reports of chronic colitis due to *Aeromonas* infection which can mimic ulcerative colitis [7,8,37] or ischemic colitis [38].

Plesiomonas

Plesiomonas shigelloides is associated with ingestion of raw oysters or travel to Mexico. Symptoms include watery diarrhea; one-third of cases have colitis [39,40].

Vibrio parahaemolyticus

This causes an illness similar to *Samonella gastroenteritis*, with occasional presesntation as dysentery [41].

Tuberculosis

Intestinal tuberculosis is most commone in the ileocecal area or jejunum (75%). Associated pulmonary involvement may be present in less than half of cases. Chronic abdominal pain is the most common symptom (80–90%) [42,43]. It may be difficult to differentiate intestinal tuberculosis from CD as both may have granulomas on mucosal biopsy [44]. Diagnosis requires identification of the organism by acid-fast stain, culture or PCR of biopsy specimens.

Neisseria gonorrhoeae

Gonorrhea is usually accompanied by a mucopurulent discharge and very distal inflammation (5–10 cm above the dentate line) [45]. A smear of the anal mucus is usually diagnostic with intracellular Gram-negative diplococci. Cultures of anal swabs can be innoculated directly onto Thayer Martin media. Biopsies of rectal mucosa are nonspecific but may show mild inflammation [46].

Chlamydia trachomatis

The non-LGV strains of *C. trachomatis* can cause ulcerative proctitis [47,48]. The LGV strains can also cause a proctocolitis which may be mistaken for CD, because biopsies show epithelioid granulomas such as those seen in CD [27]. Other clinical manifestations include rectal strictures and fistula formation or abcesses. Many patients are asymptomatic. Diagnosis may be difficult but the organism can be isolated in cultures. Direct immunofluorescent staining and enzyme immunosorbent assays can detect the presence of *C. trachomatis*.

Syphilis

Syphilitic infection of the anorectal area is uncommon but can present with proctitis [49], an anal mass or an ulcer [50]. Diagnosis is best made by darkfield examination of a swab taken from the lesion or by serology.

Parasites

Amebiasis

The protozoan *Entamoeba histolytica* invades the colon, with a predilection for the ileocecal area, causing ulcers, typically "flask-shaped" ulcers in normal surrounding mucosa. Presenting symptoms include abdominal pain, fever, non-bloody diarrhea or dysentery. Asymptomatic carriage is common. Serology [enzyme-linked immunosorbent assay (ELISA)] will be positive with invasive disease. The diagnosis is made by identifying the parasite in fresh stool specimens (90% will be positive for trophozoites) or in biopsy specimens. Because amebic colitis can be chronic, it may be mistaken for IBD, either UC or CD [51]. For this reason, it is important to exclude amebiasis. In such patients, serology can be helpful [52].

Blastocystis hominis

B. hominis is a large protozoan formerly classified as a yeast. Sporadic cases report an associated colitis that responds to therapy [53–56], but other studies show no correlation of the organism's presence in stools of patients with gastrointestinal symptoms [57,58]. It may be a pathogen in immunosuppressed patients.

Balantidium coli

Rare cases of chronic colitis associated with the parasite *B. coli* have been described, with resolution after tetracycline therapy [59,60].

Strongyloides

Although *Strongyloides* usually affects the small intestine, rarely it can involve the colon and should be considered in the differential diagnosis of colitis, especially in endemic areas of the United States. The diagnosis is made on colonic biopsy when Strongyloides larvae are identified, usually near eosinophilic infiltrates in the lamina propria [61].

Viruses

Herpes simplex virus type II

Herpes simplex virus type II involves the very distal rectum, infecting the squamous epithelium. Symptoms include severe anal pain, often associated with constipation, urinary hesitancy or retention (possibly due to neural involvement) and ulcerative lesions with vesicles [62,63]. Diagnosis can be made by anal culture or less often by recognizing diagnostic intranuclear inclusions in biopsy specimens.

Fungi

Most fungal infections occur in immunosuppressed patients, with the exception of histoplasmosis, which can involve the colon and small intestine, and also other areas of the gastrointestinal tract when it disseminates. Perianal ulcers have been previously described and can lead to an incorrect diagnosis of CD [64]. In South America, paracoccidiomycosis (South American blastomycosis) can cause a granulomatous inflammation that resembles CD. In both of these infections, organisms can be recognized in biopsy specimens. *Candida* occurs ubiquitously but can invade in immunosuppressed patients in whom the infection may be diagnosed at autopsy, with detection of colonic ulcers.

Other causes of colitis

Ischemic colitis

Ischemic colitis refers to colon injury due to compromised intestinal blood flow. Ischemic colitis is usually a disease of the elderly, but young people can also develop significant ischemia. The most common cause of ischemic colitis is hypotension (e.g. from sepsis, hemorrhage, volume depletion or impaired left ventricular function). Other conditions that predispose patients to ischemic colitis include long-distance running, vasculitis, hypercoaguable state and certain medications [including anti-hypertensives, non-steroidal anti-inflammatory drugs (NSAIDs), digoxin, oral contraceptives and pseudoephedrine] [65–67]. However, it is not uncommon that a cause is not identified.

Most patients present with acute onset, cramping abdominal pain followed by an urge to defecate with passage of bright red or maroon stools. Blood loss is usually not hemodynamically significant [68].

Laboratory studies are usually normal in mild cases. More severe cases with marked ischemia or necrosis may produce a leukocytosis, metabolic acidosis or elevated lactate. Plain abdominal radiographs may show distention, thumbprinting or mural thickening [69]. Computed tomography (CT) may demonstrate segmental, circumferential wall thickening.

Endoscopic findings vary with the severity of ischemia and include: superficial ulceration, mucosal friability, edema and patchy erythema [70,71]. More extensive injury may result in extensive ulceration, pseudomembrane formation or a dusky gray or black discoloration of the mucosa. The splenic flexure and rectosigmoid junction are most often involved, but any portion of the colon may be affected. Rectal ischemia, rare due to abundant collateral blood supply, can occur in severe cases [72]. The histologic changes of ischemic colitis are nonspecific and include minimal mixed inflammatory infiltrate, superficial necrosis, granulation tissue and degeneration of crypts [73]. The "ghost-like appearance" of partially destroyed crypts is, however, characteristic of ischemic colitis (Plate 21.7).

Most patients with ischemic colitis improve clinically within 24–48 h with supportive care, with endoscopic and radiographic abnormalities resolving within several weeks. The diagnosis is often straightforward in the setting of a classic clinical presentation. However, diagnostic confusion with IBD due to overlapping endoscopic and histologic findings can occur [66]. Endosocpic findings that suggest a diagnosis of ischemia rather than IBD include normal rectum, sharply defined involved segments and rapid resolution on serial examinations [74].

Diverticular colitis

Diverticular colitis refers to chronic mucosal inflammation that is confined to a segment of colon containing diverticula. The clinical presentation is usually subacute, with intermittent hematochezia (without hemodynamic compromise or anemia), abdominal pain, diarrhea and/or constipation. Endoscopy reveals patchy or confluent erythema, friability and granularity adjacent to diverticular orifices. Mucosal abnormalities are, for the most part, confined to the sigmoid colon, with sparing of the rectum and proximal colon [75–77]. Biopsies show focal chronic active colitis: increased mixed inflammatory infiltrate of the lamina propria, cryptitis, crypt abscesses, crypt architectural distortion and basal plasmacytosis [78]. Small granulomatous foci next to inflamed crypts have been described and, therefore, are not diagnostic of CD in this clinical setting [77].

Distinguishing this condition from IBD may be difficult as the clinical presentation, endoscopic and histologic findings are often very similar. The lack of rectal involvement helps to exclude ulcerative coltis (Table 21.4). The distinction between diverticular colitis and CD in a segment of colon with diverticulosis is often more challenging. Evidence of CD elsewhere (e.g. terminal ileal or perianal involvement) should be sought before making a definitive diagnosis of CD based only on biopsies of an area of the sigmoid colon containing diverticula.

Further confusing the differentiation of diverticular colitis from IBD are that symptoms respond to treatments used for IBD (5-aminosalicylates, antibiotics or steroids) and a small subset of patients eventually develop classic

UC, with involvement of the rectum and left colon away from the area of diverticula [77].

Diversion colitis

Diversion colitis is a chronic inflammatory disorder that develops in segments of colonic mucosa that have been surgically excluded from the fecal stream (e.g. Hartmann's pouch). Although many patients are asymptomatic, some patients develop cramping abdominal pain, bleeding or mucous discharge. Endoscopic and histologic changes eventually develop in all patients to some extent. The endoscopic features include diffuse mucosal erythema, friability, nodularity (caused by lymphoid hyperplasia) and apthous ulcers. Histologic findings are characterized by a chronic lymphoplasmacytic inflammatory infiltrate, lymphoid follicular hyperplasia, superficial erosions, cryptitis and crypt abscesses [79–81]. Crypt architectural distortion may also be present [80]. These endoscopic and histologic findings can make distinguishing diversion colitis from CD or UC difficult. In diversion colitis, however, endoscopic and histologic abnormalities can dramatically improve with local application of short-chain fatty acids [82] and with restoration of bowel continuity.

Soliltary rectal ulcer syndrome

Solitary rectal ulcer syndrome (SRUS) is an uncommon chronic disorder affecting the rectum. Typical symptoms include bright red blood per rectum, straining during defecation and a sense of incomplete evacuation. Associated rectal prolapse is common. The endoscopic appearance is variable, ranging from patchy rectal erythema to single or multiple ulcerated or polypoid lesions. Lesions are most often located on the anterior wall of the rectum, within 10 cm of the anal verge. Histologic features include a thickened muscularis mucosae, replacement of the lamina propria with smooth muscle and fibrosis, mild crypt architectural distortion and chronic inflammation [83] (Plate 21.8). Although the finding of distorted crypts, in conjunction with the clinical and endoscopic findings, is suggestive of IBD, the presence of collagen in the lamina propria can be helpful in distinguishing solitary rectal ulcer syndrome from IBD.

Microscopic colitides

Microscopic colitis is characterized by chronic watery diarrhea, normal endoscopy and diagnostic histologic changes. It encompasses two possibly related disorders: collagenous colitis and lymphocytic colitis. The peak incidence of collagenous colitis and lymphocytic colitis is at around 60–65 years of age [84]. In addition to diarrhea, symptoms may include abdominal pain, weight loss, fecal urgency and nocturnal stools. Endoscopic examination is essentially normal, although some nonspecific mucosal findings such as erythema, pallor or edema have been reported in up to one-third of patients [85]. Histologic changes in collagenous colitis include a mixed inflammatory infiltrate composed of lymphocytes and plasma cells within the lamina propria, intraepithelial lymphocytes, damage to the surface epithelium, preservation of crypt architecture and expansion of the subepithelial collagen to at least 10 μm (Plate 21.9). Lymphocytic colitis is histologically similar to collagenous colitis except with a higher number of intraepithelial lymphocytes and a normal thickness (Plate 21.9) of the subepithelial collagen layer [86].

Although the distinction between microscopic colitis and IBD is usually clear, a small proportion of patients with microscopic colitis may show IBD-like histologic features, such as active crypt inflammation, surface erosion or ulceration and crypt architectural irregularity [87]. Therefore, the presence of some histologic features normally associated with IBD should not exclude a diagnosis of microscopic colitis.

Medication-associated colitis

A number of medications can cause colonic injury resulting in colitis. The diagnosis is often one of exclusion as the clinical, endoscopic and histologic findings are nonspecific and the temporal relationship between drug administration and effect can vary widely. Offending medications include NSAIDs, methyldopa, isotretinoin, penicillamine, gold salts, oral potassium chloride and cyclosporin A [88,89]. Chemotherapeutics including 5-fluorouracil and irinotecan are also associated with colitis.

NSAIDs have the potential to produce a colitis that mimics IBD, but also to trigger a new presentation or flare of IBD. Many types and formulations (parenteral, rectal and intramuscular) of NSAIDs have been associated with colonic injury. The duration of NSAID use prior to the onset of symptoms can range from weeks to years [90,91]. Clinical presentation may include abdominal pain, diarrhea with or without blood and/or iron deficiency anemia [92]. Obstruction, perforation and hemorrhage can also occur [91]. Endoscopically, NSAID-associated colitis can demonstrate patchy or diffuse erythema, edema, friability, apthous ulcers and/or exudates. Single or multiple sharply demarcated ulcers with adjacent normal-appearing colonic mucosa are typical [92,93]. Although lesions can occur throughout the colon, the ileocecal area and ascending colon appear most often involved. The extent of disease can range from segmental involvement to pancolitis.

Histologically, NSAID-associated colitis demonstrates focal active colitis: patchy mild to moderate inflammation (either lymphoplasmacytic, neutrophilic or mixed), slight crypt disarray (variation in crypt distribution or size) without crypt distortion and focal erosions. Granulomas are not usually seen; however, NSAID-induced granulomatous inflammation has been reported [94]. Lesions typically heal upon withdrawal of NSAIDs, usually within a few weeks.

Radiation colitis

Up to 50% of patients treated with pelvic radiation will develop acute radiation colitis, usually within 2 weeks of initiation of therapy. Symptoms include abdominal cramping, tenesmus, diarrhea and rectal bleeding. Chronic radiation colitis can occur 6 months to decades after radiation therapy and occurs in only about 5% of patients. The most common symptoms are rectal bleeding, rectal ulcers and diarrhea. These occur due to a progressive vasculitis leading to small vessel thrombosis [95]. The histology resembles ischemia, with superficial necrosis and hyalinized blood vessel walls. The most important risk factor for chronic radiation colitis is the total dose of radiation.

Colitis with systemic disease (vasculitis)

Any systemic vasculitis can affect the colon and cause colitis, including polyarteritis nodosa and systemic lupus erythematosus. Colonoscopic petechial lesions in the colon have been described in patients with Henoch–Schönlein purpura [96]. These lesions resemble the skin lesions. Vasculitis has been demonstrated by biopsy.

Churg–Strauss syndrome is a rare entity with asthma, hypereosinophilia, necrotizing vasculitis and extravascular granulomas [97]. Colonic involvement is rare, but multiple colonic ulcers can occur [98]. There are a few cases published describing colitis in patients with Wegener's granulmatosis with endoscopic findings of ulcerating mucosa but histology demonstrating nonspecific findings for IBD [99].

Sarcoidosis

Gastrointestinal sarcoidosis can clinically present like IBD with endoscopic and histologic appearances of CD in biopsies demonstrating chronic inflammatory infiltrates, crypt abscesses and non-necrotizing granulomas. The correct diagnosis of sarcoidosis can usually be made when the usual extraintestinal signs of sarcoid are manifest [100]. Unlike CD, there is no transmural inflammation, lymphoid aggregates or strictures [101].

Behçet's syndrome

Behçet's syndrome consists of uveitis and oral and genital ulcers. There can also be gastrointestinal involvement in the form of deep flask-shaped ulcers in the background of chronic inflammation that may demonstrate granulomas, thus raising the possibility of CD. Some feel that Behçet's syndrome is indistinguishable from CD [102]. Not only can the prevalence of Behçet's syndrome vary significantly with geography, but also the presence of gastrointestinal involvement. In Japan the prevalence is 1:10,000 whereas in the North America it is 1:500,000. In a large study from Turkey involving 1000 patients with Behçet's syndrome, none had colitis [103].

Neutropenic colitis (necrotizing enterocolitis in cancer patients)

This is a complication of cytotoxic drug therapy often for leukemia or lymphoma. It is usually due to clostridial infection (*Clostridium septicum*, *Clostridium perfringens* or *Clostridium paraperfringes*) whose toxins cause hemmorrhagic necrosis. This diagnosis needs to be considered in any neutropenic patient with fever, abdominal pain and diarrhea,which can be bloody. It can progress to peritonitis, septicemia, shock and death. Endoscopically the bowel is edematous with ulceration and hemorrhages. Histologically, there is submucosal edema, hemorrhage, necrosis and very few inflammatory infiltrates. Perforation can occur in 5–10% and in this case series the mortality rate was 28% [104].

Eosinophilic colitis

Eosinophilic infiltration of the mucosa of the gastrointestinal tract can involve the stomach, small intestine or colon. When ileocecal involvement occurs, it may mimic CD [105,106]. Symptoms are nonspecific but include abdominal pain and cramps, diarrhea and rectal bleeding. Biopsies show intense eosinophilic infiltration, more marked than the increase in lamina propria eosinophils which can be seen in IBD. Peripheral blood eosinophilia is fairly marked.

Conclusion

Colitis represents a nonspecific inflammatory response of the colon. Distinguishing among various forms of colitis can be difficult, yet is essential for initiation of appropriate treatment. A detailed history (including duration of symptoms, specific risk factors and extra-intestinal involvement), physical examination, stool studies and endoscopic/histologic findings can be extremely helpful in differentiating IBD from other forms of colitis. Initial evaluation should include stool culture for enteric pathogens (including *C. difficile*) and three ova and parasite examinations. If these are negative, flexible sigmoidscopy or colonoscopy with biopsy should be pursued.

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Chapter 22
Disease Management in Chronic Medical Conditions and its Relevance to Inflammatory Bowel Disease

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Summary

- The major reason behind the need for implementing disease management (DM) is the varitation in physician practice.
- DM involves medical decision making driven by data, and consists of identification of the patient group, intervention according to best practice guidelines, education to lessen physician variability and to improve patient compliance and a measurement of outcomes to ensure that the result is beneficial.
- Proper implementation of this evidence-based approach has been difficult and slow, especially in the care of patients with IBD where insufficient data are available to allow predictive stratification of patients or selection of management options for both acute and maintenance phases of the diseases.
- Another reason that implementation of DM is difficult is that the concept of DM was in part based on business models, but health is not the same as money, as it is neither stable, nor can it be traded across time or individuals.
- Yet another reason why DM programs have been under-utilized is that the cost benefits of intervention programs are incompletely estimated, in part because the value assigned to quality of life varies so much between individuals.

What is Disease Management?

Historically, disease has been managed on a one-on-one arrangement between physician and patient. As managed care systems developed in response to the need to regulate total spending on healthcare, a need arose to assist the process of medical decision-making that could be driven by data and not by personal experience and knowledge alone. Managed Health Care aimed at maximizing the value of medical services to all parties, the payer as well as the consumer. It included initiatives for preventive services and flexible algorithms to optimize treatment of diseases. Many terms have been used to describe the holistic approach to care of homogeneous groups of patients, whether identified by a common disease (e.g. diabetes mellitus, hypertension), a common geographic base (e.g. medical center or city) or a common single payer base (e.g. country or insurance program). Disease Management (DM) and Pharmacy Benefit Management (PBM) have been perceived and developed as components within Managed Health Care. Disease Management focuses on specific (often common) illnesses and includes both the identification of the population at risk and therapeutic guidelines. Because DM (and also PBM) were based on data and included elements of control and/or decision-making for the provider involved, an information infrastructure was an essential element. Although computers are enablers rather than essential elements of DM, other terms that imply computer use for managing medical care include "integrated care (IC)", "clinical decision support systems (CDSS)", "evidence-based care", "managed care" and "care management". Integrated or managed care and care management are meant to comprise a comprehensive program, like DM, whereas evidence-based care and CDSS are usually directed more towards operational issues within the healthcare system. Care management guidelines have been suggested and introduce a major role for allied healthcare professionals (nurses, social workers) [1,2]. This system has been used to develop guidelines for care of specific illnesses (e.g. dementia [3]). Similar systems employing allied professionals have been developed in the UK (e.g. community matrons).

This chapter discusses why comprehensive management solutions were needed for healthcare (referred to hereafter as DM), how they developed initially and have become modified to include sociological, quality of life and reimbursement issues, why the drivers for DM

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development were (and continue to be) economic, how DM works and how it can be implemented, including the role of practice guidelines and the development of analytical model systems. DM was initially directed to those chronic conditions that accounted for the largest portion of healthcare expenses, i.e. diabetes, depression, coronary artery disease, congestive heart failure, osteoporosis, osteoarthritis, benign prostatic hypertrophy, asthma, cancer and peptic ulcer disease. These are mostly diseases that have (or had) a large impact on hospital costs. Thus, most of gastroenterology, aside from gastrointestinal bleeding or peptic ulcer disease [4], was excluded from the initial focus of DM. This chapter outlines the progress that has been made to date in development of practice guidelines in gastroenterology and on the concept of DM as it applies to inflammatory bowel disease (IBD).

Why was a management solution needed for healthcare?

When governments or large healthcare organizations have imposed standards or recommendations of care, it has been usually in areas of public health or preventive medicine (e.g. vaccines, fluoridated water). In recent decades, as the largest payers have incurred increased financial risk, economic pressures have developed to direct/control management of diseases in a population of patients, rather than in individuals. The reasons for this shift in emphasis have been many [5]. The most important of these reasons have been increasing expenses, the resultant increased financial risk to both payers and patients, the more widespread availability of information on the cost of healthcare and increased patient and payer education. These factors have created a market demand for better value without sacrificing quality of care. The two models that developed initially for DM were the "carve-out" and "primary care-based" models. Subsequent models have expanded the physician-based model to include a fully integrated model (health and social care services) for a closed network that includes multidisciplinary team responsible for quality outcomes [6].

Because drug costs were easily identified and a large component of total healthcare costs, pharmaceutical companies were among the first groups to find it in their interest to become involved in DM programs. Drug spending represented a significant percentage of total healthcare spending (ranging from ~8.8% in the USA to 16.8% in France) and the data on prescription drug use were relatively easy to identify. Other problems associated with drug use also added to the total expense of healthcare, the resolution of which would lower the costs. These problems included prescription errors [7], complications of drug therapy [8] and non-utilized drugs once prescribed [9]. The most common prescription errors were lack of knowledge of the drug, lack of information about possible drug interactions or allergies and incomplete or poorly legible prescriptions [4]. The largest expense by far related to drug complications in the USA was from hospital admissions [10]. Similar figures for levels of error have been reported from Australia, Israel and the UK [11]. In Sweden, the average difference in cost between prescribed and dispensed medications was about 20% [12].

To address these issues and to save money, PBM companies were created to manage the reimbursement of pharmaceutical products, normally only including prescription drugs (a "carve-out" model). A prerequisite for an effective PBM is a well-established network of pharmacies and an infrastructure enabling interventions at the point of service (e.g. Caremark or Medco in the USA). These companies were very successful in driving down the costs by negotiating with pharmaceutical manufacturers for price discounts, by providing services to payers such as drug utilization review (DUR), formulary management, prior authorization and physician profiling [13]. Many of these features both improved care and controlled costs. However, improved management by itself is probably not sufficient to control prescription errors; a standardized order form or mechanism is also an important variable. In the USA, the standardized order form has proven of value in reducing error rate and cost [14]. In the UK, a Government white paper, "An Organization with a Memory (2000)", set as a target by 2005 a 40% reduction in the number of serious errors due to prescribed drugs [15]. Some progress has been made with the centralized healthcare system in the UK. Near miss or dispensing errors occurred at a rate of ~0.25% in 35 community pharmacies [15]. A much higher error rate (2.5%) was found in nine national Health Service (NHS) Trust pharmacies dispensing psychiatric prescriptions, but most of these errors were due to prescription writing [16]. Although not directly compared, these studies suggest that electronic prescriptions could be very helpful in managing prescribed drugs. The goal of the NHS is to have all prescriptions ordered by GPs sent electronically. However, issues that still stand in the way are security of patient-identifiable information and reservations from GPs about sharing patient records with pharmacists [17]. Getting the prescription correct is only the first half of the equation; the patient has to understand and properly use the medication. The US FDA regulates the content of the prescription label, but not the format, which is left up to each state. The highly regulated part of the prescription, the package insert, is not usually provided to the patient and, if it were, would not help the patient very much. Consumer medication information is not at all regulated and is often not very good, even when available. When indigent populations were tested for comprehension of the label, the level of literacy was a major factor in poor understanding [18]. This problem points up again the importance of the role of the physician or healthcare professional in communicating with the patient. Even if the patient understands, correct use of the

medication is not guaranteed. These issues point out the difficulty in providing a seamless and error-free program just for prescription drug delivery. Difficulties in providing such standards to a whole program of disease management have proven even more difficult.

PBMs could address a broad spectrum of problems and their economic value became apparent early. The DM strategy started later and focused on common chronic illnesses that accounted for a majority of the hospitalized care. Initially the idea was to deliver "comprehensive" DM for these disorders. DM aimed to assist in identifying and monitoring long-term care of patients with chronic diseases at high risk for hospitalization (e.g. Crohn's disease, diabetes, coronary artery disease, congestive heart failure, osteoporosis, depression, asthma, cancer, benign prostatic hypertrophy, osteoarthritis and peptic ulcer disease) [19]. Most programs have aimed at providing assistance to the physician, based on evidence-based algorithms and or on nationally accepted guidelines for management of specific disorders. Up to this point, there was little direct involvement with physicians, who were not themselves at financial risk from the changes that were introduced. About 200 companies in the USA were offering disease management programs by 1999, many of them associated with pharmaceutical companies and many acting as contracting firms to deliver services [20]. The DM Purchasing Consortium and Advisory Council (www.dismgmt.com) was established as a central organization for the DM industry. Although many claims are made for improved efficacy and reduced costs, the results of analyses are usually proprietary, not public and the studies are rarely randomized, blinded or peer reviewed. When such studies are published in reviewed journals, the results with a control group are often centered on adherence to medication, but efficacy data are limited to the intervention group alone [21]. Although one major rationale for DM programs was cost control, it is not clear that such control can be delivered, unless the treatments for the chronic illness themselves lead to cost reductions [22]. Enthusiasm for such DM programs has waned in the last decade, although they are still offered by many companies, in particular the large PBMs, Caremark and Medco. In addition, concern has been raised that "carving out" services may contribute to fragmentation of patient care [20].

The countries in which the concepts of DM or managed care have been most studied have been the USA and the UK. However, their healthcare delivery systems could hardly be more different. The USA has almost no national healthcare policy, whereas the UK supports a national healthcare service, the NHS. The key person in managing diseases or costs is the primary care physician. In the USA, primary care includes family physicians, internists, pediatricians and gynecologists (for women), but there is no organizational communication between these groups [23]. Managed care reforms increased prominence for the primary physician in controlling costs, but the absence of a national policy has hampered further growth of their ability to influence the system. In the UK, budgets for primary and secondary care have been separated for nearly 50 years, allowing GPs to achieve a position of importance in the system. Utilization management has been achieved by the dual and non-overlapping system of community GPs and hospital-based specialists. The formation of the Primary Care Trusts in the last decade has encouraged the formation of a primary healthcare team, including nursing and social services, in the overall care of the patient [24]. In the USA, training programs have delivered exceptional quality, but have remained hospital-based and procedure orientated, a system that is reinforced by the fee-for-service payment system. In the UK, the GP's computerized record, although perhaps less complete than its US counterpart, nonetheless encourages continuity of care and avoids duplication of studies. Although it is difficult to compare general practice with specialty practice, there is evidence that improved access to primary care provides better health and lower costs in the entire healthcare system [25].

In these diverse backgrounds, the DM concept has struggled to find a place. The Balanced Budget Act of 1997 in the USA included a possible change in reimbursement to providers who do not follow standards of care. Medicare has the option to enforce these standards and withhold payment when they are not followed [26]. In the UK, the white paper on the NHS created Primary Care Groups who would be responsible for accepting some of the financial risk, in that they would be cash limited, but able to move money from one service to another [27,28]. These groups will be responsible for commissioning secondary care and tackling the variations in quality of care [29]. The plans that have developed related initially to primary care physicians, the group most often targeted for DM programs, but more recently have also involved specialty groups. Because of the magnitude of the problems, the most practical and successful approaches have been very focused. "Comprehensive" DM, if it becomes a widespread reality, will be comprised of a group of smaller, more focused and successful programs that all deal with a given disease entity or within a closed network system.

What was the Disease Management solution originally proposed?

The concept of Managed Health Care (also called Integrated Care), of which DM was a component, developed in the 1980s as an adaptation of the reorganization of industrial management skills (also called total quality management, business process design, etc.). In this paradigm, businesses committed to placing customer needs first, decentralizing responsibility, placing reliance on

well-defined economic/statistical tools and quality team work and measurement of results. The first programs "borrowed" from industry in the early 1990s were designed to cut costs by using physician oversight, including gatekeepers, second opinions and utilization review. The experience in the Managed Health Care industry regarding the concepts of total quality management was applied in part to the components of managed care. In the latter part of the decade, as more quality tools were developed, so also were clinical pathways, algorithms, guidelines, automated prompts and financial incentives. None of these programs by themselves, however, are equated with DM, although most are included in it.

DM involves medical decision-making driven by data. The data must be captured and transformed into actionable information for use by providers to improve efficiency and effectiveness of healthcare delivery. In its comprehensive form it involves an integrated, systematic approach to patient care, based on identifying at-risk patients, capturing clinical data, intervening in the course of the illness and improving outcomes. Implicit in such a program is that the information and analysis of decision-making will help to change the behavior of patients, providers and payers. Although DM was meant to be comprehensive, its goals were so broad that all programs have been comprised of fragments of overall disease care.

In the USA, group-model and staff-model Health Maintenance Organizations, such as the Group Health Cooperative of Puget Sound and the Kaiser Health Plan, have developed in-house programs of DM (the "primary care-based" model) [4]. This model provides electronic systems that allow viewing of a spreadsheet for specific diseases, e.g. diabetes. Fully integrated models have developed in the USA for even more specific groups, e.g. the frail elderly. The two models that have been produced for this group of patients include the social health maintenance organization (Social HMO) and the Program of All-inclusive Care for the Elderly (PACE) [6]. The components of the integrated healthcare system are outlined in Table 22.1. They include a closed population defined by enrolment, a package of healthcare and social care services defined by contract, a financing system dependent on prepaid Medicare and Medicaid capitation in part and accepting full risk for all services, emphasis on primary non-institutionalized care and micro-management techniques to ensure quality care and control costs. These techniques include DM and utilization review. These programs combined acute and long-term care services and are still works in progress. It is not clear whether such fully integrated models can be replicated in other countries, but for such systems to succeed, they must reflect the interests and perspectives of the major stakeholders.

A review of the quality of clinical care in general practice in the UK concluded that most of the care did not attain acceptable standards of practice [30]. In part due to such data, the UK government has set specific standards for quality improvement in medical care, as part of policy of the NHS. In the UK, the mechanism for providing integrated care systems has been the Primary Care Trusts that have combined primary and community health services [24]. The NHS Plan suggested making new funds available for social services to allow co-localization of services and home care teams. In particular, plans were developed for the frail elderly population that would include a register of nursing homes [31]. These changes were suggested at the same time as employment of community matrons was becoming a key feature in case management policy. The Evercare approach to caring for frail elderly patients has been used throughout Europe and also in the UK and USA. In the USA, when patients became ill, intensive domiciliary nursing was used [32]. In the UK, however, the focus was on reduced hospital admission. When case management strategy was added to the Evercare program in the UK, no further fall in hospital admissions was seen, because of the addition of high-risk patients resulting from increased case finding [32]. Although it is clear that integrated systems for the frail elderly have been initiated in both the USA and the UK (and also in Europe), there is as yet no evidence that the programs have yielded better outcomes or lower costs.

For DM to work requires the collection and management of large volumes of patient data. Because many elements of this information-based system are foreign to providers, development of such programs must include education of and support from providers for successful implementation. The provider, therefore, is a crucial

Table 22.1 General components of DM and its operationalization for integrated care.

Steps involved	Factors needed for integrated care
Identify and classify patients	Ability to identify and target at-risk populations
Provide treatment guidelines to physicians	Interaction of society guidelines with government policy and healthcare administration
Educate patients	Alignment with patient needs regardless of limitations on eligibility and services
Deliver interventions and support services to patients	Bundling of necessary services Networking between institutions and providers Ensuring continuity of care and coverage
Measure clinical and financial outcomes	Coordinating multidisciplinary providers Clinical, administrative and financial information sharing Stakeholder involvement in planning
Ensure funding	Align funding for health and social care Overall responsibility for quality and costs

Derived in part from [6].

element in the planning and administration of DM programs. Reluctance to change or participate is a danger that the medical profession must overcome if the promise of information-based decision-making is to be achieved. As a way to start the process, health plans have begun to incorporate evidence-based medicine into portions of the healthcare system that are overseen by medical management, including therapeutic review, secondary prevention programs, adherence measures comparing practices, pay-for-performance programs linking physician adherence to guidelines for financial incentives and patient compliance programs [33]. A study by the RAND Corporation found that adherence to evidence-based guidelines for 30 conditions in 12 communities between 1996 and 1998 showed appropriate use of recommendations only 54% of the time [34]. The process of evidence-based medicine is well established and involves combining a systematic review of the information related to diagnosis and treatment of a condition with the clinician's training and experience [35]. Both components are important, so that physicians are encouraged to participate in the process. A study covering most of 2003 reviewed evidence-based processes in 89 health plans in the USA [33].

Disease and case management were a problem that was involving nurse case managers, similar to the community matrons in the UK. Evidence-based medicine is being used more often for coverage, denial management (based on "standard of care") and pay-for-performance, as well as for the more traditional roles of pharmacy benefits management and provider profiling. Reducing health costs, not disease management *per se*, is still the major driver for these uses of evidence-based medicine. It seems clear that the healthcare systems want very much to get into DM more vigorously, but the decentralized nature of the system, with multiple levels of financial incentives, has prevented a consistent approach to the problem of care for chronic diseases. Older physicians seem less able to accumulate new information and are at risk for providing lower quality care, at least when looked at largely in North America [36]. Another problem is how to assess when standards are achieved and inequities are diminished. For this problem, evidence-based methods are being used, most extensively (in 48 countries) in the CIET cycles (Community Interventions and Epidemiological Technologies) [37]. This cycle analyzes existing data from cluster surveys to discuss with local and national health workers and includes a buy-in from stakeholders concerning what to measure. The process is reiterative and gradually builds up expertise in epidemiology, decision-making and community understanding. These methods have been used more in poorer nations to address issues such as access to healthcare services, food security, prenatal health and HIV/AIDS, but there seems no reason why such evidence-based analysis cannot be incorporated some day into management of chronic diseases.

Why were economic evaluations an important driver to the development of Disease Management?

DM concepts developed at the same time as did the search for alternative methods of health resource allocation. Thus, it was originally thought that DM would save money. One fear commonly expressed was that the profit motive would interfere with the delivery of quality healthcare. These fears have been mollified by the need for physician groups in the USA to control healthcare costs, as reimbursement decreases and expenses increase. As DM programs have been developed with physicians, however, their involvement focuses naturally on the healthcare aspects. "Reduction in the costs of care is often a beneficial product of such programs, but it is a secondary goal and, importantly, not always achieved" [38]. Because economic forces continue to be important drivers of healthcare policies and of managed care programs, it is useful for the physician to understand some aspects of medical economics [39,40]. What follows is not meant to be comprehensive, but to take aspects of this large field that apply to DM concepts, explain some of the methods used and put them into perspective for the physician.

The concept of DM was relevant to care needed by a population with a given disease, instead of focusing on individually taken decisions during multiple physician–patient interactions. Thus, health was considered a commodity and DM principles (as Managed Health Care principles) were adopted from the business world. However, health differs from money. One cannot trade health across time or individuals and it is not stable, but affected by illness severity. Moreover, many health outcomes are irreversible. Other aspects of a market-based approach do not fit well with healthcare, because most health risks are unique and individual and values placed on those risks differ widely among individuals. Thus, health can be converted to monetary terms only with difficulty. Although the problem was difficult, the analogies noted above have been accepted for healthcare and the field of health economics has been the result.

In economic situations in which competitive markets do not exist (healthcare is a good example), cost–benefit/effectiveness analysis aims to provide the rationale for decisions that supply-and-demand forces resolve in competitive markets. However, economists and biomedical researchers do not examine the problem in the same way. Those trained in econometrics often use retrospective data and models to allow conditional estimates of the effects of an intervention (e.g. tax) or an outcome (e.g. profits). These models provide unbiased and robust estimations to predict effects in populations or groups. Those trained in biomedical sciences rely mostly on randomized controlled observations to achieve the same results, but then these results can only be applied to the more narrow group

Table 22.2 Disadvantages of PRCT or meta-analysis for cost-effectiveness analysis.

- Limited target population
- Low study power (small numbers)
- Lack of timeliness with economic factors in healthcare
- Control arm may not represent a practical or local standard-of-care alternative therapy
- Short time frame for study
- Efficacy of treatment does not consider inappropriate use
- May miss rare but important adverse events

studied. This dichotomy has prevented much communication between economists/planners and medical researchers/providers. "These two groups seldom meet in practice, and if they did, they would seldom agree about proper rules of inference" [41].

There are many disadvantages in using prospective randomized controlled trials (PRCTs) or meta-analysis for cost-effectiveness analysis and one must be wary of interpreting such studies that have economic evaluations placed on them. These disadvantages are listed in Table 22.2. Hence it should not be surprising that when PRCTs are performed to provide data for economic analysis, the answers may be very different from those of apparently similar studies. For example, the Washington University Heart Failure DM trial found that intervention provided an increased percentage of patients free of rehospitalization in 90 days compared with usual care (64.1 and 53.6%, respectively) [42]. However, a multicenter Veteran's Administration DM trial found an increase in readmission rate among patients with congestive heart failure [43]. The only practical solution to this dilemma is a DM program with locally derived and applicable data. That characteristic has and will impede the wide application of generic DM programs.

Can Disease Management really lower healthcare costs?

In addition to the theoretical difficulties in comparing population-based economics and practice by selected providers, the healthcare industry is so complex that economic evaluations are not easy, even when estimated for a population. Three kinds of economic analysis have been used, depending upon the outcome to be studied [26]. Cost identification (or minimization) analysis simply estimates the costs to produce an intervention. For DM, this analysis is used to identify interventions where cutting costs that would seem to have little impact on outcome, such as provision of materials (sutures, guidewires) at lower cost. However, simple as this seems, estimating costs of healthcare services can be very difficult and is limited mostly to procedures or tests. For example, the cost of drugs and dispensing of drugs may include manufacturer rebates or consumer co-payments. Charges for hospital costs may equal charges only by chance, as some components are discounted and others are charged more heavily. Non-hospital institutional charges, such as nursing homes, are usually not known and may vary widely. Non-institutional labor costs, such as physicians, are usually not included in any cost analysis. There is still little agreement on how to value physician time. Costs borne by patients are usually unknown, including lost income, home care and care visits to doctors.

The most common strategy for DM programs is to provide care by teams, a plan that involves healthcare professionals other than physicians and that utilizes changes in information systems. One obstacle to implementing such a plan is the traditional fee-for-service financing that is the norm in the USA. The Society of Internal Medicine Task Force on the Domain of General Internal Medicine has recommended that general internists should work in teams, coordinating communication with patients and other team members [44]. In addition, the Task Force advocated abandoning current financing of physician services, so that reimbursement could be offered for team services that occur outside of face-to-face visits. The suggested change would involve either (a) payment for time supervising long-term care and managing teams, (b) a form of capitation (patient management fee) with reimbursement for specific services or (c) a salary with incentives for productivity, quality and improved outcomes. The Task Force favored plan (c). If this were initiated, it would approach the problem of containing costs for comprehensive programs by altering the financing of the system. The experience thus far shows that the solution to the problem of cost containment is much more complex and will require a system-wide approach.

Pay-for-performance has been initiated in a number of programs and is in theory supported by practicing physicians in the USA [45]. Broad application of this approach will probably depend upon adoption of the electronic medical record, a process that has proceeded at a very slow pace in the USA. Problems with such programs that still need to be worked out include the target of the incentive (will it just make good doctors wealthier?), the target of the payment (physicians, management units or patients with decreased co-pay?), the type and amount of payment (cash or credits?) and the development of acceptable standards of performance. These are all major problems that need to be resolved before such an incentive program can succeed. A review of 17 studies that have reported on pay-for-performance programs found that most programs showed partial or positive effects on quality of care, but the effects were small [46]. None of the studies addressed the issue of optimal duration of incentives, nor did they assess cost-effectiveness. In 2005, 84 health plans sponsored some sort of purchasing initiatives, perhaps the easiest way to start; Medicare has initiated projects focused on

hospitals, physicians and DM programs; and employers have started bonus programs for cost-effective programs. Some collaborative programs have been initiated (e.g. the Human Resources Policy Association), but there has yet to be a national policy on pay-for-performance and it is not clear whether the program will survive, at least in its current forms. The 2006 Congress did pass a bill that authorizes Medicare to pay doctors a bonus for reporting data on the quality of their care (reported in an editorial in the *New York Times*, 26 December 2006).

The NHS in the UK adopted a pay-for-performance program in April 2004, called the Quality and Outcomes Framework (QOF). This plan was initiated to reverse the trend in which GPs who did the best job actually took home less money, because they hired more staff and nurses on their fixed capitation income. In this plan, physicians were scored on 146 indicators of quality, each of which had a financial bonus associated with it [47]. The program was very successful in delivering bonuses, but went well over budget. The data from the plan have been well documented, because the electronic medical record in the UK provides data reliability and validity, unlike many sites in the USA. Criticism of the QOF plan has focused on its high cost, the view that more money is being paid to physicians for what they were already doing, the ability to exclude some patients who had complex problems that would not allow accurate assessment of quality and a shift in professional ethos that would lead to physicians doing only what they were paid to do, as in the USA. Moreover, because of the need to simplify the assessment of quality, there has been a move towards focus on diseases rather than on patients, a problem that is antithetical to the concept of DM. The lesson for the NHS is that it should have initiated a program that was smaller, moved more slowly to reduce risks to physicians and payers and concentrated on issues where there was the largest expectation of health improvement. Thus, in this example, the NHS seems to want to become more like the system in the USA. There is other evidence that the QOF program has had little impact thus far on the total cost of healthcare. Primary Care Trusts are the purchasing units of the NHS to pay for patient care management by GPs, but there is great disparity between those trusts that overspent and those that developed a surplus. The factors that cause these differences are complex (related to patient density, income and increased demand for services), but are not related to total points in the QOF program [48].

Issues with economic analysis of Disease Management programs

The cost of intervention programs is often incompletely estimated, because many of the factors listed above are not included. Putting a dollar value on the benefits is even more difficult, as estimates must be made for nearly all such benefits. For example, incremental costs (those saved by an intervention) can only be estimated, but such costs vary widely based on local factors and estimates cannot easily be transferred from one situation to another. "Sunk" costs (those that must continue regardless of the intervention, such as depreciation) are not always included and are hard to estimate. The largest "savings" estimated from an intervention usually are due to decreased hospitalization costs. For example, in asthma a DM program led to an eight-fold increase in drug spend (on steroid inhalers) but a nearly 90% decrease in costs of emergency room visits and hospitalizations [49]. However, hospitals have large sunk costs invested in the buildings and personnel. Unless downsizing occurs as a result of the intervention, much of the estimated healthcare savings calculated as a benefit will not be realized in practice. Finally, discounting is often included in the economic analysis to account for the loss of income if the money spent (or saved) had been invested. Unfortunately, there is no way to assess accurately the discount rate for each study [50]. The rate of 5% is most often used, but rates from 0 to 6% have been employed. The rate selected may have an important impact in the proposed benefits gained from an intervention.

Cost-effectiveness analysis is used to compare the cost per outcome when the effectiveness of two programs differs, but the same outcome measure is used. Cost-utility analysis is a variant of cost-effectiveness when the measure of effectiveness is assessed and valued by the patient. Outcome measures that have been used to study effectiveness of an intervention include health-related quality of life (HRQL) scales [51], generic health status measures, such as the Sickness Impact Profile (SIP) [52], and the Short Form with 36 Items (SF-36) [53]. Many studies use disease-specific health status measures instead of or in addition to more generic scales. Patient preferences are sometimes used to determine which intervention is better suited to achieve the same outcome. However, there are many methods used in such utility measures, standardized to a single weighted measure, a quality-adjusted life-year (QALY) [54]. Most scales are selected as the choice of the author, not the patient [50]. Most commonly study- or disease-specific rating scales are used (65%), followed by generic systems (21%) and time trade-off (17%) [50]. The last asks the patient to choose between a less healthy longer life or a shorter life in better or perfect health, such as for home total parenteral nutrition (TPN) patients [55]. Although cost-effectiveness analysis is recognized as not reflecting every important aspect involved in healthcare decisions, the information is used widely to decide about healthcare resource allocation [56]. Hence these data should be as excellent as possible. The physician must be aware of the methods used and how the analysis is performed, because "Any (decision) option can be made to look cost-effective if it

is compared to a sufficiently cost-ineffective alternative" [57]. The "straw man" so well known from clinical studies is equally as prevalent in cost-effectiveness studies. The best rule for using cost-effectiveness studies for decision-making is to use only those options whose incremental cost-effectiveness ratios are lower than all other more expensive options that produce similarly good outcomes.

Cost–benefit analysis measures costs and benefits in dollars to assess the overall economic value of a program in which effectiveness and outcomes are measured by different parameters. Cost-effectiveness analysis treats all patients as having the same value for their health and does not convert quality of life measures into monetary terms. Translating these effectiveness measures into economic equivalents for cost–benefit studies introduces yet another layer of difficulty into the analysis. Benefits have traditionally been valued in ways that the insurance-like character of public programs and private payers understand. Thus, a value of benefit from an intervention usually equals the sum of the willingness to pay of all the persons whose welfare is affected. In such an economic paradigm, all measures of benefits (e.g. additional wages, years of life) are only valid if they are proxy to the willingness to pay. Thus, economists acknowledge the assessment methods for utility (based on consumer satisfaction with commodities or outcomes) that consider economic trade-offs (e.g. time trade-off, standard gamble). On the other hand, healthcare researchers prefer relative rankings without immediate economic relevance (e.g. symptom rating scales).

Because it is less difficult, cost-effectiveness analysis has been used more often than cost–benefit analysis (e.g. [58,59]). However, there are significant problems inherent to cost-effectiveness studies that should be understood by all who use their results [57]. Because such studies often access variables from created or existing patient record databases, which have built-in biases, omitted factors may be confounded with treatment or other variables. The cost-effectiveness ratio is measured usually at one point in time and is not usually related to population parameters. As noted above, certainty about costs and values of benefits is unwarranted. Sensitivity analysis performed to identify factors that produce variance is often incomplete. Both cost-effectiveness and cost–benefit analyses address only the absolute costs, and not whether the benefits are worth the cost. Neither takes into account cost shifting, or variations in the delivery of healthcare. Finally, the power of the studies is often too low.

A few of these points are worth mentioning in more detail. The outcome measures used have ranged from randomized controlled trials (RCTs) (level 1) to opinions by experts (level 3) [60], but most involve RCTs. Thus, their conclusions may not be applicable to a wider patient population. Cost shifting has been well demonstrated when comparing population costs of smokers versus non-smokers [61]. Although cost savings were estimated for the 45–70 year age group, most of these costs would probably be shifted to the surviving patients over age 70 years. The power of a study becomes important when one realizes that there is no absolute cut-off to determine what degree of cost savings is clinically meaningful. There is no *a priori* reason for assuming that 10–15% differences, as usually applied to clinical problems, would be reasonable either economically or clinically [62]. Moreover, to achieve a 10–15% difference in costs often requires a very large sample, because cost data are likely to have large variances, to be highly skewed and to require log transformation [63]. Thus, the sample size to achieve a meaningful cost difference may be much greater than that needed to find a clinically important difference. More care needs to be taken in prospectively deciding what size of cost difference would be worth achieving and what study power would be needed to achieve such a difference, based on estimates of the variance in the cost projections. There is no agreed cut-off number to determine when an intervention is cost-effective, but looking at the decisions made by the National Institute for Health and Clinical Excellence (NICE) in the UK, one can infer that an intervention is recommended if the cost is <£30,000 per QALY gained [64]. In the USA, the figure of $50,000 per QALY gained is often used. Even when cost-effectiveness data are available, e.g. on drug usage, these data are often not applied, because they cannot be properly evaluated or do not seem to apply to the setting in which they might be helpful [65].

Factors that influence drug use and cost

The high cost of biologicals has led more healthcare organizations in the USA to use the guidelines of the Academy of Managed Care Pharmacy, because economic analyses are incorporated into the analysis of these guidelines. The Veterans Health Administration, state Medicaid programs and many managed care organizations use these guidelines. It is worth noting that the Center for Medicare and Medicaid Services does not use cost-effectiveness analyses in their decisions regarding funding [66]. A number of studies have examined the cost-effectiveness of anti TNFα preparations. Medicare policy covers infliximab reimbursement and in addition allows purchase of the drug from the physicians and provides an infusion fee for the physician (BCBS Rhode Island 2006; see www.bcbsri.com/BCBSRIWeb/plansandservices/services/medical_policies/TumorNecrosisFactorTNFinhibitors.jsp). The other antibodies (adalimumab, etanercept) are FDA approved, but must be purchased at a pharmacy and subject to applicable co-payments. It is not surprising, therefore, that rheumatoid arthritis patients with public (Medicare) insurance received infliximab preferentially to those

without Medicare/Medicaid coverage [67]. This reflects an advantage for the patient as well as for the physician. When cost-effectiveness of the preparations was examined, all three biologicals (infliximab, etanercept, adalimumab) were cost-effective, using the ceiling of $50,000 per QALY gained, but not all studies fell below that ceiling [64]. The two studies with Crohn's disease that were accepted into this analysis showed cost-effectiveness that was not acceptable, $355–377 per QALY gained for symptomatic perianal fistulae [68] and $78–933 per QALY gained for moderate–severe disease resistant to standard therapy [69].

Results in other countries have been similar. Infliximab and etanercept were shown to provide cost-effective benefits for patients with rheumatoid arthritis in the UK and Sweden [70]. However, the range of cost per QUALY varied by 10-fold and the largest component of costs was indirect (productivity lost). Many of the other cost-effectiveness analyses do not measure indirect costs and so may have exaggerated the cost per QALY. Another source of variation in cost is the managed care costs for a closed system (e.g. patient administration, medical follow-up, transportation costs) in addition to the cost and administration of the drug. In three centers in France this variation was from 16,000 to 24,000 [71]. One advantage of comprehensive cost-effectiveness analysis such as that performed by NICE is that use of the drug is linked to utilization guidelines. When these guidelines for use of anti-TNF therapy were followed in the NHS in Northern Ireland, clinical practice was more uniform [72]. Moreover, such guidelines were observed and agreed to by colleagues in the Republic of Ireland that has no proscribed rationing. Thus, adherence to guidelines may possibly occur independent of financial restrictions. Issues involving physician implementation of DM and of practice guidelines are discussed in the following sections.

Why is Disease Management important for physicians and vice versa?

The major reason behind the need for implementing DM is the variation in physician practice. There is much information to be used, but even the relatively small number of evidence-based decisions that can be made are not necessarily widely or uniformly applied. When physician variation is quantified, as for example by indices related to total medical expenses, a wide scatter with a range of ±30% was found [41]. Lack of knowledge of the drug was the major reason in prescription errors by physicians [4]. Even diagnostic precision should be improved, as reflected by the fact that the ability of clinicians to achieve consensus on a diagnosis from a given data set is limited [73]. Even using similar historical criteria, the probability of a given diagnosis may vary greatly depending on the clinical setting [74]. Therefore, any system that improved such variability should be welcome.

The reasons for practice variation are multiple [75]. There are first and foremost variations in the characteristics of the patients. The variability of the physician/provider includes not only how the physician responds to these characteristics, but also what differences there are locally in availability and/or access to services and what local differences there are in thresholds for actions. These provider-related issues involve processes that could be made more efficient by computer assistance, although the acceptance of such assistance by physicians depends heavily on the ease of use of the interface [76]. The need for local modification of these processes, however, is easily forgotten when introduction of DM programs is widely offered.

DM is best suited to decision points that are supported by evidence-based medicine [35]. However, doctors do not make most decisions based on "hard" scientific evidence. They also retain a moral commitment to intervene on behalf of their individual patient and not in response to a population-focused best-case scenario. Finally, personal intuition and physician expertise are encouraged for their advantages in professional esteem and marketplace advantage. After all, if all physicians looked and performed the same, what would there be to choose between them? "The model of the clinician . . . encourage(s) individual deviation from codified knowledge on the basis of personal, first-hand observation of concrete cases. This deviation is called 'judgment' or even 'wisdom' " [77]. The healthcare system needs to change, in part due to increased inefficiency, but also due to increased cost and information overload. The problem has been stated clearly, but it is unclear how this is to be accomplished. "For the organization to break even they have to somehow control what the individual doctor does. The real trick is, can those entities . . . find collegial methods to sort of control utilization (of medical resources) without the rancour?" [78].

Decision-making in Disease Management needs accepted guidelines

The process of DM consists of four main steps: (1) identify the target patient group, (2) intervene to introduce preventive or curative treatment according to validated therapeutic guidelines and/or best evidence, (3) educate to improve physician performance and patient compliance and (4) measure outcomes to ensure that the result is beneficial (Table 22.1). Typically, guidelines are produced by medical experts, but as local practice may vary in many ways (including those that do not alter the medical decision), these guidelines must be locally adapted and adopted. However, the existence of guidelines does not

easily translate into a useable platform for DM programs. More than 2500 guidelines exist, most of them produced by special interest groups (e.g. national societies) and they have not been demonstrated to have an impact on medical practice. It is clear that guidelines are not completely objective documents, aside from bias or self-interest of the societies producing them. Judgment is unavoidable in making decisions about guidelines where facts are not sufficient [79]. Moreover, when clinical experience does not match published evidence, experience and beliefs usually dominate. This action is understandable, but means that all guidelines are flawed and especially so if there was an anticipated regulatory use for them. General practice groups (in Canada, for example) have made a number of initiatives to translate this mass of information into usable form. First, the Ontario Medical Association has formed a Guideline Advisory Committee (GAC) that produces a list of priority topics, the guidelines are obtained and then they are judged by a guideline-scoring instrument to choose the top guideline for that topic. In this way, 60 guidelines have been identified and synopses of the guidelines with the reference placed on the GAC website, www.gacguidelines.ca. Another approach has been to use the Delphic method to identify performance indicators for family practice in Ontario [80]. High consensus could be reached in only ~50% of the indicators studied. However, this approach would combine guidelines with DM decision nodes and/or pay-for-performance financing. These or related approaches need to be taken to the formation of practice guidelines by specialty societies, because many of those guidelines produced up to 1998 were not adequately developed [81]. Some specialty groups are responding to obtain new data regarding the benefit of guidelines. Nephrology groups and pharmaceutical companies have initiated the ACORD (Anaemia CORrection in Diabetes) and IRIDIEM (Individualized Risk profiling in DiabEtes Mellitus) studies to assess the impact of best practice guidelines that support early intervention and aggressive treatment of hypertension, hyperglycemia, proteinuria, hypercholesterolemia and anemia [82]. The World Gastroenterology Organization (WGO-OMGE) has established a task force to identify existing guidelines, select topics and panels that would represent caregivers in the appropriate countries and deliver and implement those guidelines [83]. This task force correctly identifies the fact that "guidelines are not an end in themselves but a means to improving clinical care".

What is the current status of guidelines for IBD? The British Society of Gastroenterology has published guidelines for management of IBD in adults [84]. The American College of Gastroenterology (ACG) has updated its guidelines for ulcerative colitis [85]. The American Society of Colon and Rectal Surgeons has published practice guidelines for the surgical treatment of ulcerative colitis [86]. Guidelines in Crohn's disease have been more confused and the new classification of IBD at the 2005 World Congress of Gastroenterology has been viewed as a positive step in creating a consensus [87]. However, many controversies still exist and it may be another decade before a consensus classification is achieved [88]. At the same time as this classification was being worked out, the European Crohn's and Colitis Organization (ECCO) produced its consensus on the management of Crohn's disease [89].[1] Perhaps because of the ferment in the field, the AGA and the ACG have not published recent guidelines. The ACG guidelines for management of Crohn's disease in adults date from 2001 [90]. The AGA Clinical Practice Committee has limited its scope thus far to perianal Crohn's disease [91]. Few of these guidelines have been prospectively assessed for their effect on outcomes. When the ACG guidelines for Crohn's disease and ulcerative colitis were used as the standard of care, it was found that patients referred to a specialty center often did not receive "optimal" care [92]. This may be in part because the diseases and decisions regarding them are very complex, but the art of communicating with the patient is equally so [93]. Hence there will be many variations in judgment during such studies, even if guidelines are agreed upon and implemented. When the target for decision making is much more restricted than the entire illness, the chances for improved care are better. When the AGA and ACG guidelines for diagnosis and management of osteoporosis in patients with IBD were used to identify patients at high risk, they detected patients with a high incidence of osteopenia [94]. However, it was not clear how much better the detection would have been if the guidelines had not been used.

Does Disease Management work when guidelines are applied?

Developing guidelines for best practice in a given condition is not sufficient to produce a system in which good decision-making can be maximized. NICE commissioned a study to examine the published guidelines to determine the quality of decision-making models that could be used in assessment of health technology [95]. This process of decision-making modeling is complex and requires rigor in planning and execution (Table 22.3). However, such rigor will be necessary if the role of guidelines is to be properly evaluated in determining outcomes and costs of healthcare interventions. Comprehensive computer-based decision support systems (e.g. the Texas Medication

[1] In 2009 the World Gastroenterology Organization published a consensus summary for diagnosis and management of IBD (Bernstein CN, Krebsheier FM, Cohen H *et al.* Guidelines for the diagnosis and management of IBD in 2010. *Inflamm Bowel Dis* 2009, epub ahead of print, doi 10.1002/ibd.21048). This report was based in part on the consensus reached for IBD practice in China, published in 2008 (Ouyang Q, Hu PJ, Qian JM *et al.* Consensus on the management of IBD in China in 2007. *J Dig Dis* 2008;**9**:52–62).

Table 22.3 Attributes of good practice in decision-making modeling.

Dimension of quality	Attributes of good practice
Structure:	
Statement of decision problem	Objective should be defined Decision-maker should be identified
Statement of scope	Relevant costs and consequences clear Scope should be specified and justified Outcomes consistent with objective Iterative approach to including clinical events
Rationale for structure (guidelines used here)	Structure consistent with health condition Treatment pathways reflect biology of disease Not dictated by existing service provision Structure consistent with existing evidence
Structural assumptions	Transparent and justified Match needs and purposes of decision-maker
Strategies	Explore all options Justify excluded options
Data:	
Data identification	Justify any choices for specific data input Systematic, not necessarily comprehensive
Baseline data	Based on natural history Probabilities should be calculated properly
Treatment effects	Use of meta-analysis where proper Justify choice of analytic methods
Quality of life weights	Use appropriate measures

Data from [95].

Algorithm Project) have been developed for depression that provide support in areas of diagnosis, treatment, follow-up and preventive care [96]. This system has reported early encouraging results in the setting of primary care [97]. Similar programs have been developed in Berlin (Berlin Algorithm Project) and for treatment-resistant depressed patients (STAR*D) [98]. A study using guidelines applied only to data capture has been applied to care of IBD patients. The guidelines used were the Mayo Practice Guideline Score (MPGS), a 15-point assessment of documentation of various aspects of care. This score was used at a tertiary medical center in the USA and the IBD Quality of Life score was used as an outcome measure [99]. The MPGS was higher in the intervention group, but the final IBDQ was not different from the control group, even though the IBDQ score was higher than baseline in both groups. This study shows that practice patterns of obtaining data can be altered by education, but a more complete decision-making model will be needed to assess properly the effects on clinically relevant outcomes.

There are many studies showing that the application of clinical guidelines does improve the process of clinical practice. Because the concept of DM was meant to affect populations of patients in a setting of comprehensive care, the majority of the studies have been confined to general practice sites and issues [100]. When outcomes are examined, these usually also improve, although the results of the studies are not particularly robust [101]. The "bottom line" to be extracted from these studies is that DM *can* work in office practice and that when it does, physicians appreciate the results [102].

The meta-analyses of Grimshaw and Russell [101] combined the application of guidelines to assessment of both physician compliance and patient outcome and were carried out in a general medical or primary care setting. These studies focused on hypertension and diabetes mellitus, conditions with well-publicized guidelines and fairly standard outcomes measures and ideal for DM. Studies varied in size, although many have been fairly large. A highly structured continuing education program to impart guidelines regarding management of burns in the emergency room was employed and demonstrated a benefit among patients admitted to hospital in decreased early complications (30 vs 45% among patients whose physicians were not instructed) [103]. This was a large study involving 298 physicians and over 2500 patients.

Sixty physicians were provided not only with computer reminders, but also on-line information on treatment protocols and their patients' blood pressure percentile [48]. In this study of over 3000 patients, such reminders led to a diastolic pressure of <90 mmHg for more days out of the year (323 vs 255 days for controls). Another review of many studies showed a wide variation between countries in the ability to reach the blood pressure standards of that country [30]. In diabetic studies, improvement in physician compliance was marked. Compliance in the ICU produced better insulin dosing, but only modest changes in the frequency with which targeted blood glucose concentrations were achieved [104]. Glucose control in the ICU was improved using nutrition support guidelines, but the more complex outcome of enteral nutritional adequacy was not improved by the use of educational and web-based tools [105].

Preventive medicine is another area in which guidelines should produce non-controversial applications and improved results. Computer-based reminders to 115 physicians (12,467 patients) led to better compliance in providing occult blood testing and mammography for cancer screening, weight reduction diets and programs and influenza and pneumococcal vaccines, compared with controls [106]. Moreover, hospitalizations and emergency room visits were decreased among the vaccinated patients. Studies in the UK on smoking cessation used short courses to physicians to improve delivery of information to the patient [107]. In all studies, it was clear that continuing educational programs substantially changed the way in which physicians counseled smokers. As a result, there was a small improvement in long-term abstinence, especially among patients who wanted to quit. However, in the USA, where patterns of practice tend to be more variable, computer prompts about smoking advice were noted by physicians, but referrals for treatment were not as frequent as recognition of the prompts [108].

Although DM is considered usually only for chronic conditions, programs have been applied successfully to medically self-limited conditions. The North of England Study is unique in that the GPs in the study developed their own guidelines, which were mailed to all physicians, but only the study group had educational programs related to them [109]. Although the control group that received guidelines may also have improved its performance, there was still improved compliance in the study group and in one condition (recurrent wheezing) there was clear improvement in outcome.

Depression is a disorder also amenable to DM, as it is underdiagnosed and treated. After establishing baseline data, the Swedish Committee for Prevention and Treatment of Depression launched an educational program for the diagnosis and treatment of depression for all GPs on the island of Gotland and the immediate effects were evaluated after 2 years. The direct benefits were fewer days in hospital and a reduced (by half) rate of suicides [110]. The amount of money saved depended on the value placed on a life, but however calculated, the indirect benefits of the program corresponded to up to a significant portion (>10%) of the total health expenditure. A study in five general practices in the UK also showed short-term benefit (6 weeks) on depression scores from computer-generated patient-specific guidelines, but the effect was gone by 6 months [111]. When computer-generated algorithms were used for 1 year, the benefit in depression outcomes was retained during the course of the study [97]. A review of 36 studies testing the effectiveness of organizational and educational interventions for treatment of depression has been reported, using examples from primary care settings [112]. Modest improvements in medication adherence and depression outcomes were found, although these benefits were no longer found to be so robust at a 24 month follow-up. Simple guidelines or educational strategies were not effective in most studies. The successful programs used nurse case management and more integrated care between primary physicians and consultants. Hence it would appear that management programs will have lasting effects only if they are repeated and/or continued.

Some studies on depression confirmed that after a brief educational program, primary care physicians improved recognition and treatment of late-life depression and demonstrated improved symptomatic control [27]. When the diagnosis was confirmed before the intervention was applied, the value of standardized pharmaco- or psychotherapy was dramatic in 8 months, with complete recovery of 70% in the study group versus 20% in controls [113].

Case management has been used successfully to produce better adherence to published dementia care guidelines, leading to better quality of life [3]. This study and the depression studies reviewed by Gilbody *et al.* [112] stress the concept of case management and the use of allied professionals, such as nurses and social workers. From these structures, it is apparent that the usual fee-for-service financing is not appropriate. The Vickrey study [3] was performed with a relatively homogeneous population of well-educated, non-institutionalized patients with health insurance. The variable results with depression studies do not provide a cause for the variability, but it is not clear yet whether these good results using case management approaches can work in other settings, nor is it clear how long such interventions will need to be provided. However, even if disease outcome were not altered in the long run, the programs might very well be valued for their ability to teach better adherence to good guidelines, save time or simply manage record keeping better. Although DM is difficult to develop and implement, the underlying concepts are sound and supported by the available evidence.

How can Disease Management best be implemented?

The most difficult task in DM programs involves implementation. Multiple methods and interventions have been used, even in a single study. A meta-analysis of multifaceted interventions in general practice (61 controlled studies) revealed that multifaceted interventions were most effective. Information linked to performance (feedback, physician and patient reminders) was the most effective single intervention [114,115]. When three or four interventions were used together, the strategy was nearly always successful, although the number of studies was small in that group and does not allow a firm conclusion. The same group reported subsequently on 235 studies showing a wide range in positive results, both within and across interventions [116]. In this larger analysis, there was no relationship between the number of component interventions and the effects of the interventions (Table 22.4). Another review examined 17 controlled trials estimating the effect of interventions on the referral of outpatients to secondary care [117]. Effective strategies included dissemination of educational materials either with a structured referral sheet or using consultants for educational activities. What did not work were uni-dimensional techniques, such as distribution of referral guidelines or discussion with an adviser. A review of 39 studies in chronic care management of diabetes found that 32 of the studies showed improvement in at least one process or clinical outcome measure due to the intervention [2]. However, because of the small number of studies, it was not possible to say whether a greater number of interventions led to greater effectiveness. Hence there is not a sufficient database on which to base decisions about how well guideline dissemination or implementation strategies will work in different circumstances. In addition, the data do not agree whether single interventions provide as much

Table 22.4 Effectiveness of strategies for implementing changes in primary/secondary care.

Strategy used	Studies (No./significant*)	Improvement	
		Strength	Median (range) (%)
Single vs no intervention:			
Educational materials	18/0	Modest (n.s.)	8.1 (3.6 to 17)
Educational meetings	3/0	None	−3.6 (1 study)
Audit and feedback	10/1	Modest	7 (1.3 to 16)
Patient-directed intervention	7/0	Moderate/large	20.8 (10 to 25.4)
Reminders	38/3	Moderate	14.1 (−1 to 34)
Multifaceted vs no intervention:			
Education + outreach	68/1	Modest	6 (−4 to 17.4)
Education material/meetings	10/0	Small/modest	1.9 (−3 to 5)
Education + audit/feedback	4/0	Modest	7.4
Reminders + patient-directed intervention	6/0	Moderate/large	(1.3 to 20)
Combination of 3	14/0	Small	(1.4 to 3)
Multifaceted vs intervention controls:			
Reporting dichotomous process	55/0	Small	3.9 (−5.7 to 33)
Reporting continuous process	18/0	Mixed	(−20 to 257)

*Versus no intervention.
Data from [116].

benefit as multiple interventions. The strategy to keep the intervention simple seems reasonable; the challenge is how to link interventions together into a meaningful pattern to deal with an entire disease.

Computer methods are enablers of DM concepts, but are not essential to the concepts themselves. However, many of the studies in DM have used electronic media to provide either information or reminders. A few have even used the computer to compile or calculate patient data that are then fed back to the physician or provider. A review of such studies using computer-based clinical decision support systems (CDSSs in current jargon) in 1994 found 29 controlled trials [118] (Table 22.5); a similar review in 1998 found 65 papers that examined the effect of CDSSs [119] and one in 2006 reported on 257 studies [120]. The use of computers, however, does not ensure a better outcome. This may be related to the fact that there is a wide range of factors that affect changes in clinical practice and none was dominant [121]. The most frequent reasons for change were organizational (e.g. hospital management, staffing, improved services), education, contact with professionals, availability of technology and clinical experience. The use of computers could be considered either as a new technology or as an organizational change. Other reasons for change, such as education and contact with professionals and patients, can also be delivered through the computer. Hence the computer has a great potential for changing practice.

Table 22.5 Computerized decision support systems can improve clinical performance and patient outcome (1982–1992)

Strategy used	Results of 29 PRCTs	
	Improvement	No improvement
Computer-assisted diagnosis	1	4
Computer-assisted drug dosing	3	1
Preventive care reminders	4	2
Computer-aided active medical care	7	2
Fewer errors in test ordering	3	0
Response rate to clinical events	3	0
Adherence to hypertension protocol	1	2
Effect on patient outcome	2	3

Modified from [118].

Information provided by computer is more accessible, more thoroughly indexed, potentially more up-to-date, more linked to related data sources and can be incorporated into a local decision support system that includes decision-making advice and warnings. Thus, it is good at identifying patients, at intervening by providing pathways and reminders, at educating patients and physicians to maintain the program and at measuring outcomes: all the key features required for a DM program. As with all DM programs, such a system would not dictate decisions, but would provide the basis for making more consistent and logical decisions. The electronic medical record (EMR) is not an essential part of a computer-based program, but it can make the practice of medicine more efficient. The fully implemented EMR can be interfaced with the laboratory, enabling transfer of reports directly to the record. Prescriptions can be transmitted directly to the pharmacy at the time of the patient's visit and the pharmacist can confirm that the prescription was filled and picked up. Referral

letters can be generated automatically, based on the data from the most recent patient encounter. The record can be programmed to note potential drug interactions and can produce patient recall letters for preventive services. Patient educational information can be provided automatically at the end of the patient visit. Also, the potential of this EMR can increase markedly once patients also come on-line and the confidentiality concerns are overcome.

Even without an EMR, there is strong evidence that CDSSs can improve physician performance, as shown in Table 22.5, summarizing studies up to 1992 [118]. Other studies have utilized the computer outside the doctor's office, a setting in which its use is much more familiar. For example, the pharmacy can track antibiotics during acute hospitalization and produce reduction in overdose, allergies, mismatches and adverse events [122]. Anticoagulation is another area of drug control in which nurses can provide excellent follow-up via computer, leading to a longer time in the therapeutic range [123]. In the primary care setting, regulation of anticoagulation using a CDSS has been remarkable, with acceptable INR control from 23% of the time to 86% [124].

Applying evidence from these clinical trials to the care of individual patients is a very large challenge [102]. Knowing when results obtained from the process of "evidence-based medicine" apply to a given patient still requires a great deal of skill and experience and must be individualized. Hence guidelines for deciding how and when this should be done can only be process driven, with or without computer assistance. This is true even in system such as the NHS in the UK, where such implementation is now a national priority. Evidence-based indicators linked to interventions that improve outcomes have been suggested [125] as an adjunct to primary care practices to help them find a way to succeed. Over the last 40 years, decision support systems have developed and have proven to improve the organizational aspects of clinical practice. The four features of these systems that predicted improvement were the automatic provision of decision support as part of the clinical workflow, providing recommendations and not just assessments, providing the support for decision-making in real time and place and the use of the computer for delivering the support [126]. Another large review was performed examining the effect of health information technology (electronic medical records, decision support, electronic results, electronic prescribing, consumer health information, knowledge retrieval systems, etc.) on the quality and efficiency of healthcare [120]. Of 257 studies, about 25% derived from four academic institutions that had internal systems and only nine others involved multifunctional commercial systems. The major benefits found were increased adherence to guideline-based care, better surveillance/monitoring and decreased prescription errors. Decreased utilization of care was documented, but the economic benefit of such a change was incompletely documented. This review emphasized again the patchy nature of the comprehensive approach to decision support and to the lack of evidence for generalization of the systems. The effect on organizational benefits was also confirmed, but improvement of clinical outcomes was not well documented.

Indirect evidence suggested that systems that used computers should provide the best chance to show an effect on clinical outcomes. However, when this hypothesis has been examined, the answer has been surprisingly inconclusive. A review of 100 studies up to 2004 that documented both practitioner performance and clinical outcomes found that the former was improved, but the effect on outcomes was inconsistently studied and the results when studied suggested little effect (Table 22.6)

Table 22.6 Effect of computerized decision support systems on practitioner performance and patient outcomes (1976–2004).

	Improvement in 100 studies*			
	Practitioner performance (97 trials)		Clinical outcome (52 trials)	
Strategy used	Yes	No	Yes	No
Diagnosis	4	6	0	5
Cancer screening, other prevention	16	5	n.a.	n.a.
Diabetes management	5	2	0	3
CV management and prevention	5	8	1	11
Other active health conditions	6	3	3	5
Prevention of unnecessary healthcare utilization	7	1	1	3
Anticoagulant dosing	8	5	2	6
Drug dosing and prescribing	11	5	0	11

*Improvement is defined as a statistically significant positive effect on at least 50% of the outcomes measured. Most studies had inadequate power to detect a significant improvement in patient outcomes. n.a. = not available.
Data from [127].

[127]. Another review of 26 studies using computer-based patient record systems (CBPRSs) performed between 2000 and 2003, however, showed no more consistent effect on clinical outcomes [128]. The best results were for the use of CBPRSs on preventive care. Positive experiences/satisfaction from either physicians or patients were reported no more often than in studies showing no benefit. None of the six studies analyzing the effect of CBPRSs on clinical outcome showed a benefit.

What is the evidence for Disease Management programs in IBD?

The process of establishing a meaningful DM protocol takes considerable commitment and energy [129]. In addition, the complexity of the disease has left most patients cared for by specialists, who, because of their better education in dealing with fewer diseases, may feel less need for such programs. Published studies comparing the process of care between gastroenterologists and GPs have revealed that gastroenterologists do deliver better care for gastrointestinal bleeding and diverticulitis and in general provide better, more accurate diagnosis in other disorders [130]. No such comparisons have been published for the treatment of IBD. However, one study tested a training program for consultants in 19 centers in the UK to provide a patient-centered approach to care [131]. A guidebook for patients was developed with patients prior to the study and a written self-management program was developed along with improved access lines for patients to obtain advice. After 1 year there were one-third fewer hospital visits and 20% fewer clinical relapses. Cost-effectiveness analysis favored patient self-management over standard care. Drugs account for about 6% of the total cost of care in Crohn's disease [19]. Infliximab has been shown to be potentially cost-effective over 6 months by reducing the length of hospital stay and use of diagnostic procedures [132]. Longer studies will need to confirm these data. In addition to the studies regarding the use of anti-TNF antibodies [64,72], the use of azathioprine or methotrexate was found to be cost-effective in New Zealand when compared with no immunotherapy [133].

Studies of patients with ulcerative colitis show that annual surveillance if dysplasia is diagnosed, coupled with colectomy, has a cost-effectiveness ratio (incremental) similar to that for cervical cancer screening [134]. A mailed brochure improved appointment keeping for colonoscopy [135], consistent with the expected benefits from organizational improvement. However, colonoscopy done at centers that offer an integrated healthcare delivery system still produce complications and at a rate at least as high as that for other screening procedures [136]. A cost analysis of the role of colonoscopy versus sigmoidoscopy for initial evaluation of ulcerative colitis has shown that, based on the physician preference for knowing disease location, colonoscopy is most cost-effective [137]. However, the real question of how important it is to know precise disease location was not addressed.

Nutrition is another area in which DM related to IBD has been studied. In a single gastroenterology ward in Berlin, a standardized intake approach identified more patients with malnutrition, leading to earlier nutrition support intervention and allowing more rationale estimate of costs [138]. Improved healthcare quality was not studied. One must always be alert to ask if the outcome will really be affected by the intervention. The same is true for a study analyzing lifetime cost–utility for patients with inactive Crohn's disease [139]. A small incremental benefit for mesalamine was found using QALYs as the utility measure, but also a small incremental cost. The conclusion was that long-term maintenance should not be discouraged on a cost–utility basis. More correctly, the authors pointed out that the real question is the long-term prognosis of such patients. After all, if mesalamine has no long-term benefit or if only patients with clinically active disease are benefited, then a population-based study will not be helpful. The same kind of careful clinical questions have been raised in a study of the cost of endoscopic screening for intestinal precancerous conditions [140].

One factor necessary for a successful DM program is the identification of a homogeneous group of patients. In addition to all the problems in correctly classifying IBD patients, there is the issue of whether irritable bowel syndrome (IBS) can occur in patients with IBD. There seems little doubt that IBD patients can have symptoms similar to IBS, but whether they have two distinct disorders is not at all clear. There can be a long prodromal period of gastrointestinal symptoms before the diagnosis of IBD is made. One study showed that this prodromal period was longer for patients with Crohn's disease than for ulcerative cholitis [141], whereas another found no difference according to diagnosis [142]. However, the presence of symptoms does not diagnose either disease and the diagnosis of IBS relies on the recurrent occurrence of symptoms, including abdominal pain. It is difficult to determine the precise nature of these symptoms in retrospect, as has been done to this point. Reviewing the data has led some experts to accept the presence of both diagnoses and to recommend different treatments for both disorders in the same patient [143,144]. The problem is not so troublesome when the symptoms precede the diagnosis of IBD, as it is known that inflamed bowel can escape detection by imaging or endoscopy. When the diagnosis of IBS is given to a patient in presumed remission for IBD, the issue becomes more complex. Should this trend continue, inclusion of such patients (with both diagnoses) in IBD care management programs may confuse the definition of the patient

population and the ability to determine treatment-related outcomes.

Conclusion

What are the reasons why there has been so much difficulty in implementing computerized practice guidelines for long-term care of chronic diseases? The reasons are as many as the issues are complex. Initially physician skepticism and lack of computer expertise seemed important, but these obstacles have been largely overcome in highly computerized societies. Surely the need for multi-step programs is a major factor. When programs are established, the major stumbling blocks are usually related to workflow integration [145]. While these issues may seem soluble, there are others that may not succumb to DM, whether computer based or not. One such problem is that of competition between physicians. DM requires group rationality with cooperation between services, yet behavior emphasizing individual advantage is common in medicine [146]. How to resolve this dilemma is unclear. Perhaps as more knowledge accrues and care programs become more evidence based, there will be less room for individualism. However, this process will take time and is not necessarily all to the benefit of the patient. Most DM programs assume a level playing field for data capture, but that does not take into account the art of history taking and clinical observation, skills that separate one clinician from another, and will probably always do so. Another (related) problem is that of clinical uncertainty, on the part of both the physician and the patient [147]. This uncertainty has been evidenced in the production of guidelines, where clinical experience usually overcomes evidence, when the evidence is conflicting [148]. When such uncertainty is carried forward in programs that appear to allow relatively restricted choices, it is not surprising that it becomes difficult to document improvement in such a difficult area as clinical outcomes. One is left with the conclusion that the profession cannot give up on concepts of DM, as they are logical and attempt to be evidence based. However, one must also realize that we may have to settle for modest benefits such as practice organization and data retrieval.

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Chapter 23
Outcomes, Disease Activity Indices and Study Design

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Summary
- In clinical trials, randomization minimizes the chance of bias from imbalance of known or unknown confounding factors.
- A broad range of outcome measures have been developed for IBD clinical trials, particularly in ulcerative colitis.
- Choice of the type of outcome measure, the definition of response or remission, the allocation distribution and the placebo or comparator response rate all affect the efficiency of the trial.
- Most pre-marketing clinical trials are too small to detect rare but serious adverse effects.
- Because of the limitations of clinical trials to answer safety questions fully, observational studies are often used to assess the safety of drugs and devices.

Introduction

Clinical studies that assess pharmacological therapies are frequently classified as Phase I, II, III or IV. Phase I trials are usually dose-finding studies intended to obtain safety data at various doses. Phase I studies are typically the first time that the medication is consumed by humans. In this type of study, varying doses of a drug are assessed for the maximum dose tolerated. In addition to dose finding, another objective of phase I studies may be to evaluate the treatment mechanism.

The primary purpose of a Phase II study is to assess preliminarily the efficacy and safety of a drug at a fixed dose or doses. Information from a Phase II study provides the probability of benefit of a drug and additional safety data. Results from a Phase II trial provide information helpful in deciding whether a Phase III trial should be pursued and, if so, what dosing regimens should be employed.

The pivotal Phase III clinical trial compares a new treatment of interest with other therapies or placebo with the primary purpose of determining the efficacy of a new treatment. For regulatory purposes in the United States, two Phase III studies are usually required. Phase III studies may test the superiority or equivalence of a new treatment to a control group.

A Phase IV study is a post-marketing study (i.e. completed after the drug is approved for marketing). Phase IV studies may be needed to assess the relationship between a new medication and side effects, to assess for new indications or to test new dosing regimens. The design features for Phase III and IV clinical trials will be the primary focus of this chapter.

Randomization

To avoid selection bias, a form of treatment allocation employed in clinical trials is randomization of subjects to treatment groups. By randomizing subjects to treatment groups, the investigator avoids inadvertent or advertent selection of subjects by severity of illness or other prognostic factors to a specific treatment arm. When the trial is sufficiently large, randomization helps to assure that the study groups are balanced on both known and unknown confounding factors. This helps to assure that any difference observed between the treatment groups is a result of the intervention and not some other factor.

There are several methods for randomizing subjects. Simple randomization, i.e. "coin flip", may lead to an imbalance in the number of subjects allocated to treatment arms, particularly in small studies. To assure balance in the number of subjects in the treatment arms, other methods of randomization may be employed, such as constrained randomization and stratified randomization [1]. Constrained randomization is also known as "block randomization" and the block sizes are small multiples of the number of treatment arms (two treatment arms, block sizes = 4, 6, 8, etc.). Randomization of treatment occurs within each block, such that with a block of four subjects in a two-arm

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Table 23.1 A randomization scheme of two treatments, two strata and block size of four.

Subject ID	Stratum (sex: female = 1, male = 2)	Treatment (treatment = T, placebo = P)	Block
1	1	T	1
2	1	P	1
3	1	P	1
4	1	T	1
5	1	T	2
6	1	P	2
7	1	T	2
8	1	P	2
9	2	P	1
10	2	T	1
11	2	T	1
12	2	P	1

trial, two patients would be randomized to each arm. In stratified randomization, separate randomization occurs within each stratum. In some trials, a randomized block design is utilized within each stratum; an example is provided in Table 23.1.

Blinding/masking

To avoid bias in assessment of outcomes, patients, investigators and analysts may be blinded regarding the treatment arm to which a subject is assigned. This is also referred to as masking. Single blinded usually refers to the patient being blinded to the treatment assignment, double blinded usually refers to the patient and the investigator being blinded to the treatment assignment and triple blinded may refer to the patient, investigator and analyst [person(s) assessing the data] being blinded.

Placebo versus active comparator

Having a control group in a clinical trial allows for comparison of patient outcomes between the new treatment arms. There are several types of control groups: placebo, no treatment control, different active treatment or different dose of new treatment. The choice of control group depends on several factors, most importantly the question that is to be answered in the trial. If effective therapy is known to exist for a particular disease, a placebo control arm may not be ethical and would necessitate an active drug control group. At times, even when an effective treatment is known, a placebo-controlled design may still be ethical if treating patients who are refractory to or intolerant of the available therapy. Similarly, for studies of maintenance therapy, all patients may be initially treated with the active medication. Then among responders, some are randomized to continue therapy and others to withdrawal of the active therapy (with or without a placebo).

The choice of type of control group is also based on the inference of the investigator. The investigator may wish to determine whether the new treatment is better than current therapy for active disease, which therefore necessitates an active control group. In studies assessing dose response and efficacy of new treatment, there may also be more than one control group in a study. For example, the high-dose group may be compared with placebo and with a low-dose group.

Sample size considerations

Several components determine the sample size of a clinical trial and include type of outcome data, the hypothesis to be tested (superiority, inferiority or equivalence trial) and error rate in study findings investigators are willing to allow.

Primary endpoint data can be measured as counts or continuous variables. Counts may be further divided into dichotomous, categorical, ordinal and counts over time. With dichotomous outcomes, one may also consider time to event or survival analysis.

Another factor contributing to sample size is the inference of the investigator for the new treatment compared with the control. Is the investigator testing superiority, inferiority or equivalence? By convention, clinical trials are designed to have 80 or 90% power (power $= 1 - \beta$, type II error or $\beta = 0.2$ or 0.1) to detect a difference between groups. This means that there may be a 20 or 10% likelihood of not detecting a difference when there truly was a difference based on having 80 or 90% power, respectively. The type I error $= \alpha$ is set at 0.05 and means that there is less than a 5% likelihood of detecting a difference between the groups as large as or larger than that observed when there truly is not a difference. By convention, the α level is set low to reduce the likelihood of a false-positive study. Typically, the sample size required for a given level of statistical power is greater for trials designed to test equivalence than trials that test superiority (discussed further below).

One of the most influential factors in sample size determination is the size of the clinically significant difference estimated between a new treatment and control. For example, in studies assessing the induction of remission of a drug compared with control, what are the expected remission rates of the new drug and control groups? Usually control data are based on prior studies and the new drug data from earlier clinical trials (Phase II). The difference in remission rates is a parameter applied to the sample size calculations (see Equation 23.1). In superiority trials (testing newer treatment is more effective than control treatment), the greater the difference in effectiveness

hypothesized, the smaller is the sample size needed for the trial. However, if the investigator selects a small sample size based on a large hypothesized difference in event rates, there is greater risk of missing a smaller but potentially clinically important difference (i.e. a type 2 error). Hence trials should ideally be designed to have sufficient power to detect the minimal clinically important difference.

$$\text{sample size} = \frac{f(\alpha, \beta) \times P \times (1 - P) \times 2}{(p_0 - p_1)^2} \quad (23.1)$$

where p_0 is the incidence in the control group, p_1 is the incidence in the treatment group, P is the average of p_0 and p_1, $f(\alpha,\beta)$ is a mathematical function of α and β, $\alpha = 0.05$ (type I error) and $\beta = 0.20$ (type II error).

Superiority versus equivalence trials

Many inflammatory bowel disease (IBD) pharmacological drug trials test the superiority of a new therapy compared with the control treatment, usually standard therapy or placebo. The null hypothesis is that the new treatment and the control treatment are not different, whereas the alternative hypothesis is that the treatments are different. However, a negative superiority study does not mean the treatments are by definition equivalent. The new treatment could be equivalent or even inferior to the alternative therapy.

At other times, one may wish to test whether two therapies are equivalent in terms of efficacy. For example, a drug or therapy may be developed that may be as effective as current treatment, but may have fewer side effects, cost less, etc. In this example, the investigator would need to design the trial for equivalence rather than superiority of a new treatment. A slight variation on equivalence trials is the non-inferiority trial, where one hypothesizes that the new therapy is equal or superior to the alternative therapy. Equivalence and non-inferiority trials usually require larger sample sizes than superiority trials.

Induction and maintenance trials

IBD, ulcerative colitis and Crohn's disease are diseases that relapse with waxing and waning disease activity. The overarching objective of IBD therapies is to induce a remission in patients with active disease and then maintain remission. Pharmacologic clinical trials for IBD may select IBD populations with active disease with the goal of inducing a remission or decreasing activity of disease (clinical, endoscopic and/or histologic). These trials are also known as induction trials. IBD populations with inactive disease (in remission) may also be selected for pharmacologic trials for the outcome of maintaining remission. These trials are also known as maintenance of remission trials. In these trials, a medication of interest may be given to maintain a remission. There are also medication withdrawal studies in which maintenance of remission is assessed after a drug is stopped.

Outcome measures specific to IBD trials

Disease activity may be assessed clinically, endoscopically and histologically. For research purposes, disease activity indices have been developed to provide an objective measure of disease activity. Although one could rely on the investigator or patient's opinion as to whether they have improved, this would potentially have low reproducibility either within the rater or between raters. Disease activity measures are an attempt to increase reproducibility within and between raters.

Crohn's disease

The most widely used clinical disease activity index for Crohn's disease is the Crohn's disease activity index (CDAI). The CDAI is a clinical activity index developed in the 1970s by investigators from the National Cooperative Crohn's Study [2]. Over 100 predictors of disease severity were tested in a multivariable regression model as compared to the physician's assessment of the disease activity. Eight variables were found to best predict the physician's rating of disease severity with coefficients simplified to a weight for each variable. Three of the variables are based on a 7 day patient diary. Table 23.2 provides the variables of the CDAI and scoring method. The scores range from 0 to 662. Score cutoffs were then correlated with physician global assessment (very well, fair–good, poor and very poor). A score range of 100–200 was found to be discriminating for patients with minimally active disease. The investigators chose the midpoint of 150 as the score cutoff to differentiate patients that they assessed as "very well" or in remission (<150) to patients that were "fair–good" (>150). Additionally, a score >450 was associated with a very poor physician global assessment or very active disease. As such, a CDAI score <150 is considered remission, 150–219 mildly active, 220–450 moderately active and >450 severely active [3]. In the development of the CDAI, a drop in the score also correlated with clinical response as rated by the physician investigators. The CDAI has been used in numerous trials and a decrease in CDAI of 70 or 100 points has frequently been utilized to demarcate response as a clinical endpoint in clinical trials.

There has been criticism of the CDAI since several variables are based on subjective responses, many of which

Table 23.2 Crohn's Disease Activity Index [2].

Variable	Descriptor	Score	Multiplier
Number of liquid stools	Sum of 7 days		2
Abdominal pain	Sum of 7 days ratings	0 = none 1 = mild 2 = moderate 3 = severe	5
General well-being	Sum of 7 days ratings	0 = generally well 1 = slightly under par 2 = poor 3 = very poor 4 = terrible	7
Extraintestinal complications	Number of listed complications	Arthritis/arthralgia, iritis/uveitis, erythema nodusum, pyoderma gangrenosum, aphthous stomatitis, anal fissure/fistula/abscess, fever >37.8 °C	20
Antidiarrheal drugs	Use in the previous 7 days	0 = no 1 = yes	30
Abdominal mass		0 = no 2 = questionable 5 = definite	10
Hematocrit	Expected – observed hematocrit	Males: 47 – observed Females: 42 – observed	6
Body weight	Ideal/observed ratio	[1 – (ideal/observed)] × 100	1 (not <–10)

overlap with irritable bowel syndrome. In addition, the CDAI employs a 7 day patient diary that is inconvenient to use in clinical practice. There is also inconsistency in how investigators score the CDAI [4]. Nonetheless, because of the validation work that went into the development of the CDAI and its long history of use, it is considered the gold standard clinical activity index for Crohn's disease.

The CDAI was developed in a population of adults and was thought to be inadequate for a pediatric and adolescent population because it contained several subjective items and excluded linear growth that may reflect disease activity in a pediatric/adolescent population. In the 1990s, the Pediatric Crohn's Disease Activity Index was developed. Changes to the CDAI included adding linear growth as a variable reflecting active disease, removing the use of antidiarrheals from the index, decreasing the weight of patient-reported symptoms (abdominal pain, general well-being and diarrhea) and adding more laboratory data. The variables in the PCDAI are listed in Table 23.3. The total score ranges from 0 to 100 with a score of <10 defining inactive disease, 11–30 mild disease and >30 moderate to severe disease [5].

Another clinical disease activity index, the Harvey Bradshaw Index (HBI), was created to simplify the CDAI [6]. This index utilizes 1 day measurements and excludes the variables body weight, hematocrit and use of antidiarrheals, which were in the CDAI. The final variables in the HBI are listed in Table 23.4 and the sum of the variables equals the total score. The variables are not weighted as in the CDAI. The total score ranges from 0 to 19 with an HBI < 5 defined as remission, 5–7 as mildly active disease, 8–16 as moderately active disease and >16 as severely active disease. Response has been defined as a decrease of 3, 4 or 5 points, whereas relapse has been defined as an HBI ≥5 and increase in HBI by 2, 3 or 5 points [7]. The

Table 23.3 Pediatric Crohn's Disease Activity Index [5].

Variable	Score
History (over 1 week):	
Abdominal pain	0–10
Stools per day	0–10
General well-being	0–10
Laboratory tests:	
HCT (stratified by age)	0–5
ESR	0–5
Albumin	0–10
Examination:	
Weight	0–10
Height	0–10
Height velocity	0–10
Abdominal examination	0–10
Perirectal disease	0–10
Extraintestinal manifestations:	
Fever >38.5 °C for 3 days over the past week, arthritis, uveitis, erythema nodosum, pyoderma gangrenosum	0–10

Table 23.4 Harvey–Bradshaw Index [6].

Variable	Score
General well-being	0 = very well 1 = slightly below par 2 = poor 3 = very poor 4 = terrible
Abdominal pain	0 = none 1 = mild 2 = moderate 3 = severe
Number of liquid stools daily	*N*
Abdominal mass	0 = none 1 = dubious 2 = definite 3 = definite and tender
Complications	1 point for each item = arthralgia, uveitis, erythema nodosum, aphthous ulcer, pyoderma gangrenosum, anal fissure, new fistula, abscess

HBI correlates well with the CDAI (the gold standard) although it is not as discriminating [7]. The advantage of the HBI is that it may be used for retrospective studies as opposed to the CDAI, which requires prospective data (i.e. 7 day diary information). In addition, the HBI is less labor intensive and costly due to the smaller number and type of variables in the index, which may render it more practical to use in prospective pilot studies.

The Perianal Disease Activity Index (PDAI) was created to measure the clinical severity of perianal Crohn's disease. The index has five categories: discharge, pain/restriction of activities, restriction of sexual activity, type of perianal disease and degree of induration. Each category is scored from 0 to 4 and the total score is a sum of all five category scores (total score range 0–20). Although the index has been used in clinical trials, further research is needed to validate a cutoff to define remission and a change in score to define response.

Out of the necessity to standardize assessment of fistulizing disease in Crohn's disease, a fistula drainage instrument was created for an infliximab trial for the treatment of fistulizing Crohn's disease [8]. Since its development, this fistula assessment tool has been used in several controlled trials involving patients with fistulizing Crohn's disease. Response is defined as a decrease of drainage from baseline in the number of open draining fistulae of >50% for at least two consecutive visits. Complete response or remission is defined as closure of all fistulae that were draining at baseline for at least two consecutive visits. "Closure" of an individual fistula is defined as no fistula drainage despite gentle finger compression. Whether the PDAI or the fistula draining assessment is the superior instrument for patients with perianal fistulizing Crohn's disease has yet to be determined.

Corticosteroids are primarily used to treat disease flare. Due to significant morbidity with long-term corticosteroid use, corticosteroids are not preferred for maintenance of remission. Unfortunately, subjects may develop corticosteroid-dependent disease. In general, Crohn's disease patients who relapse soon after discontinuation of corticosteroids are considered corticosteroid dependent; more precise definitions have been created for the evaluation of subjects included in clinical trials. In an early study by Munkholm *et al.* of corticosteroid use in Crohn's disease subjects, corticosteroid dependence was defined as relapse of Crohn's disease within 30 days after prednisone treatment is completed in subjects who had a partial or complete response to prednisone 40–60 mg per day [9]. In a consensus review by international experts of Crohn's disease clinical trial endpoints, the European Agency for the Evaluation of Medical Products (EMA) defined corticosteroid dependence as patients requiring daily corticosteroids to control symptoms [3].

Clinical disease activity indices do not incorporate the use of corticosteroids. Many trials have incorporated an additional endpoint of corticosteroid-free remission. The definition of corticosteroid-free remission, however, is not clear. The FDA has defined this as the absence of corticosteroid use for 6 months and the EMA has defined it as the absence of corticosteroids for 3 months [3]. The review of international experts of Crohn's disease clinical trial endpoints recommends a minimum of 6 months without corticosteroids and relapse, defined as a CDAI score $\geq$150 and an increase in the CDAI score of $\geq$70 points [3].

Another measure of Crohn's disease activity is evaluation of mucosal disease severity by endoscopic evaluation. The Crohn's Disease Endoscopic Index of Severity (CDEIS) was developed and validated to evaluate for endoscopic healing. The CDEIS includes assessment in five separate segments from ileum to rectum (ileum, right colon, transverse colon, left colon and rectum), the "number of deep ulcerations", the "number of superficial ulcerations", segmental surfaces involved with disease per 10 cm and segmental ulcerated surface per 10 cm. In addition, the presence of non-ulcerated stenosis or ulcerated stenosis in any segment is included in the score. Each variable is weighted. Total scores range from 0 to 44. A cutoff has not yet been defined for endoscopic remission score although higher scores represent more severe endoscopic disease severity [7,10–13].

A simplified version of the CDEIS was developed due to the cumbersome assessment of mucosal disease that the index requires. The Simplified Endoscopic Activity Score for Crohn's Disease (SES-CD) has four variables: ulcer size, ulcerated surface, affected surface and presence of narrowings, which are each scored from 0 to 3. Each variable is assessed for five segments from the ileum to

rectum (ileum, right colon, transverse colon, left colon and rectum) [14]. The SES-CD correlates with the CDEIS ($r = 0.920$). For each endoscopic variable, the score ranges from 0 to 15 except stenosis, which ranges from 0 to 11. Total score ranges from 0 to 56. As in the CDEIS, a higher score represents more severe mucosal disease and a cutoff score for endoscopic remission has yet to be determined.

Another endoscopic disease severity index developed and utilized for the evaluation of postoperative endoscopic recurrence of Crohn's disease is the Rutgeerts Score [15]. Scores range from 0 to 4 with scores of 3 or 4 having a higher likelihood of relapse.

The clinical symptoms of Crohn's disease do not always correlate with the severity of mucosal disease, with low clinical index scores reported in the presence of continued mucosal ulceration. Further studies are needed to determine the prognosis of subjects in clinical remission but with mucosal ulceration. Endoscopic mucosal healing in clinical trials is commonly used as a secondary endpoint and clinical remission as the primary endpoint.

Histologic indices have been developed for Crohn's disease but these indices have poor correlation with clinical assessment [16,17]. For this reason, histologic indices are not recommended as an endpoint in clinical trials.

Ulcerative colitis

Unlike the case with Crohn's disease, numerous outcome measures have been developed and used in studies of ulcerative colitis patients. When evaluating these various activity indices, several general issues merit consideration. First, it is important to recognize that these activity indices generally are non-specific and therefore patients with other disorders, such as irritable bowel syndrome, could attain high scores even in the absence of any inflammation. Also, almost none of the outcome measures in ulcerative colitis have been prospectively validated. Finally, the scores of the indices are typically derived by incorporating various signs and symptoms for which there are no standardized definitions. For these reasons, one should exercise appropriate caution when interpreting the results of a study of ulcerative colitis patients using an activity index. Whether or not a certain treatment is deemed effective could be dependent upon the definitions of the variables used, which determine the total score of such an index. Hypothetically, it is possible that if a different index were used, a completely different conclusion regarding the efficacy of a particular therapy may be reached; however, examples of this are not common. Further detailed discussion of the specific outcome measures used for assessing disease activity in ulcerative colitis will be carried out according to the type of disease activity measured by the index: clinical activity, endoscopic activity, combined clinical and endoscopic activity and histologic activity. In all cases, higher scores correspond to higher levels of disease activity.

At least 11 purely clinical activity indices have been developed for ulcerative colitis patients (Table 23.5) [18–28]. The first instrument to be developed was by Truelove and Witts in 1955 and included six variables: bowel frequency, rectal bleeding, temperature, pulse, hemoglobin concentration and erythrocyte sedimentation rate (ESR) [18]. However, this index is not quantitative and therefore although potentially useful in identifying active disease, it is not helpful in examining changes in disease activity. The Powell–Tuck Index (also known as the St Mark's Index) [19], although quantitative, has never been validated. However, a total score of <3.5 points for the combined seven self-reported items has been shown to correlate with Patient-Defined Remission with a sensitivity and specificity of 93 and 75%, respectively [28]. The Clinical Activity Index (also known as the Rachmilewitz Index) [20] has been validated in one study by Rutgeerts [29], and hence may be a reasonable choice of clinical activity index for an ulcerative colitis trial. The Seo Index (also known as the Activity Index) is a somewhat complex prediction model, in which scores of <150, 150–200 and >200 points correspond to mild, moderate and severe disease, respectively, as categorized by the Truelove and Witts Severity Index [18,21]. The authors showed in subsequent studies restricted to patients with severe disease that a score of <180 points after 2 weeks of intravenous corticosteroids predicted remission, while a score of >200 points predicted a need for colectomy [30,31]. In addition, a score of <120 points has been shown to correlate with Patient-Defined Remission with a sensitivity and specificity of 82 and 96%, respectively [28].

The Simple Clinical Colitis Activity Index is based largely on the Powell–Tuck Index [25], and at a score of <2.5 points it has been shown to correlate with Patient-Defined Remission with a sensitivity and specificity of 82 and 79%, respectively [28]. The much-mentioned Patient-Defined Remission is a survey asking patients whether or not they feel that they were in remission, to which a "yes" or "no" answer is given [28]. This instrument also includes a seven-point Likert scale regarding whether a patient's condition has improved or worsened since their last visit. Patient-Defined Remission was shown to have a sensitivity of 86% and a specificity of 76% for remission defined as absence of rectal bleeding combined with a modified Baron endoscopic score of 0–2 points. Neither the Lichtiger Index (also known as the Modified Truelove and Witts Severity Index) nor the Ulcerative Colitis Clinical Score has been validated. Similarly, the global scales, such as the Physician Global Assessment, the Investigators Global Evaluation and the Improvement Based On Individual Symptom Scores, have never been validated. An additional clinical index, the Pediatric Ulcerative Colitis Activity Index, has just been developed for

Table 23.5 Clinical Activity Indices for Ulcerative Colitis*.

Name	Variables	Score range	Remission score
Truelove and Witts Severity Index	Stool frequency, bleeding, temperature, pulse, hemoglobin, ESR	N/A	N/A
Powell–Tuck Index	Well-being, abdominal pain, stool frequency, stool consistency, bleeding, anorexia, nausea/vomiting, abdominal tenderness, extraintestinal, temperature	0–20	0
Clinical Activity Index	Stool frequency, bleeding, physician's global assessment, abdominal pain, temperature, extraintestinal, laboratory	0–29	≤4
Seo Index	Bleeding, stool frequency, ESR, hemoglobin, albumin	50–250	N/A
Physician Global Assessment	Symptom relief on Likert scale	1–6	1
Lichtiger Index	Stool frequency, nocturnal stools, bleeding, incontinence, abdominal pain, general well-being, abdominal tenderness, need for antidiarrheals	0–21	≤3
Investigators Global Evaluation	Symptom score on Likert scale	0–4	0
Simple Clinical Colitis Activity Index	Stool frequency (day), nocturnal stools, urgency, bleeding, general well-being, extraintestinal	0–19	N/A
Improvement Based On Individual Symptom Scores	Bleeding, patient functional assessment, stool frequency, abdominal pain, sigmoidoscopic grade, physician's global assessment	0–3 for each item	0 for bleeding, stool, flexible sigmoidoscopy, global assessment
Ulcerative Colitis Clinical Score	Stool frequency, bleeding, patient functional assessment, physician's global assessment	0–12	≤1
Patient-Defined Remission	In remission: yes/no question	Yes/no	Yes

*Abbreviations: ESR, erythrocyte sedimentation rate; N/A, not applicable.

pediatric patients with ulcerative colitis [32]. This index, which includes six clinical parameters (abdominal pain, rectal bleeding, stool consistency, stool frequency, nocturnal stools and activity level), was validated in a different cohort of patients and found to be highly reliable [32].

With respect to endoscopic activity, at least nine indices have been developed and include the Truelove and Witts Sigmoidoscopic Assessment, Baron Score, Powell–Tuck Sigmoidoscopic Assessment, Endoscopic Index, Sigmoidoscopic Index, Sigmoidoscopic Inflammation Grade Score, Mayo Score Flexible Proctosigmoidoscopy Assessment, Sutherland Mucosal Appearance Assessment and Modified Baron Score [33–36]. Many of these indices focus on mucosal friability and bleeding. Baron *et al.* examined interobserver variability in describing endoscopic findings and showed that interobserver agreement was in fact best with respect to mucosal friability [33]. None of these endoscopic indices have been validated, however, hence their true value is uncertain.

The combination of clinical and endoscopic indices was first proposed as a variation of the Powell–Tuck Index with the inclusion of an 11th item for sigmoidoscopic score (0–2 points) [19]. However, since the endoscopic subscore of this scale accounts for only 2 out of 22 points, it is not surprising that a recent study by Higgins *et al.* demonstrated that the addition of the endoscopic component added little in the assessment of disease activity, as similar conclusions were reached by two purely clinical scales, the Seo Index and the Simple Clinical Colitis Activity Index [37]. Two other indices have been developed that have incorporated measures of both clinical and endoscopic activity but with a more proportionate emphasis on the endoscopic component [35,36]. The first, the Mayo Score, includes four variables: stool frequency, rectal bleeding, findings on flexible proctosigmoidoscopy and physician's global assessment [35]. Each variable carries a score of 0–3, with a total possible scale score of 12. Although this index has not been validated, various modifications of the Mayo Score have been used to assess disease activity in multiple studies, including the relatively recent ACT 1 and 2 trials [38–42]. The second index, the Sutherland Index (also known as the Ulcerative Colitis Disease Activity Index), consists of four items similar to those in the Mayo Score: stool frequency, rectal bleeding, mucosal appearance and physician's rating of disease activity [36]. As in the Mayo Score, each variable carries a score of 0–3, with a total possible score of 12. The Sutherland Index also has not been validated, but a score of <2.5 points has been shown to correlate with the Patient-Defined Remission [28].

The use of a histologic index, at first glance, seems to be an attractive option to measure disease activity in ulcerative colitis, as this disease has the unique feature of rectal involvement in most cases and sigmoidoscopy with biopsy has been shown to be a safe and relatively simple

procedure. Unfortunately, multiple studies have revealed that the correlation between histologic activity and clinical activity is low [43–47]. For this reason, the consensus opinion among experts is to use histologic activity as a secondary endpoint in studies of ulcerative colitis treatment [48]. A number of histologic indices have been developed and, although none is ideal, two such indices, the Geboes Index and the Riley Index, have been validated and shown to be highly reliable [49,50]. The Geboes Index incorporates six elements: architectural changes, chronic inflammatory infiltrate, lamina propria neutrophils and eosinophils, epithelial neutrophils, crypt destruction and erosions or ulceration [49]. Scores range from 0 to 5.4 points in this index. The Riley Index also encompasses six slightly different elements: round cells in the lamina propria, polymorphonuclear cells in the lamina propria, crypt abscesses, mucin depletion, surface epithelial integrity and crypt architectural irregularities [50]. In this index, scores range from 0 to 24 points.

Quality of life

By far the most commonly used instrument to assess health-related quality of life in IBD is the Inflammatory Bowel Disease Questionnaire (IBDQ). The IBDQ, a physician-administered questionnaire assessing patients' quality of life during the previous 2 weeks, was originally developed in 1989 by Guyatt *et al.* in a cohort of Canadian patients [51]. It consists of 32 questions, which are divided into four domains: bowel symptoms (10 questions), systemic symptoms (5 questions), emotional function (12 questions) and social function (5 questions). Each question receives a score of 1–7 points (with higher scores indicating better quality of life) and thus the total score ranges from 32 to 224 points. The original instrument has been validated and shown to be highly reliable [52]. The IBDQ has also been adapted and validated in many other countries, including The Netherlands, England, Korea, Spain, Sweden, Greece and China [53–61]. In addition, two other forms of the questionnaire, a self-administered version and a shortened 10-question version, have been validated and found to be reliable [62,63]. The IBDQ has also been used extensively in studies of IBD treatment, especially in Crohn's disease, including a number of randomized controlled trials [48].

Optimizing clinical trial efficiency

Clinical trials in patients with IBD are expensive and labor intensive. In addition to the work involved in following a clinical trial protocol, patient recruitment can be a limiting factor in the timely execution of clinical trials. As a result, consideration of methods used to increase the efficiency of clinical trials is justified. This section focuses on several modifiable characteristics of clinical trials that may influence the efficiency of a study.

Type of outcome measure

The type of outcome measure, specifically continuous versus binary, can have an enormous impact on not only the sample size needed for a study, and therefore the cost, but also the conclusion reached. It is well recognized that studies using dichotomous outcome variables typically require larger sample sizes to show a difference in efficacy of two treatments than do studies that use continuous outcome measures [64]. For example, in a study of Crohn's disease patients using the CDAI, a larger sample would be needed to show a difference in response or remission rate (binary outcome measure), even if defined by the CDAI, than to show a difference in mean or median CDAI score (continuous outcome measure). Thus, from an efficiency standpoint, using changes in mean or median CDAI score appears to be more desirable. However, one must keep in mind that change in mean or median CDAI score is really a group data determination, whereas response or remission rate is an individual data consideration. One could argue that individual data are more valuable when making a determination about a particular treatment intervention and that binary outcome measures therefore should be used whenever feasible. It is also possible that a situation may arise in which the mean or median CDAI score may not change appreciably, which would lead one to conclude that two different treatments were equal, while the rate of response or remission may change significantly, which would lead one to conclude that one treatment is superior to the other. In this case, two completely different conclusions may be reached about the same treatments just by altering the type of outcome measure. Again, as the binary outcome definition yields more clinically useful information because it deals with individual data, this method should be utilized whenever possible.

Placebo response

Even though placebos are often thought of as inert agents, such as "sugar pills", they have been noted to lead to improvement in a variety of both subjective and objective outcome measures in several different medical conditions, such as anxiety, depression, insomnia, pain, asthma, obesity, hypertension and even myocardial infarction [65–67]. In some of these disorders, placebo response rates of up to 40% have been reported. The general question of the influence of placebos was formally addressed by Hrobjartsson and Gotzsche via a meta-analysis of randomized controlled trials comparing placebo with no treatment [68]. In this study, 32 trials (with a total of 3795 patients) with binary outcomes and 82 trials (with a total of 4730 patients) with continuous outcomes were included. The authors found no significant differences in response rates for

studies with objective or binary outcomes but small benefits with placebo for studies with continuous subjective outcomes and for pain treatment. A related issue addressing adverse effects reported by patients receiving placebos, known as the "nocebo phenomenon", was reviewed by Barsky *et al.*, who noted that roughly 25% of patients freely report adverse effects with placebos, but when actively asked about side effects, up to 71% of patients report them [69]. In some cases, a higher incidence of adverse effects is discovered in placebo groups compared with the active treatment groups. The authors also explained that although the mechanism underlying the nocebo phenomenon is yet to be determined, certain factors, such as conditioned learning (from prior experiences with medications), expectation of adverse effects and situational and contextual influences (such as the setting in which a medication is prescribed and the nature of patient–doctor relationship, respectively), appear to be associated with this phenomenon.

The anticipated rate of remission or response among patients receiving placebo is important in planning the sample size for a study. Sample size requirements are driven largely by what is deemed a clinically meaningful difference in the rate of the outcome. For example, an absolute increase in remission rates of 20% or a doubling of the remission rate may be considered clinically meaningful. When this clinically meaningful difference is expressed in terms of an absolute increase in the outcome rate, lower rates of the outcome in the control group will generally translate to greater statistical power for a given sample size or smaller sample sizes for a given level of statistical power. Hence an accurate estimate of the outcome rate in the control group is critical to avoiding underpowered trials. Likewise, unexpectedly high rates of remission or response among patients receiving placebo risk a "negative" study even with an effective therapy due to inadequate statistical power.

With respect to the placebo response in IBD, two meta-analyses by Su *et al.*, one in Crohn's disease and the other in ulcerative colitis, addressing this issue have been published recently [70,71]. The first included 23 randomized, placebo-controlled trials in Crohn's disease patients in which the CDAI was used to define response and/or remission [70]. Among the studies defining response as a decrease in CDAI score of $\geq$100 points and >70 points, the authors reported a pooled response rate of 19% [95% confidence interval (CI) 13–28%; range 0–46%] and 26% (95% CI 19–34%; range 0–45%), respectively, but with significant heterogeneity between studies. For remission, they found a pooled remission rate of 18% (95% CI 14–24%; range 0–50%), but again with significant heterogeneity. Although univariate analysis suggested that longer follow-up duration, higher number of follow-up visits and lower disease severity at study entry may lead to an increased placebo remission rate, multivariate analysis showed that only study duration was significantly (but weakly) positively associated with the placebo remission rate (odds ratio 1.19, 95% CI 1.06–1.33). The other meta-analysis by Su *et al.* included 40 randomized, placebo-controlled trials in ulcerative colitis patients in which the most commonly used activity indices were the Mayo Score or the Sutherland Index [71]. The authors reported a pooled response rate of 28% (95% CI 23–33%; range 0–67%) and a pooled remission rate of 13% (95% CI 9–18%; range 0–40%), but with significant heterogeneity for each. Studies that used less stringent outcome definitions had higher placebo response and remission rates. Similarly to the meta-analysis of Crohn's disease patients, univariate analysis in this study suggested that longer follow-up duration, larger number of follow-up visits, longer disease duration and lower disease severity at study entry were positively associated with the placebo remission rate. Unfortunately, multivariate analysis could not be conducted due to the relatively small number of studies using the same outcome definition.

Allocation distributions

In a randomized controlled trial, the investigators must decide on the ratio of active treatment to placebo. Ratios of 2:1 and 3:1 are not uncommon, for at least two reasons. First, higher ratios of active treatment to placebo may improve trial recruitment, as they are more appealing to patients and physicians who want their refractory patients to have access to a novel treatment. Second, in pre-marketing studies, having higher numbers of patients who receive active treatment may help a company fulfill the requirements of the US Food and Drug Administration (FDA) for a minimum number of exposed patients needed before a drug can come to market. However, when the ratio of active treatment to placebo is increased from 1:1 to 2:1 or 3:1, the total sample size for a given statistical power and detectable difference is also increased. Hence investigators must weigh the risks and benefits of a 1:1 ratio, which increases statistical efficiency, and a 2:1 or 3:1 ratio, which may improve patient recruitment and facilitate approval by the FDA.

Definition of response and remission

As discussed in detail above, the various outcome measures used in Crohn's disease and ulcerative colitis are not without flaws, as the majority are non-specific, have not been validated, have not been compared with each other in randomized controlled trials and incorporate signs and symptoms that have not been standardized. For these reasons, studies relying on these activity indices to determine response and remission are prone to potential outcome misclassification bias, as no gold standard test for determining disease response and remission has ever

been developed for either Crohn's disease or ulcerative colitis. Fortunately, however, outcome misclassification bias in a randomized controlled trial is likely to be non-differential or random, between the two groups being compared. Thus, any bias that could result would likely be towards the null and would also likely be relatively minor.

Measuring safety in clinical trials

Limitations of clinical trials

All clinical trials are required to assess safety as at least a secondary outcome. Phase I clinical trials are designed specifically to assess safety. Phase II clinical trials collect additional safety information, while also helping to select likely effective dose(s) for study in Phase III clinical trials. Phase III studies are designed to determine the efficacy of the medication or intervention, but also provide additional safety information. However, despite the fact that all pre-marketing clinical trials collect safety data, there are often large gaps in the knowledge base regarding safety when new medications or devices are first approved for marketing.

There are several reasons for these knowledge gaps. Patients who enroll in clinical trials are often different from those who are treated in a clinical setting. Clinical trials typically have strict entry criteria that exclude patients with certain comorbidities. In addition, off-label use of medications sometimes includes treatment of patients with either disease characteristics that were not included in clinical trials (e.g. fistulae) or diseases that were not studied (e.g. use of a medication approved for Crohn's disease to treat ulcerative colitis). When a medication or device is used in a new population, unanticipated adverse effects are possible. For example, pregnant women and people with severe kidney or liver disease are often excluded from clinical trials. Hence the incidence of birth defects among offspring of women (or men) who are taking a medication will not be apparent until after the drug is marketed. Likewise, if dose adjustments are required for patients with liver or kidney disease, this may not become apparent until such patients are treated.

Another limitation of clinical trials to establish safety is the requirement for relatively short duration of study. Most medications for IBD are used chronically, with patients treated for years or even decades. However, for practical and financial reasons, few medications are studied in clinical trials for more than 1 year. Most adverse effects of a medication would be expected to occur as frequently or more frequently in the first year of therapy than in later years, making this less of a concern. However, certain toxicities, such as cancer, cirrhosis or chronic renal injury, may require long periods of exposure or even post-exposure time to become evident. Clinical trials rarely can detect such associations.

Safety data from clinical trials are sometimes of lower quality than primary outcome data. In conducting a clinical trial, investigators typically collect information on all potential adverse effects. As such, the data collection forms do not necessarily focus on specific outcomes. Since the adverse events are secondary outcomes, the level of detail is often less than that collected for the primary outcome. For some outcomes, such as death, this is not a problem. However, for other outcomes, it can potentially be problematic.

Because the investigators collect safety data on all potential outcomes, interpretation of safety data requires more subjective skill than interpretation of primary outcome data. There are an unlimited number of potential adverse effects, although most of these will be relatively rare. As such, clinical trials are generally underpowered to determine whether an adverse event is more common among the treated group than the control group. A simple rule of thumb (the 1000:3–3000:1 rule) is that assuming an equal number of exposed and unexposed subjects, there will be 90% power to detect a doubling of risk with 1000 exposed subjects if the adverse event occurs in 3% or more of the control patients and with 3000 exposed subjects if the adverse event occurs in 1% or more of the control patients. Since many drugs come to market with 3000 or fewer exposed subjects and often far fewer unexposed control subjects, the power to detect a doubling of risk for adverse events that occur in less than 1% of unexposed subjects is typically low. Hence strict reliance on p values when interpreting safety data can be misleading.

Another challenge in interpreting safety data results from the many comparisons that are performed. Because data are collected on all adverse events, there are an unlimited number of possible statistical comparisons. This increases the possibility of a type 1 error, where an association is observed as a result of chance. Thus, interpretation of safety data requires one to consider the biological plausibility, the consistency of the finding with other studies of the same drug or similar drugs, the magnitude of the association and the potential consequences of the outcome.

Observational studies to examine safety

Because of the limitations of clinical trials to answer safety questions fully, observational studies are often used to assess the safety of drugs and devices. These studies typically confer the advantages of being less expensive, more quickly executed, having larger sample sizes and including less homogeneous populations than those studied in clinical trials. A detailed discussion of all possible observational study designs is beyond the scope of this chapter. However, we will briefly review the commonly used designs.

Case reports, case series and spontaneous reporting systems

Case reports and case series are characterized by the lack of a control group. As such, they are useful for describing a clinical condition, but are generally not helpful in establishing causality. Rather, they are a source of new hypotheses.

Spontaneous reporting systems, such as MedWatch, collect case reports of adverse events among patients treated with medications or devices. These collections of reports can serve as a signal for a possible association that may require further study. In rare cases, no further study may be required because the adverse event is so extremely rare in the absence of the exposure that causation is assumed. However, this is the exception to the rule.

Advantages of spontaneous reporting systems are the low cost and the ability to collect large numbers of reports. However, there are major limitations to these systems. Because anyone, including the lay public, can report cases to these systems, the quality of the reports is variable. In addition, because the number of people treated with a medication is generally unknown, spontaneous reporting systems cannot be used to calculate incidence rates. Likewise, because there is no control group, it is not possible to know from spontaneous reporting systems whether the frequency of an adverse event in the exposed population is higher than would be expected in the absence of the exposure. Thus, although case reports and case series that accumulate in spontaneous reporting systems are useful for hypothesis generation, they typically are not useful to test hypotheses.

Analytic observational studies

The two most commonly employed observational study designs for assessing drug safety are the cohort study and the case–control study. Cohort studies are analogous to clinical trials, but without systematic treatment assignment (e.g. randomization). Rather, treatment assignment is the result of usual clinical care. Cohort studies can be conducted prospectively or with retrospective analysis of previously collected data. The analysis of a cohort study allows for the determination of incidence rates, rate differences and relative risk. Another advantage of the cohort design is the ability to study multiple outcomes in a single study.

Case–control studies are often thought of as the opposite of a cohort study. Case subjects are identified on the basis of having the condition of interest. The index date is typically the date of onset of the condition. Control subjects are identified who do not have the condition at a certain point in time, their index date. Then, for both case and control subjects, exposure to the medication or other factors of interest prior to the index date is determined. A positive association is identified when the prevalence of the exposure of interest is more common among the case subjects than the controls. The association is typically measured with an odds ratio. A disadvantage of the case–control design is the inability to calculate incidence rates; a major advantage is the ability to study multiple exposures in a single study.

Case–control studies are often considered to provide less strong evidence than cohort studies because of the retrospective nature (i.e. both the exposure and the outcome have occurred when the data are collected). However, this theory is not entirely correct since many cohort studies are also retrospective. When a case–control study is conducted among a well-defined cohort, it is referred to as a nested case–control study. In this setting, the case–control study should not be viewed as inferior to a cohort study; rather, it is a more efficient method to analyze the data from the cohort study.

Randomized controlled trials are generally considered as the gold standard of epidemiologic evidence. The main reason for this is that randomization reduces the risk for bias from confounding. A confounder is a variable that is associated with both the exposure and the outcome. Failure to account for a confounder will result in a biased estimate of the association. For example, if one studies the association between alcohol consumption and Crohn's disease, a positive association might be observed. However, since smoking is associated with Crohn's disease and heavy alcohol consumers are generally more likely to be smokers, it would be necessary to account for smoking when assessing the association between alcohol consumption and Crohn's disease.

Other limitations of observational studies include selection bias and information bias. These biases refer to systematic errors in the way that the data are collected (information bias) or the patients are selected (selection bias), such that the measured association is biased. Clinical trials can also be subject to information bias if the data collection instruments are not used properly or are inaccurate. For example, errors in calculation of the CDAI could bias a study away from seeing a positive effect of the intervention. Randomized clinical trials are generally not subject to selection bias, since patients are assigned at random to the treatment strategy. A common misconception is that the strict inclusion criteria applied in clinical trials are a source of selection bias. This is not correct for the reason noted previously. Rather, the inclusion criteria may reduce the generalizability of the findings to other populations. However, this is not a selection bias.

Meta-analysis

As discussed previously, individual clinical trials are generally underpowered to assess the association of the new therapy with rare adverse events. One approach to this problem is to pool the results from multiple clinical trials, thereby increasing the statistical precision. This is commonly referred to as a meta-analysis. Although this

technique is useful to increase the statistical power, it is limited by the quality of the data that are used in the analysis. Thus, a meta-analysis of poorly conducted trials will give the impression of a highly precise estimate, albeit potentially of low-quality data. Another caveat pertaining to meta-analysis is the need to examine whether the results from the various trials are consistent (i.e. homogeneous). When the results are heterogeneous, it is the investigator's challenge to identify reasons for the heterogeneity. Reasons might include factors such as the duration of the studies and different inclusion criteria. In the setting of heterogeneity, one needs to be extremely cautious about pooling results. Meta-analysis has also been applied to observational data, in which case particular attention must be given to assessing the quality of the studies that are included, the consistency of the outcome definition and the presence of heterogeneity.

Balancing efficacy and safety

Ultimately, it is the physician's responsibility to select therapies for their patients that optimize the balance of potential benefit to potential harm. Physicians do this daily in a subjective manner. For the purpose of developing optimal treatment algorithms, researchers will often try to provide quantitative measures of risk and harm.

One approach to this is to calculate the number needed to treat and the number needed to harm. The number needed to treat represents the number of patients who would need to be treated with one therapy rather than an alternative therapy so as to have one additional patient achieve the beneficial outcome of interest. This is calculated as the reciprocal of the risk difference (1 divided by the difference in rates of the outcome with the two strategies). The number needed to harm is calculated analogously but in reference to the number of patients who must be treated to have one additional patient experience an adverse outcome.

While number needed to treat and number needed to harm are helpful, by themselves they cannot completely answer the question of how best to balance risks and benefits, since not all outcomes are of the same importance. For example, one might accept a high risk of stroke to prevent or abort an acute myocardial infarction, but only a very low risk of stroke to treat a minor infection. To address the imbalance of different outcomes, some investigators have utilized a method of mathematical modeling where different outcomes are given different weights based on the severity of the condition. This is commonly referred to as decision analysis. Using mathematical modeling based on the probability of each outcome occurring, the decision analysis provides an estimate of which choice (i.e. therapy) on average will provide the greatest life-years or utility for patients facing the clinical decision. However, decision analyses are difficult to conduct and are subject to several important limitations. Most importantly, the results of the decision analysis are only as good as the mathematical model and the data that are used as inputs. Therefore, when interpreting a decision model, it is necessary to consider carefully the quality of the data that went into the model. Lastly, because decision models use expected value decision making (i.e. average outcomes for a large population of patients), it is possible to recommend a preferred strategy that provides huge benefits to a small number of subjects, but most patients would actually do better by selecting the alternative strategy. The science of decision modeling is rapidly evolving and improved methods are likely to be developed in the course of the next decade.

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Chapter 24
Non-targeted Therapeutics for Inflammatory Bowel Diseases

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Summary

- Glucocorticoids influence about 20% of the human genome and their effects spare no organs and tissues. Glucocorticoid receptors are present in almost all body cells. Glucocorticoids affect the growth, differentiation and function of lymphocytes, the distribution of cellular subsets and the production of cytokines. Glucocorticoids downregulate the oxidative burst reaction of neutrophils and reduce IL-8 secretion leading to a reduced immigration of neutrophils into the mucosa.
- Non-response to glucocorticoids may be mediated by mutations of the receptor, a reduced number of glucocorticoid receptors or a downregulation of receptor expression; however, primary (hereditary) abnormalities in the glucocorticoid receptor gene make only 2.3% of patients "resistant" to glucocorticoid therapy. In the majority of patients glucocorticoid resistance seems to be acquired and localized to the sites of inflammation.
- Four important factors determine the effects of glucocorticoids: the ligand structure, the glucocorticoid–receptor concentration, the coactivator or corepressor concentration/availability and glucocorticoid modulatory elements (GMEs). An important impact of the elucidation of these basic mechanisms for the steroid treatment of patients with inflammatory bowel diseases can be expected.
- Peroxisome proliferator-activated receptor gamma (PPARγ) and alpha (PPARα) have been shown to have NF-κB-inhibiting activities and could be possible tools for the treatment of inflammatory bowel disease in the future. They are further examples for a multi-site treatment approach.
- NF-κB transcription factors play an important role in the inflammatory process of inflammatory bowel disease. Both PPARγ and glucocorticoid receptor antagonize NF-κB activation, but by different pathways, and could have additive effects.

Introduction

Both genetic susceptibilities and environmental factors play a role in the etiology of inflammatory bowel diseases (IBDs). In recent years, many of the factors have been identified that contribute to the chronification of inflammation in Crohn's disease and ulcerative colitis, finally leading to a number of new therapeutic concepts.

Among the factors contributing to the pathogenesis of IBD are cytokines and chemokines [1,2] and also adhesion molecules [3], which are relevant for the transfer of immune-cells into the intestinal mucosa. In recent years, inhibitors or neutralizing antibodies to a number of those factors have been clinically studied in IBD patients [3–8]. The strategy behind this approach was a targeted treatment trying to eliminate just one specific pathogenic factor. So far, only the specific inhibition of TNFα has found its way into clinical practice [5,8–11]. Many attempts to develop a specifically targeted therapy eliminating or neutralizing one specific factor have failed. This may be due to the fact that many, if not all, of the cytokines, chemokines or signaling molecules involved have "back-up systems". A therapeutic intervention at a particular, singular, very specific point in the complex network of mediator-interactions is less likely to be successful than a multi-site targeted anti-inflammatory strategy.

Even if the etiology of inflammatory bowel disease could eventually be completely elucidated, a causative therapy might not be possible and a multi-site anti-inflammatory strategy still could be preferable. Therefore, the improvement of "classical" multi-site targeted anti-inflammatory therapies and the development of new concepts for this approach are of great importance for the future management of patients with IBD.

The improvement of "classical" concepts and therapies is necessary as so far no therapeutic strategy has proved successful in all patients. Clinically, we observe

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"resistance" of a certain percentage of patients to any particular therapy [1,12,13]. An important goal for the future must be a better understanding of mechanisms leading to resistance to classical multi-site anti-inflammatory strategies. This would allow the early identification of patients who are not likely to respond to a treatment modality. Specifically, those patients would profit from alternative strategies or early "aggressive" therapies. To understand the mechanisms of resistance to a particular multi-site targeted anti-inflammatory therapy, we first have to understand the molecular and cellular mechanisms involved in an effective therapy. Important insights into a number of those mechanisms of drug action have been obtained in recent decades.

A classical example of a multi-site targeted anti-inflammatory treatment is therapy with glucocorticoids. Therefore, the principles of glucocorticoid therapy, the structure of the effect-mediating receptor, the molecular mechanisms of action and the effects on the cellular levels will be highlighted in this chapter. The glucocorticoid receptor is a member of a family of receptors, the so-called nuclear receptor superfamily, which share structural and functional similarities. Other members of this family are the **p**eroxisome **p**roliferation-**a**ctivated **r**eceptors (PPARs). Ligands to PPARγ have recently been shown to be effective in animal models of colitis. The inhibition of the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) is likely to be one of the most important targets of glucocorticoid therapy in addition to PPAR-mediated effects. Therefore, the mechanisms and pathways of NF-κB activation and the consequences of NF-κB inhibition will also be explained.

Multi-site targeted therapy by glucocorticoids

Glucocorticoids are used for the suppression or reduction of inflammation in a wide variety of diseases such as rheumatoid diseases, allergic diseases, IBD and autoimmune diseases in general [14–18]. In many of these diseases, they are still the standard or first-line therapy due to their high efficiency [18]. Glucocorticoids have been proven to be the first choice in the treatment of acute flares of IBD in several major studies [18–25]. The systemic administration of glucocorticoids (oral or i.v.) during acute flares of Crohn's disease or ulcerative colitis is followed by a multitude of different effects in different body cells [26–29]. Among the intended effects of therapeutically administered glucocorticoids is the downregulation of the expression of multiple cytokines and their receptors [30], chemokines and their receptors [31,32], kinins and their receptors [33], adhesion molecules and inflammation-associated enzymes such as inducible nitric oxide synthase (iNOS) [34–36] and inducible cyclooxygenase (COX-2) [37,38]. Glucocorticoids influence about 20% of the human genome and their effects spare no organs and tissues [14].

The mechanisms by which glucocorticoids suppress or reduce inflammation have been investigated in detail [14].

Glucocorticoid action is mediated by the glucocorticoid receptor

Most, if not all, of the effects of naturally occurring glucocorticoids such as cortisol on the one hand and synthetic corticosteroids such as prednisolone and its methylated or acetylated derivatives, triamcinolone, dexamethasone and beclomethasone on the other are mediated by binding of those molecules to cytosolic glucocorticoid receptors [14]. Glucocorticoid receptors are present in almost all body cells at concentrations between 2000 and 30,000 binding sites per cell [39,40].

The glucocorticoid receptor is a member of the so-called "nuclear receptor superfamily", which also includes the peroxisome proliferator-activated receptors (PPAR) alpha, gamma and delta, the thyroid hormone receptor and many others [41,42].

The glucocorticoid receptor consists of 777 amino acids and was cloned in 1985 [43–46]. The majority of research during recent decades has focused on the mechanisms of action of one isoform of GR, GRα. However, recently a number of additional human GR (hGR) isoforms have been reported. Multiple hGR isoforms are generated from a single hGR gene via mutations and/or polymorphisms, transcript alternative splicing and alternative translation initiation [47,48]. Each of the hGR isoforms subsequently may be subject to post-translational modifications. The nature and degree of these post-translational modifications in turn affect receptor function [48]. The receptor compositions and relative receptor proportions within a cell may determine the specific response to glucocorticoids: the specific effects of induction of gene expression or repression. The GR gene encodes two 3′ splicing variants, GRα and GRβ, from alternative use of two distinct terminal exons (9α and 9β). Each of the two mRNAs is translated from at least eight initiation sites into multiple GRα and possibly GRβ isoforms [49]. This is followed by 16 possible GR monomers and 256 different homo- or heterodimers [49]. The translational GRα isoforms may be produced at specific variations depending on the tissue and cell type. They have varying intrinsic transcriptional activities and influence different patterns of glucocorticoid-responsive genes.

The major splice variant of the normal glucocorticoid receptor (GRα) discovered around a decade ago has been termed GRβ, as mentioned above [50–54]. GRβ does not regulate gene expression under glucocorticoid control but is thought to be an antagonist of glucocorticoid action [50,55–61].

Alternative translation-initiation sites within exon 2 are the major reason for the production of isoforms of GR

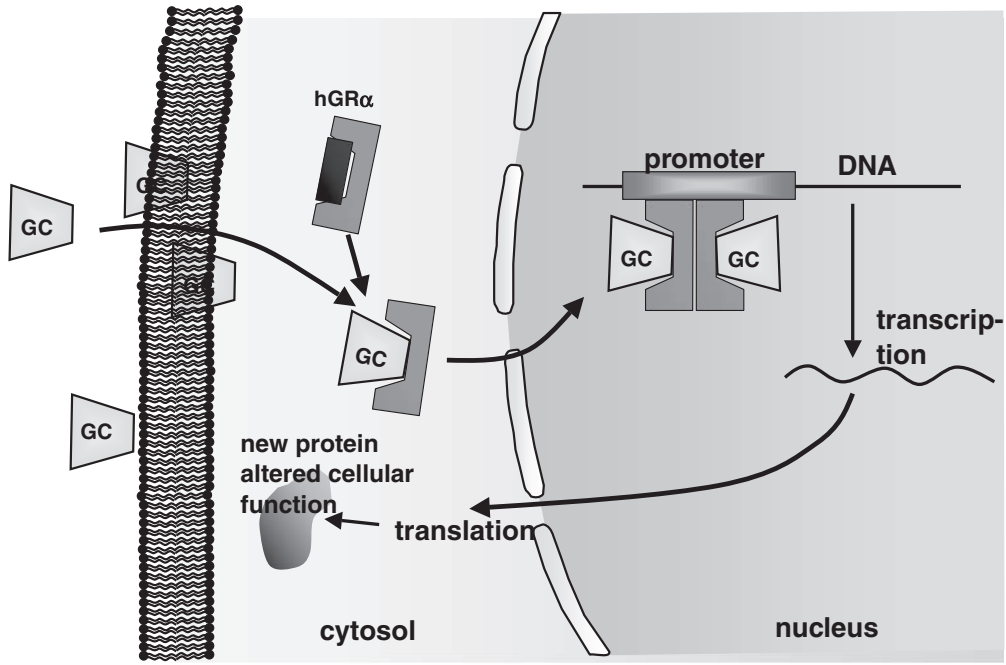

Figure 24.1 Cellular mechanisms of glucocorticoid receptor action. After binding of glucocorticoids to its receptor, hsp90 is released from the complex. This is followed by an exposure of the nuclear localization signal, homodimerization and a rapid translocation of the activated GR–glucocorticoid complex to the nucleus, where it bind to response elements (glucocorticoid response elements, GREs) and mediates transactivation or transrepression of gene transcription.

[62]. Translation from the first methionine codon in GR mRNA produces proteins that consist of 777 amino acids (GR α-A) and 742 amino acids (GR β-A). Translation from a second methionine produces proteins with 751 amino acids (GR α-B) and 716 amino acids (GR β-B). Important functional differences between the A and B isoforms have been reported: glucocorticoid receptor α-B has roughly twice the biologic activity of glucocorticoid receptor α-A in gene expression studies *in vitro* [62]. The finding that the A and B isoforms are expressed at different ratios in various tissues suggests that they may have distinct functions.

The system of multiple GR splicing forms with different activity has become even more complex as post-translational modifications of the receptor have been discovered: The human glucocorticoid receptor (hGR) has five serine residues that are phosphorylated under different conditions by cyclin-dependent kinases and mitogen-activated protein kinases (MAPKs) [62]. The phosphorylation of several of the serines is dependent on the binding of ligands such as cortisol to the glucocorticoid receptor, whereas other serines are phosphorylated in a ligand-independent manner. The phosphorylation of serines has effects on GR transcriptional activity. For example, the glucocorticoid receptor is found primarily in the cytoplasm and is inactive when phosphorylated at serine 203, but it actively transcribes DNA when phosphorylated at serine 211 [63]. GR can be further modified by the covalent attachment of ubiquitin to the receptor after cortisol binding inducing degradation of GR by the proteasome [64]. In addition, sumoylation (the attachment of small, ubiquitin-related modifiers) of the glucocorticoid receptor may potentiates its transcriptional activity [47,65]. Little is known about the effect of those post-translational modifications on the repression of gene transcription, interactions with other transcription factors or non-genomic signaling pathways [62].

The interaction of glucocorticoids with the normal GRα isoform is followed by activation and dissociation of GR from its inhibitory protein complex [43,66] and by translocation of the receptor into the nucleus, where the complex of steroid and receptor interacts with promoter regions of different genes [67–70]. This is followed by either an increase or decrease of gene transcription (Figure 24.1).

This process is very similar within the whole family of nuclear receptors, which show a high degree of genetic similarity of 40–90% identical amino acid sequence [45,70,71].

An important problem for the therapy of patients suffering from IBD and in general chronic inflammation or autoimmune diseases is the occurrence of glucocorticoid non-responders or "glucocorticoid-refractory" patients. Non-response to glucocorticoids may be mediated by mutations of the receptor, a reduced number of glucocorticoid receptors or a downregulation of receptor expression [72]. Studies demonstrated that primary (hereditary) abnormalities in the glucocorticoid receptor gene make only 2.3% of patients with asthma relatively "resistant" to glucocorticoid therapy [73,74]. "Resistance" to the beneficial

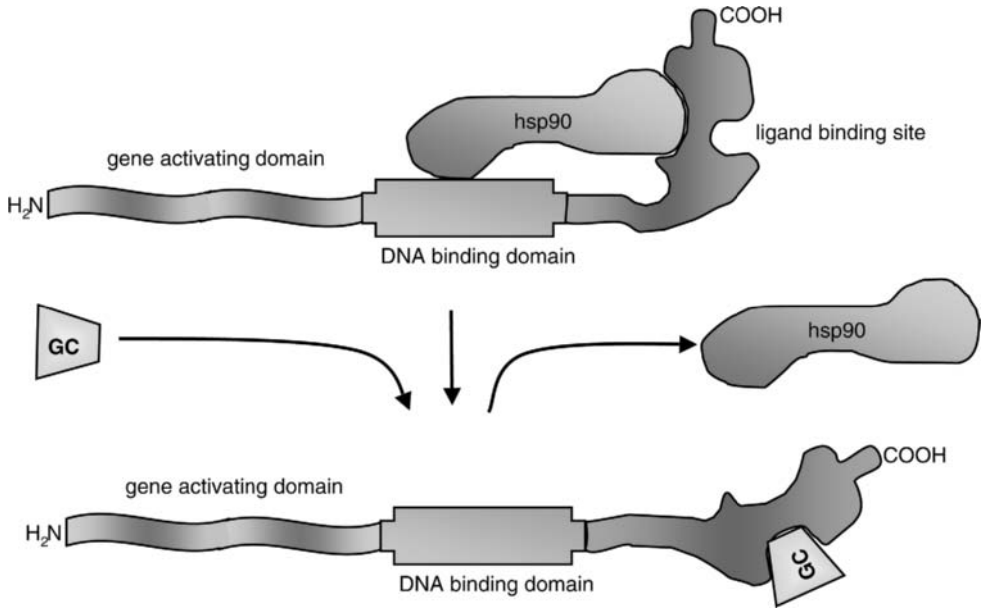

Figure 24.2 Molecular structure of the glucocorticoid receptor. The N-terminal part of GR contains a transactivation domain that plays an important role in gene regulation. The DNA binding site (DBD) consists of two zinc finger domains and contains an invariant pattern of eight cysteines arranged in two groups of four. Between the DBD and the carboxy-terminal ligand binding domain (LBD) is a so-called "hinge region". This region of the GR protein contains a nuclear localization signal, a binding region for heat shock protein 90 (hsp90) and a second transactivating domain. The C-terminal LBD not only represents the specific binding sites for glucocorticoids but also serves as a homodimerization domain. Additionally, it interacts with other proteins regulating GR activity. The inactivated GR is bound to a protein complex of approximately 300 kDa including two molecules of heat shock protein 90 (hsp90). The hsp90 proteins cover the nuclear localization site preventing the unliganded GR from localizing to the nuclear compartment. After binding of the specific ligand to the LBD, the tertiary structure of the molecule changes followed by a release of hsp90 from the complex.

clinical effects of glucocorticoid therapy in patients with IBD, therefore, is probably rarely related to primary (hereditary) glucocorticoid resistance [61]. In the majority of patients with rheumatoid arthritis or asthma, the glucocorticoid resistance seems to be acquired and localized to the sites of inflammation, where it reflects high local cytokine production, which interferes with glucocorticoid action [74,75]. Another basic mechanism that could be the reason for glucocorticoid resistance, the competition for co-factors, will be explained in detail later.

Glucocorticoid levels and binding affinities (K_d) vary among patients and have been correlated with patient response [76]. A certain threshold level of glucocorticoid receptor expression is necessary for glucocorticoid responsiveness. In patients with rheumatoid arthritis, a decrease of systemic glucocorticoid-receptors in leukocytes has been described [14,74]. However, the glucocorticoid receptor density did not correlate with inflammatory disease activity.

Molecular structure of glucocorticoid receptors

The mechanisms mediating the glucocorticoid response via glucocorticoid receptors are complex. Regulation of gene expression and GR actions occur on several levels in the body and the single cell. However, the glucocorticoid-mediated anti-inflammatory mechanisms are a classical example of the transmission of basic research to the bedside. It is important first to have a look at the structure of the normal GR isoform, GRα.

GRα is a phosphorylated 92 kDa protein, which is bound into a complex with other proteins in its inactivated form. Like most members of the nuclear receptor superfamily, GR contains a DNA binding site consisting of two zinc-finger domains [77–82]. This area contains an invariant pattern of eight cysteines arranged in two groups of four, so as to coordinate the binding of two zinc atoms [77, 78,81,83,84]. The DNA binding domain is located in the middle of the GR molecule (Figure 24.2) [80,85,86].

The two zinc-finger motifs in the DNA binding domain induce the formation of a tertiary structure that interacts with specific DNA (promoter) sequences organized in glucocorticoid receptor response elements (GREs) [39,67,80]. Between the DNA binding domain and the carboxy-terminal ligand binding domain is a so-called "hinge region". This region of the GR protein contains a nuclear localization signal, a binding region for heat shock protein 90 (hsp90) and a second trans-activating domain (activation function 2, AF2) [87–95].

The carboxy-terminal ligand binding domain not only represents the specific binding sites for glucocorticoids but also serves as a homodimerization domain [88,96].

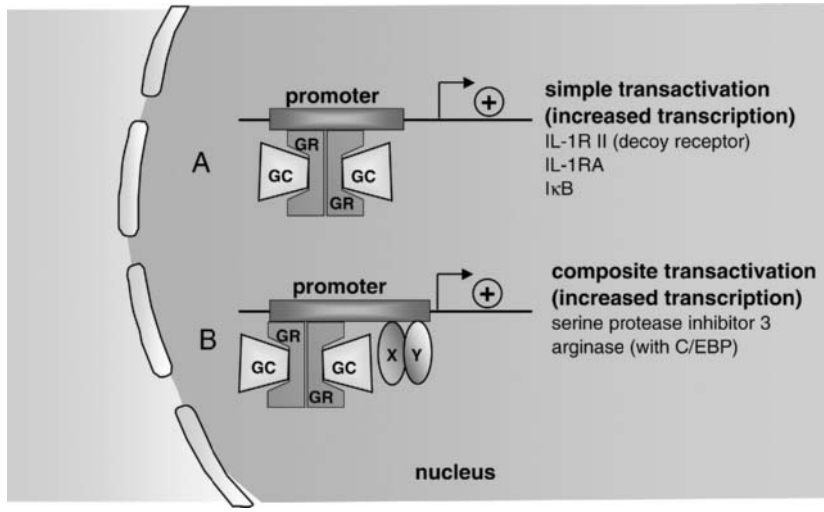

Figure 24.3 Transactivation of gene transcription by glucocorticoid receptor. (A) GR binds as a homodimer to classical GRE to activate transcription of response genes. The GRE has a palindromic motif (consensus sequence: **GGT ACA** NNN **TGT TCT**). A direct transactivation of gene transcription by GR has been described for IL-1 type II, IκB, IL-1 RA or lipocortin I. (B) For other genes such as serine protease inhibitor 3 or arginase, a cooperative transactivation involving GR and other transcription factors such as C/EBP has been described.

Additionally, it interacts with other proteins regulating GR activity.

The N-terminal part of GR contains a transactivation domain (activation function 1, AF1) that plays an important role in gene regulation [83,97,98]. This structure may be especially important during transactivation activity. AF-1 has been reported to interact with the basal transcriptional machinery but also with other adaptors or co-activators [99].

The inactivated GR is bound to a protein complex of approximately 300 kDa. It includes two molecules of hsp90, a 59 kDa immunophilin protein and other inhibitory proteins [100,101] (Figure 24.2). The hsp90 proteins cover the nuclear localization site preventing the unliganded GR from localizing to the nuclear compartment. After binding of the specific ligand to the ligand-binding domain, the tertiary structure of the molecule changes followed by a release of hsp90 from the complex. This leads to an exposure of the nuclear localization signals and a rapid translocation of the activated GR–glucocorticoid complex to the nucleus, where it can bind its response elements (GREs).

Molecular mechanisms of glucocorticoid receptor action

Activated and nuclear translocated glucocorticoid receptor can act in two principle ways: it can mediate *transactivation* of gene transcription leading to an increased mRNA expression or *transrepression* followed by a downregulation of shut off of a certain gene product.

Transactivation

In the nucleus, glucocorticoid receptors can bind to classical GREs to activate transcription of the response gene [68,69,102,103] (Figure 24.3, A). The GRE has a palindromic motif (consensus sequence: **GGT ACA** NNN **TGT TCT**). Usually GR binds this or similar DNA sequences cooperatively as a homodimer (Figure 24.3, A). For homodimerization, interaction of a group of five amino acids known as the dimerization or D loop is needed. They are located within the DNA binding domain of the GRα molecule and are essential for dimerization and transcriptional activation.

A direct transactivation of gene transcription by GR has been demonstrated for IL-1 type II receptor that binds IL-1 without induction of signal transduction thus preventing cells from activation and inflammatory reaction [104,105] (Figure 24.3, A). Another important protein whose transcription is transactivated by GR is IκBα, the inhibitory protein for the pro-inflammatory transcription factor NF-κB (see below).

For other genes such as serine protease inhibitor 3 or arginase a cooperative transactivation involving GR and another transcription factor such as C/EBP or AP-1 was shown (Figure 24.3, B). This means that binding of activated and translocated GR is necessary but not sufficient for the increased transcription of these genes. However, as soon as both GR and the cooperating transcription factor (C/EBP or AP-1) are bound to the promoter, increased gene transcription takes place.

GR-mediated transactivation of genes is also involved in the induction of apoptosis by dexamethasone [106–117]. In addition to these transactivating actions, which usually take several hours, GR have more rapid transrepressing activities.

Transrepression via DNA binding

In addition to transcription-inducing GREs, the existence of negative GRE sites (nGRE) mediating a negative regulation of transcription (transrepression) via glucocorticoids has been postulated [40,41,118–120]. However, the consensus binding site is variable and described for only a few genes [121]. Binding to a promoter sequence and subsequent transrepression by GR have been shown for the

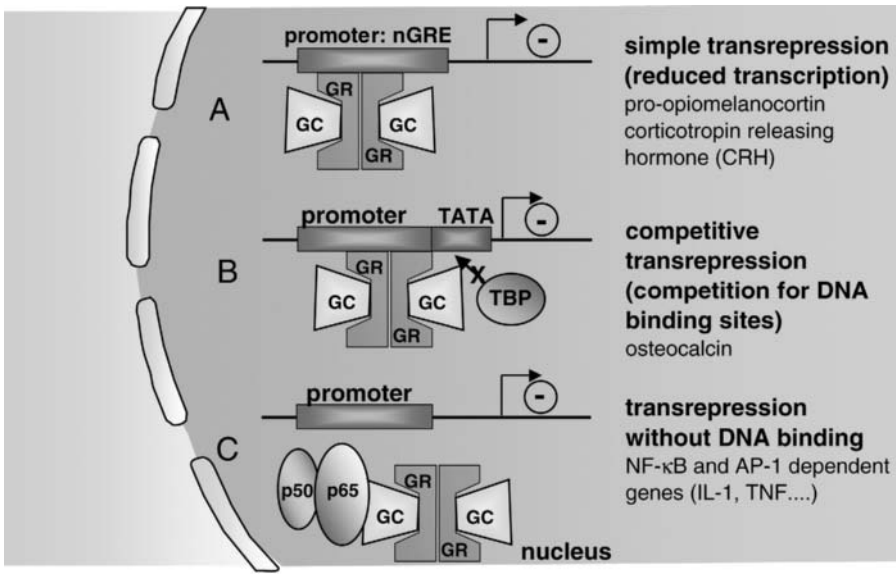

Figure 24.4 Transrepression of gene transcription by glucocorticoid receptor. (A) Binding to a negative glucocorticoid response element (nGRE) and subsequent transrepression by GR have been shown for the pro-opiomelanocortin (POMC) gene, an ACTH precursor and CRH, both allowing a negative feedback circle. (B) GR and transactivating transcription factors can compete for binding sites in promoters. In the osteocalcin promoter, GR overlaps the TATA box. Activation and nuclear translocation of GR can therefore prevent the binding of the basal transcription factor, TATA binding protein (TBP), which is necessary for the recruitment of RNA polymerase II and initiation of transcription. (C) For the p65 subunit of NF-κB and GR, a direct physically interaction has been demonstrated, leading to a transrepression of transcription of the NF-κB-dependent gene without DNA binding of GR. This interaction effect does not impair the DNA-binding ability of NF-κB.

pro-opiomelanocortin (POMC) gene, an ACTH precursor allowing a negative feedback circle [122–124] (Figure 24.4, A). Another promoter containing a negative GR binding site (at –278 to –249 of the promoter) is the corticotropin-releasing hormone (CRH) promoter [125–127]. Thus it seems that direct transrepression activity is reserved for the negative feedback circle in the hypothalamic-pituitary-adrenal axis.

Glucocorticoid receptors and transactivating transcription factors may also compete for binding sites in promoters [128–131]. In this case, the presence of GR blocks transactivation by another factor and does not actively transrepress transcription itself (Figure 24.4, B). In the osteocalcin promoter bound GR overlaps the TATA box. Activation and nuclear translocation of GR therefore can prevent the binding of the basal transcription factor, TATA binding protein (TBP), which is necessary for the recruitment of RNA polymerase II and initiation of transcription [132–134]. This may be one of the reasons for the occurrence of osteoporosis during long-term glucocorticoid therapy.

Transrepression without DNA binding

In addition to the competition for binding sites in promoters, a transrepression mechanism for GR has been described that does not involve DNA binding (Figure 24.4, C). The promoters of most pro-inflammatory genes contain binding sites for the pro-inflammatory transcription factor NF-κB or AP-1 [135]. In the genes regulated by both factors, nGREs are not found. However, glucocorticoids are able to transrepress rapidly AP-1- or NF-κB-induced transcription of these genes. This in fact might be the most important mechanism triggering their therapeutic efficacy in the treatment of inflammatory diseases.

It is not completely clear whether direct protein–protein interaction between GR and AP-1 or NF-κB is sufficient for transrepression [136,137] or whether other mechanisms are involved [138].

For the interaction of NF-κB and GR, a direct or semi-direct physically interaction of the p65 subunit of NF-κB and GR has been postulated [119,139–141]. This interaction seems to occur without impairing the DNA binding ability of both transcription factors [142,143]. The data indicate that co-activating or co-repressing proteins may play a major role in that function [138].

Pro-inflammatory transcription factors such as AP-1 and NF-κB on the one hand and GR on the other could compete for a co-activator molecule called CREB binding protein (CBP) (for more details, see [144]). CBP plays an essential role in the activation of transcription by numerous transcription factors/transcriptional activators [145–147]. CBP binds and is necessary for co-activation of CREB, AP-1, **s**ignal **t**ransducer and **a**ctivator of **t**ranscription (STAT) proteins and NF-κB and also nuclear receptors such as GR, PPARs, progesterone receptor and retinoid receptors [148,149]. It is likely that CBP and the related p300 are not

acting as single co-activator proteins but are present in the nucleus in a complex of proteins [148].

Factors determining transactivation or transrepression

The factors determining whether glucocorticoid binding to its receptor induces more transactivation or more transrepression activity on genes are not completely understood. Clearly, specific DNA binding sites play a role. Still, the question arises of why some ligands are able to induce mainly transrepression actions whereas others may mainly induce transactivation.

In experiments modeling the GR ligand-binding domain, it was shown that tyrosine 735 may interacts with the D ring of dexamethasone and that the substitution of D ring functional groups results in steroids with reduced ability to direct transactivation [150]. A substitution of tyrosine 735 by phenylalanine (Tyr735Phe) did not reduce the ligand binding affinity and did not alter transrepression of NF-κB, but reduced transactivation. These data suggest that tyrosine 735 is important for ligand interpretation and transactivation [150]. In addition, it has been suggested that GR does not necessarily have to form homodimers to modulate gene expression. nGREs have been identified in keratin gene promoters that bind GR as four monomers [151]. Thereby, a specific set of co-repressors is bound, including histone acetyltransferase and CBP but not SRC-1 and GRIP-1.

In the regulation of glucocorticoid receptor action, so-called glucocorticoid modulating elements (GME) in promoters and proteins binding to them (GMEBs) have attracted attention. A GME in the gene tyrosine aminotransferase (TAT) was identified in 1992 [152]. The glucocorticoid modulating element was identified as a 21-basepair sequence of the rat TAT gene. It is located at –3.6 kb and 1 kb upstream of the GREs [153,154]. It modulates both the dose–response curve of agonists bound to the glucocorticoid receptor and the residual agonist activity of GR-bound antisteroids. The expression of GME activity involves the binding of two novel proteins (GMEB-1 and GMEB-2) [155,156].

Taken together, it seems that four important factors determine the dose–response curve and the preference of transactivation or transrepression of glucocorticoids: The ligand structure, the GR concentration, the co-activator or co-repressor concentration/availability and the glucocorticoid modulatory elements (GMEs). An important impact from the elucidation of these basic mechanisms on the steroid treatment of patients with IBDs can be expected.

Regulation of glucocorticoid receptor expression

To complicate further the system of transactivation, transrepression with DNA binding and transrepression without DNA binding involving co-activators and co-repressor, the expression of the GR gene itself is regulated by activated GR. The GR (GRα) represses its own synthesis in a hormone-dependent manner [157,158]. The reduction in cellular receptor levels is followed by insensitivity of the cells to glucocorticoids. It is dose and time dependent and reversible upon hormone withdrawal. For this down-regulation of GR expression by GR itself, DNA binding seems to be crucial [159–161].

The phosphorylation status of GR also may be important for this feedback function. Deletion of phosphorylation sites from the GR protein by site-directed mutagenesis resulted in a GR that cannot repress its own transcription upon binding of glucocorticoids [162].

GRβ

In contrast to GRα, the shorter GRβ isoform that is generated by alternative splicing lacks a ligand binding domain. GRβ is identical with GRα through the first 727 amino acids but differs in the carboxyl terminus, where it lacks the last 50 amino acids found in GRα but contains an additional 15 non-homologous amino acids [54,163–165]. As GRα, GRβ is widely expressed in adult human tissues but is primarily localized to the cell nucleus independent of the presence of ligand [54]. GRβ still binds to DNA and may therefore potentially interfere with the action of GRα [166,167]. It has been speculated that GRβ might be an antagonist of GRα action as it blocks DNA binding sites without suppressing gene transcription [54,164]. However, this inhibition seems to require increased GRβ expression relative to GRα. It has been studied frequently in transfection assays with a strong overexpression of the β-isoform. In human tissues, in contrast, the α-form of GR is much more abundant.

Glucocorticoid resistance in asthma patients has been described to be associated with elevated *in vivo* expression of the glucocorticoid receptor β-isoform [53,59,168]. However, conflicting data have also been obtained showing no overexpression of GRβ in resistant patients [169,170].

Regulation of GR action in IBD

The regulation of GR action and the mechanisms involved in its function are complex. It has always been a question whether it would not be possible to evaluate which patient would respond to glucocorticoid therapy and which patient would be refractory *before* starting drug administration. In addition, elucidation of the mechanisms leading to a steroid refractory state would raise the potential of direct targeting or modulating the individual steroid response.

Investigations on possible mechanisms of steroid-refractory IBD so far have not provided conclusive data: hsp90 has some features of an inhibitory protein of GR and is bound to its inactive form (see the section Molecular structure of glucocorticoid receptors). The expression of human hsp90 in patients with Crohn's disease and

ulcerative colitis was studied, but no differences between patients and controls were found, making a role of hsp90 in glucocorticoid-refractory inflammatory bowel disease unlikely [171].

The number and dissociation constant (K_d) of GR in peripheral blood mononuclear cells of six non-responders of glucocorticoid treatment with ulcerative colitis, five responders and ten healthy controls was determined in another study [172]. A significant increase in the number of binding sites and the dissociation constant in non-responders compared with responders was found. Surprisingly the number of binding sites was highest in glucocorticoid non-responders.

When GR levels were determined in the cytosol to ensure that only free receptor and not already steroid-associated, translocated receptor molecules were quantified, the situation seemed to be different. The dexamethasone binding in cytosol from peripheral blood mononuclear cells (PBMNCs) of corticosteroid-treated IBD patients was significantly lower compared with controls and not glucocorticoid-treated IBD patients [173]. Systemic GR levels in non-treated IBD patients did not differ significantly from controls. There was no difference in the binding affinity of patients and controls with an obvious lower binding maximum indicating a reduced receptor number in the steroid-treated patients group. In contrast to the findings in PBMNCs, mucosal GR levels of IBD patients were significantly decreased in both steroid-treated and non-reated patients compared with controls [173].

The reduced binding of [^{3}H]dexamethasone in cytosol from steroid-treated IBD patients is most likely due to a feedback regulation of GR in the cells by the ligand. This assumption is supported by a number of studies [76,174]. In IBD, there seems to be no difference in systemic GR levels between non-glucocorticoid-treated patients and control persons in general. This indicates that localized inflammation in the intestinal mucosa is not followed by a systemic depression of glucocorticoid receptor levels in leukocytes. However, IBD patients with severe inflammation or severe extraintestinal manifestations have not been studied.

Honda *et al.* studied the expression of GRα and GRβ in PBMNCs of patients with ulcerative colitis and controls [57]. They found expression of GRβ in only 9.1% of patients with steroid-sensitive disease whereas it was present in 83.3% of steroid-resistant patients as detected by polymerase chain reaction (PCR). They concluded that the determination of GRβ expression could provide a tool to predict steroid responsiveness of UC patients [57]. In contrast, our own recent data show expression of GRβ transcripts in all patients investigated at levels not different form controls.

Despite these studies, most of the questions regarding the mechanisms of steroid-refractory IBD are still unanswered [175,176]. It is not clear whether changes in glucocorticoid binding are just an epiphenomenon or a cause of different disease courses. It is not clear whether GRβ expression or decreased GRα levels are crucial for the treatment success.

Future studies on the glucocorticoid receptor expression in IBD have to answer several important questions: Are glucocorticoid receptor levels at the onset of the disease predictive for the success of glucocorticoid therapy? Are low levels correlated with the development of a steroid-refractory disease? The question of whether measurement of glucocorticoid-receptors in the mucosa can be predictive for success of the therapy has to be answered for the future management of patients with IBD. Patients with low glucocorticoid receptor levels could then be primarily treated with other drugs such as azathioprine.

Cellular mechanisms of glucocorticoid action in inflammatory bowel disease

GRα or GRα isoforms activated by ligand binding mediate glucocorticoid action and induce transactivation or transrepression of genes and also inhibition of pro-inflammatory transcription factors such as NF-κB and AP-1. The question arises of what effects the ligand-induced activation of glucocorticoid receptors has on a cellular level or, more precisely, what effects can be observed in different cell types. A simplified view could focus on two major principles (Figure 24.5). First, the cellular effects induced by a downregulation of pro-inflammatory transcription factors. As mentioned this is facilitated by a so far not completely understood antagonism to transcription factors like NF-κB and AP-1. Second, the induction of apoptosis of activated immune cells, which limits the immune response and as a consequence is also followed by reduced levels of circulating pro-inflammatory factors. Only some of the effects on some of the relevant cell populations in the intestinal immune system can be highlighted.

Lymphocytes

Glucocorticoids affect the growth, differentiation and function of lymphocytes, the distribution of cellular subsets and the production of cytokines [29,177–180]. In the chronically inflamed mucosa, the number of lymphocytes is strongly increased. Most of these cells are activated T helper cells.

Glucocorticoids reduce lymphocyte proliferation and induce apoptosis in these cells [17,29,107,181]. Studies indicate that mainly transcriptional transactivation functions are required for this glucocorticoid-mediated apoptosis [182]. Interestingly, intestinal intraepithelial lymphocytes (IELs) may be resistant to steroid-induced apoptosis, which could be due to the expression of high levels of the anti-apoptotic protein Bcl-2 and Bcl-x [29,183].

GR-mediated inhibition of cytokine secretion in inflammatory diseases may be mediated not only by direct

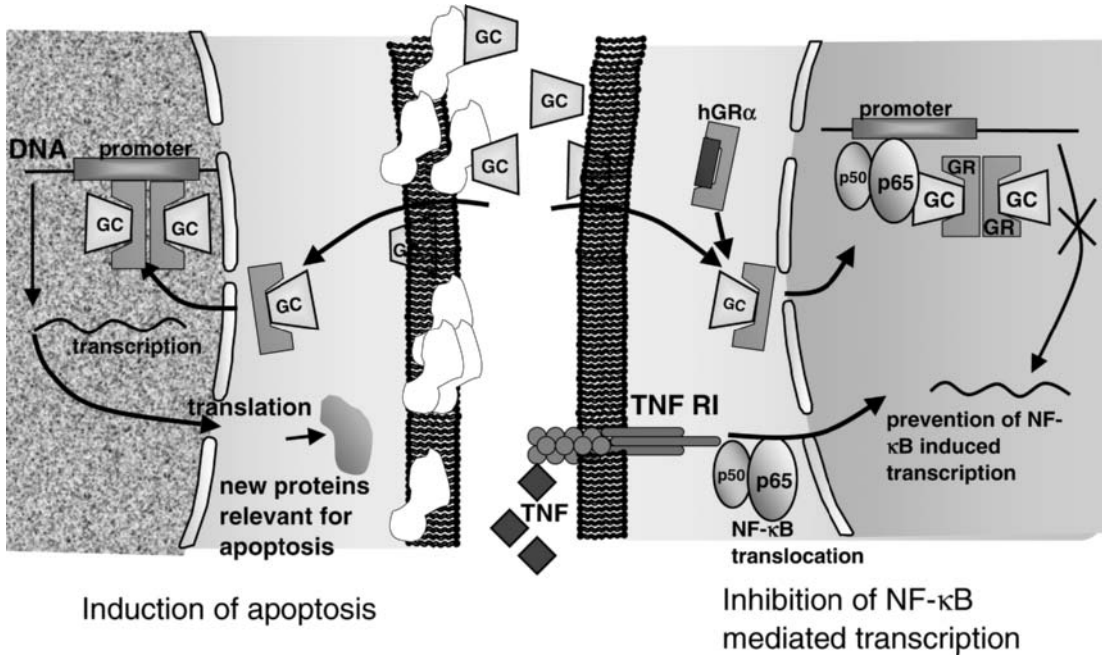

Figure 24.5 Glucocorticoid-induced anti-inflammatory mechanisms. Two major principles mediate the anti-inflammatory effect of glucocorticoids. After penetrating the cell membrane and binding to the glucocorticoid receptor, nuclear translocation occurs. The complex then can transactivate genes that are involved in the induction of apoptosis. The induction of apoptosis in activated lymphocytes, eosinophils, basophils and other cell types reduces the overall amount of circulating cytokines and inflammatory mediators and allows reconstitution of the intestinal mucosa. On the other hand, the heterodimer of GR interacts with translocated NF-κB and prevents transcriptional activation by this pro-inflammatory transcription factor. As NF-κB activation has anti-apoptotic effects in a number of cell types, antagonism to its action also has pro-apoptotic effects, indicating the interconnection of both mechanism of glucocorticoid action.

action on lymphocyte and monocyte/macrophage NF-κB but also in addition indirectly through promotion of a T helper cell type 2 (Th2) induction [184] with increased levels of Th2 cytokine (IL-4) and reduced levels of Th1 cytokine (IL-12) secretion [185]. In addition, dexamethasone can inhibit IL-12-induced phosphorylation of STAT4 without altering IL-4-induced STAT6 phosphorylation [185]. Both result in a blockade of pro-inflammatory T helper cell type 1 (Th1) cytokine expression.

A further example of the multi-site mechanisms involved in glucocorticoid action is illustrated by another monocyte/macrophage–lymphocyte interaction. Glucocorticoids downregulate the T cell co-stimulatory molecules B7-1 and B7-2 on macrophages which are essential for clonal T cell expansion in reaction to antigen-presenting cells [186]. On the other hand, co-stimulatory molecules can prevent cells from glucocorticoid-induced apoptosis [187]. It is interesting in this context that normal intestinal macrophages express no co-stimulatory molecules, whereas there is a clear upregulation in IBD [188].

Macrophages

Macrophages are known to play an important role during inflammation in many different tissues [189,190]. Intestinal macrophages represent one of the largest compartments of the mononuclear phagocyte system in the body [191]. They are localized preferentially in the subepithelial region and constitute 10–20% of mononuclear cells in the intestinal lamina propria [192]. Macrophages are able to secrete pro-inflammatory cytokines which are known to be regulated by NF-κB such as IL-1β, TNFα, IL-6, IL-8 andMCP-1 [189].

In normal mucosa, only very few macrophages express activation-associated markers such as CD14, CD16, HLA-DR, CD11b and CD11c [193], supporting a concept of anergy in the normal mucosa. Several findings indicate a phenotypic change of the intestinal macrophage population in IBD. Mahida *et al.* demonstrated the presence of CD16 (FcγIII receptor) in IBD by immunohistochemical methods and in isolated cells [194]. CD54 (ICAM-1) expression increased from 7 to 70% in ulcerative colitis and to 46% in Crohn's disease [195]. Inflammation-associated intestinal macrophages express Toll-like receptors, Fc receptors and co-stimulatory molecules [188,190,193,196–200], which enable them to stimulate T cells and partially prevent them from glucocorticoid induced apoptosis (see above).

The number of macrophages is clearly increased in both Crohn's disease and ulcerative colitis mucosa [201–203]. The activated macrophage population secretes a multitude of inflammatory mediators such as prostaglandins,

leukotrienes, cytokines such as TNF, IL-1 and IL-12, chemokines such as MCP-1 and IL-8 ands tissue-damaging reactive radicals and tissue-degrading enzymes [2,204–209]. The transcription, translation and secretion of almost all molecules mentioned above can be reduced or inhibited by the administration of glucocorticoids. In the promoters of most of those genes, NF-κB binding sites can be found, indicating that the glucocorticoid-mediated inhibition of NF-κB activation may be the most important mechanism for their anti-inflammatory potential.

Intestinal epithelial cells

Intestinal epithelial cells (IECs) play an active role in the intestinal immune system [2,210–212]. Epithelial cells are able to respond to damage or bacterial invasion by secreting cytokines and chemokines [2,213,214]. Among the cytokines secreted by intestinal epithelial cells are inflammatory mediators such as IL-6 and IL-8.

The induction of cytokine production in human intestinal epithelial cells following stimulation is usually associated with activation of the transcription factor NF-κB. Jobin *et al.* demonstrated that adenovirus-mediated transfection of HT-29 and Caco-2 cells with an NF-κB superrepressor caused a reduced induction of inducible nitric oxide synthase (iNOS), IL-1β and IL-8 by IL-1 or TNF, indicating involvement of the NF-κB system in regulation of these genes [215]. In the inflamed mucosa, activation of NF-κB has been demonstrated in intestinal epithelial cells whereas activation was absent in non-inflamed mucosa [206].

Glucocorticoids downregulate the expression of IL-6, IL-8, iNOS and class II molecules in intestinal epithelial cells and restore epithelial cell physiology [216–219]. This is again likely to be mediated by the inhibition of NF-κB activation. On the other hand, they impair cell proliferation and therefore mucosal repair functions. This becomes especially relevant after surgical procedures. It has been shown that administration of glucocorticoids before surgical procedures is associated with up to a 20-fold increased risk of infectious complications [220]. This may be due to impaired closure of wound defects partially associated with impaired epithelial repair mechanisms.

Other cells types

As GR are expressed in most if not all cell types, effects of glucocorticoid therapy on all cell types involved in intestinal inflammation have been found. During the course of IBD, increased numbers of eosinophils and mast cells are present in the intestinal mucosa [221]. These cells are activated and secrete different tissue-damaging proteins and also cytokines and chemokines [222,223]. Again, NF-κB has been found to be a major factor in activation of eosinophils [224] and glucocorticoids have been shown to downregulate the production and secretion of the pro-inflammatory proteins [225–228]. In addition, the number of mucosal and circulating eosinophils is decreased by glucocorticoids. This is probably mediated by an induction of apoptosis in these cells [226].

In addition to the number of eosinophils and mast cells, the number of basophils is increased in IBD mucosa. Glucocorticoids reduce the number of basophils similarly as described for eosinophils [228].

Neutrophils are a major component in active lesions in ulcerative colitis and to a lesser extent in Crohn's disease [1,229]. They are recruited under the influence of the neutrophil-chemoattractant IL-8 [230,231]. An important feature of these neutrophils is their ability to induce the so-called "oxidative burst" reaction involving the NADPH oxidase system leading to secretion of oxygen radicals that not only kill surrounding bacteria but also damage surrounding tissue. Glucocorticoids act in several different ways in neutrophils [232–235]. They downregulate the oxidative burst reaction and reduce IL-8 secretion, leading to reduced transfer of neutrophils into the mucosa [235]. On the other hand, a increased release from the bone marrow followed by leukocytosis is induced.

Another important feature of the treatment with glucocorticoids is the downregulation of the expression of adhesion molecules on endothelial cells, mononuclear cells and epithelial cells [236–238], which again may be mainly mediated by antagonistic effects to NF-κB.

Glucocorticoid therapy in combination with other anti-inflammatory drugs in inflammatory bowel disease

Despite recent discussion on "top-down" therapy or early "immunosuppressive" approaches (glucocorticoids of course are also immunosuppressants), an established therapy for acute flares of IBD still is the systemic application of glucocorticoids. The effectiveness of a glucocorticoid regimen has been shown in numerous multi-center trials [18–22,24]. Initial remission rates in patients with acute flares of Crohn's disease under a standard therapy vary from 60 to 80%, which is higher than under treatment with sulfasalazine or 5-aminosalicylic acid (40–50%).

Recently evidence has been found that indeed some of the anti-inflammatory effects of 5-aminosalicylic acid (5-ASA) and sulfasalazine in IBD patients may be mediated by the inhibition of the pro-inflammatory transcription factor NF-κB. 5-ASA and sodium salicylate inhibit activation of NF-κB by blocking IκB kinases (IKKs) [239], which are key enzymes in NF-κB activation [240–242]. Similar results were found for sulfasalazine [243,244]. The inhibition of both IKK alpha and beta has been shown [243].

The lack of additional effects of a combination of glucocorticoid and salicylate therapy indicates that the glucocorticoid effect on NF-κB inhibition may be superior. However, long-term studies show that despite the high initial response rates, only 44% of the patients initially treated with glucocorticoids have a long term remission. About

25–35% of the patients become "steroid dependent", indicating that steroid treatment cannot be completely tapered and omitted. About 20% of glucocorticoid-treated patients turn out to be primarily "glucocorticoid resistant" [245].

Peroxisome proliferator-activated receptor gamma (PPARγ)

Expression and function of PPARγ

GR is a member of the steroid or nuclear receptor superfamily. Other members of this family have attracted increasing attention in recent years. Particularly peroxisome proliferator-activated receptor gamma (PPARγ) and alpha (PPARα) have been shown to have NF-κB-inhibiting activities and could be possible tools for the treatment of IBD in the future. They are further examples of a multi-site treatment approach.

PPARs are like the glucocorticoid receptor ligand-activated receptors. They have been discovered as regulators of lipid and lipoprotein metabolism [246,247]. However, in recent years, it has been shown that they also regulate cellular proliferation, differentiation and apoptosis [247–249], features that may be very important during repair processes after or during intestinal inflammation. Three family members are known; besides PPARα and -γ, PPARδ is encoded by a separate gene and expressed in most tissues. PPARs form heterodimers with the retinoid X receptor and bind to PPAR response elements (PPREs) in the promoters of target genes.

A high expression of PPARγ is found in adipose tissue, the adrenal gland, the spleen and interestingly the colon [250–254]. PPARγ also is expressed in differentiated macrophages whereas there is no expression in monocytes [177,247]. In activated macrophages, a significant upregulation of PPARγ was found [255]. Activation of PPARγ inhibits inducible nitric oxide synthetase (iNOS), gelatinase B and scavenger receptor A genes. This inhibition is partially mediated by an antagonism to the transcription factors AP-1, STAT1 and NF-κB [255]. In addition to macrophages and adipocytes, colonic epithelial cells express high levels of PPARγ mRNA and protein [256].

Anti-inflammatory properties of PPARγ

When activated, PPARγ molecules form heterodimers with another transcription factor of the nuclear receptor superfamily, the retinoid X receptor (RXR). Heterozygous PPARγ and RXRα knockout mice display a significantly enhanced susceptibility to 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis compared with their wild-type littermates, indicating a role for the RXR/PPARγ heterodimer in the protection against colon inflammation [257]. A synergistic effect of PPARγ and RXR ligands was observed.

Interestingly, the pro-inflammatory genes that are repressed by PPARγ overlap with but are not identical with the genes that are downregulated by GR. The PPARγ-mediated effects in an experimental setting of Toll-like receptor stimulation were independent of NF-κB and interferon regulatory factor (IRF) – in contrast to GR action [138]. This indicates that glucocorticoids and ligands of PPARγ could have additive therapeutic effects.

In addition to their NF-κB antagonistic properties, ligands for PPARγ similar to GR have been proven to induce apoptosis in a number of different cell types and cell lines [247,248,258–260].

The eicosanoids 13-hydroxyoctadecadienoic acid (13-HODE) and 15-hydroxyeicosatetraenoic acid (15-HETE) and also 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2) have been identified to be natural occurring ligands of PPARγ [261]. Thiazolidinediones (TZDs) are high-affinity synthetic ligands of PPARγ frequently referred to as "PPARγ agonists". TZDs are currently used as insulin-sensitizing agents in the treatment of type II diabetes mellitus. Due to the anti-inflammatory properties of PPARγ, the therapeutic efficacy of eicosanoids and TZDs has been evaluated in different models of inflammation [262,263].

Treatment of colitis with PPARγ agonists

PPARγ has attracted interest among gastroenterologists [264] as it could be consistently demonstrated that PPARγ ligands are capable of reducing the mucosal damage and prevent or downregulate the inflammatory response in several murine models of intestinal inflammation [257,265–267]. Further evidence for an anti-inflammatory role of TZD–PPARγ ligand rosiglitazone was found in IL-10-deficient mice in which rosiglitazone delayed onset of colitis [251] and in TNBS-induced colitis in rats in which it reduced mucosal ulceration and TNF secretion [248]. Overexpression of PPARγ by an adenoviral construct in mucosal epithelial cells in mice was associated with amelioration of experimental inflammation [268].

However, not only TZDs could become important for therapeutic use of PPARγ effects: Activation of PPARγ by conjugated linoleic acids also protected mice from experimental colitis [269]. This effect was not seen in mice with a colonic knockout of PPARγ. As linoleic acids in the gut are mainly food-derived bacterial metabolites, this finding raised the possibility of positive effects of food supplements on intestinal inflammation mediated via PPARγ. This finding linked the PPARγ-mediated effects with homeostasis of intestinal microflora and the epithelial barrier. In normal mucosa, PPARγ in intestinal epithelial cells could recognize luminal bacterial metabolites and then set the threshold of NF-κB activity.

Another aspect of the therapeutic potential of PPARγ agonists is the prevention of colitis-associated cancer. Ligand activation of PPARγ in colon cancer cells caused a reduction in linear and clonogenic growth [270]. Human

colon cancer cells transplanted into mice showed a significant reduction in growth when the animals were treated with TZDs. Furthermore, specific colitis-associated colon carcinogenesis is suppressed [271].

Rousseaux *et al.* presented evidence that the therapeutic effect of 5-ASA may be mediated by PPARγ [272]. 5-ASA treatment had beneficial effects on colitis in wild-type mice but not in heterozygous PPARγ knockout mice [272]. In epithelial cells, 5-ASA increased PPARγ expression and promoted its translocation from the cytoplasm to the nucleus, where it induced a modification allowing the recruitment of co-factors for the regulation of transcription.

Interestingly, a reduced expression of PPARγ during ulcerative colitis but not in Crohn's disease has been reported [273]. A pilot study in patients with active ulcerative colitis refractory to standard medical therapy has shown some beneficial effects of TZDs [274].

In a recent multicenter, randomized, double blind, placebo-controlled clinical trial, 105 patients with mildly to moderately active ulcerative colitis were treated with rosiglitazone 4 mg orally twice daily or placebo for 12 weeks [275]. At week 12, rosiglitazone-treated patients were more likely to achieve clinical response (44 vs 23% for placebo-treated patients; $p = 0.03$) and more likely to be in clinical remission (17 vs 2% for placebo-treated patients; $p = 0.01$). It appears that RRARγ agonists could be effective for the treatment of patients with ulcerative colitis and represent a successful multi-sited targeted therapy.

Inhibition of NF-κB-mediated transactivation as a central target of IBD therapy

The discussion of glucocorticoid receptor functions and PPARγ effects showed that one of the most important anti-inflammatory mechanisms mediated by glucocorticoid therapy is the inhibition of the pro-inflammatory transcription factor NF-κB. Therefore, the NF-κB system needs a more detailed consideration.

Molecular mechanisms of NF-κB activation

Transcription factors of the NF-κB/Rel family form dimeric complexes which control the expression of a variety of inducible genes involved in inflammation and proliferation [240,276,277]. The prototypic heterodimeric complex NF-κB consists of the subunits p50 and p65 (RelA) (Figure 24.6). The inactive NF-κB dimer is present in the cytosol bound to inhibitory proteins, termed IκB.

The activation of NF-κB by inflammatory cytokines and microorganisms requires the release of IκB from the complex (Figure 24.6). The release of IκB is induced by phosphorylation of IκB at two conserved amino-terminal serine residues by a multi-protein IκB kinase (IKK) complex containing IκB kinase alpha (IKK-α) and beta (IKK-β) as two catalytically active subunits and a regulatory subunit, IKKγ or NEMO (Figure 24.6) [240,242,278,279]. NEMO

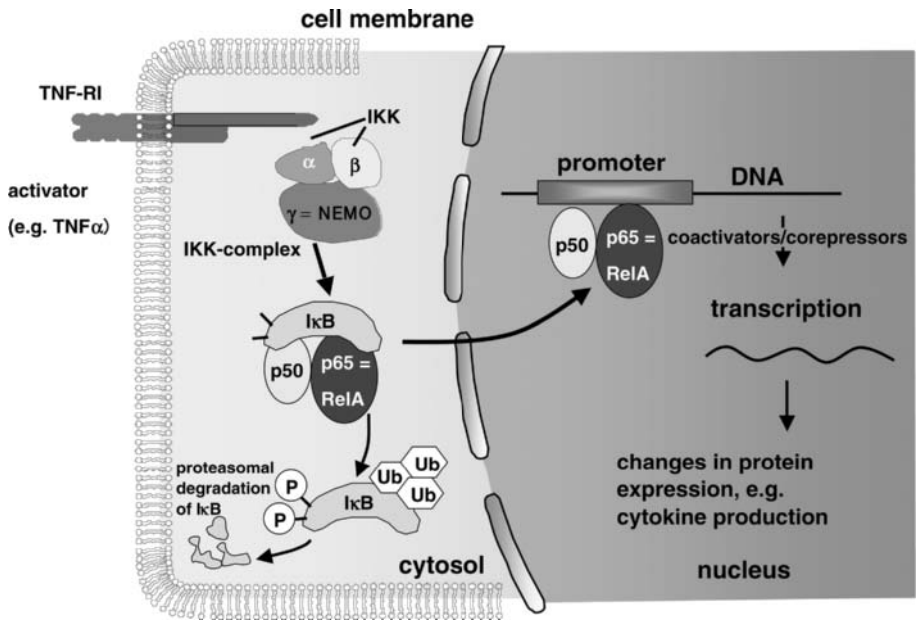

Figure 24.6 Principles of NF-κB activation. In the cytoplasm, the NF-κB complex is associated with the inhibitory protein IκB. Cell membrane-bound receptors activate the IκB-kinase complex that contains the two active IκB kinases IKK-α and IKK-β, which are phosphorylated and themselves phosphorylate of IκB at serine 32 and serine 36. This is followed by release of IκB from the NF-κB complex. Consecutively a rapid proteasomal degradation of IκB and a translocation of the activated NF-κB into the nucleus take place. In the nucleus, the activated NF-κB dimer interacts with cofactors and corepressor and regulatory NF-κB elements in promoters leading to alteration in transcription rates and altered cell function. When the degradation of IκB is blocked by inhibitors of the proteasome complex, IκB re-associates with the NF-κB dimer and NF-κB activation is inhibited.

is essential for NF-κB activation. NEMO dysfunction in humans is the cause of incontinentia pigmenti and hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID).

The activation of the IKK complex is followed by a polyubiquination of the IκB proteins by a specialized E3 ubiquitin ligase complex (E3IκB), which makes them accessible to proteolytic degradation by the 26S proteasome. The removal of IκB proteins activates and exposes nuclear localization signals (NLS) followed by translocation of the activated NF-κB into the nucleus. There, the activated NF-κB dimer interacts with regulatory NF-κB elements in promoters and enhancers (Figure 24.6).

NF-κB activation in inflammatory bowel diseases

There is evidence that NF-κB transcription factors might play an important role in the inflammatory process of IBD. It was shown that the administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-κB abrogates colitis in IL-10-deficient mice and in the TNBS–colitis model [280]. Inhibition of the proteasome complex which is responsible for the rapid degradation of IκB, prevented NF-κB activation and attenuated the colonic and splenic injury and inflammation in the PG/PS model [281]. In the DSS model of chronic colitis, the DSS-induced intestinal inflammation was characterized by an increase in NF-κB activity [282]. Blocking NF-κB activation by administering gliotoxin, a fungal product, was accompanied by a significant suppression of intestinal inflammation and mRNA expression of TNFα and IL-1α *in vivo* [282].

In mucosal biopsies from patients suffering from inflammatory bowel diseases, activation of NF-κB was demonstrated to be mainly localized in two cell types in the intestinal mucosa by double-labeling techniques, (1) in lamina propria macrophages and (2) in epithelial cells [206].

A variety of genes are induced in the inflamed mucosa which have been shown to be regulated by NF-κB, including the genes encoding TNFα, IL-1β, IL-6, IL-8, macrophage colony-stimulating factor (M-CSF), macrophage granulocyte colony-stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Some of these gene products, such as TNFα and IL-1, are also able to activate NF-κB, leading to a positive autoregulatory loop.

NF-κB-activation and innate immunity

NOD1 and NOD2 and also Apaf1 are members of a family of intracellular proteins that contain an N-terminal **ca**spase **r**ecruitment **d**omain (CARD) [283–286]. NOD1 and NOD2 both respond to bacterial wall constituents with NF-κB activation independent of TLR4 and MyD88 function (Figure 24.7). After binding of bacterial wall components to a leucine-rich domain in the NOD1 or NOD2 protein the CARD domain of NOD interacts with the corresponding CARD domain a protein called RICK (also called RIP2 and CARDIAK) (Figure 24.7). RICK is a protein kinase that is able to activate NF-κB in an IKK-dependent pathway. Based up these results, NOD1 and NOD2 are likely to be members of an intracellular recognition system for bacterial wall constituents regulating NF-κB activation among other cellular functions.

Ligation of another family of pattern recognition receptors, the Toll-like receptor (TLR) family, is also followed by IKK complex activation and subsequent NF-κB translocation to the nucleus (Figure 24.7). TLRs are members of the IL-1 receptor family of proteins. They recognize bacterial, fungal and viral products such as bacterial lipopolysaccharide (LPS) (TLR4), flagellin (TLR5) and bacterial DNA (TLR9). An important adaptor protein involved in IL-1R family members-induced signaling is MyD88 (Figure 24.7).

As NF-κB is activated by pattern recognition receptors such as TLRs and NOD2 and NOD2 variants lead to defects in innate immune and epithelial barrier functions, it was speculated that inhibition of NF-κB might also be associated with impaired innate immunity. Indeed, evidence for a protective role of NF-κB has been found. Recently, it was demonstrated that intestinal epithelial cell-intrinsic IKKβ-dependent gene expression is a critical regulator of responses of dendritic cells and T cells in the intestinal mucosa [287]. Mice with an epithelial-specific deletion of IKKβ showed a reduced expression of the cytokine lymphopoietin in the intestinal mucosa and, after infection with the gut-dwelling parasite *Trichuris*, failed to develop a pathogen-specific Th2 response associated with persisting infection. In addition, these animals showed increased production of IL-12/23p40 and TNF, leading to severe intestinal inflammation. This work pointed to an important role of IKKβ-dependent gene expression in the intestinal epithelium at least during acute infection (Figure 24.8).

Further evidence was found for an important role of NF-κB for intestinal epithelial integrity and the interaction between the mucosal immune system and gut microflora. In a mouse model of epithelial cell-specific inhibition of NF-κB through NEMO, severe chronic intestinal inflammation occurred associated with increased apoptosis of colonic epithelial cells, impaired expression of antimicrobial peptides and translocation of bacteria into the mucosa [288]. Deficiency of MyD88 is associated with a lack of TLR-induced signaling (see Figure 24.7). MyD88 deficiency prevented the development of intestinal inflammation, indicating that TLR ligation is essential for disease pathogenesis in this mouse model.

These findings demonstrate that a complete lack of NF-κB activation in intestinal epithelial cells is associated

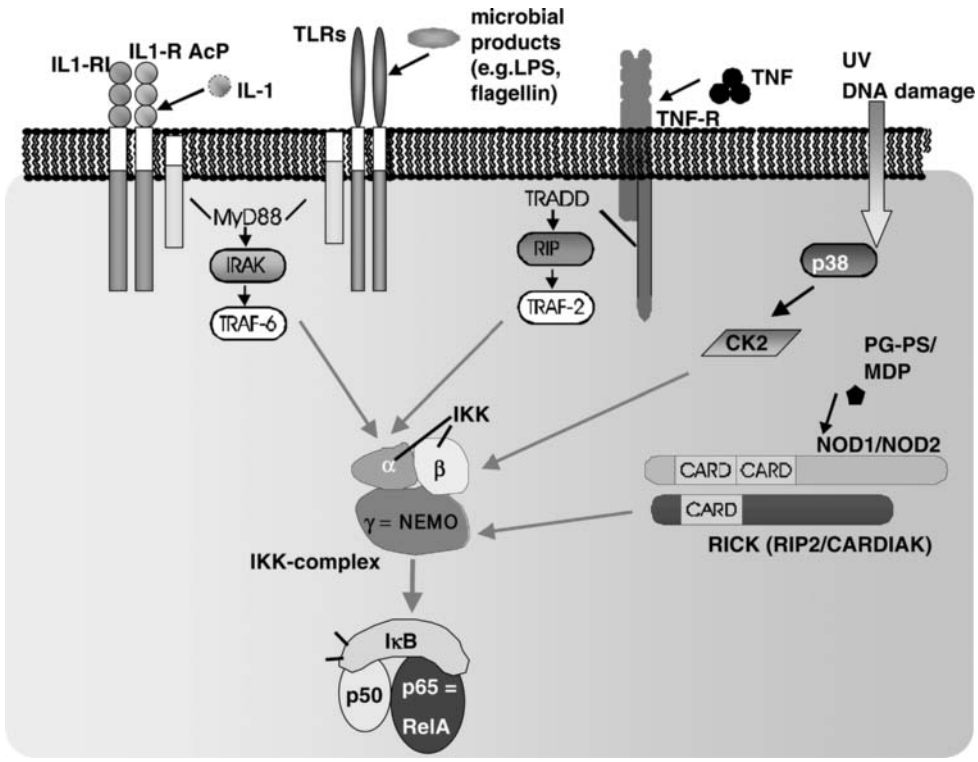

Figure 24.7 Pathways of IKK complex and subsequent NF-κB activation. TNF-RI and the IL-1 receptor family members (e.g. IL-1 RI or TLRs) utilize distinct but analogous signaling cascades, resulting in IKK activation, IκB degradation and finally NF-κB translocation and activation. Both bind adaptor proteins (TRADD associates with TNF-RI, MyD88 associates with IL-1RI and TLRs). This is followed by recruitment of a seronine–threonine kinase (RIP and IRAK, respectively) that are phosphorylated. Both pathways then utilize TRAF factors (TRAF-2 and TRAF-6) leading to IKK activation. After binding of PG-PS or MDP to the LRR domain of NOD1 or NOD2, their CARD domain interacts with the CARD domain of RICK followed by IKK complex activation. DNA damage causes IKK complex activation via the p38 kinase pathway.

with mucosal inflammation and provide evidence for a dual role of NF-κB activation (Figure 24.8). NF-κB activation may be essential for epithelial cell protection and innate defense mechanisms, especially in a state of acute challenge such as bacterial translocation. In this situation, an acute inflammatory response might be necessary to eliminate the invading microbiotics (Figure 24.8). It has to be kept in mind that TNF neutralization is also of disadvantage in most models of acute infection of acute inflammation. We therefore knew already that an inflammatory response is of crucial importance for the protection of the human body.

The beneficial effects of NF-κB inhibition during chronic inflammation could be explained by predominantly negative effects of the transcription factor for mucosa integrity in this situation.

Side effects of NF-κB blockade: evidence for a dual role of NF-κB

We all are aware of the immunosuppressive side effects of a systemic glucocorticoid therapy that can lead to fatal consequences in the case of infections [220]. One of the reasons for the observed immunosuppression is the induction of apoptosis in activated lymphocyte and monocyte populations. As the inhibition of NF-κB is one of the most important mechanisms of glucocorticoid-mediated effects, it is clear that NF-κB inhibition may contribute to the side effects.

The disruption of the p65 gene in mice is followed by embryonic lethality at 15–16 days of gestation and a massive degeneration of the liver by programmed cell death or apoptosis at this early time point [289]. TNF-mediated gene induction is absent in those mice, whereas a basic NF-κB-dependent transcription of genes is conserved [289]. This indicates that the p65 subunit is essential for inducible, but not basal, transcription in NF-κB-regulated pathways. Mice lacking the p50 subunit, in contrast, show no developmental abnormalities [290]. However, they exhibit multifocal defects in various forms of immune responses, e.g. a lack of proliferation of B cells in response to bacterial lipopolysaccharide and a defect in basal and specific antibody production [290].

From these observations, it can be concluded that a systemic complete blockade of NF-κB activation could be a dangerous treatment approach. In a DSS model of colitis, treatment with $2 \times 20\,\mu g$ per day of the NF-κB inhibitor

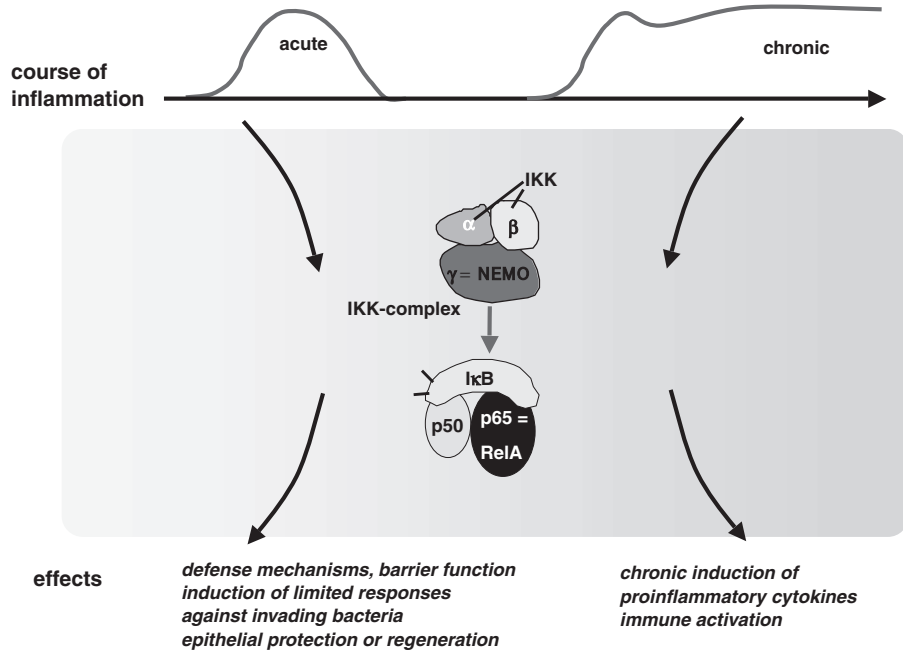

Figure 24.8 The dual role of NF-κB. Recent evidence suggests that a lack of IKKs and NEMO function might be associated with intestinal inflammation. Obviously NF-κB activation plays a role in mucosal protection, epithelial barrier function, epithelial repair and activation of the innate immune system (as is obvious from its connection to the TLR- and NOD-mediated recognition of bacterial products). This may be crucially important during acute bacterial translocation, especially for defense mechanisms. During chronic inflammation, negative effects of NF-κB activation may be predominant, explaining the positive effects of NF-κB inhibition during chronic inflammation.

gliotoxin was followed by reduced survival of the mice despite improved histology in the colon [282].

Therefore, it may be better to achieve a local (mucosal) NF-κB inhibition. Curcumin, a substance derived from the *Curcuma longa* plant, which is used as a spice in India, inhibits IκB degradation and thereby NF-κB activation [291]. As the absorption rate of curcumin from the gut is low, a local NF-κB inhibition could be potentially achieved with this drug. Like "topical" steroids such as budesonide, this "local" NF-κB inhibition would avoid systemic side effects.

Another possibility that has been discussed would be the development of tissue-specific IKK inhibitors [292]. However, so far there is no clear evidence for a clear tissue specificity of IKK activation pathways.

Overall, NF-κB seems to play a key role in the treatment of chronic and acute inflammatory diseases and is the final target of a number of successful multi-site therapeutic approaches. Inhibition of NF-κB on different cellular levels provided therapeutic alternatives in the past and will provide new alternatives to glucocorticoid therapy in the future. On the other hand, NF-κB activation may be crucially important in epithelial cells for barrier integrity and regeneration (Figure 24.8).

The understanding of the mechanisms involved in NF-κB activation on the one hand and glucocorticoid receptor and PPAR-mediated suppression of NF-κB activation on the other is essential for future therapy development in the field of multi-site targeted therapies for IBD.

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Chapter 25
Targeted Treatments for Inflammatory Bowel Diseases

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Summary
- Whereas older therapies for IBD were developed before their precise molecular targets were known, current drug development strategies aim to identify potential drug targets first and design ways to alter their activity pharmacologically.
- Monoclonal antibodies that have been humanized using advanced biotechnology offer the potential to inhibit selectively a multitude of cell surface and extracellular targets in IBD.
- Cytokines and their receptors represent one important class of selective targets with high potential value in IBD.
- Targeting cell surface-expressed proteins on immune cells such as lymphocytes and monocytes can lead to apoptosis of those cells, with profound inhibition of immune responses in IBD.
- Drugs that target leukocyte extravasation constitute an important emerging category of promising agents for IBD.

Introduction

Although there has been significant progress in the understanding of the pathogenesis of inflammatory bowel disease (IBD) in recent years, the precise etiological factor or factors have not yet been precisely determined. Increasing knowledge has provided a picture of complex interplay between environmental, genetic and inflammatory factors in which there is inappropriate, dysregulated mucosal immune responses to luminal antigens in a genetically susceptible individual.

Conventional treatment of IBD to date has been based on 5-aminosalicylates, corticosteroids and a variety of immunosuppressive drugs including azathioprine, 6-mercaptopurine, cyclosporin and methotrexate. Conventional therapies are based on non-specific suppression of the immune process, with many of the undesirable effects of these drugs occurring as a result of broad, unfocused immunosuppression. The goal of the treatment of chronic inflammatory disease is to suppress pathological inflammation only in the areas affected, without inactivating normal inflammatory mechanisms that are required for host defense. With increasing understanding of the pathogenic processes in IBD, there are greater opportunities for finding specific new therapeutic targets, with the possibility of obtaining more precise control of aberrant inflammatory processes. The success of infliximab, a monoclonal antibody (mAb) targeted at a single mediator, tumor necrosis factor-α (TNFα), as a therapeutic modality in IBD has encouraged the research and development of multiple agents targeting diverse mechanisms involved in the perpetuation of the aberrant inflammatory processes.

Site-specific therapeutics are agents which are directed specifically at a target molecule. Site-specific therapeutics currently in use in IBD are biological agents, such as monoclonal antibodies and recombinant proteins. At present, several small molecule drugs with specific molecular targets are also under development for IBD. Site-specific therapies for IBD and their clinical development phase are listed in Table 25.1. In this chapter, we explore the rationale for the use and mechanisms of action of both established and experimental site-specific therapeutics for IBD and discuss them under the following topics:
- IBD pathogenesis and rationale for site-specific therapeutics
- types of site-specific agents
- individual site-specific agents
- drawbacks of site-specific agents.

IBD pathogenesis

Ulcerative colitis (UC) and Crohn's disease (CD) have been the subject of extensive epidemiological studies which

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Table 25.1 Site-specific therapies for IBD and their development phase*.

Method	Target	Agent	Method of action	Development
Inhibition of pro-inflammatory cytokines	TNFα	Infliximab	Chimeric anti-TNFα mAb	FDA approved for CD and UC
		Adalimumab	Human anti-TNFα mAb	FDA approved for CD
		Certolizumab pegol	Humanized anti-TNFα Fab′ fragment	Phase III (FDA application submitted)
		Etanercept	P75 TNFR	Failed Phase II
		Onercept	P55 TNFR	Failed Phase II
		CDP 571	Humanized anti-TNFα	Failed Phase II
		CNI 1493	Small-molecule MAP kinase inhibitor	Phase II
		BIRB 796	Small-molecule MAP kinase inhibitor	Failed phase II
	IL-6	Tocilizumab	Humanized anti-IL-6R mAb	Phase II
Inhibition of Th1 polarization	IL-2	Basiliximab	Anti-IL-2R	Phase II
		Daclizumab	Anti-IL-2R	Phase II
	IL-12	ABT 874	Human anti-p40 subunit mAb	Phase II
		STA 5236	Small-molecule inhibition of transcription	Phase II
	IFN-γ	Fontolizumab	Anti-IFNγ	Phase II
		IFNβ	rHuman protein	Failed Phase II
		IFNα	rHuman protein	Failed Phase II
Anti-inflammatory cytokines	IL-10	Ilodecakin	rHuman protein	Phase II
	IL-11	Oprelvekin	rHuman protein	Failed Phase II
Inhibition of T cell activation	CD40	Ch5D12	Human anti-CD40 mAb	Phase I
	CD40	IDEC-131	Human anti-CD40L mAb	Withdrawn due to prothrombotic effects
	CD4	cM-T412	Chimeric anti-CD4 mAb	Withdrawn due to T cell depletion
		BF-5	Murine anti-CD4 mAb	Withdrawn due to T cell depletion
	CD3	Visilizumab	Humanized anti-CD3 mAb	Phase II
Inhibition of leukocyte adhesion	α4-Integrin	Natalizumab	Humanized anti-α4 integrin mAb	Phase III
	$\alpha 4\beta$7-Integrin	MLN-02	Humanized anti-$\alpha 4\beta$7 integrin mAb	Phase II
	ICAM-1	Alicaforsen	ICAM-1 antisense oligonucleotide	Failed Phase II

*Abbreviations: mAb, monoclonal antibody; TNFR, tumor necrosis factor receptor; IL-6R, interleukin 6 receptor; rHuman protein, recombinant human protein; IFN, interferon; ICAM-1, intracellular adhesion molecule-1.

have demonstrated multiple risk factors associated with disease development, particularly environmental and genetic factors. Currently, evidence suggests that genetic susceptibility leads to an exaggerated acquired immune cell response to luminal antigens, which may be precipitated or reactivated by external environmental factors. It is likely that there are several coexisting initiating mechanisms, leading to the final common process of inflammation.

In patients with IBD, regulation of immune function is impaired, replaced by uncontrolled inflammation, probably occurring as a result of abnormal immune responses to luminal antigens. The precise initiating factors in this process are uncertain, although several have been proposed:

- Luminal antigens may play a role in the initiation of inflammation, either as a result of persistent pathogenic inflammation [1,2] or as a result of dysbiosis [3].
- Intestinal epithelial cells have disordered patterns of TLR expression leading to impairment in innate immune mechanisms and production of chemoattractant factors. Abnormal function of NOD2 in epithelial cells may also contribute by impairing the ability to eliminate microorganisms from the intestinal mucosa. Innate immune receptors may also contribute to inflammation through abnormal activation processes leading to atypical antigen presentation and T cell activation [4].
- Dendritic cells are abnormally activated in IBD and may inappropriately induce T cell responses to commensal

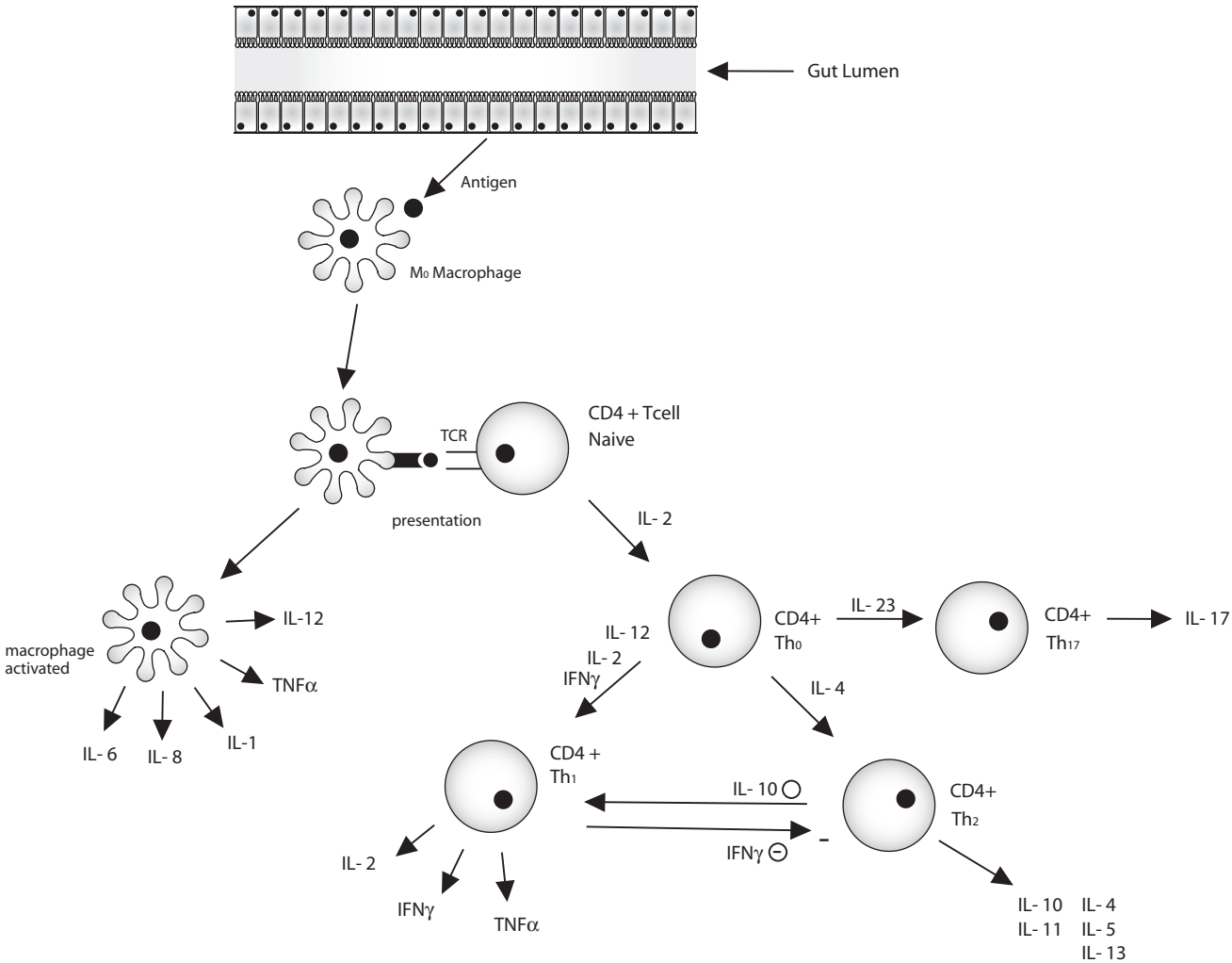

Figure 25.1 Therapeutic targets in the inflammatory cascade in IBD. One current model of IBD pathogenesis invokes a genetically-determined aberrant T cell-mediated response to luminal antigens leading to establishment and maintenance of a pathological inflammatory process. In this scenario, drugs aimed at inhibiting T cell activation, inhibiting T cell polarization, inhibiting pro-inflammatory cytokines or administration of anti-inflammatory cytokines may interrupt the pathological process.

bacteria. Furthermore, immature, regulatory dendritic cells appear to be diminished in number in IBD. These mechanisms could serve as initiating and maintenance mechanisms for pathogenic inflammation.

Irrespective of reasons for initiation, the end result is the production of an aberrant adaptive immune response, characterized by antigen presentation to and activation of naïve $CD4^+$ Th0 cells, activation and clonal proliferation of effector T cell subtypes, upregulation of pro-inflammatory cytokines, migration of inflammatory cells from the vasculature and further proliferation of inflammatory cells in a self-sustaining pathogenic inflammatory process (Figure 25.1).

Increasing knowledge of the precise processes underlying the inflammatory processes allows for multiple theoretical points at which to abrogate this process:

- inhibition of pro-inflammatory cytokines
- inhibition of T cell activation
- modulation of T cell polarization
- use of anti-inflammatory cytokines
- inhibition of leukocyte adhesion.

Classes of site-specific therapeutics

Monoclonal antibodies

General features of antibody structure

Antibody structure can broadly be divided into two regions: the antigen binding region, which is variable between antibodies of different specificity (V region), and the region which determines effector activity of the antibody, the constant region (C region). The C region has five

forms, each of which has specific functions regarding activation of immune mechanisms and is class and species specific. Antibody isotypes are comprised of IgG, IgA, IgD, IgM and IgE, each with further subtypes according to specific actions. Monoclonal antibodies for treatment of IBD are all of the IgG subtype.

IgG antibodies are large protein macromolecules, of approximately 150 kDa, and are made up of two types of polypeptide chains: heavy (H), of approximately 50 kDa, and light (L), of approximately 25 kDa, each antibody consisting of two heavy and two light chains (see Figure 25.2). The heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to one light chain, also by disulfide bonds. There are two subtypes of light chains, lambda (λ) and kappa (κ), only one of which is found in any given antibody. There are five classes of heavy chains which determine the isotype and function of the antibody. Gamma (γ), alpha (α), delta (δ), mu (μ) and epsilon (ε) are the heavy chains of IgG, IgA, IgD, IgM and IgE, respectively. Each chain is made up of a number of similar sequences with a discrete folded protein structure. Each heavy chain has four and each light chain has two protein domains. The first protein domain at the amino terminal end of each heavy and light chain is highly variable and forms the V region, governing antigen specificity. The remaining domains form the C region. The C region determines the function of the antibody. For example, IgG1 mediates complement-dependent and antibody-dependent cytotoxicity, IgE mediates signaling in mast cells and basophils. The C region also contains binding sites for the neonatal Fc receptor (FcRn), which mediates cell surface binding and endocytosis and protects the antibody from breakdown.

Within the protein domains of the V region there are areas of high variability between antibody molecules, termed hypervariable regions or complementarity-determining regions (CDRs). In the complete antibody molecule, the hypervariable regions from the heavy and light chains are brought together and form a single hypervariable site which is the antigen binding site [5]. This determines the specificity of the antibody for its target.

Production of monoclonal antibodies

The possibility of a directed drug for the specific treatment of disease was envisaged by Ehrlich in the 19th century, which he referred to as a "magic bullet". As antibody structure and function were investigated and understood, it became apparent that these large molecules offered the opportunity to realize Ehrlich's vision. Early research on antibody function was carried out on gamma-globulins produced in large amounts by immortal myeloma cell lines. Significant technical difficulties lay in the production of specific monoclonal antibodies, requiring the combination of the specificity of a B cell with the antibody production capability of a myeloma cell. This problem was first overcome by Kohler and Milstein, with successful fusion of mouse B cells with a mouse myeloma cell line [6] to produce a hybridoma, an immortal cell line capable of antibody production. This method has been refined and rationalized for production of engineered antibodies on an industrial scale.

Monoclonal antibody production consists of several steps (see Figure 25.3). First, a population of B cells producing antibodies against the antigen must be induced by immunization of mice. The animal is immunized at three or four sites subcutaneously or intramuscularly with contemporaneous intraperitoneal injection. Immunization is repeated at 2–4 week intervals until an adequate antibody titer is measured in the serum. At that time, a final challenge of antigen is given to stimulate production of committed B cell precursors, the cells of choice for hybridoma formation. Animals are euthanized 2–3 days later and lymph nodes and the spleen are harvested. Lymphocytes are separated and mixed with myeloma cells in a polyethylene glycol (PEG) solution, which allows fusion of B cells with myeloma cells, producing hybridomas. The cells are cultured in a selective growth medium containing HAT (hypoxanthine–aminopterin–thymidine), which permits only the growth of fused hybridomas. These are then seeded on plates at low density (<1 cell per well) to allow isolation of pure clones of unique hybridoma lines. Many different hybridoma clones are generated as each antigen has many different antibodies against it that is produced by distinct B cell populations, each directed at different antigenic epitopes. Hybridoma clones are screened to identify the production of antibody with the desired specificity, affinity for target and immunological activity [7].

A number of methods have been developed to streamline the process of monoclonal antibody development. For example, the use of phage display libraries allows more rapid assessment of a much larger number of potential antibodies. In phage display, gene segments encoding antibody variable regions are incorporated into the genome of bacteriophage viruses. These viruses are used to infect bacteria and the viruses (phage) that result from this process express the variable regions and display the antigen binding domains on their surface. To assess function of different variable regions, the antigen of choice is fixed to a surface after which phage containing solution is applied, a process referred to as "panning". Phage that bind to a specific antigen can be isolated and the genes encoding the binding site can be isolated and used to construct a complete antibody molecule by joining them to genes encoding the constant region of an antibody. The reconstructed antibody gene sequences can be introduced to a cell line which will produce the complete antibody [7].

These methods result in the production of complete murine antibodies, which have substantial limitations due to the development of anti-mouse antibodies when

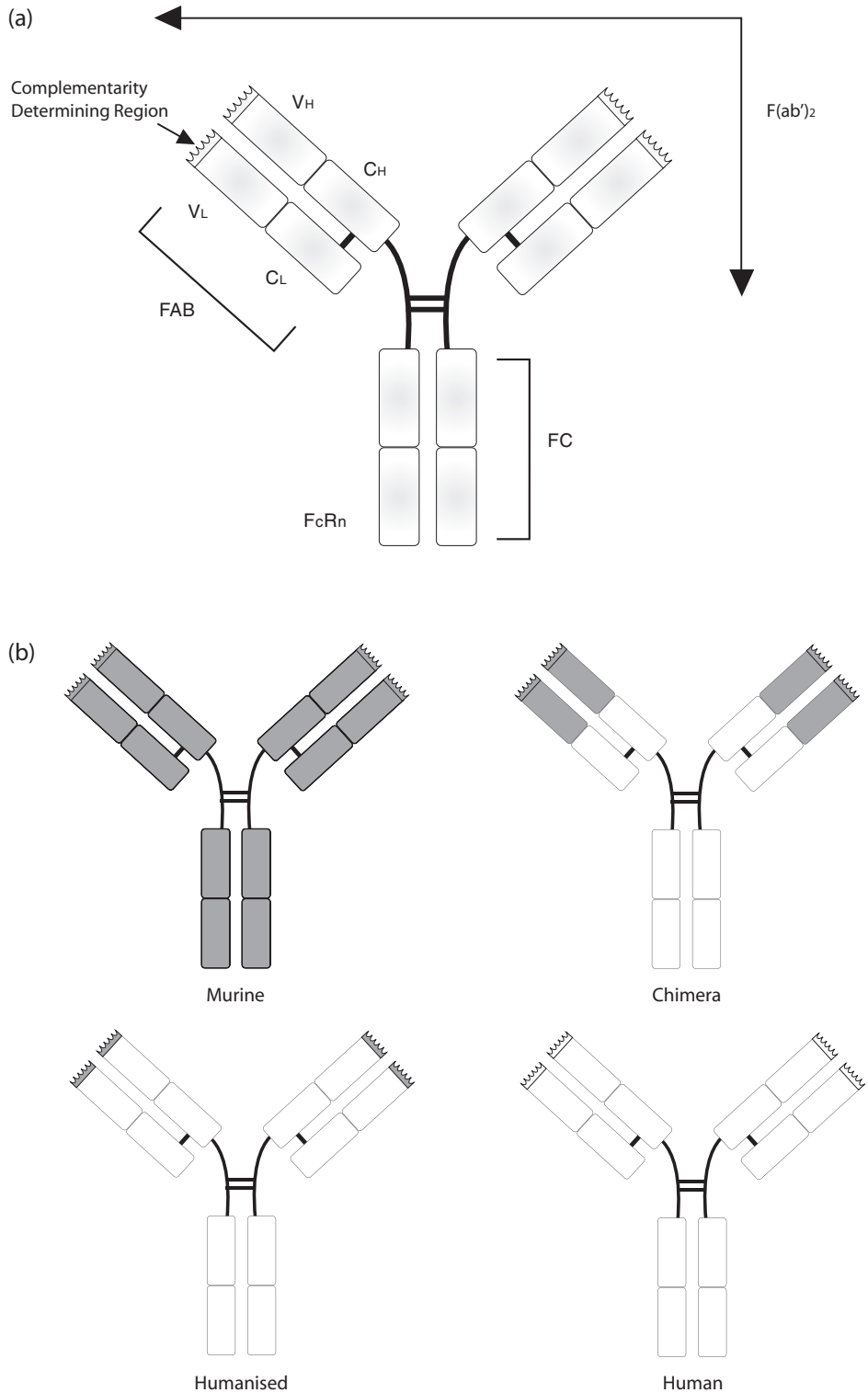

Figure 25.2 Monoclonal antibodies. (a) General structure of an antibody. Antigen specificity is determined by the Fab region, whereas activity is determined by the Fc region. (b) Types of therapeutic monoclonal antibodies in which the murine portion is illustrated in grey and the human portion in white.

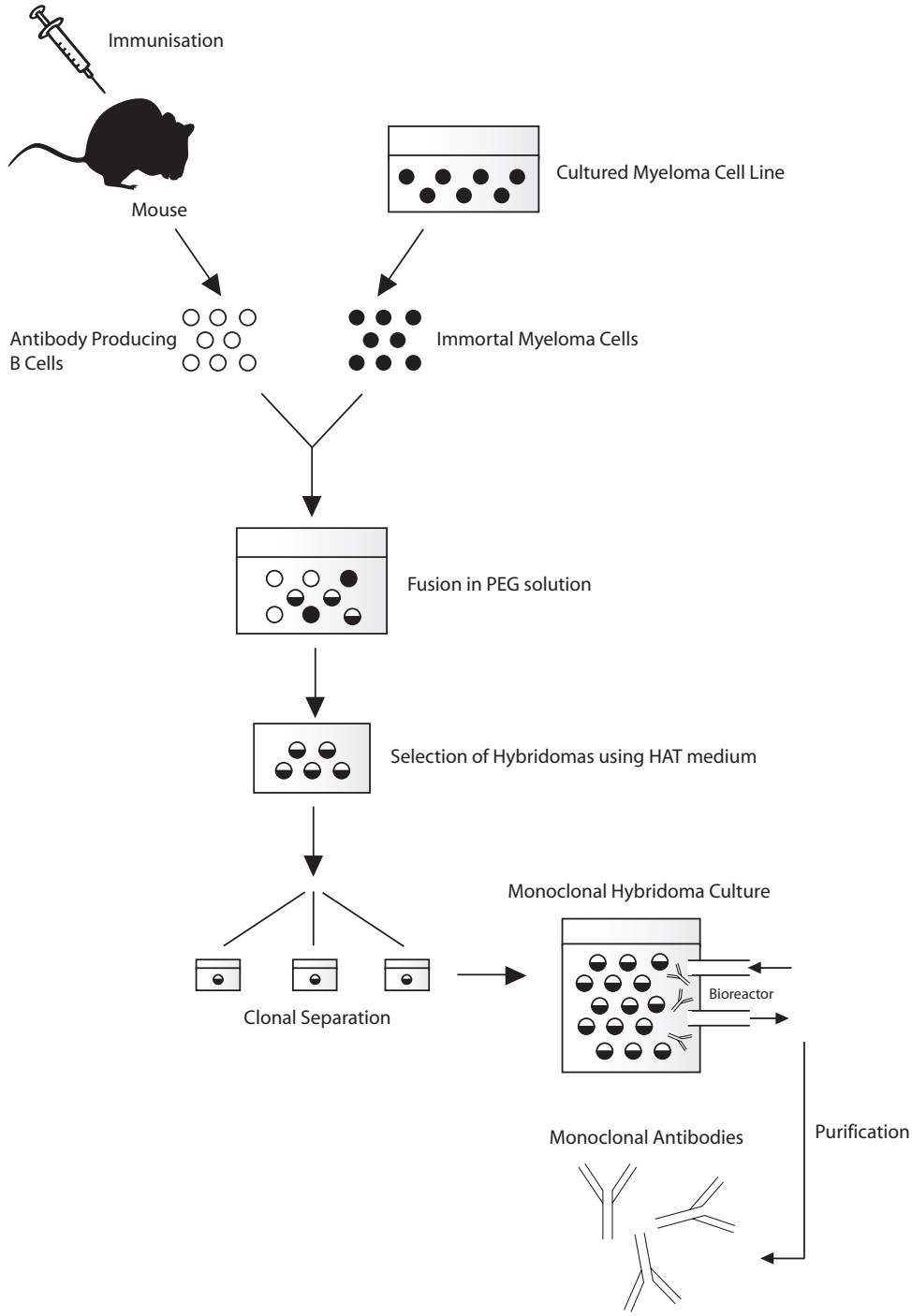

Figure 25.3 Production of monoclonal antibodies. Mice are immunized with the target antigen and, after a period of time, B cells are isolated. B cells are then fused with immortal myeloma cells in a polyethylene glycol solution to form hybridomas. Selection of hybridomas takes place in a selective HAT growth medium. Hybridomas are seeded at low concentration to ensure growth of a individual clones producing a single antibody directed at the target antigen. The antibodies produced in this method are screened for specificity and activity. Clones producing antibodies with the desired characteristics are cultured intensively in bioreactors and the monoclonal antibodies are purified from the growth medium.

injected into humans. Antibodies that develop against the Fc portions of the injected antibody result in rapid clearance with resultant impaired clinical response, along with a high likelihood of infusion reactions. Muromonab (OKT3), a murine monoclonal antibody to CD3 for use in acute renal allograft rejection, was the first engineered monoclonal antibody, approved for use in 1986. OKT3 stimulates a robust human anti-murine antibody responses and cytokine release syndrome, but despite this limitation, OKT3 is still useful clinically.

In an effort to overcome immunogenicity of murine antibodies, chimeric antibodies have been developed. Chimeric human antibody molecules are combinations of human C regions with murine V regions. Fully murine antibodies to the target antigen are produced as described above. The V region heavy and light chain genes are isolated and cloned. These are then spliced to specific locations on the genes encoding the heavy and light chains of human C region and incorporated into a plasmid vector. This vector is then used to transfect either a mammalian cell line or a non-antibody-producing myeloma cell line. The cell line is cultured and the antibody can be harvested from those cells.

Humanized antibodies further decrease the non-human component to reduce the likelihood of immunogenicity complicating therapy. The CDRs of human antibodies are replaced with murine CDR sequences known to have a high affinity for the target antigen. By reducing the CDR antibody sequences to be grafted on to the human antibody structure, it is predicted to reduce immunogenicity compared with murine or chimeric antibodies. CDR sequences are selected on the basis of affinity and likelihood of inducing an immune response. Two methods are commonly used to produce candidate CDRs for selection [8]. Both involve the creation of libraries of CDRs or complete V domains originating from CDRs of murine antibodies. The first approach is the creation of large numbers of CDR-displaying phage, by inducing random mutations in the DNA sequence using polymerase chain reaction (PCR). This varies the conformation of the final CDR. These are incorporated into phage as described above and tested for affinity, those with the greatest affinity being further investigated. Smaller libraries are produced by varying single amino acids either randomly or at focused areas of the V chain structure. The resultant sequences are then selected for antigen affinity. After selection of the sequences, these are grafted on to human antibody sequences and transfected into cell lines.

Fully human antibodies have become possible with the development of transgenic animals engineered to produce human antibodies [9]. Genes for human heavy and light chains have been introduced to the murine germline after deletion of the native antibody genes. These transgenic mice are challenged with the target antigen and fully human antibody-producing hybridomas can be produced. These are tested for affinity and specificity and may be modified with engineered amino acid changes [10,11]. Complete antibody molecules require mammalian cells for production to ensure that proteins undergo the correct post-translational modifications and folding. Examples of cell lines in use include Chinese hamster ovary, murine lymphoid cell lines and hybridomas. Industrial-scale production requires intensive cell culture methods with the use of various forms of bioreactors, with strong emphasis on optimization of cell line, media and harvesting techniques [11]. Transgenic technology may allow the use of animals as functional bioreactors (molecular "pharming"). Transgenic chickens have been produced which produce monoclonal antibodies in eggs, as have cows which secrete monoclonal antibodies in their milk [12].

Mechanism of action of mAbs

The mechanism of action of an individual monoclonal antibody or antibody fragment is determined by the presence or absence of necessary domains for effector functions or the addition of extra domains either to induce monoclonal antibody associated biological activity or to mediate drug delivery.

The best understood mode of action of monoclonal antibodies is binding to and neutralization of, or interruption of signaling pathways mediated by, target antigens. To be effective, the antibody must cause sufficient blockade of the target antigen to prevent function. This activity is determined by the specificity of the antigen-binding region and also the affinity of the binding region for the target antigen. The avidity or the strength with which the monoclonal antibody binds to the target antigen may also be an important determinant of efficacy. Complete monoclonal antibody molecules may induce cytotoxic effects against target cells. Antibody-dependent cell-mediated cytotoxicity (ADCC) occurs when antibody binds to membrane-bound antigen and the Fc domain of the antibody engages Fc receptors on effector cells. FcR engagement leads to activation of effector cells and induction of apoptosis in target cells. Complement-dependent cytotoxicity (CDC) may also mediate target cell death. After binding to the target antigen, the antibody undergoes a conformational change, revealing complement binding sites in the C region which bind C1q, a component of complement C1, leading to activation of the classical complement cascade and to the formation of the membrane attack complex, which forms pores in the cell surface leading to target cell lysis. While ADCC and CDC have been demonstrated in association with mAbs used in the treatment of inflammatory diseases [13] and malignancy, the importance of this activity in clinical efficacy is uncertain at this time. Biological effects of specific antibodies used in the treatment of IBD are discussed below.

Monoclonal antibodies have been modified in cancer chemotherapeutics to deliver directed cytokines, toxins,

radioactivity and catalytic domains for prodrug–drug conversion, although such tactics have no role in IBD at this time.

Pharmacokinetics of mAbs

Monoclonal antibodies are hydrophilic, high molecular weight proteins with marked difference in pharmacokinetics compared with conventional drugs, which are small lipophilic chemicals. Numerous factors, including structure, the type and extent of disease being treated and individual patient variation, affect monoclonal antibody pharmacokinetics.

Antibodies exhibit low tissue penetration due to their high molecular weight and largely remain confined to the bloodstream and extracellular fluid. Therefore, volumes of distribution are low. Due to size, antibodies are unlikely to enter cells by diffusion but may enter by fluid phase or receptor-mediated endocytosis. Antibodies appear to cross the placental barrier readily.

Clearance of mAbs from the circulation is poorly understood, but probably represents degradation by the reticulo-endothelial system. Monoclonal antibody administered by intramuscular or subcutaneous injection may be catabolized locally, reducing bioavailability. Fluid phase and receptor-mediated endocytosis are possible mechanisms by which antibodies are removed from the circulation. Once endocytosed by reticulo-endothelial cells, antibodies are either released into the interstitial space or accumulate in lysosomes where they undergo enzymatic degradation. The metabolism of monoclonal antibodies varies according to individual differences in antibody structure and in inter-individual variations. The $T_{\frac{1}{2}}$ of IgG in humans is ~21 days, except in the case of IgG3, which has a shorter $T_{\frac{1}{2}}$ of ~7 days. IgG1, IgG2 and IgG4 have longer $T_{\frac{1}{2}}$ due to the presence of FcRn-binding domain, which reduces catabolism. Antibody fragments lack this domain and also tend to have a shorter $T_{\frac{1}{2}}$ than intact mAb. The $T_{\frac{1}{2}}$ of monoclonal antibody increases with levels of humanization: murine (1.5 days) < chimeric (10 days) < humanized (12–20 days) < fully human (15–20 days). Various protein structures (epitopes) in mAbs can induce host immune reactions. The idiotype is a unique antigenic structure carried by the V region and can induce anti-idiotypic antibodies. These antibodies increase clearance through complex formation and can also cause steric hindrance, leading to inactivation of the active site. Allotypes are epitopes carried on the κ and γ chains, which vary from individual to individual according to polymorphisms in the genes encoding the constant regions of the chains. Administration of monoclonal antibody to an individual who is homozygous for opposite polymorphisms may result in the formation of anti-allotypic antibodies. Of more significance are epitopes displayed on murine regions of the V domain, relevant only in chimeric mAb. Immunoglobulin formation against monoclonal antibody usually alters pharmacokinetics by increasing clearance, reducing serum levels and impairing targeting.

Weight affects plasma monoclonal antibody concentration and therefore most monoclonal antibodies are administered using weight-based dosing. Other possible sources of variation in monoclonal antibody pharmacokinetics include disease type and activity, patient-specific antigenic load and polymorphisms in target antigens which affect binding. Antigenic load refers to the amount of antigen, either soluble or membrane bound, which is available for binding. If membrane bound, the antigenic load depends on antigen density per cell and numbers of expressing cells. Should the antigen be present in excess, a high fraction of the monoclonal antibody will be bound, leaving unbound antigen able to continue the pathological process. For example, in the case of infliximab, high expression of TNFα has been described as a factor contributing to poor response in both rheumatoid arthritis (RA) and CD [14]. Whether this is a function of disease activity or is genetically related is uncertain. Polymorphisms have been described in the locus encoding TNFα, some of which are associated with increased TNFα production. These loci have been found to affect response to therapy in RA but not in CD [15]. FcγRIIIa (CD16a) is a receptor expressed on NK cells, macrophages and monocytes which binds the Fc portion of infliximab and may be involved in mediating infliximab-induced cell lysis. Certain variants in this receptor have been shown to affect the metabolism and efficacy of anti-TNFα [16]. Variants have also been described in the locus for TNFα-1 and TNFα-2 but polymorphisms affecting outcome efficacy have only been described in association with TNFα-1 [17].

Routes of administration of mAbs

mAbs are administered by intravenous (i.v.), intramuscular (i.m.) or subcutaneous (s.c.) injection. The i.v. route allows administration of a large quantity of antibody with immediate systemic delivery. Methods of absorption after s.c. or i.m. injection are unclear, but may be via the lymphatic system and therefore tend to be slow. For example, the $T_{\max}$ (time to maximum concentration) for the s.c.-administered receptor fusion protein etanercept varies widely, from 25 to 78 h. There are no successful oral, transdermal or pulmonary formulations of mAbs in clinical use.

Recombinant proteins

Recombinant proteins are administered therapeutically to mimic or reinforce the activity of endogenously produced proteins which are either absent (e.g. insulin in type 1 diabetes mellitus) or inadequate in amount to satisfactorily control a pathological process (e.g. anti-inflammatory cytokines in IBD). Recombinant proteins are structural analogues to native proteins but may be modified in the production process to increase bioavailability, reduce clearance or increase activity [18].

Production and mechanisms of action of recombinant proteins

The term "recombinant protein" describes the production of non-native proteins by a eukaryotic or prokaryotic cell line through insertion of coding DNA sequences into the cell which transcribes and translates the DNA to produce the protein. The method of DNA insertion into the producing cell line must ensure the fidelity of the sequence to be inserted and must produce a protein of adequate activity and stability and in sufficient amounts [19].

Recombinant protein production requires identification and characterization of the protein to be produced and cloning of the cDNA sequence. cDNA is purified and cloned into a vector, which is then used to transfect a target cell line. Individual clones are grown in culture and assayed, either individually or in pools, for activity of the target protein or by immunoassay. By this process, it is possible to exclude non-transformed cells or cells which produced a non-functioning version of the protein. This leads eventually to identification of a clone producing the desired protein [19]. Current information about human protein sequences and the development of easy and rapid cDNA production by PCR have streamlined this process.

cDNA sequences may also be modified, by either directed or random mutagenesis to change the properties of the produced protein. For example, a recombinant protein may bind to multiple receptors inducing undesirable effects. Mutagenesis allows alteration of the amino acid sequence, which may maximize binding to a desired receptor to exclusion of others. It may also be used to modify stability or solubility or be used to insert amino acid sequences to accumulate recombinant protein in intracellular inclusion bodies or to encourage exocytosis of the protein for ease of harvesting.

Recombinant proteins are usually produced in mammalian cell lines which are capable of post-translational modifications necessary for proper protein folding [19]. cDNA is inserted into target cells using a variety of vectors. Essential components of a vector include a promoter to drive expression, initiation and termination sequences and the capacity to integrate into the host genome if a stable cell line is required. Other components include genes to aid selective growth in culture. For example, antibiotic resistance genes allow cells to grow in antibiotic-containing media, ensuring the production of clones in which the cDNA is satisfactorily incorporated and functional. Bacterial cell transformation can be achieved with plasmids (self-replicating circular dsDNA maintained in bacteria separate from the main chromosome) or bacteriophage viral vectors. Foreign gene transfer into mammalian cells is mostly by viral vectors and cell lines have been established that are modified to maximize the likelihood of target gene incorporation and activity.

After development of the recombinant cell lines, they are cultured intensively in bioreactors to maximize protein production. Isolation of recombinant protein is dependent on the cell type used and the protein in question. If the protein is produced and secreted, the growth medium may be exchanged and the protein purified from it. If the protein is not exocytosed or is membrane bound, the cells must be physically or chemically disrupted, after which the protein is purified. Protein purification involves several methods, including centrifugation, sequential filtering and dialysis. As is true for monoclonal antibody production, recombinant proteins have also been produced in transgenic animals, producing recombinant proteins in cows' milk and in chicken eggs.

Proteins may be artificially modified to alter certain features, such as bioavailability and activity [18]. For example, PEGylation is the addition of polyethylene glycol (PEG) to a protein to increase its molecular weight and alter its pharmacokinetics, specifically to decrease its clearance and prolong $T_{\frac{1}{2}}$. PEG is a polymer of ethylene oxide that is water soluble, lacks significant toxicity and immunogenicity and is readily cleared.

Pharmacokinetics of recombinant proteins

Most recombinant proteins are administered parenterally, by either i.v. or s.c. injection, depending on the molecular weight of the molecule. t-PA, a prototypical recombinant protein, has a molecular weight of 65 kDa and does not reach therapeutic levels unless administered i.v. For recombinant proteins administered by s.c. injection, the method of entering the systemic circulation and thus the time to maximal concentration (T_{max}) are dependent on the molecular weight. Smaller proteins are absorbed by the capillary circulation. Larger molecules (>16 kDa) are usually absorbed via the lymphatics, which have slower flow rates [20]. The site of administration may also affect absorption [21].

Delivery of the recombinant protein to the site of action is dependent on the physicochemical structure of the individual molecule, including any modifications such as PEGylation that are introduced during manufacture. In the vascular space, the recombinant protein may be bound to circulating plasma proteins. Binding to plasma proteins may also lead to modification of the bound protein, inhibiting or stimulating activity and altering clearance [18].

Metabolism of recombinant protein occurs by catabolic processes similar to those for endogenous proteins. Proteolytic enzymes degrade recombinant proteins to amino acid fragments. Recombinant cytokines [TNFα, interferon (IFN)-γ, interleukin (IL)-1β, IL-4 and IL-6] may affect the cytochrome P450 system, but the implications of this effect have not been thoroughly elucidated.

Variations in the pharmacokinetics of recombinant proteins occur due to several factors, including rate, time and method of drug delivery and induction of immunogenic reactions. Immunogenicity of recombinant proteins is dependent on the route of administration (s.c. and i.m. > i.v., due to precipitation of protein at the injection site), dose, frequency and duration of administration and the

presence of modifications to the recombinant proteins [22]. As mentioned above, some proteins are linked to PEG, a hydrophilic polymer, a modification which reduces immunogenicity, possibly by shielding the recombinant protein in aqueous solution. Binding antibodies may block the active site of the molecule (neutralizing) or other epitopes (non-neutralizing), affecting the activity and clearance of the proteins.

Antisense oligonucleotides

For protein production to proceed, DNA must be transcribed to form a pre-messenger RNA (mRNA) which is modified within the nucleus to produce mature mRNA (see Figure 25.4). The mature mRNA exits the nucleus and binds to ribosomes where protein is translated amino acid by amino acid, as specified by the codon sequence of the mRNA. Further post-translational protein modifications take place within the cell which alter the proteins functions.

Antisense oligonucleotides downregulate or abolish production of target proteins by interfering with the pre-translation mRNA sequences. Antisense oligonucleotides are short (13–25 nucleotides), single-stranded deoxyribonucleic acid sequences that are complementary to sequences on the target mRNA to which they bind by Watson–Crick base pairing [23]. Upon binding to the target mRNA sequence, within either the nucleus or cytoplasm of the cell, they either induce degradation of the target mRNA via specific mRNAses (usually mRNase H) or cause steric blockade which prevents ribosomal binding. This method results in the inhibition of a single step in a regulatory cascade by inhibition of production of a single protein. The usefulness of this therapeutic approach to date has mostly been in the research field, although it has undergone testing as a form of cancer therapy, as a therapy for viral illnesses and for regulation of inflammatory diseases, including IBD. The only antisense oligonucleotides to have received FDA approval to date is formivirsen for the treatment of cytomegalovirus retinitis in AIDS patients [23].

Production and mechanism of action of antisense oligonucleotides

Antisense oligonucleotide design requires characterization of the mRNA sequence for the targeted protein. Knowing the sequence of the mature mRNA in question, it is possible to generate short strands of complementary DNA. Simple production of complementary sequences is insufficient to achieve effective targeting. The likelihood of satisfactory interaction and therefore inhibition is governed by several factors, including secondary and tertiary structure of the mRNA transcript, stability of the interaction (thermodynamic stability) and location of the target region on the mRNA transcript, specifically proximity to important functional motifs, e.g. the start site. Various methods are available to test potential sequences [24]. The use of computer modeling allows for a more targeted approach in the design of antisense oligonucleotides. This approach uses modeling programs to predict likely secondary structures of mRNA and to identify potential accessible sites which are well conserved, are likely to have satisfactory activity (if RNase activity is required) and are likely to bind the antisense oligonucleotides with sufficient strength. Oligonucleotide arrays may also be used. Oligonucleotide sequences complementary to the target mRNA are applied to a prepared surface after which labeled transcripts are hybridized, and subsequently washed off. Analysis of the array allows quantification of the binding intensity and those sequences which demonstrate strong binding may be further investigated for antisense activity [24].

Phosphorothioate oligonucleotides are somewhat more stable than conventional nucleic acids and are often used for therapeutic antisense applications.

On entering the cell, the antisense oligonucleotides may mediate inhibitory activity either within the cytoplasm or within the nucleus (Figure 25.4). Within the nucleus, they bind to the 5′-end of pre-mRNA, preventing 5′-cap formation. Alternatively, antisense oligonucleotides may bind elsewhere and prevent mRNA splicing. Both effects prevent the formation of mature mRNA suitable for translation. RNase-mediated degradation of the target mRNA may occur within either the nucleus or the cytoplasm. Steric blockade of ribosome complex formation occurs in the cytoplasm [25].

Pharmacokinetics

The route of administration of antisense oligonucleotides depends on the disease process. Antisense oligonucleotides can be given by local application or injection (s.c., intravitreous) i.v. or rectally. When systemically administered, antisense oligonucleotides are highly bound (>95%) to plasma proteins. Clearance is in a dose-dependent manner with a $T_{\frac{1}{2}}$ of 30–60 min. Antisense oligonucleotides are degraded by exonucleases and excreted renally within 1–5 days.

Class toxicities of antisense oligonucleotides are dependent on modifications to the nucleic acid rather than being sequence dependent. Important toxicities include activation of the complement cascade and inhibition of the clotting cascade [23].

Current and emerging site-specific therapeutics for IBD

Inhibition of pro-inflammatory cytokine signaling

TNFα inhibitors

TNFα has multiple known functions, including regulation of acute and chronic inflammation, control of infection and regulation of embryonic development. Inflammation

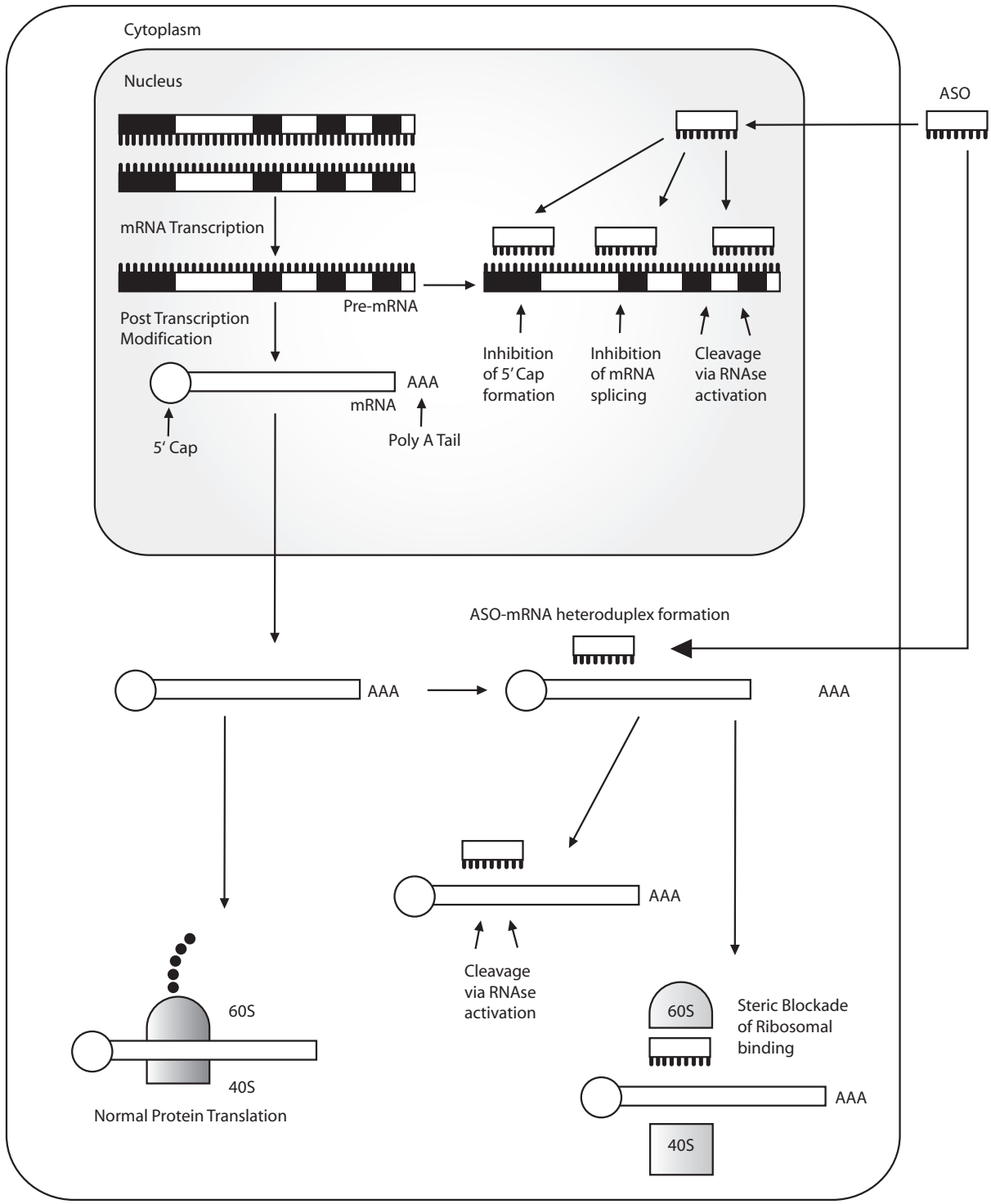

Figure 25.4 Mechanism of action of antisense oligonucleotides (ASOs). ASOs act to inhibit target protein production either in the nucleus or in the cytoplasm. Within the nucleus, ASOs hybridize to target pre-mRNA and prevent formation of mature mRNA by inhibition of 5′ cap formation, pre-mRNA splicing or cleavage of pre-mRNA by induction of RNAse. Within the cytoplasm, ASOs act by cleavage of mature mRNA or steric blockade preventing binding of ribosomes, thereby inhibiting protein translation.

associated with elevated levels of TNFα is a feature of CD and UC. TNFα in IBD patients functions as a pro-inflammatory cytokine with multiple effects, including induction of further pro-inflammatory cytokines such as IL-1 and IL-6, upregulating endothelial adhesion molecules and expression of chemokines to facilitate leukocyte transmigration. Furthermore, TNFα stimulates activation and proliferation of T cells, neutrophils and macrophages. TNFα and its receptors have been the subject of intensive research and there are several potential approaches to its blockade.

Production and secretion of TNFα in IBD is mainly by monocytes/macrophages but also by activated T and B cells, epithelial cells and endothelial cells. Stimulation of cells by TNFα leads to activation of mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) pathways, resulting in increased expression of TNFα mRNA, resulting in a positive feedback loop. TNFα is translated as a 26 kDa protein which is initially expressed on the cell surface and then is cleaved by a membrane-associated enzyme, TNFα-converting enzyme (TACE), to produce a soluble 17 kDa protein (Figure 25.5). Both membrane-bound (mTNFα) and soluble TNFα (sTNFα) are biologically active only as homotrimers. mTNF has a cytoplasmic domain which mediates juxtacrine signaling (signaling occurring as a result of cell-to-cell contact) and has overlapping and distinct functions with the soluble form [26].

The biological response to TNFα in effector cells is mediated through two receptors; Type I (TNFR1, also known as p55, CD120a) and type II (TNFR2, p75, CD120b). Both are members of the TNF superfamily of receptors and their extracellular domains share structural and functional homology. Their intracellular domains have distinct structural features and mediate activity through some overlapping and distinct pathways. TNFR1 has an associated death domain which can bind accessory proteins to mediate its distinct pro-inflammatory and pro-apoptotic signaling pathways. TNFR1 is believed to initiate the majority of TNFα activities and to a certain degree TNFR2 may function as a cofactor which increases TNFα binding to TNFR1, although it does have its own distinct activities [26]. TNFR1 binds TNFα irreversibly, but TNFR2 binds TNFα reversibly, allowing a possible mechanism called ligand passing, whereby TNFR2 increases the TNFα concentration at the cell surface before passing it on to TNFR1. However, TNFR2 is also known to bind mTNFα more efficiently than TNFR1 [27]. This TNFR2–mTNF interaction allows information to be passed from the secreting cell to the effector cell, but also allows signals to be passed back to the secreting cell, a process termed reverse signaling [28] (Figure 25.5). Upon TNFα binding to TNFR1 or TNFR2, intracellular signaling is mediated through several kinase-dependent pathways, resulting in the activation of important signaling pathways, including NF-κB and MAPK.

Potential therapeutic targets for modulation of TNFα activation include suppression of TNFα synthesis, inhibition of membrane-bound or soluble TNFα, inhibition of TNFR1 or TNFR2, inhibition of TACE, inhibition of post-receptor signaling and inhibition of NF-κB. At this time, there are several therapies that directly inhibit TNFα, but there is little experience with other forms of modulation of the TNFα pathway.

TNFα inhibitors

Infliximab

Efficacy and safety

Infliximab was the first biological agent approved for the treatment of IBD. It is an IgG1κ chimeric murine:human monoclonal antibody (Figure 25.2) directed against TNFα and binds to both the soluble and transmembrane forms [29] (Figure 25.5). It was first demonstrated to be a potential therapeutic agent for the treatment of IBD in an open-label study published in 1995 by Van Dullemen *et al.* [30]. Targan *et al.* carried out the first randomized controlled trial of infliximab versus placebo as an induction regime in resistant CD, demonstrating significant differences in response and remission rates in treated patients [31]. The ACCENT-1 study showed the efficacy of infliximab as a maintenance drug after single-dose induction, demonstrating improved maintenance of remission and response at 48 weeks in treated patients versus placebo [32]. The role of infliximab as a treatment for fistulizing CD was demonstrated by Present *et al.* in a randomized controlled trial comparing an induction regimen of infliximab versus placebo. The treatment arms had significantly improved reduction in draining fistulae and complete fistula closure versus placebo [33]. The efficacy of infliximab as a maintenance treatment for control of fistulizing disease after induction of a clinical response was subsequently shown in the ACCENT-2 trial, in which patients who responded to an induction regimen were randomized to maintenance infliximab or placebo. Treated patients had a significantly greater response at study completion and a significantly longer interval to loss of response [34].

The role of infliximab as a treatment in UC was subsequently demonstrated in the Acute Ulcerative Colitis Trials (ACT 1 and 2) [35]. Both studies compared infliximab with placebo for induction and maintenance in the resistant UC ACT-1 to 54 weeks and ACT-2 to 30 weeks. The results demonstrated significant benefits in response, remission, mucosal healing and steroid discontinuation for infliximab over placebo from week 8 through to week 30. At this time, infliximab is indicated for management of CD resistant to conventional therapies [31,32], management of fistulizing CD [33,34] and in the treatment of steroid-resistant UC [35].

Infliximab is generally well tolerated. However, infusion reactions and infections, particularly reactivation of latent tuberculosis, are significant problems. The risk of

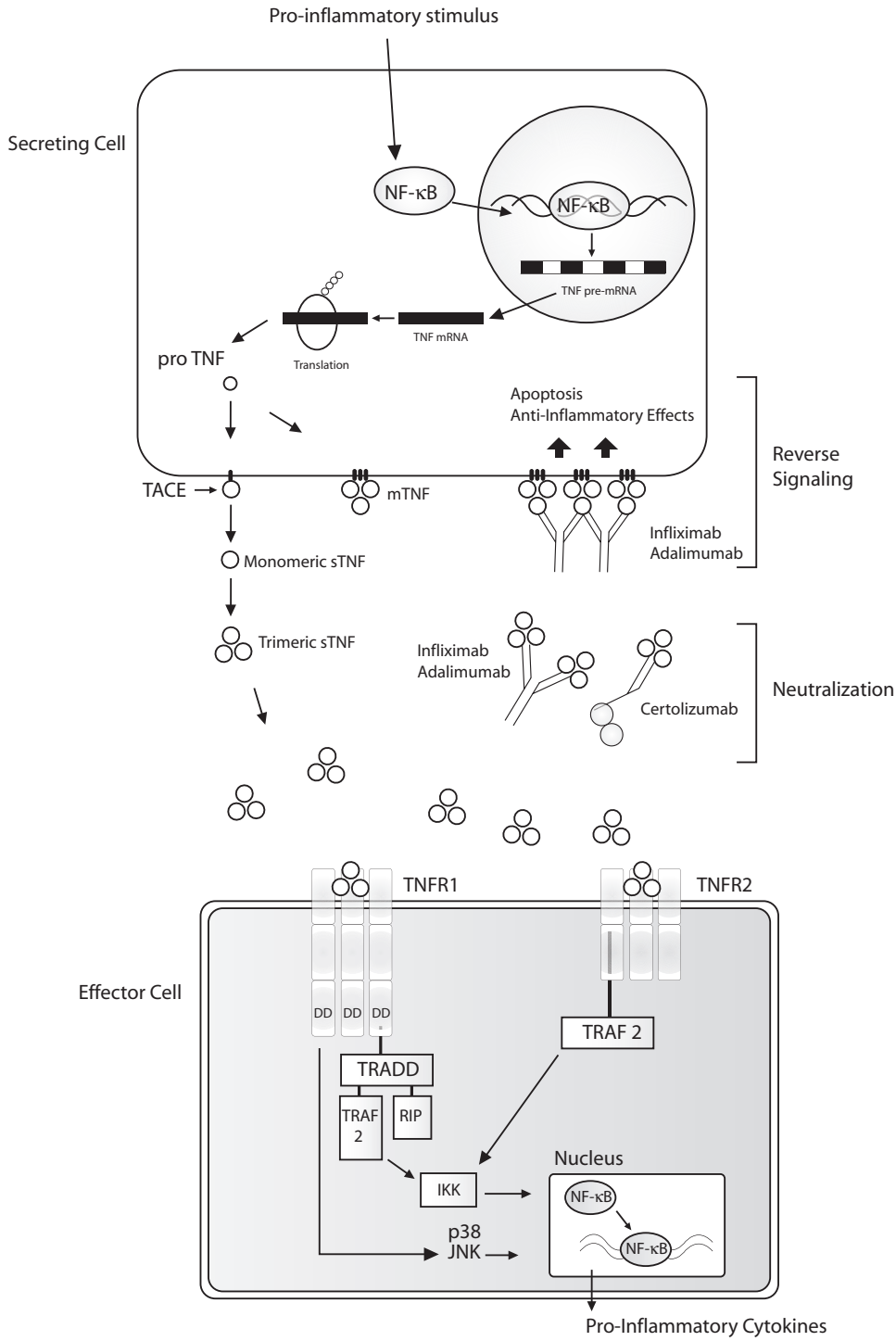

Figure 25.5 TNFα production, activity and targets for inhibition. Current knowledge of pathways of TNFα production, secretion and activity present multiple possible approaches for suppression of TNFα-mediated inflammation in IBD. Strategies currently in clinical use include blockade of TNFα activity by binding to soluble sTNFα (neutralization) and modulation of the activities of membrane-associated mTNFα (reverse signaling). Other potential strategies include suppression of TNFα synthesis, reduction in secretion of TNFα by TACE inhibition, inhibition of TNFR1 or TNFR2, inhibition of signal transduction by MAP kinases and NF-κB. Abbreviations: DD, death domain; IKK, IκB kinase; mTNF, membrane bound TNF; sTNF, soluble TNF; RIP, receptor interacting protein; TACE, TNFα-converting enzyme; TNFR1, TNF receptor-1; TNFR2, TNF receptor-2; TRADD, TNFR1-associated death domain; TRAF-2, TNFR-associated factor-2.

infusion reactions is increased in the presence of antibodies to infliximab and is associated with episodic as opposed to continuous use.

Despite the efficacy of infliximab in the treatment of IBD, its precise place in therapy remains to be defined. Infliximab is currently applied mostly in the "step-up" fashion in individuals resistant to conventional treatments including steroids and immunosuppressants. Some advocate a "top-down" approach with early introduction in individuals who may be unlikely to respond to conventional therapy.

Mechanism of action

Despite the demonstrated clinical efficacy of infliximab in randomized controlled trials, there remains uncertainty as to its clinically relevant mechanisms of action and as to why some other anti-TNFα agents, such as etanercept and onercept, have had less success in IBD. Infliximab shares with these agents the capacity to bind and neutralize TNFα, hence this ability alone is insufficient to explain its clinical efficacy. It is likely that other factors, such as structural and binding features and the ability to induce apoptosis in immune cells, linked with the capacity for reverse signaling are the determinants of the efficacy of infliximab in IBD.

Infliximab is a complete antibody molecule with two antigen binding sites and an effector region (Figure 25.2). It recognizes the monomeric components of trimeric TNFα and binds to each individually. Given that it has two antigen-binding sites it is capable of binding two molecules of TNFα at a time, allowing binding of two TNFα monomers within a single homotrimer, binding of two distinct homotrimers or binding of up to three molecules of infliximab to a single homotrimer. Once bound, infliximab is unlikely to dissociate, allowing the formation of stable infliximab–TNFα complexes comprising multiple infliximab molecules and multiple TNFα homotrimers [36]. Crosslinking of mTNF by infliximab also allows reverse signaling (see below). Etanercept (Figure 25.6), by comparison, binds reversibly to one homotrimer at a time, has little affinity for mTNF [36,37] and is not capable of polymer formation. Therefore, the maintenance of stable sTNFα complexes with a high degree of TNFα blockade and the capacity for polymeric interactions with mTNFα may contribute towards the efficacy of infliximab.

Infliximab treatment *in vivo* has been observed to reduce levels of pro-inflammatory cytokines [38] (particularly IL-1 and IL-6), to reduce mediators of leukocyte migration [38,39] and to lower significantly populations of immune cells in the intestinal mucosa of IBD patients [40], including T lymphocytes, monocytes and neutrophils. Although these findings could be explained by TNFα blockade leading to a general reduction in inflammation, they are more likely to be due to infliximab-induced apoptosis of immune cells. Indeed, infliximab has been demonstrated

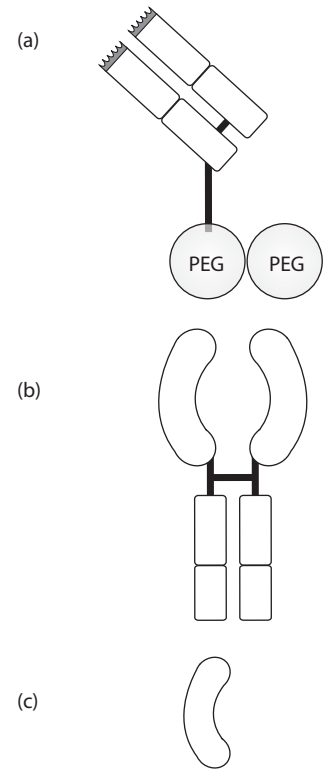

Figure 25.6 Schematic representation of biological anti-TNFα agents other than monoclonal antibodies. (a) Certolizumab pegol is a humanized monoclonal antibody Fab fragment against TNFα, linked to PEG. (b) Etanercept is a p75 TNFα receptor (TNFR2) fused to an IgG1 Fc antibody fragment. (c) Onercept is a p55 TNFα receptor (TNFR1).

in vivo [41] and *in vitro* [13,37,42] to induce apoptosis of T cells, monocytes, neutrophils and macrophages. More recently, a study by Van den Brande *et al.* demonstrated real time observation of *in vivo* apoptosis using radiolabeled annexin V (which binds to phosphatidylserine residues on apoptotic cells) after infliximab infusion for CD. This technique allowed the assessment of the degree of apoptosis, which was found to correlate with treatment response [43]. Although there is controversy regarding apoptotic versus nonapoptotic pathways in the control of other inflammatory disorders [44], it appears, on the basis of this evidence, to be a significant determinant of efficacy in IBD. Current research is investigating the precise mechanism of apoptosis induction by infliximab in IBD patients.

Infliximab has been demonstrated to induce apoptosis of monocytes *in vitro* [13] and *in vivo* in monocytes and T cells [37,42]. Infliximab appears to induce apoptosis in activated monocytes via a caspase-dependent pathway mediated by caspase-9 and -3 with pro-apoptotic protein (*bax* and *bak*) induction, mitochondrial activation and cytochrome *c* release [42]. Caspase-3 activation has been described in treated lamina propria T cells [41] from patients with CD. Infliximab-induced apoptosis does not occur

on resting immune cells and appears to be selective for activated immune cells, which express mTNFα in large amounts. Reverse signaling via infliximab–mTNFα interaction has been demonstrated to induce effects in a T cell line expressing mTNFα, including apoptosis, inhibition of proliferation, cell cycle arrest and IL-10 production [45]. Apoptosis was associated with caspase-3 activation and induction of *bak* and *bax*. In the same study, etanercept was not found to be capable of inducing any of these effects and demonstrated no evidence of reverse signaling.

Infliximab has also demonstrated the ability to induce non-apoptotic anti-inflammatory effects *in vivo* [46]. The study by Ringheanu [46] demonstrated that infliximab was able to suppress production of pro-inflammatory cytokines (specifically IL-1 and IL-6) by activated monocytes *in vitro*, apparently via reverse signaling.

On the basis of current evidence, the clinical efficacy of anti-TNFα agents is related to interplay of structure, mechanism and extent of binding and ability to induce directly biological effects such as apoptosis in target cells. However, the clinical effects of certolizumab pegol (Figure 25.6) may force further revisions of this model of the pharmacological actions of anti-TNFα agents (see below).

Pharmacokinetics

Infliximab is administered i.v. It is primarily distributed within the intravascular space and has a median $T_{\frac{1}{2}}$ of 8–9.5 days. The volume of distribution and clearance are not dose dependent. Clearance does not appear to be related to age, weight or gender but is known to be affected by the presence of antibodies to infliximab (ATI) [29]. Blood levels of 6-TG were transiently raised in patients co-treated with infliximab and azathioprine, accompanied by a decreased leukocyte count and raised red blood cell mean corpuscular volume [47]. The mechanism was postulated to be related either to enhanced azathioprine absorption or to suppression of thiopurine methyltransferase (TPMT) activity. Interestingly, raised 6-TG and macrocytosis was associated with a good clinical response to infliximab.

Adalimumab

Efficacy and safety

Adalimumab is the second biological agent to be approved for use in the treatment of IBD. It is a fully human IgG1 monoclonal antibody (Figure 25.2) produced in mammalian cells. The efficacy of adalimumab as an induction regime in the treatment of CD was demonstrated in the CLASSIC-1 trial [48], a randomized controlled trial comparing placebo against induction regimens of adalimumab in anti-TNF naïve patients. This trial demonstrated a significant difference in the remission rate between placebo and the 160/80 mg induction regime at week 4 (12 vs 36%). CLASSIC-2 was a follow-on study which demonstrated the efficacy of adalimumab administered weekly or every other week versus placebo as a maintenance regime in responsive patients. Both treatment cohorts had significantly improved remission rates compared with placebo at most interval time points and at study completion. The CHARM trial [49] also demonstrated a significant difference between adalimumab weekly or every other week versus placebo in maintaining remission and response after an induction regime. Importantly, steroid-free remission and fistula closure were significantly increased in the treatment arms compared with placebo. Response was independent of the use of concomitant immunosuppression and was found to be superior in anti-TNFα naïve patients.

Adalimumab has also been noted to be useful for induction therapy in infliximab-resistant and intolerant patients in several pilot studies [50–52]. In one randomized controlled trial [53], the GAIN study, patients previously treated with infliximab were randomized to placebo versus an adalimumab 160/80 mg induction regimen. Assessment at 4 weeks demonstrated increased remission and response in adalimumab-treated patients over placebo. Adalimumab has also been investigated for maintenance treatment in patients who had become unresponsive to or intolerant of infliximab in an open-label study [54].

The commonest side effects in the randomized controlled trials attributable to adalimumab were injection site reactions. Rates of serious infections were low (1.7–4%) and tuberculosis was diagnosed in two patients in CHARM. The rate of antibody development to adalimumab was low (4%) in CLASSIC-II.

As with infliximab, the precise role of adalimumab in CD therapy has not been fully defined. Adalimumab has demonstrated efficacy for induction and maintenance therapy and may have a role in those requiring biologic therapy who are infliximab intolerant. An obvious advantage over infliximab is its s.c. route of administration, which may reduce ancillary costs associated with treatment and improve patient convenience.

Mechanism of action

Adalimumab was developed using a phage display library technology and consists of human-derived heavy- and light-chain variable regions with human IgG1κ constant regions (Figure 25.2). Like infliximab, adalimumab binds to TNFα with high affinity and forms stable polymeric complexes with sTNFα [55] and mTNFα and prevents its interaction with TNFR1 and TNFR2. Adalimumab has been observed to induce complement-dependent cytotoxicity [56] and to induce caspase-dependent apoptosis to a similar degree to infliximab *in vitro* [57,58] and *in vivo* in a mouse model [58]. The mechanism by which adalimumab induces apoptosis is uncertain at this time.

Pharmacokinetics

Adalimumab is administered s.c. and is distributed mainly in the extracellular space. The volume of

distribution is dose dependent, although clearance is not, ranging from 12 to 14 ml h^{-1}. Clearance is increased by the presence of anti-adalimumab antibodies and is decreased with increasing age and concomitant use of immunosuppression. $T_{\frac{1}{2}}$ is approximately 14 days (range 10–20 days) and bioavailability is estimated at 64% after a 40 mg dose.

CDP 571

CDP 571 is a humanized (95%) IgG4 monoclonal antibody directed against TNFα. It has been investigated to date in the management of CD. A Phase II dose-finding trial demonstrated induction of a short-term clinical response at 2 weeks with a dosage of 10 mg kg^{-1} in active CD [59]. A Phase III study again demonstrated the clinical response at 2 weeks but did not demonstrate a significant difference at 28 weeks from placebo of CDP571 10 mg kg^{-1} administered 8 weekly [60]. Two further trials investigating a role for CDP571 as a steroid-sparing agent in steroid-dependent CD failed to demonstrate a significant benefit [61,62].

Non-mAb anti-TNFα agents

Three non-mAb TNF-neutralizing proteins have been investigated for therapeutic activity in IBD (Figure 25.6). Etanercept is a human p75 TNFα receptor (TNFR2) bound to an IgG1 Fc fragment that is administered by s.c. injection. Its mechanism of action is to bind and neutralize soluble and membrane-bound TNFα, but etanercept is not capable of inducing apoptosis in TNFα-expressing cells. As discussed above, it has efficacy in the treatment of rheumatoid arthritis, but does not induce clinical benefit in the treatment of IBD [63]. Onercept is a recombinant soluble p55 TNFα receptor (TNFR1) with a similar mechanism of action against TNFα. It also has not shown efficacy in the treatment of CD [64].

Certolizumab pegol (CDP870)

Efficacy and safety

Certolizumab is a humanized monoclonal antibody Fab fragment against TNFα, linked to PEG. Two phase II trials were carried out to investigate the efficacy of certolizumab pegol in CD. Neither study was able to demonstrate a significant difference between certolizumab pegol and placebo, partly due to very high placebo response rates, although clinical benefit was demonstrated, in a dose-dependent manner [65,66]. A Phase III randomized controlled trial, PRECiSE I, was conducted, in which patients were randomized to certolizumab pegol 400 mg or placebo 2 weekly at weeks 0, 2 and 4 and then 4 weekly to week 24. Subjects were stratified for elevation of C-reactive protein and concomitant immunosuppressive drug use. Response was significantly higher in certolizumab pegol-treated patients than controls at weeks 4, 6 and 26. The remission rate was also significantly superior at 4 weeks (certolizumab pegol 20.1% vs control 9.6%), although not at later time points [67]. In PRECiSE II, patients who responded to induction therapy were randomized to certolizumab pegol 400 mg 4 weekly versus placebo for up to 26 weeks. The results showed a significant benefit of certolizumab pegol over placebo for response (63 vs 36%) and remission (48 vs 29%). PRECiSE III aims to treat up to 5 years; data accumulated to date [68] show sustained response and remission rates at week 80 to be 44.2 and 37.2%, respectively.

Serious adverse events were reported as 10.3 versus 7% in certolizumab versus treated patients in PRECiSE I, 5.6% versus 6.6% in PRECiSE II and 19.1% in PRECiSE III. The commonest effects included headaches and nasopharyngitis. There was no significant difference in serious infectious complications, although there was one case of tuberculosis in the certolizumab-treated group. At the time of writing, a license application for certolizumab for treatment of CD has been submitted.

Mechanism of action

Certolizumab pegol was engineered by grafting the complementarity-determining region from a murine monoclonal antibody on to a human Ig Fab′ fragment (IgG γ1κ) (Figure 25.6). The engineered Fab′ fragment retains the biologic potency of the original antibody. The Fab′ fragment is linked to two crosslinked chains of PEG.

Certolizumab pegol binds to and neutralizes TNFα. As it has no Fc region, it does not induce complement or cell-mediated effector functions. There is little information available as yet on the precise biologic effects of certolizumab, although current data demonstrate that it has a high affinity for sTNFα and mTNFα. It does not form large complexes, is not capable of crosslinking mTNFα [69] and has not been demonstrated to induce apoptosis in activated monocytes or lymphocytes [69,70]. This challenges the notion that apoptosis induction explains the clinical efficacy of adalimumab and infliximab. Further investigations of the effects of certolizumab should lead to an increased understanding of the mechanism of efficacious TNFα blockade in CD.

Pharmacokinetics

Certolizumab can be administered i.v. or s.c. and a single dose has a $T_{\frac{1}{2}}$ in healthy volunteers of approximately 17 days, with high bioavailability. Antibodies to certolizumab were demonstrated in 12.3% of CD patients at least once over 12 weeks in patients receiving 3 × 400 mg doses. Although circulating certolizumab levels were reduced in the presence of those antibodies, initial results suggest that they may not be associated with clinical efficacy [66,71].

Inhibitor of IL-6R

IL-6 is produced by T cells, B cells, monocytes, fibroblasts and endothelial cells and has multiple biological

activities, including immunoregulation, the induction of acute-phase reactants and hematopoiesis. Of interest in IBD is the role of IL-6 in immunoregulation, causing B and T cell proliferation, terminal B cell differentiation, cytotoxic T cell differentiation and macrophage differentiation (Figure 25.1).

Increased concentrations of IL-6 and soluble IL-6R correlate with disease activity in CD. However, polymorphisms in the IL-6 gene that are associated with overproduction of this cytokine have not been shown to increase the likelihood of IBD [72]. The membrane-bound IL-6R consists of a binding α subunit (80 kDa) and a signal transducing β subunit (130 kDa) (also referred to as gp130), which mediates intracellular effects of IL-6. The α subunit is expressed only on certain cell types but the gp130 subunit is expressed ubiquitously. The soluble IL-6R may form extracellular complexes with IL-6 which allows signal transmission in non-α subunit-expressing cells (trans-signaling). This IL-6–sIL6R interaction may mediate the IL-6-associated pathological activity in chronic inflammatory disorders. A theoretical benefit of immunomodulation of IL-6 activity compared with TNFα or interferon is that IL-6 does not appear to play a significant role in granuloma formation. Therefore, inhibition of IL-6 may be less likely to cause latent TB reactivation than anti-TNFα.

Anti-IL-6R (tocilizumab) is a humanized IgG1 monoclonal antibody which has been investigated in rheumatoid arthritis, juvenile idiopathic arthritis, Still's disease and CD. One pilot randomized trial has been carried out to date in CD in which 36 patients with active disease were randomized to anti-IL-6R monoclonal antibody 8 mg kg^{-1} 2 weekly, alternating 8 mg kg^{-1} and placebo 2 weekly or placebo for 12 weeks. The response rate was 80, 42 and 31%, respectively, with a significant difference between 2 weekly dosing and placebo. Remission rates were 20, 25 and 0%, respectively (not significant) [73]. There was no improvement on endoscopic or histologic examination. No significant difference in adverse events was noted between the groups. This was a small study and, in view of the observations of early response, requires further investigation in larger trials.

Inhibitors of IL-2R

IL-2 is a 15.5 kDa protein produced by activated T cells that is a critical mediator of the initiation and maintenance of adaptive immune responses. It has autocrine activity leading to expansion of antigen-specific T cells and also local paracrine activity stimulating growth and proliferation of a number of other cells, including B cells, NK cells, monocytes and neutrophils. Together with IFN-γ, IL-12 and IL-18, it leads to T cell activation and to Th1 polarization (Figure 25.1). Its effects are mediated through the IL-2 receptor (IL-2R), the structure of which varies according to cell type and activity, with varying affinity for IL-2 depending on the composition of the subunits. The high-affinity IL-2R is a heterotrimeric transmembrane receptor consisting of α, β and γ subunits, (IL-2Rαβγ). The β and γ subunits mediate signal transduction through multiple pathways. These include MAPK, JAK/STAT and phosphoinositide-3-kinase (PI3K), whose downstream targets affect cell cycle control. Although the α subunit is not necessary for mediation of activity on all cell types, it is required in activated T and B cells, offering a specific target for control of T cell activation in IBD. Antagonism of IL-2 activity is a rational therapeutic approach as a mechanism of T cell inhibition and also as a treatment to counteract pathogenic activation of adaptive immunity.

Two anti-IL2R monoclonal antibodies have been investigated to date in UC. These antibodies were originally developed to prevent and treat graft rejection following renal transplantation. Basiliximab is a chimeric murine monoclonal antibody and daclizumab is a humanized (90%) monoclonal antibody with murine complementarity-determining regions, both against the IL-2Rα subunit. Daclizumab was assessed in an open-label study of 10 patients with steroid-refractory UC, each of whom received 1 mg kg^{-1} on days 1 and 29 by i.v. injection. Daclizumab caused a significant improvement in clinical activity, endoscopic and histologic scoring at week 8 [74]. This was followed up with a larger study which randomized 159 patients with moderately active disease to placebo, daclizumab 1 mg kg^{-1} at weeks 0 and 4 or 2 mg kg^{-1} at weeks 0, 2, 4 and 6. End of study remission rates were 10, 7 and 2% and response rates were 44, 25 and 33%, respectively, [75]. These differences were not significantly different. Given the promising results of the initial study, these results were disappointing, suggesting a lack of sustained clinical efficacy.

Basiliximab has been evaluated in two open-label studies as a co-treatment with corticosteroids for induction of remission in moderate to severe steroid-resistant UC [76,77]. In the first study, a single dose of basiliximab 40 mg, in addition to corticosteroid, was given to 10 previously steroid-resistant patients; 9/10 achieved remission by week 6, but 8 of those relapsed over 24 weeks of follow-up, requiring reintroduction of steroids. A second study was carried out to assess further the durability of response in which 20 more patients were enrolled; 50% achieved remission by week 6 and 65% were in remission at week 24. Reduction in steroid dosage was observed in moderate and severe treatment groups, with particular improvement in the moderately active group. The role of basiliximab in IBD therapy needs to be further defined, but it would appear to be an efficient method of restoring steroid sensitivity or reducing requirement in UC.

Inhibitors of IL-12

IL-12 is produced by antigen-presenting cells (dendritic cells) and macrophages after activation by bacterial challenge or by the action of pro-inflammatory cytokines. The

main functions of IL-12 are to stimulate T cell and NK cells and to promote cell-mediated immunity by inducing a Th1 response. IL-12 promotes differentiation of naïve and resting T cells to IFNγ-producing cells and positively reinforces IFN-γ production (Figure 25.1).

IL-12 is active as a heterodimer of a 40 kDa (IL-12p40) subunit and a 35 kDa subunit (IL-12p35) [78]. The p40 subunit is common to a related cytokine, IL-23, with which it shares functions but which also induces memory T cell proliferation and enhances cytotoxic T cell activation. The role of IL-23 in IBD is unclear at this time but recent evidence suggests a possible role in disease pathogenesis. The IL-12 receptor is a heterodimer of IL-12Rβ1 and IL-12Rβ2 and is expressed on activated T and NK cells. Inhibition of IL-12 activity has demonstrated efficacy in various animal models of IBD with improvement or resolution of inflammation and suppression of IFN-γ production [79].

One Phase II study of anti-IL-12 antibody in IBD has been reported. The antibody used (ABT-874) was a fully human IgG1γ monoclonal antibody against the p40 subunit of IL-12. Seventy-nine patients with moderate CD were randomized to seven s.c. injections of placebo or ABT-874 at a dose of 1 or 3 mg kg^{-1} in two cohorts; cohort 1 received one injection followed by a 4 week interval and cohort 2 received all injections weekly without interruption. No significant differences were noted in treatment or follow-up in cohort 1. Response rates in the ABT-874 3 mg kg^{-1} group in cohort 2 were significantly better than placebo at the end of treatment (75 vs 25%), but not at the 18 week follow-up. Monoclonal antibody treatment was observed to decrease production of downstream effectors of inflammation (i.e. IFN-γ and TNFα) in addition to IL-12, which was associated with an increased likelihood of response. The authors did allow that some of this effect may be due to simultaneous suppression of IL-23 activity as the monoclonal antibody was directed against the common p40 subunit. The differences observed at follow-up, although not significant, were present and warrant further investigation of anti-IL-12R.

Both IFN-γ and TNFα are required for the formation and maintenance of granulomas. Therefore, one concern with the use of IL-12R monoclonal antibody, with its associated suppression of IFN-γ and TNFα, is increased risk of the reactivation of granulomatous infection [80,81]. To date, no serious infectious adverse events have occurred in studies of ABT-874 and the only significant difference in adverse events was the development of injection site reactions in the treated patients.

An oral small-molecule inhibitor of IL-12 is currently under investigation. STA-5326 was discovered to suppress IL-12 production in a small-molecule library screen. In an animal model of IBD, STA-5326 was demonstrated to suppress IL-12 and IL-23 production with inhibition of IFN-γ production and suppression of the Th1 response. It acts at a transcriptional level to suppress production of the IL-12 p35 and p40 subunits (suppressing IL-23 production also) [82]. An open-label phase II trial in CD has demonstrated clinical activity with 70 and 100 point reductions in CDAI of 80 and 64% with maximum dosage (70 mg daily) [83]. A randomized controlled trial is currently under way.

Inhibitor of interferon-γ

IFN-γ is an immunoregulatory cytokine that is produced exclusively by immune cells (T cells and NK cells) in response to bacterial challenge or pro-inflammatory cytokine stimulation. IFN-γ upregulates MHC I and II expression on APC, increases cytokine secretion (including TNFα and IL-12), upregulates leukocyte–endothelial interaction and activates macrophages and B and T cells (Figure 25.1). This cytokine also has autocrine effects on the producing cells, further increasing its production through positive feedback. Broadly, IFN-γ causes Th1 polarization and stimulates cell-mediated immunity.

Fontolizumab is a humanized IgG1 monoclonal antibody against IFN-γ, which has demonstrated efficacy in rheumatoid arthritis. Two Phase II studies investigating fontolizumab for the treatment of IBD have been completed. The first study randomized 45 patients with moderate to severe CD to placebo or fontolizumab (0.1, 1 or 4 mg kg^{-1}) single dose. At 4 weeks, responders were randomized to 50% of initial dose or placebo, to be administered monthly for 3 months. A dose-dependent response was noted in the treatment group at day 29, although there was no significant difference between the treatment and placebo arms, possibly due to a high placebo response rate. *Post hoc* analysis of patients with raised CRP did demonstrate a slight difference between placebo and the 1 and 4 mg kg^{-1} treatment groups Significant reductions in C-reactive protein and histologic activity were noted in the 4 mg kg^{-1} group, suggesting that higher doses may be effective [84]. A second Phase II study randomized 133 patients to placebo or fontolizumab 4 or 10 mg kg^{-1}, for one or two injections. No significant difference was noted at day 28, with a high placebo rate observed again. However, there was a significantly higher response rate at day 56 in those who received a second dose of fontolizumab. *Post hoc* analysis of a subgroup of patients with raised CRP at induction demonstrated a significantly better response rate to active drug than placebo. Further investigation of fontolizumab is ongoing.

Anti-inflammatory cytokines

IL-10

IL-10 is an 18.5 kDa cytokine with complex immunoregulatory activities that affect innate and adaptive immunity. IL-10 generally downregulates inflammation, but in certain circumstance can also exert immunostimulatory effects. It is active in the form of a 37 kDa homodimer and signals via its specific cell surface receptor (IL-10R). IL-10R is a member of the receptor tyrosine kinase family.

Expression of IL-10R is variable according to cell type and state of immune activity. IL-10 downregulates inflammatory activity by suppressing pro-inflammatory cytokine production (IL-1, IL-6, IL-12, IFN-γ, TNFα and IL-8) and the antigen-presenting capacity of monocytes/macrophages (Figure 25.1) [85]. IL-10 reverses Th1 polarization, as observed in CD patients, and also has a role in stimulating intestinal electrolyte absorption and consolidating epithelial integrity.

IL-10 was investigated as a therapeutic modality for IBD using recombinant human IL-10 (rhuIL-10), known as ilodecakin. A placebo-controlled dose–response study in 46 patients reported that i.v. rhuIL-10 was of benefit in steroid-resistant CD in a non-dose-dependent manner [86]. This was followed up with three large placebo-controlled studies. The first involved 329 treatment-refractory patients randomized to s.c. rhuIL-10 1, 4, 8, 20 $\mu g\,kg^{-1}$ or placebo daily for 28 days. No difference in induction of remission was seen between the groups, but there was a trend towards improvement in the 8 $\mu g\,kg^{-1}$ group [87]. The second study assessed response to rhuIL-10 1, 5, 10, 20 $\mu g\,kg^{-1}$ versus placebo. Clinical remission and endoscopic improvement were seen in 23.5% of the 5 $\mu g\,kg^{-1}$ group versus 0% for placebo. Doses greater than 5 $\mu g\,kg^{-1}$ had a lesser effect [88]. The third study [89] evaluated 373 steroid-dependent patients with rhuIL-10 4or 8 $\mu g\,kg^{-1}$ versus placebo daily for 2 weeks, then three times weekly for 26 weeks. No significant difference in steroid withdrawal or induction of remission was noted. Animal studies of IL-10 knockout animals and IL-10 treatment of experimental colitis demonstrated great promise, which unfortunately clinical trials did not bear out in this instance. A placebo-controlled dose–response trial of rhuIL-10 did not demonstrate a beneficial effect in 94 patients with mild to moderate UC [90].

Parenteral administration studies of rhuIL-10 have been discontinued and it is possible that this route of administration may have resulted in inadequate drug levels at the site of disease. Animal studies are ongoing using oral delivery systems to deliver IL-10 to increase mucosal concentrations and minimize systemic exposure. Methods which have demonstrated efficacy in murine models include *Lactococcus* bacteria and low-alkaloid tobacco species engineered to express IL-10.

IL-11

IL-11 is a 19.1 kDa protein with multiple immunoregulatory effects. It is produced by many cell types of mesenchymal and hematopoietic origin. Regulation is complex and both tissue and cell type specific. IL-11 binds to IL-11 receptor (IL-11R), which requires coexpression of cell surface gp130 for signal transduction. Recombinant human IL-11 (rhuIL-11) (oprelvekin) is currently licensed for the treatment of cancer therapy-induced thrombocytopenia. In the course of animal studies of radiation and high-dose chemotherapeutic-induced myeloablation, preservation of intestinal function and decreased apoptosis were noted in the presence of rhuIL-11 compared with severe mucositis in untreated animals. It was also observed to have pro-proliferative effects on epithelial cells after ischemia-induced bowel injury. IL-11 downregulates expression of pro-inflammatory cytokines, particularly TNFα and INF-γ and promotes a Th2 cytokine response pattern. [91] A pilot study in CD patients was carried out in which placebo or oprelvekin 5, 16 or 40 $\mu g\,kg^{-1}$ per week was given two or five times weekly. There was a trend noted towards improvement in the 16 $\mu g\,kg^{-1}$ per week (33 and 42%) group compared with placebo (7%), with retrospective analysis suggesting that steroid treatment could reduce efficacy [91]. A second study of s.c. rhuIL-11 15 $\mu g\,kg^{-1}$ per week was carried out in patients not requiring corticosteroids [92]. There was no significant difference in CDAI at week 6, although there was a significant difference in numbers achieving remission (37 versus 17%). A randomized placebo-controlled comparison of rhuIL-11 versus prednisolone for the induction of remission in active CD was conducted in 51 patients. This study demonstrated rhuIL-11 to be inferior to standard prednisolone treatment (4 vs 46% at week 4) [93].

Inhibitors of T cell activation

Anti-CD40 and anti-CD40L

CD40 is a 45 kDa transmembrane receptor expressed on the surface of immune (B cells, monocytes, macrophages and dendritic cells) and non-immune cells (epithelial, endothelial, mesenchymal cells and platelets). CD40 Ligand (CD40L) is a 39 kDa protein expressed mostly by activated $CD4^+$ T cells and activated platelets, although it may also be expressed by monocytes, NK cells, B cells, $CD8^+$ T cells, mast cells and basophils. Increased CD40L expression on platelets in IBD may be related to the increased risk of thromboembolism in those patients. CD40L is expressed at the cell surface, cleaved and circulates in a biologically active soluble form. CD40–CD40L binding leads to tyrosine kinase activation with induction of transcription factors including NF-κB. Effects on immune cells include increased Ig synthesis, increased expression of cell surface receptors related to inflammation, increased synthesis of IL-8, TNFα, MIP-1α (macrophage inflammatory protein) and IL-12. Stimulation of non-immune cells results in production of pro-inflammatory cytokines and also increased expression of T cell chemoattractant factors. Newly recruited T cells upregulate CD40 expression, leading to further pro-inflammatory cytokine release, contributing to continuation of the inflammatory process. Animal models of colitis and ileitis have demonstrated the necessity for CD40–CD40L interaction for the development of inflammation. CD40L-deficient mice display markedly reduced inflammation [94] and anti-CD40L antibody is able to abrogate colitis [95].

Two monoclonal antibodies against the CD40–CD40L system have been studied in IBD: a human IgG4 monoclonal antibody against CD40 (ch5D12) and a human monoclonal antibody against CD40L (IDEC-131). IDEC-131 studies were terminated early due to a high risk of thromboembolic events [96] related to CD40L expression on platelets. Another human monoclonal antibody (BG9588) against CD40L was studied in lupus glomerulonephritis and clinical benefit was demonstrated. However, this study was also terminated prematurely due to thromboembolic complications. An open-label study of ch5D12 was carried out in 18 patients with active CD. A single dose of 0.3, 1, 3 or 10 mg kg^{-1} was administered i.v.; 72% had a significant decrease in CDAI and 22% went into remission at day 21. Improvement in endoscopic scores was noted in five individuals and decreased T cell, B cell and monocyte infiltration was noted on post-treatment biopsy. No clear dose–response association was noted [97]. Another modality currently under investigation is the use of CD40 antisense oligonucleotide [98].

Anti-CD4

CD4 is the characteristic receptor of the T-helper cell subtype. It is a 55 kDa transmembrane receptor protein of the immunoglobulin supergene family which functions as a co-receptor with CD28 for the recognition of antigens presented in association with Class II MHC-expressing cells. Upon binding, lymphocyte-associated function antigen-1 (LFA-1) expression is upregulated on the T cell surface and interacts with intracellular adhesion molecule-1 (ICAM-1) on the antigen-presenting cell (APC) to increase the strength of the interaction and results in tyrosine kinase activation, ultimately resulting in upregulation of transcription factors leading to T cell proliferation and cytokine production.

CD4 was one of the earliest receptors regarded as a possible therapeutic target for the treatment of CD. Two open-label studies investigated cM-T412, a chimeric depleting anti-CD4 mAb, in steroid-refractory CD and UC. CDAI score reduction and endoscopic improvement were noted [99]. Two open-label studies of BF-5, a non-depleting murine anti-CD4 monoclonal antibody in two patients with CD and UC met with less success; 5 of 9 UC patients and 6 of 16 CD patients achieved remission [100,101]. Depletion of $CD4^+$ T cells was noted with these mAb and although there were no instances of opportunistic infection, further development was halted.

Anti-CD3

CD3 is expressed on all T cells and together with the T cell receptor forms the T cell receptor complex. The anti-CD3 monoclonal antibody OKT3 was the first engineered therapeutic monoclonal antibody approved for use in 1986 for the prevention of kidney transplant rejection. OKT3 is a murine monoclonal antibody and has a marked side effect profile. These adverse effects are in part due to induction of anti-mouse antibodies and also to a syndrome of adverse effects associated with transient T cell activation observed after the first dose. This cytokine release syndrome is mediated by crosslinking of CD3 via the Fab arms and the Fcγ receptor on effector cells. Partly to overcome these limitations of OKT3, visilizumab, a humanized (90% human sequences) IgG2 against the CD3ε chain of the T cell receptor was developed. Humanization reduced the immunogenicity of mouse mAbs and the Fc region was modified to reduce interaction with Fc receptors, with less T cell activation and less cytokine release. In addition to CD3 blockade, possible mechanisms of action of visilizumab include the induction of apoptosis of activated T cells secondary to sustained T cell receptor activation and increased IL-10 secretion.

Visilizumab has been investigated as a therapeutic modality in graft versus host disease, UC and CD. Open-label studies in IBD to date have been encouraging. One study in steroid-refractory UC administered 10 or 15 μg kg^{-1} visilizumab i.v. daily for 2 days. Response and remission rates at day 30 were 79 and 54% in the 10 μg kg^{-1} group and 100% in the 15 μg kg^{-1} group, with 63% of patients describing symptoms consistent with cytokine release syndrome (nausea, fever, chills and headache) [102]. A study in previously infliximab-treated patients with CD reported that with administration of 10 μg kg^{-1} i.v. daily for 2 days, six of the eight patients had clinical response and three achieved complete remission. Further evaluation is ongoing [103].

Inhibitors of leukocyte adhesion

Maintenance of an established inflammatory process requires recruitment of T lymphocytes and other leukocytes into the area of inflammation. Recruitment of leukocytes is a multi-step, highly regulated process (see Figure 25.7). The three basic steps are rolling and adherence, before endothelial transmigration into the site of inflammation. Rolling describes gradual slowing of circulating leukocytes by formation of short-lived associations between receptors on the endothelial surface and on the leukocytes, followed by formation of high-affinity binding leading to adherence. Inflammatory cytokines, including TNFα, upregulate the expression of adhesion molecules on endothelial cells in areas of inflammation. Slowing is achieved by interactions between selectins expressed on the endothelial surface (E-selectin) and on the lymphocytes (L-selectin). These low-affinity bonds are short lived and the target cells may roll from one to the next. To halt leukocyte movement, secondary adhesion molecules, termed integrins, on the leukocyte cell surface, bind to adhesion molecules on the endothelial wall. Integrins are a superfamily of leukocyte transmembrane receptors with external domains mediating cell interaction and internal domains regulating signal transduction. They are

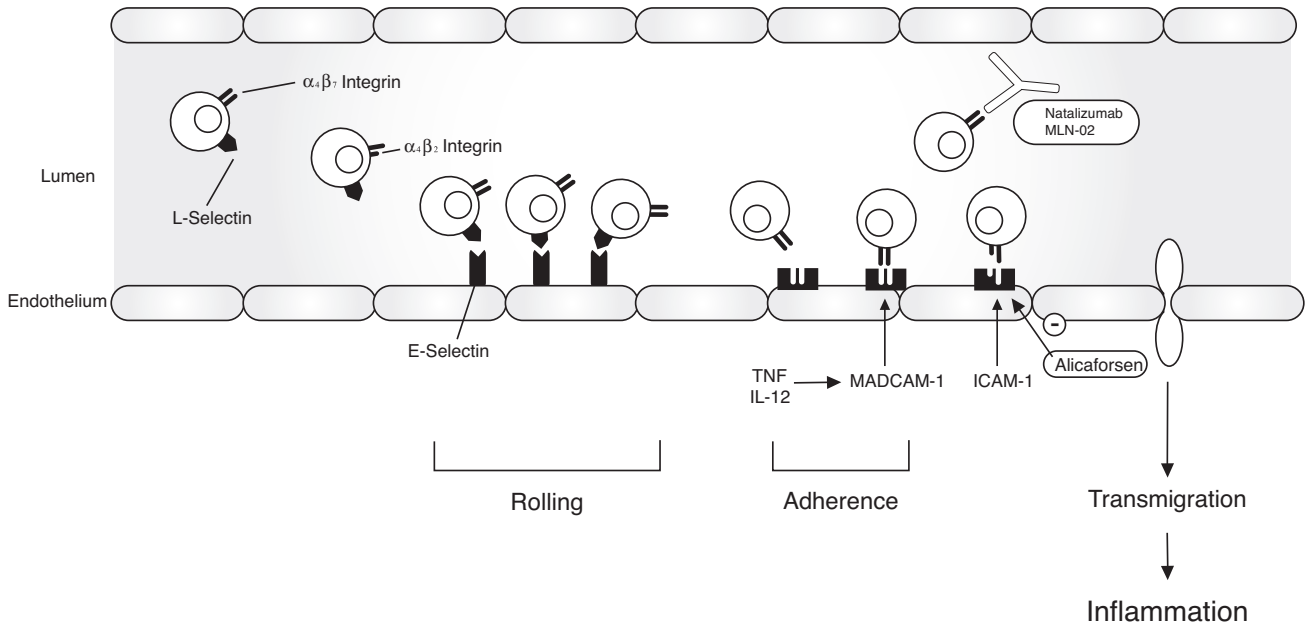

Figure 25.7 Multi-step model of leukocyte extravasation. The passage of leukocytes into areas of inflammation is mediated by adhesion molecules, comprising leukocyte-bound selectins and integrins and their complementary endothelial binding partners. After selectin-mediated slowing or rolling, integrins expressed on the leukocyte surface bind to adhesion molecules on the endothelial surface, after which transmigration takes place to the site of inflammation by diapedesis. Pro-inflammatory cytokines act as chemoattractants, upregulate expression of endothelial adhesion molecules, increasing leukocyte migration and maintaining established inflammation in IBD. Natalizumab and MLN-02 are monoclonal antibodies that bind integrins, impairing interaction with adhesion molecules. Alicaforsen is an antisense oligonucleotide which suppresses production of ICAM-1. Abbreviations: MadCAM-1, mucosal vascular addressin cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1.

heterodimers and consist of two noncovalently bound α and β subunits. Each integrin has specific receptors on the endothelial surface. α4 interacts with mucosal vascular addressin cell adhesion molecule-1 (MadCAM-1), which is highly expressed in intestinal tissue, and vascular cell adhesion molecule-1 (VCAM-1), which is ubiquitously expressed on the endothelium in areas of inflammation. β2 interacts with intracellular adhesion molecule-1 (ICAM-1). α4β1 integrin has effects in bone marrow and in the gut, including retention of haematopoietic progenitor and B cells in the bone marrow, lymphocyte adhesion and the activation of β2 integrins. α4β7, in addition to mediating adhesion, is necessary for local T cell signaling and activation and also binds to extracellular matrix proteins which may have functional effects on local dendritic cells and fibroblasts. Integrin adhesion molecule interaction is therefore an attractive site for control of inflammation. Two monoclonal antibodies and one antisense oligonucleotide have been investigated to date.

Natalizumab

Efficacy and safety

Natalizumab is a recombinant humanized (95%) monoclonal IgG4κ antibody against α4 integrin that is produced in murine myeloma cells. Initial animal studies demonstrating efficacy of α4 integrin blockade were carried out in tamarins (primates) [104]. Early studies in UC and CD suggested benefit for induction of remission and reduction of disease activity. [105,106] A Phase II randomized controlled study of natalizumab compared placebo with a number of different natalizumab dose regimens. Rates of clinical response were significantly higher in all natalizumab-treated groups at 4, 6 and 8 weeks, the highest response rate being observed in the $3\,mg\,kg^{-1}$ every 2 weeks group. There was significant improvement in IBD quality of life scores in treated patients compared with controls. The ENACT-1 trial of induction therapy in CD followed these initial positive results, but failed to demonstrate clinical efficacy of natalizumab 300 mg versus placebo at 0, 4 and 8 weeks [107]. *Post hoc* analysis of subgroups with elevated CRP, active disease despite adequate immunosuppression or absence of previous anti-TNFα treatment, suggested that any of these factors were associated with greater response and remission rates. Those who responded were offered enrolment in the ENACT-2 trial comparing maintenance natalizumab versus placebo. This trial demonstrated significant differences in disease response (61 vs 28%), remission (44 vs 26%) and steroid usage (58% not requiring vs 28%) at 36 weeks [107]. Following this, the ENCORE trial [108] investigated the use of natalizumab as induction therapy in patients with active CD demonstrated to have raised

CRP. In this cohort, natalizumab demonstrated significant benefit over placebo in response, remission and 100 point reduction in CDAI at week 4 and weeks 8–12 (48, 26, 39% vs 32, 16, 22%). A study of the efficacy of natalizumab and infliximab versus infliximab alone in the maintenance and treatment of CD unresponsive to infliximab has been conducted. The combination was well tolerated and demonstrated a non-significant reduction in CDAI compared with infliximab alone. There were no observed adverse interactions between the mAbs in this study and no significant difference in the adverse events was reported between the groups [109]. An uncontrolled study of single-dose 3 mg kg^{-1} natalizumab in 10 patients over 12 weeks in UC demonstrated significant improvement in disease activity and quality of life score at weeks 1, 2 and 4. This benefit was not sustained and 8 of the 10 patients required rescue steroids by week 8.

Safety has become a major concern with regards to natalizumab, primarily as a result of its association with three cases of progressive multifocal leukoencephalopathy (PML) (see below). With the exception of flu-like illnesses, which were more common in natalizumab-treated patients, there was no significant difference in other infection or serious infection in any of the trials. Infusion reactions were increased in natalizumab-treated patients, especially if antibodies to natalizumab were demonstrated, but the rate of antibody development was low (8–9%).

Mechanism of action

Natalizumab binds to α4β1 and α4β7 integrins preventing MadCAM-1 and VCAM-1 interaction and thereby reducing the migration of lymphocytes and monocytes from the circulation to areas of inflammation (Figure 25.7). The IgG4 isotype has no complement-activating capacity, does not bind to Fc receptors and remains in the circulation longer than other forms of IgG. It is currently licensed for the treatment of relapsing forms of multiple sclerosis under strict regulation.

Pharmacokinetics

The plasma $T_{\frac{1}{2}}$ of natalizumab in CD patients varies from 3.8 to 4.8 days, compared with 8.7 days in healthy volunteers [105]. This variation in $T_{\frac{1}{2}}$ may be related to higher circulating α4-expressing cells in the disease state, although this has not been confirmed. *In vivo* and *in vitro* studies of α4 receptor saturation after administration of natalizumab demonstrated that in CD, a minimum natalizumab concentration of 5 μg l^{-1} was necessary to achieve receptor saturation of ≥80% of α4 integrins [106]. Post-infusion increases in the B and T cell lymphocyte populations are observed in natalizumab-treated patients, probably occurring as a result of the retention of leukocytes in the vascular space.

MLN-02

MLN-02 is a humanized IgG1 monoclonal antibody specifically against the α4β7 heterodimer, which does not bind to either of the monomers. Therefore, MLN-02 should specifically inhibit leukocyte–MadCAM-1 interaction and theoretically limit its effects to the vasculature of the gut. MLN02 has been investigated in the treatment of CD and UC in two studies to date.

A total of 185 patients with CD were randomized to receive placebo, 0.5 or 2 mg kg^{-1} MLN-02 i.v. on days 1 and 29. On day 57, response rates were 41.4, 49.2 and 53.1%, respectively. Remission rates were 20.7, 29.5 and 36.9%, respectively, with a statistically significant difference between placebo and 2 mg kg^{-1} for response and remission [110]. A randomized controlled trial of MLN-02 in active UC recruited 181 patients and randomized them to placebo 0.5 or 2 mg kg^{-1} on day 1 and 29 also. Response/remission rates were 33/14, 66/33 and 53%/32%, respectively, at 6 weeks with a significant difference between treatment and placebo. High rates of α4β7 saturation were seen (>90%) at both doses at 4 and 6 weeks. Human anti-human antibodies were noted in 44% of treated patients at 8 weeks. There was an association noted between high antibody titers (>1:125), decreased α4β7 saturation and lack of clinical response. There was no significant difference in adverse events or lymphocyte counts between placebo and treated groups [111], possibly confirming specificity of action to a small proportion of the total leukocyte population.

Alicaforsen

ICAM-1 is the ligand for lymphocyte function associated antigen-1 (LFA-1 or $\alpha_L\beta_2$ integrin) and this interaction is important for leukocyte adhesion and recruitment to sites of inflammation. Alicaforsen is a 20-base phosphorothioate antisense oligonucleotide that hybridizes to a 3′ untranslated region of the mRNA encoding for ICAM-1. The heteroduplex formed results in RNase H activation and cleavage of the mRNA preventing ribosomal translation of ICAM-1. Lower ICAM-1 expression reduces lymphocyte migration into areas of inflammation (Figure 25.7). One pilot study reported positive results [112], but subsequent studies failed to report efficacy of systemic treatment. A large study randomized 299 steroid-dependent patients to receive i.v. alicaforsen 2 mg kg^{-1} or placebo three times weekly for 2 or 4 weeks [113]. Efficacy was not demonstrated in this study. There was, however, an association between drug exposure and response and a further randomized controlled study was carried out with increased dosage (300 mg i.v. three times weekly for 4 weeks vs placebo) with the primary endpoint being clinical remission at 12 weeks. This higher dose study did not demonstrate efficacy. Alicaforsen has also been investigated for the treatment of mild to moderate left-sided UC. A randomized dose-escalating study of 40 patients over 4 weeks

demonstrated a reduction in disease severity index compared with placebo [114].

Signal transduction inhibitors

Biologic agents, while effective in many IBD patients, have several potential drawbacks, which are discussed below. Another therapeutic approach is to suppress production of TNFα or to modulate the effects of TNFα and other pro-inflammatory cytokines, at the level of intracellular signaling cascades. Several small molecules have been discovered that have modulating effects on these signal pathways. Such small molecules would theoretically have several advantages over large-molecule protein-based treatments, including oral administration, increased tissue penetration, lack of immunogenicity, reduced immunosuppression and high specificity for defined portions of signaling pathways.

Many of the effects of TNFα and other cytokines are mediated by a cascade of signaling proteins referred to as mitogen-activated protein kinases (MAPK). Three MAPK families are known to be important in mammalian cells: p38 MAP kinase, extracellular signaling-related kinase (ERK) and c-Jun-N-terminal kinase (JNK). The prototypical MAPK pathway involves activation of a MAPK kinase kinase (MAPKKK) upon receptor–ligand interaction. This leads to phosphorylation and sequential activation of downstream MAPK kinase and MAP kinase which activates further protein kinases, nuclear proteins or transcription factors. In normal immune function, these pathways have multiple functions, including regulation of leukocyte recruitment and activation, cell growth, proliferation, differentiation and apoptosis. The role of MAPK in the mediation of inflammatory processes in IBD and their therapeutic inhibition at this time remains uncertain, with opinion and scientific evidence being divided [115,116].

To date, two MAP kinase inhibitors have been investigated in phase II trials of IBD. Both had previously demonstrated the ability to inhibit pro-inflammatory cytokine production and inflammation *in vitro* and in animal models. The first, CNI-1493, is a guanylhydrazone compound known to inhibit the phosphorylation of p38 MAPK and JNK. This compound was administered to 12 IBD patients randomly at a dosage of 8 or 25 mg m^{-2} i.v. once daily for 12 days. This study reported responses of 67 and 58% at weeks 4 and 8, respectively, and remission rates of 25 and 42%, respectively. Endoscopic improvement was noted in five of six patients who had repeat endoscopy. Matched biopsy specimens demonstrated a decrease in JNK expression post-therapy, but no definite observable change in MAP kinase activity. No further clinical studies have been carried out on this agent [117]. The second agent, BIRB 796, was investigated at doses of 10, 20, 30 or 60 mg twice daily versus placebo for 8 weeks in a randomized controlled trial. A total of 284 patients with moderate to severe CD were randomized and at week 8 there was no significant difference between the treated and placebo groups [118]. Further characterization of the role of MAP kinases in IBD-associated inflammation is required, along with further investigation of novel inhibitors.

Limitations of site-specific agents

Adverse affects

Immunogenicity

The use of proteins, in particular monoclonal antibodies, as therapeutic agents raises the possibility of the recipient of the protein mounting an adaptive immune response against antigens expressed on the protein. Immune-type reactions have been extensively described in the literature with regard to monoclonal antibodies, but the precise mechanism of these reactions and the clinical significance of antibodies to monoclonal antibodies with regard to safety and efficacy are uncertain at this time.

The frequency and type of antibodies to monoclonal antibodies are dependent on the structure of the monoclonal antibody and the number and quality of antigenic epitopes presented. Infliximab, a chimeric antibody with murine regions, is theoretically more likely to induce an antibody response than humanized or fully human antibodies due to inter-species variations. However, antibodies may also develop to other portions of the mAb. In the case of infliximab, antibodies may develop to murine epitopes (anti-mouse), the Fv region (anti-idiotypic) or the Fc region (anti-allotypic), cumulatively referred to as antibodies to infliximab (ATI). The estimates of prevalence of ATI in treated patients vary fairly widely, but in clinical studies appears to be of the order of 6–15% [32,33,119]. Factors which decrease the likelihood of development of ATI include concomitant use of immunosuppression [31,33,119], preinfusion use of hydrocortisone [120], use of regular maintenance regimen compared with episodic [32] treatment and use of three-dose induction compared to single-dose induction [31,32]. Although less likely with humanized or fully human mAbs, antibody development has been reported to occur at a rate of up to 12% in adalimumab-treated patients, but has been reported to be as low as 2.6% [49,121] and 8–9% [108,122] for natalizumab.

One important concern with antibodies to monoclonal antibodies is the possibility that they may mediate immune reactions with subsequent infusions. Acute and delayed-type reactions have been extensively described. Acute reactions include rash, fevers, chills, headache and shortness of breath occurring within 24 h after infusion, although most occur within 2 h. It does not appear to be true IgE-mediated anaphylaxis, since markers of mast cell degranulation are not raised and slowing of the infusion rate usually alleviates symptoms. Such reactions

may represent an anaphylactoid-type reaction. Baert *et al.* demonstrated a correlation between ATI titers and infusion reactions [123], although this was not borne out in larger studies [32,34]. Delayed-type reactions present 24 h to 2 weeks after infusion and comprise a serum sickness-like reaction with myalgia, arthralgia, headache, fevers, edema and rash. These reactions may represent a type III immune response due to antigen–antibody complex formation, but do not lower complement levels and are not associated with end organ damage. Apart from the possibility of antibody-mediated immune reactions, antibodies to monoclonal antibodies also appear to increase clearance and decrease clinical responses of the therapeutic antibody.

The development of antinuclear antibodies (ANA) in infliximab-treated patients has also been described [32,34,124]. Subtyping in one study [124] showed that 33% of patients had anti-dsDNA, 40% had anti-ssDNA and 21% had antihistone antibodies and two individuals who were antihistone and anti-dsDNA positive developed a drug-induced type lupus syndrome. ANA and anti-dsDNA may develop in up to 56 and 34% of treated individuals, respectively [32]. Although the reported development of lupus-type syndromes is rare in trials and post-marketing surveillance to date, the rate of conversion to seronegativity is unknown, as is the long-term significance of this phenomenon.

Infections

The purpose of site-specific therapeutics is to control pro-inflammatory processes, but with blockade of components of the inflammatory cascade, serious or unusual infection becomes a significant possibility because of immune suppression. Infection is the most common serious adverse effect related to TNFα inhibitors [29,56]. However, it can be difficult to establish firmly a causal relationship between the anti-TNFα agent and occurrence of infection because of potential confounders, such as concomitant immunosuppression, the use of corticosteroids and the severity of the underlying disease. Large-scale studies and analysis of patient registries in IBD or RA do not demonstrate a definitive association between the use of biologics and the occurrence of serious infectious adverse events or sepsis [32,35,49,121,125]. However, anti-TNFα agents are associated with tuberculosis, as demonstrated in several studies. All biologics approved for use in IBD carry warnings in their prescribing information about the possibility of an increased risk of infection in the course of use. In the course of clinical use, these agents should not be administered in a scenario of clinically significant infection, and in the setting of IBD care must also be exercised when using biologics to consider abscesses and perianal or other intra-abdominal infections and to exclude or treat before initiation.

Tuberculosis

Tuberculosis (TB) is the infection most strongly associated with the use of biologic agents, particularly with TNFα inhibitors. TNFα has a significant role in the response to primary mycobacterial infection and also in the maintenance of the latent state. It is important for recruitment of inflammatory cells to the site of infection and is necessary for establishment and maintenance of granulomas, the main method of immunity being bacteriostatic rather than eradication of the mycobacteria. Animal studies with TNFα deletion demonstrate the necessity of this cytokine for the primary response to mycobacterial infection [126] and blockade with monoclonal antibodies and other inhibitors of TNFα has been shown to cause reactivation of latent disease [127,128]. Impairment of granuloma integrity, with apoptosis of activated T cell as a possible co-factor, leads to dissemination of the bacilli and reactivation of TB. Reactivation of latent TB has been observed in clinical trials of infliximab [35,129], adalimumab [130,131], certolizumab [68] and etanercept. The mode and specific effects of TNF blockade may be a significant determinant of the likelihood of reactivation, since this seems to be higher in monoclonal antibody blockade of TNF (infliximab, adalimumab) than p75 receptor-mediated TNF neutralization (etanercept). The pattern and severity of disease are different in TNFα-treated subjects compared with sporadic TB in the general population and are compatible with patterns of disease seen in situations of significant immunosuppression. Keane *et al.* described high rates of extrapulmonary (56%) and disseminated (24%) TB in cases associated with infliximab, compared with 18 and 2%, respectively, in the normal population [132]. Follow-up data on the use of infliximab in the RA population [133,134] demonstrate an increased risk in the treated population, irrespective of concomitant immunosuppression, but vary in terms of relative risk from 8 to >50. Much of the current data regarding TB reactivation comes from the FDA spontaneous reporting and it is possible that there is under-reporting and under-diagnosis in the treatment population.

Because of this risk, it has been recommended to screen for TB prior to initiation of anti-TNFα agents. It is not known whether IBD is a risk factor for the development of TB infection or if latent TB infection is more common than in the general population. Tuberculin skin testing as a screening method is only useful if positive (>5 mm induration), since the possibility of anergy in IBD renders a negative test of limited utility [135]. Chest radiography should be carried out on all patients. Latent TB should be treated with isoniazid for at least 6 months or isoniazid and rifampicin for 3 months before starting anti-TNFα agents. Consideration should be given to empirical treatment in high-risk groups (people from an endemic region, prisoners and the homeless). Active TB should be aggressively treated with at least three agents and anti-TNFα treatment

deferred, although there is little consensus as to the appropriate interval at which treatment is safe.

Non-TB bacterial infections

Clinical trials of biologic agents to date [32,34,35,48,121] have not, for the most part, demonstrated a significant difference in the occurrence of mild or serious infectious complications due to the active drug. The most common reported infectious complication has been respiratory tract infections. Serious infections that have been reported to occur in biologic-treated IBD patients include pneumonia [48,136], urinary tract infection, cellulitis [136] and staphylococcal sepsis [137] and, rarely, death has occurred [34,137]. Listeriosis has also been reported, but mostly in association with concomitant immunosuppression [138]. Although there is no definite association between biologic immunomodulating therapies and infection, it has been suggested that the concomitant use of immmunosuppressants or corticosteroids may cause a synergistic increase in the risk for opportunistic infections [139].

In the treatment of CD, use of biologic agents for the treatment of fistulizing disease is associated with an increase in the occurrence of abscesses (abdominal, perianal or peristomal). One randomized controlled trial of infliximab demonstrated abscess formation in 12% of treated patients compared with 2.5% in the placebo group [33]. This point highlights the importance of ensuring adequate drainage of fistulae and abscess cavities before starting potent agents that heal inflamed fistula tracts.

Fungal infections

Opportunistic-type fungal infections, both granulomatous [138] and non-granulomatous [140–142], have been reported in association with anti-TNFα agents. Granuloma formation and maintenance of granuloma integrity are an important method of control of fungal infections, particularly histoplasmosis [143]. Invasive opportunistic infections reported in anti-TNFα-treated patients include histoplasmosis, candidiasis, aspergillosis, cryptococcosis, nocardiosis, PCP pneumonia and sporotrichosis, although they are rare. Most reported cases of histoplasmosis and cryptococcosis reported in these studies occurred in endemic areas of the United States.

Viral infections

Viral infection and reactivation, particularly with human herpes viruses including herpes simplex, varicella, cytomegalovirus and herpes zoster, has been associated with anti-TNFα treatment [139,144–146]. Cases of disseminated cytomegalovirus and varicella zoster virus with serious infections, some life threatening, have been reported after anti-TNFα treatment [34,147]. It is not known if antimicrobial prophylaxis or vaccination is of benefit in prevention of viral reactivation in the absence of other causes of immunosuppression. Varicella and cytomegalovirus infections have also been described after use of natalizumab [107].

Most notable, in the case of natalizumab, is the possibility of JC virus reactivation leading to PML. Three patients treated with natalizumab (one for CD in the course of ENACT-1 and -2, two for multiple sclerosis) were retrospectively found to have developed JC virus-induced PML in 2005 [148–150]. PML usually occurs in the setting of significant immunosuppression and is most commonly described in AIDS. All patients had been co-treated with other immunomodulatory agents (azathioprine, infliximab or IFN-β). The drug was voluntarily withdrawn pending further investigation. It was estimated that the risk for natalizumab-associated PML is 1 case per 1000 treated patients (95% CI 0.2–2.8/1000). Natalizumab has been reintroduced as a monotherapy for relapsing multiple sclerosis under strict conditions, including enrolment in a registry.

Congestive cardiac failure

TNFα levels are increased in individuals with heart failure [151], and this cytokine is directly produced by the failing heart. In view of these findings, a small study investigated whether etanercept could be of benefit in congestive cardiac failure [152] and results were positive in a 3 month trial. Following this, a larger study using infliximab was carried out which demonstrated no improvement in individuals treated at $5\,mg\,kg^{-1}$ and increased admission and all-cause mortality in those treated at $10\,mg\,kg^{-1}$. Follow-up has yielded reports of new cases of heart failure and exacerbation of pre-existing disease in the presence of anti-TNFα therapy. To August 2002, 47 cases had been reported to the FDA Medwatch system, of which 9 were pre-existing disease [153]. Of the 38 new cases, 19 had no identifiable risk factors and 10 patients were aged <50 years. On cessation of infliximab, 3 of these 10 had complete resolution, 6 improved and 1 died. Most of the long-term data with regard to congestive cardiac failure are in the setting of RA in whom there is a pre-existing increased risk of heart disease. Its relevance to IBD is uncertain.

At this time, both infliximab and adalimumab carry warnings regarding their use in patients with heart failure of any cause and infliximab $10\,mg\,kg^{-1}$ is contraindicated in the setting of congestive cardiac failure. Lower doses should be used with caution.

Malignancy

TNFα has been described to have various antitumor effects, including direct tumor cytotoxicity by induction of apoptosis, intratumoral vascular effects leading to ischaemia and tumor necrosis and by modulating antitumor immunosurveillance [154]. However, because of its potent pro-inflammatory effects, TNFα conversely may increase the risk of neoplastic transformation in the chronically inflamed intestine in IBD patients. Therefore, the

net effect of blockade of TNFα on the likelihood of developing *de novo* malignancies and of recurrence in individuals with a previous history of cancer is difficult to predict. IBD patients are at increased risk of malignancy compared with the background population as a result of chronic inflammation in the colon. Furthermore, the use of immunomodulatory agents [155] appears to increase the risk of lymphoma. Site-specific biologic agents are a relatively new mode of treatment and adequate time has not yet passed to assess conclusively their effect on the long-term risk of malignancy.

The manufacturers of infliximab and adalimumab state that there is a possible five-fold increased risk of lymphoma in treated patients [29,56], although this does include arthritis patients, who have an increased lymphoma risk at baseline. A study using a statistical model of infliximab compared with standard treatment with 100,000 patients per group suggests an increased incidence of lymphoma in the infliximab-treated group of 210/100,000 [156], although this an extrapolation of data accumulated to date. Of note, hepatosplenic T cell lymphoma has been reported in eight patients receiving infliximab. This is a rare non-Hodgkin's lymphoma most often seen in immunocompromised patients. All reported cases had been on azathioprine or 6-MP for prolonged periods, which makes it impossible to assess any role of infliximab in the causation of these malignancies.

Cumulative evidence from controlled clinical trials of biologic agents in IBD do not demonstrate a causal link between their use and the development of lymphoma or other malignancies [32,34,36]. Post-marketing surveillance with increasing numbers of patient years is more likely to demonstrate conclusively causality or lack thereof. The most comprehensive prospective long-term data on biologic therapy at this time are in the TREAT registry, which has been specifically established to assess long-term safety of infliximab. Published data from the TREAT registry do not demonstrate an increased risk of lymphoma or malignancy in infliximab-treated patients [157]. Clinical studies of natalizumab, adalimumab and certolizumab pegol in CD have not demonstrated increased risk of malignancy.

Given the uncertain role of biologic agents in the pathogenesis and progression of malignancy, caution should be exercised in their use in patients with a previous history of cancer and cessation should be strongly considered after diagnosis of a new malignancy during the course of treatment.

Neurologic

CSF and serum levels of TNFα are elevated in multiple sclerosis and the degree of elevation correlates with disease activity and progression [158]. Following this observation and the apparent protective effect in an animal model, a randomized controlled trial of lenercept [a recombinant TNF receptor p55 immunoglobulin fusion protein (sTNF–IgG p55)] was carried out in multiple sclerosis patients. Lenercept-treated patients had an increased incidence of early exacerbations. An open-label study of infliximab in two patients with rapidly progressive multiple sclerosis demonstrated increased CSF leukocytes and IgG and new lesions consistent with increased disease activity post-infusion.

The risk for demyelinating disease in IBD is increased approximately two-fold compared with the general population [159]. Definitive establishment of causality between anti-TNFα treatment and demyelination is therefore difficult to establish. Various manifestations of new-onset demyelination have been described in association with anti-TNFα treatment in IBD and RA including multiple sclerosis [160–162], optic neuritis [163–165], encephalitis, myelitis, Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy, neuropathy, transverse myelitis, seizures and leukoencephalopathy. These have been more commonly described with etanercept but have also been described with adalimumab and infliximab [166]. In most instances, the neurological symptoms resolve with cessation of treatments. All anti-TNFα agents carry warnings regarding neurologic events. Anti-TNFα agents should not be used in individuals with pre-existing diagnosis of demyelinating disorders and therapy should be discontinued immediately upon a new diagnosis of CNS demyelination.

Gastrointestinal and hepatobiliary

Gastrointestinal complications described with infliximab and adalimumab include perforation of duodenal ulcer, pancreatitis and intestinal obstruction [31,33]. Definitive causality has not been established, although known stricturing disease may be a relative contraindication to the use of anti-TNFα agents. Prescribing information for infliximab refers to severe hepatic reactions including acute liver failure, jaundice, hepatitis and cholestasis, with progression requiring transplantation in some cases. Discontinuation of infliximab is advised in the presence of jaundice or elevated serum transaminase activity to $\geq$5 times normal [29]. Reports have also described autoimmune hepatitis [167,168] and reactivation of latent hepatitis B [169,170]. In one prospective study reactivation of known hepatitis B virus occurred in two patients, one of whom died. Another patient on long-term lamivudine had no change in biochemical or clinical status during or after treatment [170]. Reactivation of HBV has been successfully treated with antivirals [171]. Treatment with anti-TNFα agents in chronic HBV carriers necessitates close monitoring before, during and after therapy.

Cost

Site-specific biologic agents have a high per-unit acquisition cost compared with other medical therapies for

treatment of IBD. Induction and maintenance treatment for IBD with infliximab requires nine infusions over a period of 1 year and for adalimumab 26 doses per year. Site-specific biologic agents require parenteral administration. It is not yet clear if i.v. administration will result in higher total treatment costs than s.c. administration.

Patients with IBD are diagnosed early in life (usually in the second or third decade) and have a near-normal life expectancy, but with substantial morbidity. From an economic point of view, this translates into long-term dependence on healthcare services, including medications, emergency and outpatient assessment, radiology, endoscopy, inpatient treatment and surgery, all of which have associated costs. At this time, the burden of economic cost to society occurring as a result of IBD is unclear. Inpatient treatment or surgery comprise the minority of IBD patients in any population, but comprise the majority of IBD-associated costs. Whether the use of biologic agents reduces costs related to IBD overall is controversial, but they do appear to reduce attendance at and use of healthcare facilities. One study compared attendances for 3 years before and 3 years after infliximab infusion and noted reduced outpatient attendances, endoscopy and radiology usage [172]. Reductions in hospitalization and surgery have been demonstrated also in the ACCENT-1 and -2 trials of infliximab, although the economic implications were not directly assessed. Longer term studies, with more precise indicators of disease assessment and economic impact of disease, will be required to determine economic benefits, or lack thereof, of biologics.

Conclusion

The advent of site-specific therapies has improved the outlook for IBD patients in addition to contributing to an increased understanding of the underlying disease processes. Although TNFα is the target which has yielded most efficacious agents to date, other drugs directed at different sites have begun to yield dividends and larger studies and clinical trials in these areas are in progress. We may soon be provided with the opportunity to use multiple agents that target different specific sites in the pathogenic cascade to treat IBD. Knowledge of which agent to use in which patients will emerge as a critical question in the near future.

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Chapter 26
Therapeutic Manipulation of the Microbiota in Inflammatory Bowel Disease: Antibiotics and Probiotics

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Summary

- The microbiota represents an essential component of the pathogenesis of IBD.
- Some components of the microbiota are required for mucosal homeostasis and protection against injury; whereas others are a potential risk factor for IBD, depending on the genetic status of the host. This provides the rationale for therapeutic manipulation or optimization of the microbiota.
- Antibiotics directed non-specifically against the microbiota may have a role in colonic rather than small bowel Crohn's disease. Their apparent efficacy may relate to increased mucosal numbers of bacteria.
- Trials of antibiotics directed at *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's disease have been limited, but negative or inconclusive, to date.
- There is strong anecdotal clinical support for antibiotics such as metronidazole and ciprofloxacin in pouchitis, but little evidence for these in uncomplicated ulcerative colitis.
- Despite the strong rationale for probiotics, they have been remarkably disappointing in clinical trials in Crohn's disease but more encouraging in ulcerative colitis. The best results have been reported in pouchitis but a favorable experience is not uniform.

Introduction

The concept of therapeutic manipulation of the gut microbiota with antibiotics or probiotics in inflammatory bowel disease (IBD) arises in the context of several potential contributions of microbes to the pathogenesis, clinical course or complications of these diseases [1–3]. The theoretical and practical possibilities for microbial involvement in IBD include (a) a classical or non-classical infection; (b) a "hit and run" triggering event; (c) co-incidental *Clostridium difficile* infection, an increasing problem in both Crohn's disease and ulcerative colitis [4]; (d) a complication of penetrating disease; and (e) direct microbial participation in the immunopathogenesis of disease.

While an infectious cause waiting to be discovered cannot be discounted, and could be accommodated by current concepts of the heterogeneity of IBD, there is a large body of evidence implicating a permissive or active role for the commensal microbiota in the pathogenesis of both Crohn's disease and ulcerative colitis, which can be reconciled with the changing epidemiology of these disorders [5]. Whereas some components of the microbiota have a pivotal role in the maintenance of mucosal homeostasis and protection against disease [6,7], and are a source of immunoregulatory signals for mucosal and systemic immune development [8–10], others may become engaged with the immune system in the pathogenesis of IBD, depending on the genetic susceptibility of the host [2,3]. Thus, the intestinal microbiota represents both a health asset and a disease liability; therein lies the rationale for therapeutic manipulation of the microbiota to enhance microbial assets and offset liabilities.

The microbiota in inflammatory bowel disease

The gut microbiota is one of a triad of interacting elements contributing to the pathogenesis of IBD, along with host genetic susceptibility factors and immune-mediated tissue damage. The identification of specific susceptibility genes for Crohn's disease and ulcerative colitis has actually highlighted to the role of the microbiota because such genes code for functional proteins at the host–microbe interface.

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The latter include sensors of the microbial environment, processing of intracellular bacteria by autophagy and regulators of host innate and acquired immunity to the microbial environment [11,12]. Disease heterogeneity arises because of the multiplicity of susceptibility and modifying genes involved and the diversity of lifestyle or environmental exposure, including variable composition of commensal microbiota.

There is no conclusive evidence for any single microbial cause or influence in either Crohn's disease or ulcerative colitis. Furthermore, epidemiologic data are at variance with a transmissible agent [5]. Some of the more consistently observed microbial alterations linked with IBD include increased total bacterial numbers within the mucosa, particularly in Crohn's disease [2]; reduced counts of fecal lactobacilli and bifidobacteria [13]; increased detection of adherent-invasive *Escherichia coli* (AIEC) in Crohn's disease [14]; increased detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) [15]; and reduced bacterial diversity by metagnomic analysis [16], including reductions in the anti-inflammatory commensal *Faecalibacterium prausnitzii* [17].

Although environmental and lifestyle factors are probably the most important modifying influences on the composition and metabolic behavior of the intestinal microbiota [5], host genetics and the immune status of the host may also alter the bacterial composition in the gut. Specific defects in mucosal immunity in different species have been linked with aberrant expansion of some, but not all, commensal organisms [18,19]. In addition, a transcription factor (T-bet) which regulates immune development and function has been reported to modify commensal bacterial populations within the murine intestine. Furthermore, deletion of T-bet appeared to lead to the emergence of a "colitogenic" microbiota with the capacity to transfer colitis [20].

In summary, there is increasing evidence for an alteration in the numbers and diversity of the microbiota in patients with IBD, and host–microbe dialogue is reciprocal. The challenge is to devise methods for optimal manipulation of the microbiota.

Antibiotic therapy of inflammatory bowel disease

There is widespread acceptance of the use of antibiotics for the treatment of IBD-related complications such as intra-abdominal abscesses, fistulae, postoperative complications and fulminant colitis. The role of antibiotics as a primary treatment for either Crohn's disease or ulcerative colitis is less certain and, in many instances, controversial. Apart from the lack of convincing evidence, there is a concern about the risk of emerging antibiotic resistance and rising prevalence of *C. difficile*-associated disease [4].

Antibiotics in Crohn's disease

Antibiotic therapy directed at a specific pathogen, as a putative cause of Crohn's disease, has been primarily antimycobacterial, against MAP. To date, such trials have been inconclusive or negative [21,22].

A diversity of antibiotic regimens directed in a nonspecific manner against the microbiota for both the induction and maintenance of remission of Crohn's disease has been studied, and is represented in Table 26.1. These studies have frequently been sub-optimal and open-label and involved a relatively small number of patients. The antibiotics most commonly used have been metronidazole and ciprofloxacin, but more recently the non-absorbable antibiotic rifamixin, which is active against anaerobic bacteria and *E. coli*, has been studied [23]. Metronidazole is a nitroimidazole antibiotic with activity against anaerobic bacteria and protozoa. It is widely used in the treatment of perianal disease and its usage in Crohn's disease was reported as early as 1975 [24]. The dosage used in studies varied from 10 to 20 mg kg^{-1}. Its side effect profile, particularly at higher dosage, includes nausea, metallic taste, gastrointestinal intolerance and peripheral neuropathy after long-term administration. Studies looking at the induction of remission demonstrate efficacy especially in colonic disease but not small bowel disease [25–27]. In terms of maintenance of remission, Rutgeerts *et al.* conducted a 3 month double-blind placebo-controlled trial comparing metronidazole 20 mg kg^{-1} with placebo in patients who had undergone terminal ileal resection [28]. Metronidazole produced a non-significant reduction in endoscopic recurrence at 3 months ($p = 0.09$) and found a reduction which was significant at 12 months ($p = 0.02$). There was also a statistically significant reduction in clinical recurrence in the antibiotic arm at year one which was non-significant at 2 and 3 years. Despite the demonstrable efficacy, there were more side effects in the antibiotic arm. Subsequently, the same group looked at ornidazole in postoperative maintenance [29]. Ornidazole is a 5-nitroimidazole derivative which undergoes rapid absorption and is thought to have fewer side effects than metronidazole. Eighty patients were randomized to either ornidazole 1 g day^{-1} or placebo within 1 week of surgery and this was continued for 1 year. Ornidazole was associated with a significant reduction in clinical and endoscopic recurrence rates compared with placebo, but there were significantly more side effects in the antibiotic arm. In summary, it appears that antibiotics have a limited role in colonic Crohn's disease but the benefit may diminish with time.

Antibiotics in ulcerative colitis

Results of trials of antibiotics in ulcerative colitis have been even less impressive than those in Crohn's disease. A summary of clinical trials is shown in Table 26.2. The majority of the studies to date looked at antibiotics in the

Table 26.1 Summary of clinical trials of antibiotic therapy in Crohn's disease.

Study design (duration)*	Antibiotic (dose)/concomitant therapy*	Trial outcome	Ref.
Induction of remission			
Open-label controlled trial (2 months)	Metronidazole (1 g) with salazopyrin or prednisolone. $N = 20$	No improvement in symptoms except for colonic disease	Blichfeldt *et al.*, 1978 [30]
Randomized crossover trial (4 months)	Metronidazole (800 mg) with sulfasalazine. $N = 78$	Significant decrease in CDAI in patients crossed over from sulfasalazine to metronidazole	Ursing *et al.*, 1982 [27]
RCT (16 weeks)	Metronidazole (20, 10 mg kg^{-1}) or placebo. $N = 56$	Reduction in CDAI at higher dose but no difference in remission rates	Sutherland L *et al.*, 1991 [26]
Open-label study (10 weeks)	Metronidazole (250 mg t.d.s.) and ciprofloxacin (500 mg b.d.). $N = 72$	68% clinical remission as per Harvey Bradshaw index and 76% clinical response	Greenbloom *et al.*, 1998 [31]
RCT (6 weeks)	Ciprofloxacin 1 g day^{-1} versus mesalazine 4 g day^{-1}. $N = 40$	No difference in remission rates in two groups (56 vs 55%)	Colombel *et al.*, 1999 [32]
Open-label study (4 weeks)	Clarithromycin 250 mg b.d. with 5-ASA or steroid. $N = 25$	Significant drop in Harvey Bradshaw index	Leiper *et al.*, 2000 [33]
Randomized placebo-controlled trial (8 weeks)	Metronidazole 1 g day^{-1} and ciprofloxacin 1 g day^{-1} or placebo with budesonide 9 mg day^{-1}. $N = 130$	No significant difference in remission rates but increased remission rate in disease confined to the colon	Steinhart *et al.*, 2002 [25]
Multicenter double-blind RCT (12 weeks)	Rifamixin 800 mg b.d. versus 800 mg day^{1} versus placebo. $N = 83$	Superior remission rates in rifamixin 800 mg b.d. group but not significant	Prantera *et al.*, 2006 [34]
Maintenance of remission			
RCT placebo controlled in prevention of postoperative recurrence (3 months)	Metronidazole 20 mg kg^{1} versus placebo. $N = 60$	Reduced early recurrence in the neoterminal ileum at 3 months and significantly reduced clinical recurrence at 12months ($p = 0.046$)	Rutgeerts *et al.*, 1995 [28]
RCT in postoperative recurrence (1 year)	Ornidazole 1 g day^{1} versus placebo post-ileocolonic resection. $N = 80$	Significant reduction in clinical recurrence ($p = 0.0046$) and endoscopic recurrence in ornidazole group ($p = 0.037$) at 1 yr.	Rutgeerts *et al.*, 2005 [29]

*RCT, randomized control trial; N = number of patients.

induction of remission in moderate to severe colitis as an adjunct to conventional medical therapy. Of all the studies summarized in Table 26.2, only tobramycin and ciprofloxacin orally showed any significant benefit. The study by Burke *et al.* looked at tobramycin 120 mg orally three times daily for 1 week versus placebo with concomitant steroid therapy in patients with an acute relapse of ulcerative colitis [41]. Tobramycin is an aminoglycoside with activity against Gram-negative organisms. At the study endpoint, 31 of 42 (74%) in the tobramycin group versus 18 of 42 (43%) in the placebo group achieved complete symptomatic remission and there was a significant improvement in the histologic scores. Lobo *et al.* followed the initial responders in the follow-up study for maintenance of remission over 1 and 2 years [42]. There was no significant difference in remission rates between the two groups, suggesting that whatever benefit tobramycin did have in the induction, its effects were short-lived. Turunen *et al.* looked at oral ciprofloxacin for 6 months in both an induction arm and maintenance of remission arm [43]. In the induction arm, there was a reduction in relapse rates in the ciprofloxacin group (21%) compared with controls (44%) which reached statistical significance. In the maintenance of remission arm, 45% of the ciprofloxacin group had relapsed compared with 60% of the placebo group ($p = 0.07$). Therefore, based on the available evidence, there is little evidence for a role for antibiotic therapy in either the induction or maintenance of remission in uncomplicated ulcerative colitis.

Antibiotics in pouchitis

It is estimated that up to 50–60% of ulcerative colitis patients following ileal pouch–anal anastomosis will have an attack of pouchitis and most of these patients will respond

Table 26.2 Summary of clinical trials of antibiotic therapy in ulcerative colitis.

Study design (duration)	Antibiotic (dose)/concomitant therapy	Trial outcome	Ref.
Prospective double-blind placebo-controlled trial (7 days)	Vancomycin 500 mg q.d.s. versus placebo with routine medical therapy. $N = 40$	No significant difference in treatment groups. Trend to a reduction in operative intervention in vancomycin group	Dickinson *et al.*, 1985 [38]
Prospective double-blind placebo-controlled trial (5 days)	Metronidazole 500 mg t.d.s. i.v. versus placebo with intravenous steroids. $N = 39$	No significant difference between treatment groups	Chapman *et al.*, 1986 [39]
Double-blind placebo-controlled trial (1 week)	Tobramycin 120 mg t.d.s. p.o. versus placebo with concomitant steroids. $N = 84$	74% in tobramycin group versus 43% in placebo group achieved symptomatic remission ($p = 0.008$) and better histological scores	Burke *et al.*, 1990 [41]
Double-blind randomized placebo-controlled trial (10 days)	I.v. metronidazole 1.5 g and tobramycin 12 mg kg^{-1} versus placebo. Concomitant steroids and parenteral nutrition. $N = 39$	No significant difference between the groups	Mantzaris *et al.*, 1994 [40]
Double-blind randomized placebo-controlled trial (6 months)	Ciprofloxacin 500–750 mg b.d. versus placebo with conventional medical treatment. $N = 83$	Reduced relapse rates in ciprofloxacin group ($p = 0.02$) at 6 months	Turunen *et al.*, 1998 [37]
Pilot study (10 days)	Rifaximin 200 mg b.d. versus placebo with steroid therapy. $N = 26$	64.3% versus 41.7% in favor of antibiotic group had an improvement in activity scores ($p =$ ns)	Gionchetti *et al.*, 1999 [44]
Prospective RCT (10 days)	Ciprofloxacin 400 mg b.d. i.v. versus placebo along with standard medical therapy. $N = 55$	No significant difference between the two groups ($p > 0.1$)	Mantzaris *et al.*, 2001 [45]

to a course of antibiotics. However, 5–10% will develop chronic pouchitis [46]. The pathogenesis is not certain, but an imbalance in the bacterial flora of the pouch, including reduced numbers of *Bifidobacteria* and *Lactobacilli*, provides the rationale for treatment with antibiotics or probiotics [47,48]. The most frequently used antibiotics are metronidazole and ciprofloxacin. Anecdotal clinical support for these antibiotics in pouchitis is uniform, although only a few randomized controlled trials have been reported. The majority are small, open-label studies as summarized in Table 26.3. The first trial was a small randomized placebo-controlled crossover study by Madden *et al.* in 13 patients with chronic pouchitis, which demonstrated a reduction in frequency of diarrhea compared with placebo, but no difference in terms of endoscopic or histologic score [49]. However, a significant proportion of patients (55%) may experience side effects with metronidazole such as nausea, vomiting, abdominal discomfort, headache, skin rash and metallic taste. In a subsequent comparative study between ciprofloxacin and metronidazole in acute pouchitis, Shen *et al.* demonstrated the superiority of ciprofloxacin in terms of reduction in pouchitis disease activity index (PDAI) scores along with a better side effect profile (33% of the metronidazole group experienced side effects versus none in the ciprofloxacin group) [50]. Gosselink *et al.* demonstrated in another open-label study that ciprofloxacin is preferable not only from a clinical point of view but also from a microbiological point of view [51]. They followed 13 patients with pouchitis and took stool cultures before, during and after a bout of pouchitis. They found a significantly greater reduction in the PDAI score in the ciprofloxacin group compared with the metronidazole group ($p = 0.04$), and that treatment with ciprofloxacin (in contrast to metronidazole) significantly reduced all coliforms, including hemolytic strains of *E. coli*, leaving anaerobes largely undisturbed.

In resistant pouchitis, Gionchetti *et al.* looked at the combination of rifamixin and ciprofloxacin for 15 days in patients who had already failed a 4 week course of standard therapy (metronidazole or ciprofloxacin) [52]. The results confirmed that 16 of the 18 patients (88%) either improved ($n = 10$) or went into remission ($n = 6$).There was also a significant decrease in the PDAI score at the end of the study with no side effects observed. This combination therapy was further validated as a treatment for chronic refractory pouchitis in a smaller study with eight patients by Abdelrazeq *et al.* [53].

In summary, in contrast to ulcerative colitis, and despite the small numbers of patients studied, there appears to be a definite role for antibiotics, particularly ciprofloxacin, in

Table 26.3 Summary of clinical trials of antibiotic therapy in pouchitis.

Study design (duration)	Antibiotic (dose)/number of patients	Trial outcome	Ref.
Double-blind randomized placebo-controlled crossover study in chronic pouchitis (7 days)	Metronidazole 400 mg t.d.s. p.o. N = 13	Significant reduction in bowel motions in antibiotic group ($p < 0.05$). No difference in endoscopy or histology score	Madden *et al.*, 1994 [49]
Open-label study in chronic treatment-resistant pouchitis (15 days)	Rifamixin 2 g day^{-1} plus ciprofloxacin 1 g day^{-1}. N = 18	Significant improvement in PDAI ($p < 0.002$)	Gionchetti *et al.*, 1999 [52]
RCT in acute pouchitis (2 weeks)	Ciprofloxacin 1 g day^{-1} p.o. versus metronidazole 20 mg kg day^{-1}. N = 16	Ciprofloxacin reduced PDAI significantly more then metronidazole ($p = 0.002$)	Shen *et al.*, 2001 [50]
Open-label study in refractory or recurrent pouchitis (4 weeks)	Metronidazole 800 mg–1 g and ciprofloxacin 1 g per day. N = 44	Significant reduction in PDAI ($p < 0.0001$) and improvement in quality of life	Mimura *et al.* 2002 [54]
Open-label study in chronic pouchitis (2 weeks)	Rifaximin 2 g and ciprofloxacin 1 g daily. N = 8	7 of 8 patients improved/remission. Significant reduction in PDAI ($p = 0.018$)	Abdelrazeq *et al.*, 2005 [53]
Open-label study in chronic pouchitis (4 weeks)	Ciprofloxacin 1 g and tinidazole 15 mg kg^{-1} daily. N = 16	Significant improvement in PDAI and quality of life scores ($p < 0.002$)	Shen *et al.*, 2007 [55]

pouchitis, because of its favorable side effect profile. The majority of patients will respond to a course of antibiotics but some with relapsing or chronic pouchitis may need continuous maintenance therapy.

Probiotics in inflammatory bowel disease

The term probiotics is commonly defined as "living microorganisms which upon ingestion in certain numbers, exert health affects beyond inherent basic nutrition" [56].

However, the definition of a probiotic is under continual revision and a less restrictive term such as *pharmabiotic* may be more inclusive. For example, non-bacterial organisms may have similar effects and the requirement that bacteria be alive may be too limited, because recent constituents such as microbial DNA and protein metabolites have anti-inflammatory, immunomodulatory or cytoprotective properties [57,58]. Genetically modified food-grade non-probiotic organisms also offer a powerful prospect for manipulating the intestinal milieu. *Lactococcus lactis* engineered for local secretion of the anti-inflammatory cytokine IL-10 and/or trefoil factor in the gut have been shown to reduce murine colitis [59,60]. Experience with IL-10 delivered by genetically modified organisms in humans is still limited, but encouraging [61].

The mechanisms of action of non-genetically modified probiotics include competitive interaction with pathogens, reduction in bacterial translocation, production of antimicrobial products and anti-inflammatory signaling; these are reviewed in detail elsewhere [58,62,63].

Probiotics in Crohn's disease

The experience with probiotics in Crohn's disease from controlled clinical trials is summarized in Table 26.4 (a miscellany of open-label studies has been excluded because of the heterogeneity of this condition and the inconsistencies in study design). Overall, in marked contrast to encouraging results in murine models, the results from human trials have been consistently disappointing and provide no encouragement for future studies with the same strains and/or dosage.

Probiotics in ulcerative colitis

Experience with probiotics in the treatment of ulcerative colitis is somewhat better than that in Crohn's disease, although the results remain inconclusive (Table 26.5). Kruis *et al.* [73] used the probiotic *E coli* Nissle 1917. Their first study in 1997 showed no difference in relapse rates between probiotic and mesalamine in the maintenance of remission, but the dose of mesalamine used was low, 1.5 g day^{-1} [73]. However, the follow-up study had improved statistical power and reported equivalence to mesalamine in the maintenance of remission [74]. Another study by Rembacken *et al.* involving 116 patients with active disease showed no statistical difference from mesalamine in the induction of remission, but showed a high relapse rate in both groups, 73% in the mesalamine group versus 67% in the probiotic group [75]. Additional small studies have claimed beneficial effects in ulcerative colitis for probiotics (Table 26.5) [76,77], but the largest using two different probiotic strains over a 52 week period found no difference for either probiotic compared with

Table 26.4 Controlled human trials of probiotics in Crohn's disease (CD).

Study type	Organism used	Trial outcome	Ref.
RCT in CD	*Saccharomyces boulardii*. $N = 20$	Decrease in diarrhea and CDAI in probiotic group	Plein and Hotz, 1993 [64]
RCT in colonic CD	*E. coli N*issle 1917. $N = 28$	Relapse rates 30% in probiotic group versus 70% in placebo (p = ns)	Malchow,1997 [65]
RCT in maintenance of remission CD	*Saccharomyces boulardii* and mesalazine vs mesalazine alone. $N = 32$	Higher remission in the probiotic plus mesalazine group	Guslandi *et al.*, 2000 [66]
RCT in maintenance of surgically induced remission CD	VSL#3 with rifamixin versus mesalamine. $N = 40$	Endoscopic remission of 80% in probiotic group versus 60% in mesalazine group.	Campieri *et al.*, 2000 [67]
RCT in maintenance of surgically induced remission	*Lactobacillus rhamnosus* GG. $N = 45$	No difference between two groups at 1 year.	Prantrera *et al.*, 2002 [68]
RCT in induction and maintenance of remission	*Lactobacillus* GG. $N = 11$	No benefit in induction or maintenance of remission	Schultz *et al.*, 2004 [69]
RCT in maintenance of remission.	*Lactobacillus* GG. $N = 75$	No benefit over placebo over 24 months	Bousvaros *et al.* 2005 [70]
RCT in maintenance of remission	*L. johnsonii*. $N = 98$	No benefit over placebo over 6 months	Marteau *et al.* 2006 [71]
RCT in maintenance of remission	*L. johnsonii*. $N = 98$	No benefit over placebo after 3 months	Van Gossum *et al.* 2007 [72]

placebo [78]. Results using a cocktail of probiotics (VSL#3) in open-label trials and those with prebiotics alone or in combination with probiotics have been reviewed elsewhere [79].

Probiotics in pouchitis

Human studies of probiotics in pouchitis are summarized in Table 26.6 [80–87]. There have been two trials looking at VSL#3 in the maintenance of remission involving a total of 76 patients. In the first trial, Gionchetti *et al.* looked at maintenance of an antibiotic-induced remission [81]. After 9 months, 85% of the probiotic arm remained in remission, whereas the entire placebo group relapsed. They also showed that all those in remission following the probiotic relapsed on completion of the trial. Mimura *et al.* confirmed the efficacy of the probiotic product in their study over 1 year, with 85% in remission in the probiotic arm and 6% in the placebo arm [82]. Patients on the probiotic also reported a higher quality of life as measured by the inflammatory bowel disease questionnaire (IBDQ). In a follow-up trial by Gionchetti *et al.* , using VSL#3 for postoperative prevention of pouchitis, patients were randomized to placebo or VSL#3 as prophylaxis after surgical creation of the pouch [83]. At 1 year follow-up, 10% of the probiotic-treated patients had pouchitis, compared with 40% of the placebo group. However, more

Table 26.5 Controlled clinical trials of oral probiotics in ulcerative colitis (UC).

Study type	Organism used	Trial outcome	Ref.
RCT in maintenance of remission UC	*E. coli* Nissle 1917. $N = 120$	No difference from mesalazine	Kruis *et al.*, 1997 [73]
RCT in maintenance of remission UC	*E. coli* Nissle 1917. $N = 327$	Equivalent to mesalamine	Kruis *et al.*, 2004 [74]
RCT active UC	*E. coli* Nissle 1917. $N = 116$	Equivalent to mesalazine	Rembacken *et al.*, 1999 [75]
RCT in maintenance of remission	*Bifidobacterium bifidum*. $N = 21$	27% relapse rate in treatment arm vs 90% in control arm	Ishikawa *et al.*, 2003 [76]
RCT in maintenance of remission	*Bifidobacteria*, *Lactobacilli*, *Enterococci* combination. $N = 30$	20% relapse in treatment arm vs 93% in placebo group ($p < 0.01$)	Cui *et al.*, 2004 [77]
RCT in maintenance of remission	*B. infantis* vs *L. salivarius* vs placebo. $N = 157$	No difference between either probiotic vs placebo over 52 weeks	Shanahan *et al.*, 2006 [78]

Table 26.6 Clinical trials of probiotics in pouchitis.

Study type	Organism used	Trial outcome	Ref.
Open-label trial	*Lactobacillus* GG and prebiotic with antibiotic. $N = 10$	Effective in inducing remission	Friedman and George, 2000 [80]
RCT to maintain remission after antibiotic-induced remission	VSL#3. $N = 40$	15% relapse in probiotic arm vs 100% in placebo arm	Gionchetti *et al.*, 2000 [81]
RCT to maintain remission in relapsing/recurrent pouchitis after antibiotic-induced remission	VSL#3. $N = 36$	Remission in 85% probiotic group vs 6% placebo ($p < 0.0001$)	Mimura *et al.*, 2004 [82]
RCT in postoperative prevention	VSL#3. $N = 40$	Acute pouchitis in 10% probiotic arm vs. 40% placebo group ($p < 0.01$)	Gionchetti *et al.*, 2003 [83]
Open label study in antibiotic-dependent pouchitis	VSL#3. $N = 31$	After 8 months only 6 remained on probiotic. No difference in PDAI	Shen *at al.*, 2005 [84]
RCT in acutely active pouchitis	*Lactobacillus* GG. $N = 20$	No benefit in clinical or endoscopic response	Kuisma *et al.*, 2003 [85]
Open-label study in prevention of acute pouchitis	*Lactobacillus acidophilus* and *Bifidobacterium lactis*. $N = 51$	Improvement in PDAI, but not histology	Laake *et al.*, 2005 [86]
Case–control study in postoperative prevention	*Lactobacillus* GG. $N = 117$	Decreased risk of pouchitis. Cumulative risk at 3 years: 7% vs. 29% ($p = 0.011$)	Gosselink *et al.*, 2004 [87]

recently, Shen *et al.* published an open-label study in 31 patients with antibiotic-dependent pouchitis using VSL#3 [84]. They found that only six patients remained on the probiotic for the 8 month duration of follow-up, and there was no difference in the PDAI from baseline in that group. There were some inherent problems with this study in that patients had to bear the cost of the medication which was self-administered with no measure of adherence.

Studies of other probiotic preparations have been less than encouraging than those with VSL#3. Kuisma *et al*. reported that although *L. rhamnosus* GG changed the pouch microflora, it had no significant effect on clinical or endoscopic response [85]. Similarly, Laake *et al.* used a fermented drink containing *Lactobacillus acidophilus* and *Bifidobacteria lactis* which was associated with an improvement in the PDAI score but not in histology [86].The only positive study with a *Lactobacillus* probiotic has been from Gosselink *et al.*, who found a decreased cumulative risk of pouchitis in 117 patients taking *L. rhamnosus* GG [87].

Conclusion

Future studies of probiotics will require improved clinical trial design with adequate study power. It is self-evident that probiotic products should fulfill all regulatory requirements. In all instances, probiotics should be derived from reputable suppliers and optimal storage should be adhered to. This is critical because of the varied results, often with the same probiotic, in different study populations. An optimal dosage and delivery vehicle needs definition. In the case of combinations or cocktails of probiotics, the primary active should be identified and the absence of bacterial antagonism should be confirmed. In addition, transit of the putative probiotic strain and survival through the alimentary tract should be established. Disappointing results in human IBD, in contrast to those in murine models, might be due to the relatively late age at which probiotics have been administered in humans. It may be that the prospects for therapeutic manipulation of the microbiota are greatest in the earliest stages of postnatal life, prior to full development of the gut and mucosal immune system.

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Chapter 27
The Role of Nutrition in the Evaluation and Treatment of Inflammatory Bowel Disease

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Summary
- IBD prevalence is associated with a "western" diet that is high in fat, meat and refined carbohydrate and low in fruit and vegetables.
- Iron deficiency is common and iron therapy should be given to all those with iron deficiency. If oral therapy is not tolerated, parenteral iron should be given.
- Vitamin B_{12} deficiency is common after ileal resection. Parenteral vitamin B_{12} should be given if >20 cm resected and monitored yearly if <20 cm resected.
- There is no diet known at present which maintains remission (excluding, arguably, total enteral or parenteral nutrition).
- Enteral nutrition is an effective short-term therapy for inducing remission in Crohn's disease, particularly in children, malnourished adults and preoperative patients. It may be less effective than corticosteroids in inducing remission but has fewer side effects and achieves mucosal healing and better growth. Low fat and very low long-chain triglyceride enteral feeds probably have the greatest efficacy.

Dietary factors in inflammatory bowel disease causation

Patients with inflammatory bowel disease (IBD) commonly ask whether a "special diet" will influence their symptoms and the course of their IBD. Unfortunately, there is little hard evidence on which to base the advice.

Epidemiology of diet in IBD

The epidemiological studies of diet in IBD are of variable quality. They have usually been underpowered and interpretation is difficult because of poor recall of pre-illness diet and variation in control groups, particularly in socioeconomic and ethnic matching. It is also difficult to pinpoint the onset of Crohn's disease (CD) and symptoms often precede diagnosis by several years, so that the definition of "pre-illness" is uncertain. This has been characterized as "a lot of data but little knowledge" [1]. Cautious interpretation of these data leads to the conclusion that IBD is more common in urban areas, the incidence rises with industrialization and is more common in "dairy-based" than "soya-based" communities. However, because there has been an enormous change in diet in a short time in the developed world, not only in food types but also in agricultural methods, food additives and processing, it is very difficult to elucidate which factors, if any, are responsible.

Geographical variations

IBD, particularly CD, is rare in countries that have not yet "westernized". One of the more intriguing observations is from Japan, where CD has increased dramatically over the recent decades. The incidence of CD in Japan correlates positively with the marked increase in average consumption of total fat, animal fat and *n*-6 polyunsaturated fatty acids (for all these, $r > 0.8$). Animal fat was the strongest independent factor on multivariate analysis [2]. However, it is clearly possible that the change in diet is only a correlate for another aspect of "westernization" that triggers IBD.

Sucrose and refined sugar

There is a remarkably consistent association between high pre-illness intake of refined sugar and risk for CD [3–8] and a dose–response relationship has been reported [9]. However, altering sugar consumption has no effect on CD [5,10,11] and CD is rare in some societies with high refined carbohydrate consumption, e.g. the Middle East. It is likely that the increased refined carbohydrate consumption is

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partly related to dietary changes in response to symptoms rather than a primary phenomenon. The association may also in part relate to the higher refined carbohydrate consumption in smokers [9].

Fast food and soft drinks

There is a significant positive association between IBD and consumption of fast foods and soft drinks [8,12,13]. However, this may again reflect some other coincidental change in diet or some other factor related to "westernization". Although politically tempting, it is not possible, on present evidence, to blame IBD on the fast food industry.

Fruit and vegetables

CD is associated with a reduced pre-illness consumption of fruit and vegetables [6,14]. This may be because high-fiber foods increase symptoms, particularly in stricturing CD, and are therefore avoided even before clinical presentation, rather than because they have a pathogenic effect.

Milk and dairy products

The hypothesis that IBD may be related to dairy products has been considered for many years. Data are very limited and largely historic. Glassman *et al.* reported a significantly higher rate of symptoms of childhood cow's milk allergy in ulcerative colitis (UC) compared with controls [15]. However, increased pasteurized milk intake (but not other dairy products) is associated with a reduction in risk of CD [odds ratio (OR) 0.82, 95% confidence interval (CI) 0.69–0.97] in some [14] but not in all studies [16].

Meat

Both CD [14] and UC [17] are associated with increased meat intake. UC relapses are associated with an increased intake of red and processed meat (OR 5.19, 95% CI 2.1–12.9) [17]. One mechanism whereby this could occur is through the sulfur and sulfate content of meat, increasing fecal sulfide, which is then metabolized by bacteria metabolism to produce mercaptides such as sodium hydrogensulfide. These substances are highly toxic and are usually detoxified by the colonic mucosa. UC patients have higher levels of fecal sulfide and this correlates with disease activity. It is possible that an increase in sulfide (due either to changes in bacteria increasing sulfide production or to defective detoxification in UC) either has a direct toxic effect in UC or possibly causes other effects via formation of thiols and inhibition of the anti-inflammatory effect of butyrate [18].

Fat

UC patients tend to have higher intakes of monounsaturated fat (OR 33.9) and polyunsaturated fat (OR 5.1) (adjusted for energy intake) [19]. This has been supported by most but not all studies. The increase in fat intake has been shown for all types of fat (animal and vegetable) [2,6] and for both UC and CD but with a stronger link to UC. The association between rising incidence of CD and rising fat intake in Japan has already been mentioned [2]. Margarine, which contains hydrogenated fats, has been positively correlated with UC and CD in most, but not all, studies [20]. Again, this may merely reflect association with other environmental changes coincident with "westernization".

Dietary factors and risk for IBD-related colon cancer

There are few data on the dietary risk factors associated with the development of IBD-associated neoplasia. Many of the dietary habits associate with increased risk for IBD are also associated with increased risk of colon cancer – high fat (particularly animal fat), low calcium and low fruit and vegetable intake. One study examined aneuploidy and diet in UC patients in remission and showed a strong positive correlation with total fat intake and a negative correlation with fruit and vegetable intake [21]. There is also some evidence for a protective effect of folate supplementation [22].

Intervention studies

Crohn's disease

Enteral nutrition is used by clinicians, particularly pediatricians, as sole treatment in some patients with active CD. Although it can be argued that there are simpler alternative therapies there is no doubt that enteral feeding can be very effective. It remains largely a mystery how this efficacy is achieved.

Several broad hypotheses remain plausible:

- Enteral (or intravenous) feeding and avoidance of normal food works by avoidance of something harmful in the normal (western) diet.
- Enteral feeding works by adding in a nutritional component that is beneficial.
- Enteral feeding works because it has beneficial effects on the intestinal microbiota.

We will consider the evidence for each of these in turn

Avoidance of harmful dietary components

Fiber

First we present an anecdote to illustrate the difficulty in designing intervention studies to investigate dietary hypotheses in CD. We conducted an extensive study of dietary reintroduction in a patient with multiple small intestinal stricturing CD who had been brought into remission with enteral feeding [23]. A variety of food components were introduced with no ill effects until vegetable fiber was introduced. This caused a prompt relapse accompanied by a sharp rise in serum C reactive protein. This relapse we presume was due to vegetable fiber obstructing behind a stricture followed by secondary

bacterial overgrowth. Remission was again obtained with enteral feeding but two attempts to reintroduce the low-fiber diet that the patient had been receiving with no problems earlier both failed. We suspect (but do not have direct evidence) that mucosal ulceration had occurred as a result of the initial relapse and that this rendered the patient intolerant of foods that he had no problem with when the mucosa was healed. If correct, this would imply that patients with active small intestinal ulceration might have a wide range of food intolerances much in the way that one might imagine rubbing food into an open wound would provoke inflammation. It therefore needs to be borne in mind that a dietary intervention might maintain remission in a patient with established mucosal healing yet fail to induce or even maintain remission in a patient who has already developed mucosal ulceration. This, of course, makes controlled dietary intervention studies very difficult to design and interpret.

Based on the epidemiological evidence that patients with CD have a low pre-illness intake of fiber, a controlled trial was performed in which one group was randomized to receive a high fiber, low refined sugar intake and a control group continued on a normal "healthy" diet [5]. The trial had to be stopped early because of a high withdrawal rate in the high fiber group and, although the results were inconclusive, the implication was that a high fiber (and low sugar) intake was unhelpful. No attempt was made to distinguish vegetable from cereal fiber. A subsequent controlled trial of low fiber intake also showed no benefit [24]. Thus, although it is common for patients to notice problems with fruit and vegetable fiber and some patients with tight strictures may have occasional partial obstructive episodes, there is no convincing evidence that overall intake of cereal or vegetable fiber affects mucosal inflammation.

Fat

Epidemiological studies (see above) have shown statistical correlations within communities and over time between increased total fat intake and increased risk for CD. An intriguing animal model has been described in which a segment of pig ileum is reversed to create an antiperistaltic segment. Pigs with the reversed segment develop ileal inflammation when fed a high fat diet but not when fed a lower fat intake [25]. It was suggested that the fat might itself be harmful to the intestinal epithelium. An alternative mechanism might be lacteal "overload" possibly compounding the lacteal obstruction that typifies CD [26].

Intervention studies testing the fat hypothesis have been contradictory. Our own collaborative study showed no difference in efficacy between two otherwise comparable enteral feeds, one with a high fat content and the other with a low fat content. However, the feeds were previously untested and the overall response rate was poor with both feeds [27]. A similar lack of effect of fat content was also noted in a controlled trial in adolescents with CD [28], but a small Japanese trial showed a dose-related effect with a substantially poorer response in those patients receiving a high fat enteral feed [29]. A UK trial of elemental feeds supplemented with different fat levels also showed less benefit with the high fat feed and the authors conducted a meta-analysis of published trials which showed a correlation between better response to enteral feeding and lower fat content [30]. Further large trials are needed to address the possible harmful effects of fat, comparing enteral feeds of proven efficacy with and without additional fat.

Protein

When intravenous feeding and avoidance of normal food were first shown to induce remission in CD [31], it was assumed that avoidance of dietary protein antigens might be an important aspect of the therapy. Early trials of enteral feeding therefore used amino acid-based feeds that lacked whole protein. These feeds proved successful, reducing intestinal protein loss [32] and reducing intestinal permeability [33,34]. Amino acid-based feeds are relatively hyperosmolar and arguably more difficult to make palatable than polymeric feeds. The results of clinical trials comparing elemental with polymeric enteral feeds in CD are mixed. Some controlled trials have shown equivalent efficacy whilst others have not (reviewed later). A recent Cochrane meta-analysis of 10 trials comprising 334 patients demonstrated no difference in efficacy between elemental and polymeric feeds (OR 1.10, 95% CI 0.69–1.75) [35]. It seems probable that the beneficial effects of enteral feeding in CD are not related to avoidance of whole protein.

Particles

An intriguing hypothesis was proposed that very small insoluble particles with nanometer dimensions (nanoparticles) might by virtue of their very high ratio of surface area to mass act as haptens if present in the diet. It was pointed out that many foods contain particulate silicates and coloring additives, particularly titanium oxide present as a white colorant, e.g. in mayonnaise [36]. It is also present in toothpaste, thus resurrecting an old hypothesis [37]. The particle hypothesis was backed up by laboratory data showing that the presence of small amounts of titanium oxide greatly enhanced lymphocyte responsiveness to bacterial lipopolysaccharide [38]. Moreover, titanium oxide could also be found in CD tissue [39]. An initial controlled trial of a low particle diet yielded promising results [40], but a subsequent controlled trial proved negative [41]. The hypothesis that nanoparticles may be a causative agent in CD remains unproven.

Emulsifiers and stabilizers

The incidence of CD has increased sharply over the past 50 years and, although the simplest dietary explanation

for this increase would probably be an increased calorie intake, suspicion has understandably fallen on food additives in processed foods. Emulsifiers and stabilizers are particularly abundant in processed foods. Stabilizers include seaweed derivatives such as carrageenan. This is a highly anionic polymer which, in degraded form, causes intestinal ulceration in animals in an intriguing model that involves synergy with bacterial membrane antigens [42,43]. However, the degraded form is not used in the food industry and there is no evidence that food-grade carrageenan causes problems [44], although there has never been any formal test in CD. It is worth noting that it or similar stabilizers are used in some polymeric enteral feeds, particularly those that are supplied ready mixed rather than as a powder, and the discrepant performance of different polymeric feeds has already been noted [30].

There is a wide range of permitted emulsifiers in foods. These are essentially detergents. Most are broken down on passage through the small intestine and their detergent effects in the distal ileum and colon may arguably be small compared with the natural effects of bile acids. There is, however, some evidence that they can increase intestinal permeability in concentrations that could plausibly occur in humans [45,46], and this is an area that merits further study.

Cinnamon and benzoates

Oro-facial granulomatosis, a condition that most typically affects children and adolescents, is fairly commonly associated with food intolerance and food additives are particularly suspect [47]. It overlaps with CD [48] and a good response has been reported to exclusion of cinnamon and benzoate, common additives in soft drinks [49].

Addition of beneficial nutritional components

Transforming growth factor beta (TGF-β)

There is some evidence to suggest that addition of enteral feeds as supplements to a normal diet may be beneficial [50–52], but it is difficult to exclude the possibility that such supplementation will inevitably lead to a reduced intake of the normal diet. Moreover, a controlled trial in a pediatric population showed that partial supplementation with enteral feeding was less effective than total replacement of normal diet with enteral feeding [53]. Nevertheless, it is possible that some of the benefits of enteral feeding may be as a result of the addition of some anti-inflammatory nutrient. It has been suggested that some of the benefit of casein-based whole protein enteral feeds may relate to their TGF-β content [54], but this is difficult to establish as many of the mucosal cytokine changes seen during enteral feeding may be a consequence rather than a cause of reduced inflammation [55]. A controlled trial of pure TGF-β supplementation in CD would be needed to test the hypothesis adequately.

Antioxidants and other anti-inflammatory food components

Enteral feeding has a variable effect on the concentration of antioxidants. Akobeng *et al.* reported increased selenium but reduced vitamins C and E and no significant change in glutathione. No controlled trials on antioxidant supplementation have been published [56]. Curcumin (present in turmeric) also has anti-inflammatory properties. One trial in UC showed an effect on maintenance of remission with UC with 2/43 receiving curcumin 1 g b.d. relapsing compared with 8/39 with placebo ($p = 0.04$) [57]. Curcumin reduces the severity of inflammation in rodent models of colitis by inhibiting the activation of NFκB and a reduction in the activity of p38 MAPK [58].

Anti-inflammatory fatty acids

There has been much interest in the possible impact of different dietary fatty acids. Omega (*n*)-3 fatty acids (i.e. with the third carbon–carbon bond unsaturated), which are abundant in fish oils, compete with n-6 fatty acids, present in many vegetable oils and reduce the synthesis of pro-inflammatory eicosapentanoids. Dietary supplementation with n-3 fatty acids reduces experimental IBD in comparison with supplementation with n-6 fatty acids [59]. Dietary fish oil supplementation has also been shown to help maintain remission in CD [60], but subsequent studies have been both positive [61] and negative [62] and a controlled trial comparing a feed high in linoleate and n-6 polyunsaturates surprisingly proved more effective than a feed high in oleate and n-3 monounsaturated fats [63]. There is still a need for further trials.

Beneficial effects on the intestinal microbiota

There is widespread agreement that the IBDs CD and UC both represent an altered reaction to the intestinal microbiota, but the nature of this reaction is unclear. There is reasonable evidence that CD, unlike UC, is associated with bacterial invasion of the mucosa. In UC, where inflammation is typically more superficial, there may still be important bacterial–epithelial interactions occurring without bacterial invasion. In both conditions there is increasing acceptance that the mucosa-associated bacteria may be the most relevant. There is increasing interest in the possible role of a type of adherent and invasive *Escherichia coli* found particularly in CD but also to a lesser extent in UC [64].

Reduced bacterial content

A simple and plausible explanation for the beneficial effects of enteral feeding could be a quantitative reduction in bacteria, perhaps particularly in the distal ileum, as a consequence of the very low residue. Studies are technically difficult and should ideally involve assessment of the mucosa-associated microbiota. Alterations in the fecal microbial profile have been demonstrated in a pediatric

study of enteral feeding [65], but there is a clear need for further studies in this area.

Prebiotic effects

A prebiotic is a dietary component which has the potential to increase the intestinal population of beneficial "probiotic" bacteria. The definition of a probiotic bacterium remains unclear, as we still have a relatively limited understanding of how they exert their beneficial effects. Possible mechanisms include interaction with Toll-like receptors via bacterial DNA, generation of short-chain fatty acids such as butyrate, secretion of anti-inflammatory proteins and competition with more pathogenic bacteria. Given these limitations, most prebiotic research has focused on dietary components which increase intestinal lactobacilli and bifidobacteria [66]. Dietary supplements which have these properties include oligofructose, galactooligosaccharides and lactulose. Prebiotics have promising effects in experimental models of intestinal inflammation [67] and deserve clinical study.

An alternative approach that we are currently investigating is the potential for soluble plant fiber (non-starch polysaccharide) to block epithelial recruitment of bacteria. In the laboratory, soluble plant fibers such as soluble plantain (banana) fiber are very effective at blocking adherence of CD mucosal *E. coli* isolates to epithelial cells [68]. It is notable that parts of the world where plantains form a staple part of the diet have low rates for IBD.

Enteral feeding as primary therapy for CD

The concept that nutritional therapy is effective in active CD is attractive for both patients and their doctors. Although there have been no placebo-controlled trials of enteral nutrition (EN), it is highly likely that enteral diets are superior to placebo (response rates 36–84% versus placebo rates in CD of 18–40%). The efficacy of enteral feeding has been shown to be equivalent to oral corticosteroids in some trials with remission rates of up to 84% in compliant patients [69,70]. There is, however, considerable variation in responses to different enteral diets, possibly because the feeds used in these studies are often very different (i.e. different "drugs") (Table 27.1). However meta-analysis shows no difference between elemental and non-elemental diets (OR 1.10, 95% CI 0.69–1.75) [35]. In seven trials comparing formulas of differing fat content (low fat <20 g per 1000 kcal versus high fat >20 g per 1000 kcal), there is no difference in efficacy (OR 1.13, 95% CI 0.63–2.01). However, there is a trend of increased efficacy in diets with very low fat and very low long-chain triglyceride (LCT) content. Eight trials comparing EN with corticosteroids showed a pooled OR of 0.33 favoring steroids. However, these meta-analysis data obscure some clear advantages of EN over corticosteroids. These include the lack of major side effects, improved nutritional status and increased growth in children [71,72]. Enteral feeding is an excellent preoperative treatment to gain short-term control of CD prior to surgery, improving nutritional status and eliminating the risk of steroid therapy (which increases postoperative mortality and morbidity). Although the data are limited, there is some evidence that EN nutrition induces mucosal healing [73,74].

Commonly, enteral feeding is used as the sole nutritional source for 3–6 weeks and then normal diet is gradually reinstated over 3–6 months. This approach is supported by some trial data [53] where the effect of 100% enteral diet was compared with a 50% enteral diet and 50% standard food. The response rates were 42 and 15%, respectively ($p = 0.005$). Unfortunately, approximately half the patients relapse within 6 months of returning to a normal diet and further studies are needed to define a diet which might better maintain remission. A reasonably evidence-based approach is to recommend a low-fat, low-fiber diet to patients who are returning to normal food after a period of enteral feeding.

Enteral nutrition in the prevention of post-operative recurrence of Crohn's disease

One of the holy grails of IBD research is to find a safe, effective therapy to prevent postoperative recurrence of CD. EN is certainly safe but there is, as yet, no adequate trial addressing the efficacy. The only evidence comes from two small non-randomized trials. Esaki *et al* studied 40 patients following surgery, 24 of whom were able to continue for remarkably long periods on >1200 kcal of EN and 16 were not [75]. EN reduced recurrence (46 versus 75%) over approximately 5 years of follow-up with higher remission rates in penetrating-type disease. Yamamoto *et al* observed 20 very well motivated patients who continued overnight naso-gastric elemental feeding (with a low-fat diet during the day) following ileo-colonic resection and compared them with 20 who had neither nutritional therapy nor food restriction [73]. Clinical recurrence at 1 year occurred in 5% (1 patient) with EN against 35% (7 patients) in the non-EN group. One year postoperatively there was endoscopic recurrence in 30% (6 patients) in the EN group and 70% (14 patients) in the non-EN group ($p = 0.027$). Clearly, further large, properly controlled trials are required; however, such trials are difficult to conduct due to the low adherence with exclusive enteral nutrition. At present, it is unclear how long such EN should be continued. It can be speculated that EN might be effective by reducing bacterial colonization around the anastomosis, thereby reducing recurrence rates.

Table 27.1 Trials comparing performances of enteral feeds used as primary therapy in Crohn's disease.

Study	Intervention	Total *n*	Duration	Primary endpoint	Results	*p*-Value
Akobeng *et al.* [56]	(a) Glutamine enriched diet ($n = 7$) (b) Standard polymeric diet ($n = 8$)	$n = 15$	4 weeks	Plasma antioxidant levels	Overall plasma selenium increased in both groups	$p < 0.001$
Johnson *et al.* [53]	(a) Partial enteral nutrition ($n = 26$) (b) Total enteral nutrition ($n = 24$)	$n = 50$	6 weeks	Remission	PEN group 15% TEN group 42%	$p = 0.035$
Leiper *et al.* [27]	(a) High long-chain triglyceride whole protein feed ($n = 27$) (b) Low long-chain triglyceride whole protein feed ($n = 27$)	$n = 54$	3 weeks	Remission and response	Remission: High LCT group 33% Low LCT group 26% Response: High LCT group 52% Low LCT group 33%	Remission $p = 0.38$ Response $p = 0.27$
Middleton *et al.* [30]	(a) Elemental diet ($n = 17$) (b) Peptide based semi elemental diet ($n = 18$) (c) Elemental diet with additional fat in low LCT ($n = 22$) (d) Elemental diet with medium-chain triglyceride ($n = 19$)	$n = 76$	3 weeks	Response (Harvey Bradshaw Index) HBI <6 Remission HBI <3	Disease activity decreased in all groups Remission rate negatively correlated with amount of energy derived from LCT	$p = 0.016$
Raouf *et al.* [23]	(a) Amino acid-based feed ($n = 13$) (b) Whole protein feed ($n = 11$)	$n = 24$	6 weeks	Remission	Both feeds effective by 3 weeks Amino acid 9/13 Whole protein feed group 8/11	NS

(*Continued*)

Table 27.1 (*Continued*)

Study	Intervention	Total *n*	Duration	Primary endpoint	Results	*p*-Value
Greenberg *et al.* [110]	(a) Total parenteral nutrition and NBM ($n = 17$) (b) Defined formula diet ($n = 19$) (c) Partial parenteral nutrition and oral food ($n = 15$)	$n = 51$	21 days (follow up 1 year)	Remission	TPN group 71% Defined formula group 58% PPN group 60%	$p =$ n.s.
Bamba *et al.* [29]	(a) Low-fat elemental diet ($n = 10$) (b) Medium-fat elemental diet ($n = 10$) (c) High-fat elemental diet ($n = 8$)	$n = 28$	4 weeks	Remission	Low group 80% Medium group 40% High group 25%	Unknown
Giaffer *et al.* [111]	(a) Elemental feed ($n = 16$) (b) Polymeric feed ($n = 14$)	$n = 30$	4 weeks	Remission	Elemental feed 75% Polymeric feed 36%	$p < 0.03$
Verma *et al.* [112]	(a) Elemental feed ($n = 10$) (b) Polymeric feed ($n = 11$)	$n = 21$	Unknown	Remission	Elemental feed 80% Polymeric feed 55%	$p = 0.1$
Rigaud *et al.* [113]	(a) Elemental diet ($n = 15$) (b) Polymeric diet ($n = 15$)	$n = 30$	6 weeks	Nutritional state	Both groups showed similar improvement	$p =$ n.s.
Ueki *et al.* [114]	(a) Elemental diet ($n = 21$) (b) Semi-elemental diet ($n = 19$)	$n = 40$	6 weeks	Response	Both groups showed similar improvement	$p =$ n.s.

Ulcerative colitis

Identical twins have lower concordance for UC than for CD, suggesting a greater role for environmental factors, yet the evidence for dietary factors is considerably weaker than for CD. Controlled trials have shown no benefit from intravenous feeding and bowel rest [76,77], which makes it difficult to argue a strong case for dietary modulation.

A study performed over 40 years ago suggested that about one in five adult patients with UC benefited from exclusion of dairy products [78]. This study has never been repeated and probably should be. Cow's milk allergy is strongly associated with infantile colitis [79]. Lactose intolerance is probably not significantly associated with UC and is not sufficient reason to advise milk avoidance during relapse of colitis [16].

An interesting hypothesis has been proposed that mucosal damage in UC results from the toxic effects of hydrogen sulfide produced by bacterial metabolism of sulfur compounds within the colonic lumen [80]. Meat intake has been shown to be the predominant determinant of fecal sulfide [81] and a high meat intake has been shown to correlate with increased risk of relapse [17,81]. It is surprisingly difficult to ascertain the incidence of UC amongst vegetarians, and this deserves further study.

Dietary supplementation with fish oil has been tried, as in CD, with perhaps more consistently positive results, either alone [82,83] or in combination with antioxidants and oligofructose prebiotics. A trial of oral supplements enriched with fish oil, fructooligosaccharides, gum arabic, vitamin E, vitamin C and selenium showed marginal effects compared with placebo only, showing a reduction in dose of steroid used over 6 months, without a significant difference in disease activity compared with placebo [84]. Aloe vera is a plant product with antioxidant properties and is arguably not a nutritional supplement, but deserves mention as one of the few herbal remedies to have shown efficacy in a controlled trial [85]. Attempts to increase fecal butyrate content by increased oat bran or *Plantago ovata* seeds in the diet have been successful [86,87], but as yet there are no convincing data to support a significant clinical effect. Current evidence does not support an effect of essential fatty acids supplementation in the maintenance of remission of UC [88].

Nutritional deficiencies in IBD

Malnutrition has been reported in 18–75% of people with active IBD, the variation depending on its definition. Malnutrition occurs due to reduced calorie intake (anorexia, vomiting) and calorie loss (diarrhea, malabsorption, short bowel syndrome) in the face of increased calorie requirements. Malnutrition is associated with impaired immune function together with poor wound healing and impaired muscle function [37]. About 70% of children with CD and about one-third of those with UC have weight loss. Malnutrition leads to growth retardation and can result in short stature in adulthood. Growth retardation affects 15–40% of children with CD [89]. Growth retardation is multifactorial but primarily due to systemic inflammation (reducing IGF-1), reduced calorie intake, malabsorption and the effect of corticosteroids. EN has been shown in two randomized controlled trials (RCTs) in CD to increase height velocity significantly compared with corticosteroids [89,90].

Iron

The prevalence of anemia in IBD is highly variable (9–74%), depending on the definition of anemia and the population studied [91], but recent studies suggest that about one-third of patients attending clinic review for CD have a subnormal hemoglobin [92]. Anemia is most frequently due to iron deficiency and is even more common in UC [91]. The main reason for iron deficiency seems to be negative iron balance due to blood loss [92]. In health, 1–2 mg of iron is absorbed per day to compensate the same level of iron lost per day. Iron intake may be reduced in IBD, particularly in CD, because of the avoidance of high-fiber cereals fortified with iron [93]. The reduced intake of non-heme iron may be further exacerbated by a low intake of ascorbic acid [93]. Rarely, iron absorption can be reduced by extensive duodenal CD.

The diagnosis of iron deficiency in the absence of a low mean corpuscular volume (MCV) can be difficult as ferritin is an acute-phase protein. However, the diagnostic accuracy of ferritin as a marker for iron deficiency in IBD improves by using a higher cut-off value of 28 ng ml^{-1} [94].

There are conflicting data on whether oral iron is less well tolerated in IBD. Most recent data suggest that oral iron therapy is tolerated as well in IBD as in non-IBD iron deficiency, and disease relapse is rarely associated with iron therapy [95]. However, a randomized open-label trial suggested that intravenous iron sucrose was better tolerated than oral iron sulfate [96].

Theoretically, high iron diets could exacerbate colonic inflammation by catalyzing the formation of reactive oxygen species and there are some data in rodent models to support this [92,97]. High iron intake has also been reported to increase the risk of colon cancer in rodent models [92,97]. However, neither exacerbation of IBD nor an increased risk of colon cancer has been observed in human IBD in association with iron supplementation.

Vitamin B_{12}

Vitamin B_{12} deficiencies are common in patients with terminal ileal resection for CD. There is some dispute about the length of resection required to produce deficiency and clearly this is dependent on the function of the remaining ileum. Patients with less than 20 cm of ileal resection do not develop deficiency, while 52% of those with longer

resection have abnormal Schilling tests [98]. Resection of more than 60 cm almost invariably results in vitamin B_{12} deficiency [99]. A practical approach is to treat with parenteral vitamin B_{12} all patients with more than 20 cm resected and measure serum vitamin B_{12} yearly in those with less than 20 cm resected. Another approach is to perform a Schilling test after resection of less than 60 cm of ileum or measure vitamin B_{12} yearly. However Schilling tests can be unreliable, usually due to inadequate urine collection.

Folate

Folate is absorbed in the duodenum and jejunum. Deficiency is often due to a combination of reduced intake of folate-containing foods (high-fiber vegetables which may cause abdominal pain), drug interactions (particularly methotrexate and sulfasalazine) and malabsorption.

Vitamin D and calcium

Vitamin D deficiency [$<15\,ng\,ml^{-1}$ 25-hydroxyvitamin D (25-OHD)] or insufficiency ($<20\,ng\,ml^{-1}$ 25-OHD) is common, particularly in the northern hemisphere [100,101]. The prevalence of vitamin D deficiency is 22–70% in CD and up to 45% in UC [102]. There have been few adequate studies in children but the overall rate in IBD is about 36% and is particularly important in this group because peak bone mass is related to fracture risk in later life. Vitamin D deficiency is multifactorial but is partly due to intestinal loss of vitamin D-binding protein. Supplementation with vitamin D (400 IU) and calcium (500 mg) is beneficial in improving bone mineral density [103].

Micronutrients

Even in patients in remission and with adequate macronutrient intake, deficient intake of micronutrients such as β-carotene, vitamins B_1, B_6 and C and magnesium are common. More than 50% of patients have low plasma concentrations of vitamin C, niacin and zinc [102].

Intestinal failure/short bowel syndrome

Intestinal failure is defined as the situation "when there is reduced intestinal absorption so that macronutrient and/or water and electrolyte supplements are needed to maintain health and/or growth" [103]. CD is the commonest reason for requiring home parenteral nutrition in the UK, accounting for about 40% of cases. Rarely, short bowel results solely from primary extensive pan-enteric disease and the majority are due to complications of surgical therapy, particularly those who have multiple unplanned laparotomies for intra-abdominal sepsis [104]. The risk of developing intestinal failure with sequential planned resections is low: 0.1% from a recent single institution series. [105] The management of short bowel syndrome is complex and requires a multi-disciplinary team [103]. Management depends on the length of remaining small bowel and whether there is remaining colon or not (jejunum–colon or jejunostomy). If there is more than 200 cm of remaining small bowel then parenteral nutritional or fluid is usually not required. However, in CD the remaining small bowel may be abnormal.

Hyperphagia occurs as more than 50% of nutrients may be malabsorbed. After massive resection, some structural and functional adaption of the small bowel can occur to increase absorption of macronutrients, electrolytes and water. Intake of long-chain triglycerides tends to increase diarrhea, reduce carbohydrate fermentation, bind magnesium and lead to increased serum oxalate levels. Although a high-carbohydrate and low-fiber diet is the ideal in short bowel syndrome, this may be difficult to achieve. A diet high in MCT can be used [106] and sunflower oil can be rubbed into the skin to deliver essential fatty acids [107].

Jejunum–colon

The predominant clinical problem is usually malnutrition. PEG feeding can be used in CD provided that there is no macroscopic gastric CD or distal obstruction [108]. If weight loss continues or there is less than 50 cm of remaining small bowel, then parenteral nutrition is required [108]. Several micronutrient deficiencies can occur, including vitamin B_{12}, zinc and selenium, and require replacement, often at high doses if the last two are given orally [103]. The colon can adapt to absorb energy in the form of short-chain fatty acids (SCFA) produced by bacterial fermentation of soluble fiber. The colon can absorb between 525 and 1170 $kcal\,day^{-1}$ from dietary fiber [109].

Jejunostomy

The predominant clinical problem is usually fluid and electrolyte management. This is because the jejunum often is a net secretor of fluid and it will secrete sodium into the lumen if oral fluids have a sodium concentration of less than $90\,mmol\,l^{-1}$. Magnesium balance can be particularly difficult. Hypomagnesemia occurs due to non-absorbed fatty acids binding magnesium and because of renal magnesium loss due to secondary hyperaldosteronism. If there is less than 100 cm of remaining small bowel, intravenous saline (often with additional magnesium) is required. If less than 75 cm remains, parenteral nutrition is needed.

High-output jejunostomy

Clearly, other causes of high output need to be excluded, including recurrent CD, intra-abdominal sepsis or drug effects. The key to management of high output is the restriction of oral hypotonic fluids (to $500\,ml\,day^{-1}$) and to give a glucose/saline solution to sip feed ($>1\,l\,day^{-1}$). The aim is to have a saline/glucose drink that has a higher sodium content than jejunostomy fluid ($90\,mmol\,l^{-1}$). Enteral feeds require addition of further sodium to achieve

a concentration of sodium above 100 mmol l^{-1}. In general, elemental feeds tend to increase output as they are hyperosmolar and contain too low a concentration of sodium. The ideal diet is one of energy dense foods with large molecules (reduces osmolarity) with a high sodium concentration (>100 mmol /l^{-1}) and an osmolarity of around 300 mOsm kg^{-1}. If output remains high, then control can be achieved by use of high doses of loperamide. Combination of loperamide and codeine may be more effective [103]. If there is less than 100 cm of small bowel remaining, then use of treatments to reduce gastric acid secretion (PPI, H2 receptor antagonists) may be effective. Somatostatin analogues such as octreotide have some effect on reducing large-volume jejunostomy output but have no effect on total energy absorption [103].

Oxalate

Renal calcium oxalate stones occur in 25% of people with short bowel syndrome who have remaining large bowel. Usually, dietary oxalate binds to calcium in the lumen and is excreted. High serum oxalate occurs in short bowel syndrome because unabsorbed fats binding calcium allow unbound oxalate to be absorbed and also because of reduced oxalate degradation by bacteria. A restricted oxalate diet should be employed, e.g. avoiding rhubarb, spinach, tea, nuts and strawberries.

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Chapter 28
Therapeutic Approaches to the Treatment of Ulcerative Colitis

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Summary
- Prior to selecting therapy, patients should be assessed for disease extent and disease severity and treatment goals should be established.
- Oral and/or rectal mesalamine (and mesalamine pro-drugs) are the optimal first-line therapy for induction and maintenance therapy in patients with mild to moderate ulcerative colitis.
- Oral and/or rectal corticosteroids are effective for induction but not maintenance therapy in patients with moderately active ulcerative colitis and can be given intravenously for patients with severe disease.
- Azathioprine and 6-mercaptopurine are effective for steroid-sparing and maintenance therapy in moderate ulcerative colitis.
- Infliximab is effective for induction and maintenance therapy and for steroid sparing in moderate to severe ulcerative colitis.

Introduction

Developing an optimal therapeutic approach for patients with ulcerative colitis requires that the treating physician consider all aspects of the patient's presentation and the results of diagnostic procedures in the context of an understanding of clinical pharmacology and the evidence from controlled clinical trials. In this chapter, results from clinical trials of medications used to treat ulcerative colitis are reviewed first and are followed by steps to an integrated therapeutic approach to the medical treatment of this form of inflammatory bowel disease.

Pretreatment evaluation of the patient

Patients with ulcerative colitis should be evaluated prior to initiating or changing the treatment regimen This evaluation consists of a detailed a medical history and the determination of age of onset, duration of disease, anatomic extent of the disease, the disease course over time, prior and current medication use (including duration and dose) and the current symptom presentation. Infectious and medication-associated causes of colitis should be excluded. Colonoscopy with mucosal biopsy and small bowel imaging should be performed to exclude Crohn's disease and provide a baseline characterization of the ulcerative colitis. In patients with an established diagnosis of ulcerative colitis, these tests should be repeated when patients relapse and fail to respond to empiric therapy with 5-aminosalicylates and/or corticosteroids, prior to instituting immunosuppressive or biologic therapy or referring the patient for surgery. Adherence to this methodical approach allows the treating physician to make observations, including a change in the proximal extent of colitis, endoscopic findings of severe colitis, features that are more compatible with a diagnosis of Crohn's disease, infectious colitis or medication associated colitis and patients with ulcerative colitis in endoscopic remission who may be experiencing symptoms of concomitant irritable bowel syndrome, that would lead to a change in therapy.

Classification of the patient according to the anatomic extent of involvement is shown in Figure 28.1 [1]. Many 5-aminosalicylate-based medications and corticosteroid preparations are delivered topically and do not distribute uniformly throughout the colon at a high concentration; therefore, it is very important to determine the extent of disease involvement. The expected site of drug delivery for various topically delivered 5-aminosalicylate formulations are shown in Table 28.1 [2]. Suppositories can only be expected to release medication in the rectum (approximately the last 10 cm of the colon) [3]. In approximately 80–90% of patients, enemas will reach the ascending colon/splenic flexure (Figure 28.2) [4,5].

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Table 28.1 5-Aminosalicylate formulations

Generic name	Proprietary name	Formulation	Sites of delivery	Unit strength
Mesalamine	North American Asacol*	Eudragit-S coated tablets (release at pH $\geq$ 7.0)	Terminal ileum, colon	400 mg
	Asacol HD, Asacol 800	Eudragit-S coated tablets (release at pH $\geq$ 7.0)	Terminal ileum, colon	800 mg
	United Kingdom, Italy, Netherlands Asacol†	Eudragit-S coated tablets (release at pH $\geq$ 7.0)	Terminal ileum, colon	400 mg
	Lialda (USA), Mezavant (Europe), Mexavant XL (UK, Ireland) (SDP 476)	Advanced, multimatrix system (MMX)	Terminal ileum, colon	1200 mg
	Salofalk‡, Mesasal, Claversal‡	Eudragit-L coated tablets (release at pH $\geq$ 6.0)	Distal ileum, colon	250, 500 mg
	Apriso, Salofalk Granu-Stix‡	Eudragit-L100, polyacrylate dispersion, povidone K (Eudragit-NE 40 D, Nonoxinol 100), simeticone	80% colon, sigmoid colon, rectum	500, 1000 mg
	Claversal Micropellets§	Eudragit L-100-55, Eudragit S-100, dispersible cellulose	Ileocecal valve, colon, left-sided colon	1500 mg
	Claversal Foam§	Eudragit L-100-55, Eudragit S-100, dispersible cellulose	Left-sided colon	5 g foam (1 g 5-ASA)
Mesalamine	Pentasa**	Ethylcellulose-coated microgranules (time-dependent release) available as a tablet, capsule or sachet	Duodenum, ileum, colon	250 and 500 mg tablets; 500 mg capsules; 1000 mg sachets
Olsalazine	Dipentum	5-Aminosalicylic acid dimer linked by azo bond, available as a gelatin capsule	Colon	250 mg
Sulfasalazine	Azulfidine, Salazopyrin	5-Aminosalicylic acid linked to sulfapyridine by azo bond, available as a tablet	Colon	500 mg (200 mg 5-ASA)
Sulfasalazine	Azulfidine/Salazopyrin EN-tabs	5-Aminosalicylic acid linked to sulfapyridine by azo bond, available as a tablet coated with cellulose acetate phthalate	Colon	500 mg (200 mg 5-ASA)
Balsalazide	Colazide, Colazal	5-Aminosalicylic acid linked to 4-aminobenzoyl-β-alanine (4ABA) by azo bond, available as a capsule	Colon	750 mg (262 mg 5-ASA)

*North American Asacol: originally developed by Tillotts Laboratories, Colpermin, UK (later changed name to Tillotts Pharma AG, Ziefen, Switzerland), then Norwich Eaton, Norwich, NY, USA, currently Procter and Gamble, Cincinnati, OH, USA. Marketed by Procter and Gamble in North America. Manufactured with original Tillotts Laboratories manufacturing process.

†United Kingdom, Italy, Netherlands Asacol: purchased from Tillotts Laboratories by Smith Kline French Laboratories (name later changed to Smith Kline Beecham and then GlaxoSmithKline), Giuliani and Byk-Gulden. Differences might exist in Eudragit-S coating thickness, excipients and manufacturing processes. No published data establishing the bioequivalence of North American Asacol and United Kingdom, Italy, Netherlands Asacol.

‡Manufactured by Dr Falk Pharma in Germany.

§Manufactured by Merckle Recordati in Germany.

**United States Pentasa: 250 mg capsule from Shire Pharmaceuticals (previously developed and marketed by Marion Laboratories which later merged into Hoechst-Marion-Roussel, then Aventis and now Sanofi). Pentasa is manufactured and distributed by Ferring Pharmaceuticals.

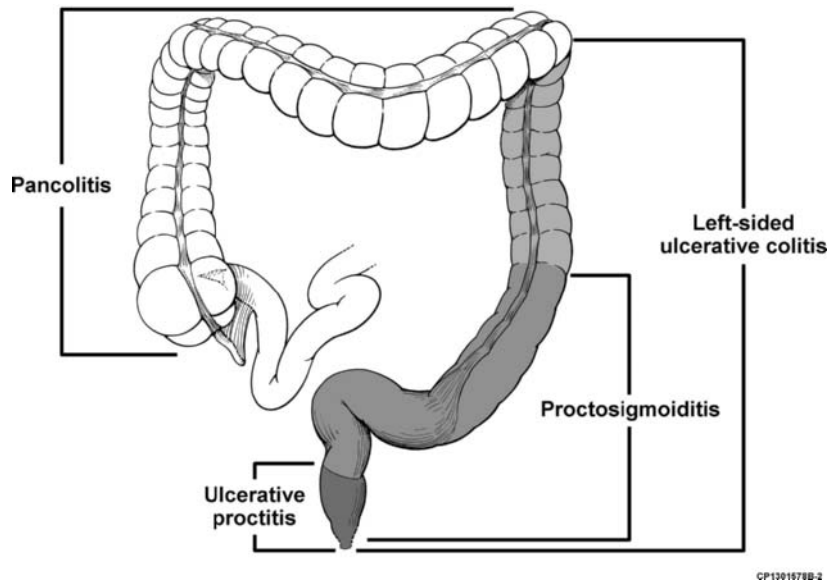

Figure 28.1 Distinguishing the various states of ulcerative colitis – proctitis, proctosigmoiditis, left-sided colitis and pancolitis – depends on both the degree of mucosal inflammation and the extent of colonic mucosal involvement. Reprinted with permission from Miner PB, Peppercorn MA, Targan SR. A rational approach to 5-aminosalicylic acid therapy in ulcerative colitis. *Hosp Pract* 1993; **28**(Suppl 3):3–24.

Determining whether patients have mild to moderately active or severely active ulcerative colitis can be aided by the use of the Truelove and Witts classification (Table 28.2) [6]. This assessment is important in determining whether or not to hospitalize the patient and whether steroid therapy is indicated. Other disease activity indices such as the Sutherland Index and the Mayo Clinic Index are more useful for distinguishing patients with remission, mildly active disease and moderately active disease for the purposes of assessing efficacy of sulfasalazine and other 5-aminosalicylate-based medications (Table 28.3) [7–9].

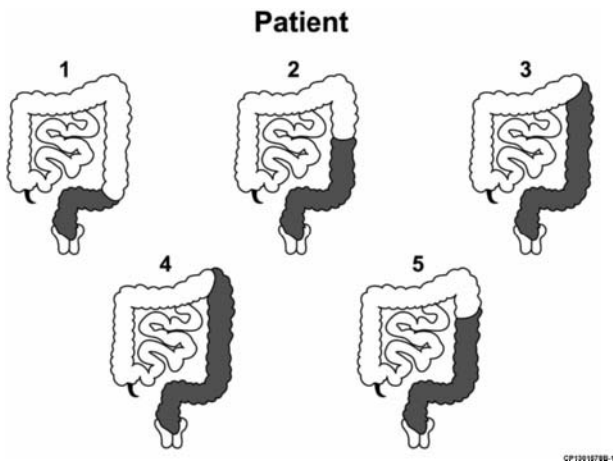

Figure 28.2 The individual colonic spread of radiolabeled low-viscosity 100 ml budesonide enemas in five patients with distal ulcerative colitis 15 min after administration. The filled areas of the colon represent areas where radioactivity was found. Modified with permission from Nyman-Pantelidis M, Nillson A, Wagner GW, Borga O. Pharmacokinetics and retrograde colonic spread of budesonide enemas in patients with distal ulcerative colitis. *Aliment Pharmacol Ther* 1994; **8**:617–22.

Goals of treatment

The primary goals of medical therapy are to induce and then maintain significant clinical improvement or remission, resulting in a reduction or resolution of the signs and symptoms of active ulcerative colitis. Secondary goals, which often occur in parallel with clinical changes, are induction of endoscopic improvement and remission (mucosal healing), steroid sparing and reduction in the rates of hospitalization and colectomy. The efficacy of various medical therapies in achieving these endpoints in patients with ulcerative colitis is reviewed in the following sections.

5-Aminosalicylate-based medications

Sulfasalazine, oral mesalamine (Pentasa, Asacol, Asacol HD, Lialda, Salofalk, Salofalk Granustix, Apriso, Mesasal, Claversal), rectal mesalamine (Canasa, Rowasa, Salofalk, Pentasa), olsalazine and balsalazide are all drugs that deliver 5-aminosalicylate to the colon (Table 28.1) [2]. The clinical pharmacology of these medications is reviewed in detail elsewhere in this book.

Sulfasalazine

In 1942, Svartz reported on both the therapeutic results and the toxic effects of a novel sufanilamide preparation, sulfasalazine, in patients with ulcerative colitis [10]. Sulfasalazine is comprised of 5-aminosalicylate linked to sulfapyridine by a diazo bond. Placebo-controlled trials demonstrated that sulfasalazine administered orally at doses of 2–6 g per day (equivalent to 0.8–2.4 g per day of 5-aminosalicylate) was effective in inducing remission in patients with mildly to moderately active ulcerative

Table 28.2 Truelove and Witts criteria for evaluating the severity of ulcerative colitis*

Variable	Mild disease	Severe disease	Fulminant disease
Stools (number per day)	<4	>6	>10
Blood in stool	Intermittent	Frequent	Continuous
Temperature (°C)	Normal	>37.5	>37.5
Pulse (beats per minute)	Normal	>90	>90
Hemoglobin	Normal	<75% of normal value	Transfusion required
Erythrocyte sedimentation rate ($mm\,h^{-1}$)	≤30	>30	>30
Colonic features on X-ray		Air, edematous wall, thumbprinting	Dilatation
Clinical signs		Abdominal tenderness	Abdominal distention and tenderness

*Moderate disease includes features of both mild and severe disease.
Data from Truelove SC, Witts LT. Cortisone in ulcerative colitis: final report on a therapeutic trial. *BMJ* 1955; **ii**:1041–8.

colitis and ulcerative proctitis [11,12]. Additional placebo-controlled trials demonstrated that sulfasalazine at doses of 2–4 g per day (equivalent to 0.8–1.6 g per day of 5-aminosalicylate) was effective in maintaining remission in patients with ulcerative colitis and that the 4 g dose was more effective whereas the 2 g dose was better tolerated [13–15]. Approximately 10–20% of orally administered sulfasalazine is absorbed systemically with the remainder passing unaltered to the colon [16]. Sulfasalazine undergoes metabolism in the colon by bacterial azo reductase enzymes to 5-aminosalicylate and sulfapyridine [17,18]. The active moiety of sulfasalazine was determined to be the poorly absorbed molecule 5-aminosalicylate and not the well-absorbed molecule sulfapyridine [16,19–22].

Adverse events occurring in patients with inflammatory bowel disease treated with sulfasalazine include headache, epigastric pain, nausea and vomiting; cyanosis, skin rash, fever, hepatitis, autoimmune hemolysis,

Table 28.3 Mayo scoring system for assessment of ulcerative colitis activity*.

Stool frequency†
0 = Normal number of stools for this patient
1 = 1–2 stools more than normal
2 = 3–4 stools more than normal
3 = 5 or more stools more than normal

Rectal bleeding‡
0 = No blood seen
1 = Streaks of blood with stool less than half of the time
2 = Obvious blood with stool most of the time
3 = Blood alone passed

Findings of flexible proctosigmoidoscopy
0 = Normal or inactive disease
1 = Mild disease (erythema, decreased vascular pattern, mild friability)
2 = Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
3 = Severe disease (spontaneous bleeding, ulceration)

Physician's global assessment§
0 = Normal
1 = Mild disease
2 = Moderate disease
3 = Severe disease

*A total Mayo ulcerative colitis activity score of 0–2 points indicates remission/minimally active disease; a score of 3–5 points indicates mildly active disease; a score of 6–10 points indicates moderately active disease; and a score of 11–12 may indicate moderate or severe disease, depending on the patient's Truelove and Witt's score.
†Each patient served as his or her own control to establish the degree of abnormality of the stool frequency.
‡The daily bleeding score represented the most severe day of bleeding.
§The physician's global assessment acknowledged the three other criteria, the patient's daily record of abdominal discomfort and general sense of well-being and other observations, such as physical findings and the patient's performance status.

transient reticulosis, aplastic anemia, leukopenia, agranulocytosis, folate deficiency, pancreatitis, systemic lupus erythematosus, sulfonamide-induced toxic epidermal necrolysis, Stevens–Johnson syndrome, pulmonary dysfunction and male infertility [23,24]. For the most part, the side effects from sulfasalazine can be attributed to the systemic absorption of sulfapyridine and they occur more commonly in patients who are genetically predisposed to "slow" acetylation of sulfapyridine to *N*-acetylsulfapyridine in the liver [23]. Headache, nausea and vomiting and epigastric pain often appear to be related to the sulfasalazine dose and it is frequently possible to desensitize patients by discontinuing sulfasalazine for 1–2 weeks and then restarting at 0.125–0.25 g per day and increasing by 0.125 g per week up to a maintenance dose of 2 g per day [24]. Sulfasalazine therapy may also lead to a paradoxical worsening of diarrhea in patients with ulcerative colitis [25].

Rectal mesalamine (5-aminosalicylate)

After it was demonstrated that mesalamine [5-aminosalicylate (5-ASA)] was the active moiety of sulfasalazine (discussed above), oral and rectal drug delivery systems were devised to avoid absorption of mesalamine in the proximal small intestine, instead targeting the colon as the site of drug release. Placebo-controlled trials demonstrated that mesalamine administered rectally as a suspension enema or rectal foam at doses of 1–4 g per day or as a suppository at doses of 0.5–1.5 g per day was effective in inducing remission in patients with mildly to moderately active left-sided ulcerative colitis, ulcerative proctosigmoiditis and ulcerative proctitis [3,26–29]. There does not appear to be a dose response across this range of mesalamine doses [28,30]. Relatively small studies comparing rectally administered 5-ASA with rectal steroids demonstrated similar efficacy rates [30]. However, a meta-analysis suggested that rectally administered mesalamine is superior to rectal steroids for inducing remission [30]. A comparison of oral mesalamine 2.4 g per day, rectal mesalamine 4 g per day and a combination of the two therapies demonstrated a benefit for rectal mesalamine and combination therapy [31]. However, another study comparing oral mesalamine 4.0 g per day and a combination of oral mesalamine 2.0 g per day and rectal mesalamine 2.0 g per day showed similar efficacy [32]. The addition of 1 g per day of rectal mesalamine to 4 g per day of oral mesalamine (Pentasa) increased remission rates in patients with extensive active ulcerative colitis [33]. Additional placebo-controlled trials demonstrated that mesalamine administered rectally as 1 or 4 g enemas or 0.5 or 1 g suppositories was effective in maintaining remission in patients with ulcerative left-sided ulcerative colitis, distal ulcerative colitis and ulcerative proctitis [34–37].

Oral mesalamine (5-aminosalicylate)

Delayed-release mesalamine (Asacol, Asacol HD)

Asacol and Asacol HD are a delayed-release tablet formulation of mesalamine that is coated with a polymer named Eudragit-S. Asacol passes to the terminal ileum and cecum where it releases at pH $\geq$ 7.0. Placebo-controlled trials demonstrated that Asacol was effective as induction treatment in patients with mildly to moderately active ulcerative colitis (1.6, 2.4 and 4.8 g per day) [8,38]. Two dose-ranging trials failed to demonstrate that Asacol 4.8 g per day was more effective than a Asacol 2.4 g per day in patients with mild to moderate ulcerative colitis, but subgroup analyses indicated that the 4.8 g per day dose was more effective in patients with moderate disease activity [39,40]. An additional placebo-controlled trial demonstrated that Asacol at doses of 0.8–1.6 g per day was effective in maintaining endoscopic remission in patients with ulcerative colitis and that 1.6 g per day was the most effective dose [41].

Sustained-release mesalamine (Pentasa)

Petasa is a sustained-release tablet formulation of mesalamine that is comprised of ethylcellulose-coated microgranules. Pentasa passes to the duodenum where it begins a time-dependent release that continues until the rectum. Placebo-controlled dose-ranging trials demonstrated that Pentasa 2–4 g per day was effective as induction treatment in patients with mildly to moderately active ulcerative colitis and that 4 g was the most effective dose [42,43]. An additional placebo-controlled trial demonstrated that Pentasa 4 g per day was effective in maintaining symptomatic remission in patients with ulcerative colitis [44].

Mesalamine pellets (Salofalk Granu-Stix, Apriso)

Mesalamine pellets are a delayed- and sustained-release pellet formulation of 5-ASA that have an outer coat of a polymer named Eudragit-L and an additional retarding polymer in the pellet core. Mesalamine pellets pass to the distal small bowel where they begin to release at pH $\geq$ 6.0 and then have sustained release that continues throughout the colon [45]. A dose-ranging controlled trial and a delayed-release mesalamine (Eudragit L coating, Salofalk) comparator-controlled trial demonstrated that a higher dose of mesalamine pellets had similar efficacy to a lower dose of mesalamine pellets and that mesalamine pellets had similar efficacy to delayed-release mesalamine (Eudragit L coating) for induction treatment in patients with mildly to moderately active ulcerative colitis (1.5 g per day mesalamine pellets similar to 3 and 4.5 g per day mesalamine pellets) (1.5 g per day mesalamine pellets with escalation to 3.0 g per day for non-response similar to 1.5 g per day delayed-release mesalamine with option to escalate to 3.0 g per day) [46,47]. To date there are no published maintenance of remission studies with mesalamine pellets in patients with ulcerative colitis.

Multi-matrix system (MMX) mesalamine [SPD476 (Lialda, USA; Mezavant XL, UK and Ireland; Mezavant, elsewhere]

MMX mesalamine is a delayed- and sustained-release formulation of 5-ASA that has an outer coat of a polymer named Eudragit-S and a multi matrix system comprised of lipophilic and hydrophilic matrices in the core. MMX mesalamine tablets pass to the terminal ileum and cecum where they release at pH $\geq$ 7.0. Once the Eudragit-S coating has disintegrated, intestinal fluids interact with the hydrophilic matrix causing the tablet to swell and form an outer, viscous gel mass. Placebo-controlled dose-ranging trials demonstrated that MMX mesalamine 2.4–4.8 g per day was effective as induction treatment in patients with mildly to moderately active ulcerative colitis and that 4.8 g per day was not more effective than 2.4 g per day [48,49]. To date there are no published placebo-controlled maintenance of remission studies with MMX mesalamine in patients with ulcerative colitis. An open-label randomized trial demonstrated that MMX mesalamine 2.4 g per day given as a single dose or in two divided doses had similar maintenance of remission rates [50].

Olsalazine

Olsalazine is a dimer, comprised of two 5-ASA molecules linked by a diazo bond. Placebo-controlled trials demonstrated that olsalazine administered orally at doses of 0.75–3 g per day was not consistently effective in inducing remission in patients with mildly to moderately active ulcerative colitis, due to a higher than expected dropout rate in olsalazine-treated patients for worsened diarrhea [51–56]. The worsened diarrhea is a result of ileal secretion [57]. Additional placebo-controlled trials demonstrated that olsalazine at doses of 1–2 g per day was effective in maintaining endoscopic and clinical remission in patients with ulcerative colitis [58,59].

Balsalazide

Balsalazide is comprised of 5-ASA linked to an inert carrier molecule by a diazo bond. A small comparative study of balsalazide 6.75 g per day (equivalent to 2.4 g per day of 5-ASA) with oral mesalamine (Asacol) 2.4 g per day in patients with active ulcerative colitis suggested that balsalazide might be more effective [60]. However, two additional larger comparative trials of the same regimens demonstrated similar efficacy [61,62]. A dose-ranging trial demonstrated that balsalazide 6.75 g per day(equivalent to 2.4 g per day of 5-ASA) was more effective than balsalazide 2.25 g per day (equivalent to 0.8 g per day of 5-ASA) [62]. Dose-ranging maintenance trials in patients with ulcerative colitis demonstrated that balsalazide 4 g per day (equivalent to 1.4 g per day of 5-ASA) was more effective than balsalazide 2 g per day (equivalent to 0.7 g per day 5-ASA) [63], that balsalazide 6 g per day (equivalent to 2.1 g per day 5-ASA) had similar efficacy to balsalazide 3 g per day (equivalent to 1.1 g per day 5-ASA) [64] and that balsalazide 6 g per day (equivalent to 2.1 g per day 5-ASA) was superior to 3 g per day (equivalent to 1.1 g per day 5-ASA) [65].

Toxicity of mesalamine, olsalazine and balsalazide

Adverse events due to mesalamine are rare in patients with inflammatory bowel disease treated with mesalamine, olsalazine and balsalazide [66]. However, infrequent but serious events including pulmonary toxicity, pericarditis, hepatitis and pancreatitis can occur. Interstitial nephritis has been reported in patients treated with mesalamine [67,68]. Nevertheless, other studies have shown that interstitial nephritis may occur in patients with Crohn's disease in the absence of mesalamine therapy [69], that renal tubular proteinuria correlates with the disease activity of the inflammatory bowel disease [70–72], that the glomerular filtration rate does not change during maintenance therapy with mesalamine or olsalazine [73] and that the frequency of renal insufficiency was low in large safety and pharmacovigilance databases for Asacol and Pentasa [74,75]. A minority of patients will experience worsening diarrhea and abdominal pain due to a hypersensitivity reaction to 5-ASA [76].

Corticosteroid-based medications

Cortisone is produced by the adrenal cortex and prednisone must be activated in the liver to hydrocortisone and prednisolone, respectively. Prednisolone and methylprednisolone have the same glucocorticoid and anti-inflammatory activity as hydrocortisone but less mineralocorticoid activity. Rectal administration of hydrocortisone, prednisolone, methylprednisolone and betamethasone, oral administration of cortisone, prednisone and prednisolone and intravenous administration of prednisolone, methylprednisolone and corticotropin are all methods of delivering corticosteroids for a systemic effect (Table 28.4). In contrast to systemically administered corticosteroids, rectal administration of beclometasone, tixicortol, budesonide and prednisolone metasulfobenzoate and oral administration of fluticasone and controlled colonic release budesonide are all methods of delivering corticosteroids directly to the colon for a non-systemic effect (Table 28.4). Topical administration of beclometasone, fluticasone, tixocortol or budesonide to the colon results in a predominately non-systemic effect because these newer corticosteroids have high affinities for the glucocorticoid receptors and undergo extensive first-pass hepatic metabolism. Topical administration of prednisolone metasulfobenzoate and tixocortol to the colon results in a predominately non-systemic effect because they are poorly absorbed.

Table 28.4 Rectal corticosteroid preparations.

Generic name	Proprietary name	Formulation	Sites of delivery	Site and mechanism of action	Daily dose (mg)	Indication
Hydrocortisone acetate	Anusol-HC 25 mg	Suppository 25 mg	Rectum	Systemic	50–100	Active proctitis
Hydrocortisone	Cortenema	Enema 100 mg/60 ml	Distal to splenic flexure	Systemic	100	Active distal UC
Hydrocortisone acetate	Cortifoam	Foam 80 mg/900 mg foam	Distal to rectum	Systemic	80–160	Active proctitis in the distal rectum
Hydrocortisone acetate	Colifoam*	Foam 125 ml/5 ml foam	Distal to splenic flexure	Systemic	125–250	Active distal UC
Hydrocortisone acetate	Proctocort	Suppository 30 mg	Rectum	Systemic	60–120	Active proctitis
Hydrocortisone acetate + pramoxine hydrochloride (local anesthetic)	Proctofoam HC	Topical Aerosol 1% hydrocortisone (~7 mg) 1% pramoxine (~7 mg)	Anus, distal to rectum	Systemic	7–28	Active procitis
Prednisolone phosphate	Predsol Enema*	Enema 20 mg/100 ml	Distal to splenic flexure	Systemic	20	Active distal UC
Betamethasone valerate	Betnesol*	Enema 5 mg/100 ml	Distal to splenic flexure	Systemic	5	Active distal UC
Prednisolone metasulfobenzoate	Predenema*	Enema 20 mg/100 ml	Distal to splenic flexure	Non-systemic: poorly absorbed	20	Active distal UC
Prednisolone metasulfobenzoate	Predfoam*	Foam 20 mg/20 ml foam	Distal to splenic flexure	Non-systemic: poorly absorbed	20	Active distal UC
Tixocortol pivalate	Rectovalone*	Enema 250 mg/100 ml	Distal to splenic flexure	Non-systemic: poorly absorbed and first pass metabolism	250	Active distal UC
Budesonide	Entocort Enema*	Enema 2 mg/100 ml	Distal to splenic flexure	Non-systemic: first pass metabolism	2	Active distal UC
Budesonide	Budenofalk Enema*	Enema 2 mg/100 ml	Distal to splenic flexure	Non-systemic: first pass metabolism	2	Active distal UC
Budesonide	Budenofalk Foam*	Foam 2 mg/25 ml	Distal to splenic flexure	Non-systemic: first pass metabolism	2	Active distal UC

*Not available in the United States.

Oral corticosteroids (systemic effect)

In 1954 and 1955, Truelove and Witts reported the preliminary and final results of a placebo-controlled trial which demonstrated that a tapering dose of cortisone beginning at 100 mg per day was effective in inducing remission in patients with mildly to severely active ulcerative colitis [6]. Studies comparing sulfasalazine with a combination of low-dose oral and rectal steroids for active ulcerative colitis concluded that steroid therapy acted more rapidly and perhaps was more effective than sulfasalazine [77,78]. A dose-ranging study demonstrated that prednisone 40–60 mg per day was more effective than 20 mg per day and that 60 mg per day was no more effective than 40 mg per day but resulted in a greater frequency of side effects [79]. A subsequent study demonstrated that a single daily dose of prednisone 40 mg was equally effective as prednisone 10 mg four times daily [80]. Placebo-controlled trials of cortisone 25 mg twice daily and prednisone at doses of 15 mg per day or 40 mg every other day failed to demonstrate a maintenance benefit for oral corticosteroids [81–83].

Intravenous corticosteroids and corticotrophin (ACTH)

Patients with severe ulcerative colitis and those refractory to oral corticosteroids are hospitalized and treated with intravenous corticosteroids. The rationale for this practice is altered corticosteroid absorption and metabolism in

patients with ulcerative colitis. Oral administration of a 40 mg dose of prednisolone resulted in a lower peak and a slower rate of decrease in the plasma concentration of prednisolone in patients with severe ulcerative colitis compared with the time versus concentration curve observed in healthy volunteers; although total prednisolone absorption was similar [84]. In contrast, intravenous administration of prednisolone to patients with ulcerative colitis resulted in serum concentrations similar to those in volunteers [85]. Continuous infusion of prednisolone resulted in greater mean serum concentrations over time compared with bolus intravenous dosing; and both intravenous dosing strategies resulted in greater mean serum concentrations than oral dosing [85]. However, a randomized comparison of 1 mg kg^{-1} per day of 6-methylprednisolone administered as a continuous infusion or bolus injection showed no difference in outcomes [86]. Uncontrolled studies have reported that approximately 60% of patients hospitalized for severe ulcerative colitis will respond to intravenous corticosteroid therapy [87–90]. Dosing strategies have included prednisolone 60 mg per day in four divided doses [87], betamethasone 3 mg twice daily [89,90] and hydrocortisone 300–400 mg per day [91,92]. Methylprednisolone 40–60 mg per day is preferred by many clinicians because it has minimal mineralocorticoid effect. There was no apparent advantage in increasing the dose of methylprednisolone to 1000 mg per day [93]. No placebo-controlled trials of intravenous corticosteroid therapy for severe ulcerative colitis have been performed.

A comparative study of intramuscular corticotropin (adrenal corticotropin hormone, ACTH) 80 U per day and cortisone 200 mg per day demonstrated a similar overall benefit in patients with active ulcerative colitis and in the subgroup of patients with a first attack, with a possible advantage for corticotropin in patients in patients with a relapse of established colitis [81]. Subsequent studies in patients with severe ulcerative colitis showed that overall the response to corticotropin 80–120 U per day is similar to that to hydrocortisone 300–400 mg per day, with trends towards better response to hydrocortisone in patients recently treated with corticosteroids and better response to corticotropin in patients not recently treated with corticosteroids [91,92].

Rectal corticosteroids (systemic effect)

Hydrocortisone, prednisolone, methylprednisolone and betamethasone administered directly to the rectum as enemas or suppositories are well absorbed (similar to an oral dose) [94]. Placebo-controlled trials have demonstrated efficacy of rectal administration of hydrocortisone 100 mg and prednisolone 5 mg in patients with active ulcerative proctitis or proctosigmoiditis [95,96]. A meta-analysis suggested that rectally administered mesalamine is superior to rectal steroids for inducing remission [30]. Rectal hydrocortisone 100 mg on 2 nights each week for 6 months did not demonstrate a maintenance benefit compared with placebo [95].

Rectal corticosteroids (non-systemic effect)

Placebo-controlled trials demonstrated that budesonide administered rectally in a suspension enema at doses of 2–8 mg per day was effective in inducing remission in patients with mildly to moderately active left-sided ulcerative colitis, ulcerative proctosigmoiditis and ulcerative proctitis; a 0.5 mg per day dose was not effective [97–101]. Relatively small studies comparing rectally administered budesonide 2.0–2.5 mg per day with other rectal steroids (methylprednisolone 20 mg, prednisolone 25–31 mg, hydrocortisone 100–125 mg) demonstrated similar efficacy rates [30]. Two larger studies demonstrated that budesonide foam 2 mg per day had similar efficacy to budesonide enemas 2 mg per day and hydrocortisone foam [102,103]. Relatively small studies comparing rectally administered budesonide 2 mg per day with rectal mesalamine 1–4 g per day demonstrated similar efficacy rates [30]. However, a meta-analysis suggested that rectally administered mesalamine is superior to rectal steroids for inducing remission [30]. Rectal budesonide 2 mg on 2 nights each week for 6 months did not demonstrate a maintenance benefit compared with placebo [101].

Oral corticosteroids (non-systemic effect)

A placebo-controlled trial demonstrated that oral fluticasone 20 mg per day was not effective in inducing remission in patients with mildly to moderately active distal ulcerative colitis [104]. A study comparing oral fluticasone 20 mg per day with prednisolone 40 mg per day tapered to 10–20 mg per day in patients with active ulcerative colitis showed a greater benefit and more rapid response in the prednisolone group [105]. A comparative study of controlled colonic release budesonide 10 mg and prednisolone 40 mg per day and tapered to 0 mg demonstrated similar response rates in the two groups, with fewer side effects in the budesonide group [106]. Oral beclometasone dipropionate combined with oral mesalamine 3.2 g per day was more effective than mesalamine alone in patients with active ulcerative colitis [107].

Toxicity of corticosteroids

Corticosteroid toxicity occurred frequently in patients with active Crohn's disease treated with prednisone at an initial dose of 60 mg per day tapered over 17 weeks. Toxicities observed included a moon face in 47%, acne in 30%, infection in 27%, ecchymoses in 17%, hypertension in 15%, hirsutism in 7%, petechial bleeding in 6% and striae in 6% [108]. A similar short-term toxicity profile can be expected in patients with ulcerative colitis.

Prolonged corticosteroid therapy at low to intermediate doses (doses frequently utilized in patients with steroid-dependent ulcerative colitis) is associated with the

potential for multiple serious side effects [109]. Hypertension occurs in up to 20% of patients [110]. New onset diabetes mellitus requiring initiation of hypoglycemic therapy occurs at a frequency 2.23 times greater than in the general population [111]. Infection occurs at a frequency of 13–20% [112] and corticosteroids are an independent predictor for serious infection and opportunistic infection [113,114]. Osteonecrosis occurs at a frequency of approximately 5% [115,116]. The frequency of steroid-associated osteoporosis may be as high as 50% [117]. Neurologic side effects occur often and can include myopathy at a frequency of 7% and psychosis at a frequency of 3–5% [118]. Ophthalmologic side effects also occur often and can include cataracts at a frequency of 22% (dose dependent) and glaucoma (frequency unclear, response genetically determined) [119,120]. These frequencies of side effects from prolonged exposure to corticosteroids were generally confirmed in a study of patients with ulcerative colitis who had undergone colectomy for medically refractory disease [121].

Immune modifier medications

The antimetabolites 6-mercaptopurine (Purinethol), its pro-drug azathioprine (Imuran) and methotrexate; the calcineurin inhibitors cyclosporin (Sandimmune, Neoral, Gengraf) and tacrolimus (FK506, Prograf); and the T-cell inhibitor mycophenolate mofetil (Cellcept) are all medications with immune modifier activity. The clinical pharmacology of these medications is reviewed in detail elsewhere in this book.

Azathioprine and 6-mercaptopurine

A placebo-controlled trial of azathioprine 2.5 mg kg^{-1} per day in combination with a tapering dose of corticosteroids in 80 patients with active ulcerative colitis showed no benefit at 1 month and a trend towards a benefit at 1 year that was not significant [122,123]. A comparative study of azathioprine 2.5 mg kg^{-1} per day versus sulfasalazine 65 mg kg^{-1} per day in patients with active ulcerative colitis showed similar efficacy for the two agents [124]. Two small placebo-controlled trials suggested that azathioprine at 1.5 and 2.0–2.5 mg kg^{-1} per day was steroid sparing in patients with steroid-dependent ulcerative colitis [125,126]. A placebo-controlled withdrawal trial in patients maintained with azathioprine 100 mg per day demonstrated a benefit for maintenance of remission [127]. A controlled trial demonstrated that azathioprine 2.0 mg kg^{-1} per day was superior to oral mesalamine 3.2 g per day and azathioprine in patients with steroid-dependent ulcerative colitis [128]. Three other controlled trials with azathioprine have also suggested benefit [129–131]. Overall, these controlled studies generally demonstrate that azathioprine (and by extension 6-mercaptopurine) are effective for steroid sparing and maintenance of remission in patients with chronically active and treatment-refractory ulcerative colitis.

Adverse events occurring in patients with inflammatory bowel disease treated with 6-mercaptopurine and azathioprine include pancreatitis (3%), fever, rash, arthralgias, malaise, nausea, diarrhea, leukopenia (2–5%), thrombocytopenia, infection and hepatitis [132–134]. In a large registry study, 6-mercaptopurine and azathioprine were not an independent predictor for serious infection [113], whereas they were an independent predictor of opportunistic infection [114]. It appears that there is not an increased risk of solid malignancies when 6-mercaptopurine and azathioprine are used as a monotherapy in inflammatory bowel disease [132,135]. In contrast, there appears to be an approximately four-fold increase in risk for non-Hodgkin's lymphoma when these agents are used as monotherapy in patients with Crohn's disease and ulcerative colitis [136]. There does not appear to be an increase in perioperative morbidity or mortality in patients with ulcerative colitis who receive azathioprine or 6-mercaptopurine and then require colectomy within a short period of time [137].

Methotrexate

Two uncontrolled reports of the treatment of ulcerative colitis with methotrexate date occurred in the late 1980s and early 1990s [138,139]. These studies suggested that intramuscular methotrexate 25 mg per week might be beneficial whereas oral methotrexate 15 mg per week appeared less promising. In patients with Crohn's disease, these uncontrolled observations regarding dose response and route of administration have been substantiated, with placebo controlled trials demonstrating efficacy for intramuscular methotrexate 15–25 mg per week but not for oral methotrexate 12.5–15 mg per week [140–143]. In patients with ulcerative colitis, a placebo-controlled trial of oral methotrexate 12.5 mg per week did not demonstrate efficacy for either inducing or maintaining remission [144]. Another placebo-controlled trial of oral methotrexate 15 mg per week also showed minimal benefit [145]. Whether intramuscular or subcutaneous methotrexate at a dose of 25 mg per week would be effective for patients with ulcerative colitis is unknown, although the uncontrolled study discussed above is encouraging [138]. Based on the currently available evidence, patients with ulcerative colitis should not be treated with methotrexate.

Cyclosporin

Multiple uncontrolled studies have reported a beneficial effect of cyclosporin administered at relatively high doses (5–15 mg kg^{-1} per day orally or 2–7 mg kg^{-1} per day intravenously) in patients with severe ulcerative colitis unresponsive to intravenous corticosteroids [146,147]. A placebo-controlled trial demonstrated that the addition of intravenous cyclosporin administered at a dose of 4 mg kg^{-1} per day as a continuous infusion to intravenous corticosteroids was effective for inducing remission in

patients with severe steroid refractory ulcerative colitis (82 versus 0% response) [148]. However, only 45% of the patients treated with cyclosporin had avoided colectomy after 6 months of follow-up [149]. Other uncontrolled studies have subsequently confirmed that colectomy rates over 5–7 years following treatment with intravenous cyclosporin are approximately 50% for patients naïve to azathioprine who receive azathioprine maintenance therapy and can approach 80–90% in patients who do not receive maintenance therapy or who have previously failed azathioprine and then receive azathioprine maintenance therapy after rescue with intravenous cyclosporin [150–153]. Two more controlled trials demonstrated that monotherapy with intravenous cyclosporin 4 mg kg^{-1} per day has efficacy comparable to intravenous corticosteroids or intravenous corticosteroids combined with intravenous cyclosporin in patients with active refractory ulcerative colitis [154,155]. Finally, a dose-finding study demonstrated that intravenous cyclosporin 2 mg kg^{-1} per day had similar efficacy to 4 mg kg^{-1} per day [156]. Overall, these studies demonstrate that intravenous cyclosporin is effective in inducing remission in patients with severe, steroid-refractory ulcerative colitis, but that the benefit is of limited duration unless the patient is naïve to azathioprine or 6-mercaptopurine and subsequently receives that agent for maintenance of remission. A placebo-controlled trial of cyclosporin enemas 400 mg per day was negative [157].

A variety of toxicities have been associated with cyclosporin treatment in patients with inflammatory bowel disease, including headache, tremor, parasthesias, seizures (predominantly with intravenous cyclosporin), hypertrichosis, gingival hyperplasia, renal insufficiency, hypertension, serious and opportunistic infections, hepatotoxicity, nausea and vomiting and anaphylaxis [146, 147,150,158,159]. There is an increased risk of opportunistic infection in patients with inflammatory bowel disease treated with intravenous cyclosporin combined with corticosteroids and azathioprine or 6-mercaptopurine; *Pneumocystis carinii* pneumonia, invasive apergillosis, lung abscess, mycotic aneurysm and overwhelming sepsis have all been reported, with death rates ranging from 1 to 2% in larger series [150,153,158,159]. There does not appear to be an increase in perioperative morbidity or mortality in patients with ulcerative colitis who receive intravenous cyclosporin and then require colectomy within a short period of time [160]. Another study reported that 20% of 99 patients with inflammatory bowel disease treated with intravenous cyclosporin had a decrease in estimated renal function greater than 30% [161]. Results from a previous study suggest that inflammatory bowel disease patients treated with intravenous cyclosporin have a significant likelihood of having histologic evidence of irreversible nephrotoxicity on renal biopsy (which to date has not been performed in patients with inflammatory bowel disease) [162].

Tacrolimus

A placebo-controlled trial demonstrated that oral tacrolimus administered at a high dose (target trough concentration of 10–15 ng ml^{-1}) or a low dose (target trough concentration of 5–10 mg ml^{-1}) for 14 days is effective in hospitalized patients with severely active ulcerative colitis that is steroid dependent or steroid refractory [163]. High-dose tacrolimus was more effective than low-dose tacrolimus. Nephrotoxicity was reported in 5% of patients in the high-dose tacrolimus group, 5% of patients of patients in the low-dose tacrolimus group and none in the patients treated with the placebo [163]. Tacrolimus has a toxicity profile that is generally similar to that of cyclosporin.

Mycophenolate mofetil

A small controlled trial reported that mycophenolate mofetil 15 mg kg^{-1} per day had similar efficacy to azathioprine 2.5 mg kg^{-1} per day in patients with chronically active Crohn's disease [164]. In contrast, a small controlled trial of mycophenolate mofetil 20 mg kg^{-1} per day versus azathioprine 2.0 mg kg^{-1} per day in patients with chronically active ulcerative colitis demonstrated a superior outcome with azathioprine [165]. Based on the currently available evidence, patients with ulcerative colitis should not be treated with mycophenolate mofetil.

Anti-tumor necrosis factor agents

Infliximab, CDP571 and adalimumab are all monoclonal antibodies to tumor necrosis factor alpha (TNFα).

Infliximab

Infliximab is a mouse/human chimeric immunoglobulin G1 (IgG1) monoclonal antibody to TNF. Two placebo-controlled trials have demonstrated that infliximab at doses of 5 and 10 mg kg^{-1} is effective for the treatment of outpatients with moderately to severely active ulcerative colitis unresponsive to conventional therapy [166]. The recommended induction regimen is three doses of 5 mg kg^{-1} administered at 0, 2 and 6 weeks. In addition, these two placebo-controlled trials demonstrated that infliximab 5 and 10 mg kg^{-1} every 8 weeks is effective for maintenance of response and remission, mucosal healing and steroid sparing [166]. One small placebo-controlled trial of infliximab in outpatients with moderately active ulcerative colitis did not demonstrate efficacy [167]. Two placebo-controlled trials have reported that infliximab is effective in hospitalized patients with severely active ulcerative colitis who are failing intravenous corticosteroids [168,169] and two additional small controlled trials have suggested that infliximab has similar efficacy to intravenous corticosteroids in patients with severely active ulcerative colitis who are just entering hospital [170,171].

Antibodies to infliximab (ATIs), also called human anti-chimeric antibodies (HACAs), occurred in 28% of patients

with Crohn's disease who received a single induction dose of infliximab compared with 6–9% of patients who received three induction doses and then systematic maintenance dosing every 8 weeks [172]. Concomitant therapy with azathioprine, 6-mercaptopurine or methotrexate was also protective [172]. Other studies in patients with Crohn's disease which used a difference assay for ATI reported independent protective effects from multiple induction doses, immunosuppressive therapy and pretreatment with 200 mg of intravenous hydrocortisone [173,174]. Patients who developed ATI had an increase rate of infusion reactions and a shortening of the duration of benefit by 50% [173,174]. In order to reduce the chance of ATI formation, it is recommended that patients have three induction doses of infliximab and then systematic maintenance dosing or that they receive concomitant therapy with azathioprine, 6-mercaptopurine or methotrexate or pretreatment with hydrocortisone [173,174]. Because of the increased risk of opportunistic infection and hepatosplenic T cell lymphoma (see below), consideration can be given to using monotherapy with infliximab.

Adverse events related to immunogenicity to infliximab include acute infusion reactions (shortness of breath, chest pain, palpitations, flushing, fever, headache and occasionally urticaria, occasionally hypotension) during the infusion [175] and delayed hypersensitivity-type reactions (arthralgias, back pain, myalgias, fever, skin rash, leukocytosis) 5–9 days after an infliximab infusion, usually after a "drug holiday" from infliximab [176]. Other adverse events associated with infliximab include development of new anti-nuclear antibodies and double-stranded DNA antibodies that are sometimes associated with drug-induced lupus, demyelination disorders, exacerbation of cardiac failure, serious and opportunistic infections and lymphoma [177]. Reactivation of latent tuberculosis is a particular concern [178]. In a large registry study, infliximab was not an independent predictor for serious infection [113], whereas it was an independent predictor of opportunistic infection [114]. The combination of infliximab and azathioprine or 6-mercaptopurine was associated with a greater risk of opportunistic infection than either agent alone [114]. Infliximab has also been associated with an increased risk of lymphoma, particularly when used in combination with azathioprine or 6-mercaptopurine [177,179]. These data have led to an evolution in clinical practice to administer infliximab as a monotherapy.

Adalimumab (D2E7)

Adalimumab (D2E7) is a fully human IgG1 monoclonal antibody to TNF. Subcutaneous adalimumab is effective for the treatment of Crohn's disease [180–182]. The approved dosing regiment is an induction regimen of 160 mg at week 0 and 80 mg at week 2 followed by maintenance therapy with 40 mg every other week beginning at week 4. Some patients require dose escalation during the maintenance phase to 40 mg weekly. A pilot study suggested that adalimumab may be effective for the treatment of ulcerative colitis [183]. Phase 3 trials of subcutaneous adalimumab for induction and maintenance of remission in patients with active ulcerative colitis are under way.

Golimumab

Golimumab is a fully human IgG1 monoclonal antibody to TNF. Subcutaneous golimumab is effective for the treatment of rheumatoid arthritis. Phase 3 trials of subcutaneous and intravenous golimumab for induction and maintenance of remission in patients with active ulcerative colitis are under way.

CDP571

CDP571 is a humanized IgG4 monoclonal antibody to TNF. Two Phase 3 trials failed to demonstrate efficacy of CDP571 for Crohn's disease [184,185]. A pilot study of CDP571 in patients with ulcerative colitis suggested benefit [186].

Other biologic agents

Natalizumab

Natalizumab is a humanized IgG4 monoclonal antibody to α4 integrin that selectively inhibits leukocyte adhesion. Natalizumab is effective for induction and maintenance of remission in Crohn's disease [187–189]. It use is restricted to patients who have failed other therapies because it has been associated with reactivation of the human JC Polyoma virus, leading to progressive multifocal leukoencephalopathy (PML) [190]. A small pilot study of natalizumab suggested a benefit in patients with active ulcerative colitis [191].

MLN-02

MLN-02 is a humanized IgG1 monoclonal antibody to α4β7 integrin that selectively inhibits leukocyte adhesion in the mucosa. A Phase 2 placebo-controlled trial demonstrated that MLN-02 is effective for active ulcerative colitis. Phase 3 studies are planned.

Alicaforsen (Isis 2302, antisense to ICAM-1)

Alicaforsen (Isis 2302) is a 20-base phosphorothioate oligodeoxynucleotide designed to hybridize to a sequence in the 3′ untranslated region of the human ICAM-1 message. The oligonucleotide–RNA heterodimer so formed serves as a substrate for the ubiquitous nuclease RNase-H with subsequent cleavage and reduction in cellular specific message content and consequent reduction in ICAM-1 expression. A small Phase 2 trial suggested a benefit of alicaforsen enemas for active distal ulcerative colitis [192], but two additional larger controlled trials did not demonstrate clear evidence of efficacy [193,194].

RDP58
RDP58 is an anti-inflammatory peptide consisting of nine D-amino acids and glycine. RDP58 blocks the p38 and JNK MAP kinase pathways and inhibits the synthesis of TNFα, interferon-γ and interleukin 12 in animal models. It is not systemically bioavailable. A Phase 2 trial of RDP58 for active ulcerative colitis demonstrated efficacy for a clinical remission endpoint [195]. A subsequent controlled trial did not demonstrate that RDP58 in combination with oral mesalamine 2.4 g per day was superior to oral mesalamine 4.8 g per day and development was discontinued (data not published).

Daclizumab and basiliximab
Interleukin 2 is produced by Th1 cells after interleukin 12, interferon-γ and interleukin 18 induce differentiation of naïve T helper cells to Th1 cells. Daclizumab is a humanized monoclonal antibody to the interleukin 2 receptor which blocks the binding of interleukin 2 to the interleukin 2 receptor. A Phase 2a study of daclizumab suggested benefit in patients with refractory ulcerative colitis [196]. A subsequent placebo-controlled Phase 2 dose-finding trial in patients with active ulcerative colitis was negative [197]. Basiliximab is a chimeric monoclonal antibody to the interleukin 2 receptor. A Phase 2a study of basiliximab suggested benefit in patients with steroid-dependant ulcerative colitis [198,199]. A placebo-controlled Phase 2 study in patients with steroid-refractory ulcerative colitis is under way.

Visilizumab
Visilizumab is a humanized monoclonal antibody to CD3 that induces activated T cell apoptosis. A Phase 1/2a dose-finding study in hospitalized patients with ulcerative colitis failing intravenous corticosteroids suggested clinical benefit [200]. A placebo-controlled trial failed to demonstrate efficacy (data unpublished).

Epidermal growth factor enemas
Human epidermal growth factor is a mitogenic peptide produced by duodenal Brunner's and salivary glands that stimulates cell proliferation in the gastrointestinal tract. A Phase 2 placebo-controlled trial of recombinant epidermal growth factor enemas demonstrated efficacy in patients with active distal ulcerative colitis [201].

Repifermin
Fibroblast growth factor 7 (also known as keratinocyte growth factor 1) is a potent stimulant of intestinal epithelial cells. Repifermin (fibroblast growth factor 10 also known as keratinocyte growth factor 2) is a homologue of keratinocyte growth factor 1. A placebo-controlled Phase 2 study of recombinant intravenous repifermin in patients with active ulcerative colitis did not demonstrate efficacy [202].

Tetomilast (OPC-6535)
Tetomilast (OPC-6535) is a novel thiazole compound that inhibits phosphodiesterase-4 and proinflammatory functions of leukocytes including superoxide production and cytokine release. A Phase 2 placebo-controlled study of tetomilast in patients with active ulcerative colitis suggested possible benefit [203]. Two Phase 3 induction trials and one Phase 3 maintenance trial failed to demonstrate efficacy (data unpublished).

Abatacept
Abatacept is a fusion protein consisting of the extracellular domain of human soluble CTLA-4 linked to the modified Fc (hinge, CH2 and CH3 domains) portion of human IgG1. Abatacept is effective for the treatment of rheumatoid arthritis. Abatacept is in Phase 3 placebo-controlled trials for induction and maintenance of remission in patients with active ulcerative colitis.

Anti-platelet activating factor agents
Platelet activating factor is elevated in the colonic mucosa and the stool of patients with ulcerative colitis. Placebo-controlled trials demonstrated that antibodies directed against platelet activating factor are not effective in patients with moderately or severely active ulcerative colitis [204,205].

Interferon α
The interferon αs are produced naturally by virally infected cells to induce resistance of the cells to viral infection. Recombinant interferon α-2a, interferon α-2b, interferon α-*n* and pegylated interferon-α are used clinically to treat HIV-related Kaposi's sarcoma, melanoma, chronic hepatitis B infection and chronic hepatitis C infection [206]. A pilot study of interferon α-2a in patients with active ulcerative colitis demonstrated possible benefit [207]. Subsequently, controlled trials with interferon α2a and pegylated α in patients with active ulcerative colitis failed to demonstrate efficacy [208,209].

Interferon β-1a
Interferon β is produced naturally by virally infected cells to induce resistance of the cells to viral infection. Recombinant interferon-β is used clinically to treat multiple sclerosis [206]. Controlled trials of interferon β-1a in patients with active ulcerative colitis failed to demonstrate efficacy [210,211].

Interleukin-10
Interleukin-10 is a cytokine produced by T-helper type 2 cells, B cells, monocytes and macrophages that has multiple immune modifier effects. A small controlled trial of recombinant human interleukin-10 (rhuIL-10) (Schering Plough Research Institute, Kenilworth, NJ, USA) in patients with mild to moderately active Crohn's

disease demonstrated a modest beneficial effect [212]. Two subsequent placebo-controlled trials in Crohn's disease were negative [213,214]. A placebo-controlled trial of interleukin-10 in active ulcerative colitis failed to demonstrate efficacy [215].

Miscellaneous agents

4-Aminosalicylate

4-Aminosalicylate (*para*-aminosalicylate, PAS) is an isomer of 5-aminosalicylate, the active component of sulfasalazine. Controlled trials with oral or rectal enema formulations of 4-aminosalicylate have shown superiority to placebo and equivalence to corticosteroids and 5-aminosalicylate [216–223].

Nicotine

Case–control studies have shown that smoking protects against the development of ulcerative colitis [224]. There are a variety of possible reasons for this protective effect [225]. Two placebo-controlled trials reported that transdermal nicotine administered at the highest tolerated dose of nicotine (up to 25 and 22 mg per 24 h, respectively) was effective for active ulcerative colitis [226,227]. A comparator-controlled trial demonstrated that transdermal nicotine at the highest tolerated dose (up to 25 mg per 16 h) had similar efficacy to prednisolone for active ulcerative colitis [228]. A comparator-controlled trial demonstrated that transdermal nicotine (15 mg per 24 h) was superior to oral mesalamine for active left-sided ulcerative colitis refractory to rectal mesalamine [229]. A placebo-controlled trial demonstrated that a lower dose of transdermal nicotine (15 mg per 16 h) was not effective for maintenance of remission [230]. A placebo-controlled trial of nicotine enemas was negative [231]. Adverse events from transdermal nicotine occur commonly and include contact dermatitis, nausea, vomiting, headaches, sleep disturbance, diaphoresis, tremor and lightheadedness [225–228,230].

Heparin

Ulcerative colitis is a hypercoagulable state and heparin has been evaluated as a possible therapy in ulcerative colitis because of its anti-inflammatory and anti-coagulant properties [232]. A placebo-controlled trial of porcine heparin 10,000 units 2–3 times daily administered subcutaneously in patients with active ulcerative colitis demonstrated efficacy [233]. In a small comparative trial in patients with severely active ulcerative colitis or Crohn's colitis, intravenous heparin had a similar response to intravenous corticosteroids [234]. In contrast, in a larger comparative trial, intravenous heparin was less effective than intravenous corticosteroids and was associated with more bleeding complications [235]. Placebo-controlled trials of low molecular weight heparin (tinzaparin, enoxaprin, reviparin) failed to demonstrate efficacy [236–238]. These results were confirmed in a meta-analysis that demonstrated that heparin and low molecular weight heparin are not effective for active ulcerative colitis [239].

Short-chain fatty acids

Bacterial fermentation of carbohydrates in the colonic lumen to *n*-butyrate and other short-chain fatty acids (SCFAs) is the major luminal source of energy for colonocytes; there is some evidence that colonocytes in patients with ulcerative colitis have reduced capacity to oxidize SCFAs [240]. Four placebo-controlled trials of SCFAs have been conducted [241–244]. Two of three studies comparing SCFA enemas with placebo [242–244] and two of two studies comparing butyrate enemas with placebo [241,242] did not demonstrate efficacy in active left-sided ulcerative colitis. A 60 g oat bran diet raised fecal butyrate concentrations in a controlled trial in patients with active ulcerative colitis, but the implication of this physiologic endpoint for clinical efficacy is unclear [245]. A placebo-controlled trial of an oral nutritional supplement enriched with fish oil, soluble fiber and antioxidants did not demonstrate efficacy for inducing remission but did demonstrate a steroid-sparing benefit [246]. Another placebo-controlled trial of essential fatty acid supplementation did not demonstrate efficacy for maintenance of remission in patients with ulcerative colitis [247].

Fish oil

Fish oil (eicosapentanoic acid and docosahexenoic acid) inhibits 5-lipoxygenase and other enzymes involved in arachidonate metabolism of leukotriene B_4. A small pilot placebo-controlled trial reported that fish oil may be effective in patients with active ulcerative colitis [248]. Subsequently, four additional placebo-controlled trials failed to demonstrate that fish oil was effective for inducing clinical response or remission in active ulcerative colitis [249–251] or for maintenance [252]. A controlled trial comparing fish oil and sulfasalazine did not demonstrate efficacy for fish oil [253]. In contrast to these negative results for clinical endpoints, fish oil did lead to a significant reduction in colonic concentrations of leukotriene B_4 [249].

Antibiotics

Given the hypothesis that luminal bacterial may play a role in the pathogenesis of ulcerative colitis, antibiotics have been evaluated as potential therapies. Placebo-controlled trials of oral tobramycin in patients with mild to moderately active ulcerative colitis demonstrated short-term induction efficacy but no maintenance benefit [254,255]. Small comparative trials demonstrated that metronidazole was less effective that sulfasalazine for the treatment of active ulcerative colitis, but suggested that metronidazole might have a maintenance benefit [256,257]. One of two placebo-controlled trials of oral ciprofloxacin in patients with active ulcerative colitis demonstrated

efficacy [258,259]. Placebo-controlled trials in patients with severely active ulcerative colitis demonstrated that oral vancomycin [260], intravenous metronidazole [261], intravenous tobramycin and metronidazole [262] and intravenous ciprofloxacin [263] are not effective. A small placebo controlled trial with the non-absorbable antibiotic rifaximin did not show a clear benefit [264]. Because the results of these controlled trials are nearly uniformly negative, antibiotics do not have a role in the treatment of ulcerative colitis, unless a documented or suspected coexisting infection with a specific infectious organism is present.

Zileuton and other leukotriene inhibitors

Large controlled trials of compounds which act to inhibit 5-lipoxygenase were negative [265–268]. Zileuton was not more effective than placebo in patients with mild to moderately active ulcerative colitis [266,267] and was less effective than 5-aminosalicylate 1.6 g per day and similar to placebo for maintenance of remission in patients with ulcerative colitis [268]. The Merck compound MK-591 was not effective for inducing clinical response and remission but did result in decreased colonic luminal concentrations of leukotriene B_4 as compared with placebo in patients with mild to moderately active ulcerative colitis [265].

Ridogrel inhibits thromboxane synthase. Although a pilot study showed that ridogrel could reduce colonic mucosal thromboxane B_2 release in patients with ulcerative colitis [269], two controlled trials did not demonstrate that ridogrel is effective in patients with active ulcerative colitis [270].

Allopurinol

Allopurinol inhibits oxygen free radical formation by blocking xanthine oxidase. A controlled trial did not demonstrate that allopurinol combined with mesalamine was more effective than mesalamine alone for maintaining remission in patients with ulcerative colitis [271].

Bismuth

Bismuth compounds exhibit both antibacterial and antidiarrheal properties. Uncontrolled studies of tripotassium dicitratobismuth enemas [272] and bismuth subsalicylate enemas [273] suggested possible efficacy in active distal ulcerative colitis. A small controlled trial showed similar efficacy for bismuth citrate enemas and 5-aminosalicylate enemas in patients with active distal ulcerative colitis [274]. The systemic absorption of bismuth after rectal administration was low.

Lidocaine/ropivacaine

Local anesthetic agents including both lidocaine and ropivacaine may have anti-inflammatory effects. Uncontrolled studies reported that 2% lidocaine enemas [275,276] and ropivacaine gel enemas [277] might be of clinical benefit in patients with active distal ulcerative colitis. A placebo-controlled of ropivacaine enemas in patient with active distal ulcerative colitis was completed, but to date the results have not been published. Systemic absorption of topical anesthetic agents after rectal administration is low [278,279].

Rosiglitazone

Rosiglitazone is a ligand of peroxisome proliferator-activated receptor gamma (PPAR γ). PPAR γ is reduced in patients with ulcerative colitis and treatment of animal models of colitis with PPAR γ ligand therapy suggested benefit, as did an uncontrolled pilot study of rosiglitazone 4 mg twice daily in patients with active distal ulcerative colitis [280]. A placebo-controlled trial of rosiglitazone demonstrated efficacy in patients with active ulcerative colitis [281].

Levamisole

Levamisole is an anti-helminthic agent that is used as an adjuvant therapy with 5-fluorouracil for the treatment of colorectal cancer and also has immunostimulatory activity. Placebo-controlled induction and maintenance of remission trials of levamisole in patients with ulcerative colitis did not demonstrate efficacy [282,283].

Disodium cromoglycate

Disodium cromoglycate is used to treat allergic diseases; its mechanism of action is through stabilization of mast cells. A small randomized pilot study in patients with ulcerative colitis demonstrated possible efficacy [284]. Subsequent larger controlled induction and maintenance of remission trials in patients with ulcerative colitis failed to show efficacy [285–288].

Apheresis

Apheresis is comprised of extracorporeal removal of cells from the circulation. Strategies for apheresis include simple centrifugation and the use of fibers or columns to remove specific components from whole blood resulting in the removal of up to four times as many cells. Two types of selective apheresis filters have been evaluated for the treatment of ulcerative colitis. In both instances, whole venous blood is perfused through an adsorption column. Adacolumn (Japan Immunoresearch Laboratories, Takasaki, Japan) is designed as a column packed with cellulose acetate beads which remove activated granulocytes and monocytes (but not lymphocytes or platelets). Cellsorba FX (Asahi Medical, Tokyo, Japan) is designed as a column that contains a filter composed of non-woven polyester fibers which removes granulocytes, monocytes, lymphocytes and some platelets.

A non-blinded, multi-center trial comparing Cellsorba apheresis as an adjunct to the current treatment regimen (including prednisone) with high-dose prednisone in

patients with active ulcerative colitis was performed [289]. Patients treated with Cellsorba apheresis were reported to show greater clinical benefit, greater decreases in clinical and endoscopic activity, greater steroid sparing and fewer adverse events than patients treated with high-dose prednisone. These data are difficult to interpret because of the lack of blinding and the absence of a sham apheresis control group.

Uncontrolled pilot studies have suggested that Adacolumn apheresis may be of clinical benefit for inducing response in patients with active ulcerative colitis [290]. However, a sham-controlled trial of Adacolumn apheresis in patients with active ulcerative colitis disease failed to demonstrate efficacy [291].

Aloe vera

Aloe vera is a herb that has many anti-inflammatory properties. A small placebo-controlled trial of oral aloe vera gel failed to demonstrate efficacy [292].

Hydroxychloroquine and chloroquine

Hydroxychloroquine and chloroquine are 4-aminoquinolones that have immunomodulatory and anti-inflammatory properties. One possible mechanism of actions is lysosomal stabilization [293]. These drugs are effective for rheumatoid arthritis. An uncontrolled study failed to demonstrate efficacy of hydroxychloroquine in active ulcerative colitis [294]. A comparator controlled trial in active ulcerative colitis showed similar efficacy for sulfasalazine and chloroquine [295].

Probiotic therapy with *E. coli* Nissle 1917, VSL#3, *Trichuris suis* and *Saccharomyces boulardi*

Two small controlled studies have reported that *E. coli* Nissle 1917 and mesalamine had similar efficacy for maintenance of remission in patients with ulcerative colitis [296,297]. These trials lacked sufficient power to demonstrate equivalence (non-inferiority) formally. An additional non-inferiority trial with sufficient power demonstrated that *E. coli* Nissle 1917 and mesalamine were equivalent for maintenance of remission in ulcerative colitis [298]. VSL#3 is a probiotic formulation that contains 10^{11} g^{-1} of viable lyophilized bacteria: four strains of lactobacilli (*L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *L. casei*), three strains of bifidobacteria (*B. infantis*, *B. longum*, *B. breve*) and one strain of *Streptococcus salivarius* subsp. *thermophilus*. An uncontrolled pilot study reported that VSL#3 might be of benefit in patients with active ulcerative colitis [299]. A controlled trial reported that VSL#3 in combination with balsalazide 2.25 g per day was more effective than balsalazide alone or mesalamine in patients with active ulcerative colitis [300].

Helminths such as *Trichuris suis* can induce T cells from intestinal mucosal to produce Th2 and regulatory cytokines [301]. A small uncontrolled study reported that the helminth *Trichuris suis* might be effective in patients with active ulcerative colitis [302]. A small placebo-controlled trial reported efficacy for induction of clinical response but not clinical remission in active ulcerative colitis [303].

Saccharomyces boulardi is a non-pathogenic yeast that has been used to treat *Clostridium difficile* colitis. An uncontrolled pilot study suggested that *Saccharomyces boulardi* might be of benefit in patients with active ulcerative colitis [304].

Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) is a steroid hormone that is effective for systemic lupus erythematosus. It has a variety of properties including inhibition of NFKβ, interleukin-6, interleukin-12 and PPARα. A pilot study reported that DHEA might be of clinical benefit for inducing response and remission in patients with active ulcerative colitis [305].

Protease inhibition with the tryptase inhibitor APC 2059

Inhibition of tryptase and other protease inhibitors could potentially block mast cell degranulation in intestinal mucosa and thus block release of inflammatory mediators. Uncontrolled pilot studies of APC2059 (a highly specific tryptase inhibitor) and the Bowman–Birk protein (a soybean extract with more broad protease inhibitor activity) showed modest clinical benefit in patients with active ulcerative colitis [306,307].

Treatment indications and algorithm and specific treatment approaches

The indications for therapy in patients with ulcerative colitis are summarized in Table 28.5 and a suggested treatment algorithm is proposed in Figure 28.3. The specific approaches to the medical treatment of patients with proctitis/distal ulcerative colitis, extensive ulcerative colitis, refractory ulcerative colitis and severe ulcerative colitis are each reviewed separately below.

Proctitis/distal ulcerative colitis

The treatment of patients with mildly to moderately active proctitis/distal ulcerative colitis may include rectal therapy, oral therapy or a combination of both. Patient preferences with regard to rectal therapy for induction of remission must be considered. Rectal mesalamine is more efficacious than orally administered mesalamine [31] or rectally administered corticosteroids [30]. Thus, for patients who will accept rectal therapy, mesalamine suppositories (500 mg b.i.d.) for proctitis and mesalamine enemas (1 or 4 g nightly) for distal colitis are the treatment of choice. For patients who prefer oral therapy, oral mesalamine 2.0–4.8 g per day, sulfasalazine 2–4 g per day

Table 28.5 Ulcerative colitis: evidence-based indications for treatment.

Drug	Dose	Mildly to moderately active		Refractory	Severely active	Remission maintenance	
		Distal	Extensive			Distal	Extensive
Sulfasalazine	Induction 2–6 g per day Maintenance 2–4 g per day	Yes	Yes	Yes*	No*	Yes	Yes
Mesalamine suppositories	Induction 0.5–1.5 g per day Maintenance 0.5–1 g per day	Yes	No	Yes*	No†	Yes	No
Mesalzine enemas	Induction 1–4 g per day Maintenance 1–4 g per day	Yes	Yes (adjunctive therapy)	Yes*	No†	Yes	No
Oral mesalamine	Induction 1.6–4.8 g per day Maintenance 0.75–4 g per day	Yes	Yes	Yes*	No†	Yes	Yes
Olsalazine	Maintenance 1–2 g per day	No[1]‡	No‡	No‡	No‡	Yes	Yes
Balsalazide	Induction 6.75 g per day (equivalent to mesalamine 2.4 g per day) Maintenance 4 g per day (equivalent to mesalamine 1.4 g per day)	Yes	Yes	Yes*	No†	Yes	Yes
Hydrocortisone enemas	Induction 100 mg per day	Yes	No	Yes*	Yes§	No	No
Budesonide enemas	Induction 2–8 mg per day	Yes	No	Yes*	Yes§	No	No
Oral corticosteroids Cortisone	Induction 100 mg per day	Yes	Yes	Yes*	No	No	No
Oral corticosteroids Prednisone	Induction 40–60 mg per day	Yes	Yes	Yes*	No	No	No
Intravenous corticosteroids Prednisolone	Induction 60 mg per day	No	No	Yes**	Yes	No	No
Oral azathioprine	Maintenance 2–2.5 mg kg^{-1} per day	No	No	Yes	No	Yes	Yes
Intravenous cyclosporin	Induction 2–4 mg kg^{-1} per day	No	No	No	Yes	No	No
Oral tacrolimus	Induction serum trough levels of 5–15 ng ml^{-1}	No	No	No	Yes	No	No
Intravenous infliximab	Induction 5 or 10 mg kg^{-1} at weeks 0, 2 and 6 Maintenance 5 or 10 mg kg^{-1} every 8 weeks	Yes	Yes	Yes	Yes	Yes	Yes

*Typically continued as a carryover of treatment for mildly to moderately active disease when additional agents are added.
†Typically discontinued because of the possibility of intolerence to sulfasalazine, mesalamine or balsalazide.
‡Diarrhea occurs frequently at higher doses in patients with active ulcerative colitis.
§Adjunctive therapy to intravenous corticosteroids.
**Some patients who fail oral corticosteroids will respond to hospitalization with intravenous administration of corticosteroids.
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and balsalazide 6.75 g per day are all equivalent first-line treatments [60–62,308,309]. Olsalazine should be avoided due to a high frequency of diarrhea in patients with active ulcerative colitis. Sulfasalazine therapy is associated with more side effects but is less expensive than oral mesalamine or balsalazide. In one study, the combination of mesalamine enemas 4 g per day and oral mesalamine 2.4 g per day was more effective than either agent alone [31]. Based on these data, many clinicians use rectal and oral mesalamine in combination to induce remission and then continue the oral mesalamine for maintenance of remission. In patients who fail to respond to oral or rectal

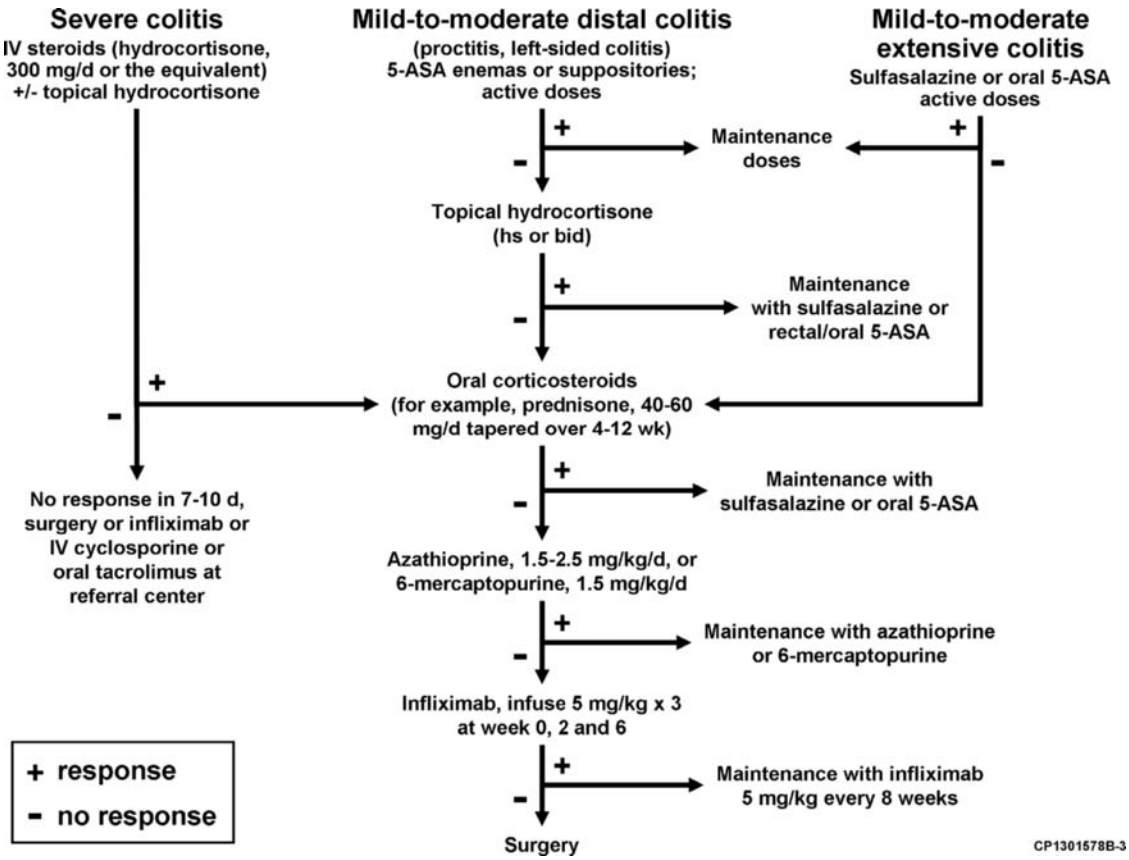

Figure 28.3 Suggested treatment guidelines for ulcerative colitis are offered in this algorithm.

mesalamine, sulfasalazine or balsalazide; rectal corticosteroids can be tried. When all of these treatment approaches are ineffective, then oral prednisone is added to the other oral and rectal therapies. The preferred initial prednisone dosing regimen is 40 mg per day administered as a single dose. The optimal tapering strategy has not been determined, but experienced clinicians will typically treat the patient with prednisone 40 mg per day for 2–4 weeks, then taper by 5 mg per week to a daily dose of 20 mg per day, then slow the taper to 2.5 mg per week until prednisone is discontinued.

Once the patients have achieved clinical remission, a long-term maintenance of remission strategy must be undertaken to avoid relapse. Again, patient preferences with regard to rectal therapy must be considered. Rectal mesalamine is more efficacious than orally administered sulfasalazine or mesalamine for maintaining remission [310,311]. Again, for patients who will accept rectal therapy, mesalamine suppositories (500 mg once or twice daily) for proctitis and mesalamine enemas (4 g nightly, every other night or every third night) for distal colitis are the treatment of choice. For patients who prefer oral therapy, oral mesalamine 1.6–4.8 g per day, sulfasalazine 2–4 g per day, olsalazine 1.0 g per day and balsalazide 3.0–6.75 g per day are all equivalent first-line maintenance treatments [308,309,312]. Sulfasalazine therapy is associated with more side effects but is less expensive than oral mesalamine, olsalazine or balsalazide. In one study, the combination of mesalamine enemas 4 g per day and oral mesalamine 1.2 g per day was more effective than oral mesalamine alone [313]; however, most patients find maintenance with both oral and rectal mesalamine unacceptable. There is no agreement among expert clinicians as to whether patients should taper rectal mesalamine to the least frequent effective dose interval or oral mesalamine, sulfasalazine, olsalazine or balsalazide to the lowest effective dose, or instead continue maintenance therapy with the same dose interval or dose required to induce remission. The former strategy is less expensive and may improve patient compliance by reducing the amount and frequency of medication administered, whereas the latter strategy may result in effective maintenance of remission in a larger proportion of patients. Rectal corticosteroids are not effective for maintaining remission and should not be used for that indication. Clinical trials have demonstrated that oral corticosteroids at low to moderate doses are not effective for maintaining remission. Nevertheless, some patients who respond to higher doses of prednisone will relapse with steroid tapering and can be maintained nearly asymptomatic by increasing the prednisone dose

back to 15–25 mg per day. These patients are classified as "steroid dependent" [314]. Because of the toxicity associated with long-term corticosteroid therapy, this is not an acceptable form of maintenance therapy and such patients should be treated for refractory disease as described below.

Extensive ulcerative colitis

Oral administration of medications that deliver 5-aminosalicylate to the colon is the treatment of choice in patients with mildly to moderately active extensive ulcerative colitis. Oral mesalamine 2.0–4.8 g per day, sulfasalazine 2–4 g per day and balsalazide 6.75 g per day are all equivalent first-line treatments [60–62,308,309]. Sulfasalazine therapy is associated with more side effects but is less expensive than oral mesalamine or balsalazide. Olsalazine should be avoided due to a high frequency of diarrhea in patients with active ulcerative colitis. There is not a clear dose response for oral medications that deliver 5-aminosalicylate [39,40,46,48,49]. There is no agreement among expert clinicians as to whether the preferred strategy is to begin with the lowest dose proven to be effective for active disease, increasing the dose in those patients who fail to respond, or rather to begin with the maximally tolerated dose and then titrate the dose downwards when the patient comes into clinical remission. When treatment with one of these agents at an optimal dose has failed, then oral prednisone is added to the oral mesalamine, sulfasalazine or balsalazide. The preferred initial prednisone dosing regimen is 40 mg per day administered as a single dose, with tapering as described above for proctitis/distal ulcerative colitis.

Once the patients have achieved clinical remission, a long-term maintenance of remission strategy must be undertaken to avoid relapse. Oral therapy with a drug that delivers 5-aminosalicylate to the colon is the treatment of choice. Oral mesalamine 1.6–4.8 g per day, sulfasalazine 2–4 g per day, olsalazine 1.0 g per day and balsalazide 3.0–6.75 g per day are all equivalent first-line maintenance treatments [308,309,312]. Sulfasalazine therapy is associated with more side effects but is less expensive than oral mesalamine, olsalazine or balsalazide. As described above for patients with proctitis/distal ulcerative colitis, there is no agreement among expert clinicians as to whether patients with extensive ulcerative colitis should taper oral mesalamine, sulfasalazine, olsalazine or balsalazide to the lowest effective dose, or instead continue maintenance therapy with the same dose required to induce remission. Clinical trials have demonstrated that oral corticosteroids at low to moderate doses are not effective for maintaining remission. As discussed above for proctitis/distal ulcerative colitis, treatment with prednisone 15–25 mg per day in patients with extensive ulcerative colitis who are "steroid dependent" is not acceptable because of the toxicity associated with long-term corticosteroid therapy. Such patients should be treated for refractory disease as described below.

Refractory ulcerative colitis

Patients mildly to moderately active ulcerative colitis who fail to respond to oral prednisone at dose of 40–60 mg per day in combination with mesalamine, sulfasalazine or balsalazide can be considered to have refractory ulcerative colitis. One potential approach to treatment is hospitalization for intravenous administration of corticosteroids. The rationale for this treatment approach is a clinical trial that demonstrated greater mean serum prednisolone concentrations with intravenous compared with oral dosing [85].

One treatment option for patients who have failed combination therapy with maximum doses of oral and rectal mesalamine and oral corticosteroids is azathioprine or 6-mercaptopurine. The prodrug azathioprine is approximately 50% 6-mercaptopurine by molecular weight, requiring a conversion factor of 2 to convert a dose of azathioprine to a therapeutically equivalent dose of 6-mercaptopurine. The doses of azathioprine and 6-mercaptopurine shown to be effective for ulcerative colitis and Crohn's disease in controlled trials range from 100 mg per day to 3.0 mg kg^{-1} per day. For patients with normal azathioprine and 6-mercaptopurine metabolism [based on normal thiopurine methyltransferase (TPMT) activity], a starting azathioprine dose of 2.0–2.5 mg kg^{-1} per day or a 6-mercaptopurine dose of 1.0–1.5 mg kg^{-1} per day is recommended. Patients with decreased TPMT activity should have their starting azathioprine or 6-mercaptopurine dose reduced by 50% to 1.0–1.25 mg kg^{-1} per day and 0.5–0.75 mg kg^{-1} per day, respectively. Patients with absent TPMT activity should not be treated with azathioprine or 6-mercaptopurine. Azathioprine and 6-mercaptopurine are slow-acting anti-metabolite drugs, requiring at least 1–2 months and perhaps 3–4 months to reach the full clinical effect. Hence concomitant therapy with corticosteroids should not be tapered below a dose of 15–20 mg per day for 2–3 months in patients who are beginning azathioprine or 6-mercaptopurine. Concomitant therapy with mesalamine, sulfasalazine, olsalazine or balsalazide is continued in most cases. Measurement of a total leukocyte count every 1–2 months as long as patients are receiving azathioprine or 6-mercaptopurine is mandatory to monitor for leukopenia. Indications for treatment with azathioprine or 6-mercaptopurine in patients with ulcerative colitis include induction of remission in steroid-refractory disease, steroid sparing in steroid-dependent disease and maintenance of remission in patients who have failed maintenance therapy with high-dose mesalamine, sulfasalazine, olsalazine or balsalazide.

Infliximab should primarily be used in patients with active ulcerative colitis refractory to corticosteroids and/or

immunosuppressive agents. The recommended induction regimen for infliximab is three doses of 5 mg kg^{-1} administered at 0, 2 and 6 weeks. Discontinuation of infliximab leads to an increased rate of HACA formation that may limit the benefit and tolerability of future therapy. Therefore, patients should be selected carefully for initiation of infliximab therapy and, in most instances, once infliximab is initiated it should be continued indefinitely in responding patients as an every 8 week 5 mg kg^{-1} maintenance therapy. Patients who initially respond to infliximab 5 mg kg^{-1} and later lose their response may benefit from dose escalation by either increasing the dose to 10 mg kg^{-1} or shortening the dosing interval for a 5 mg kg^{-1} dose to every 4–6 weeks. Concomitant therapy with mesalamine, sulfasalazine, olsalazine or balsalazide may be continued for chemoprevention against ulcerative colitis-associated colorectal cancer [315]. Prednisone should be tapered and discontinued. Infliximab is immunogenic and a strategy to minimize HACA formation, thereby minimizing the risk of loss of response and infusion reactions, should be employed in every patient treated with infliximab. Concomitant treatment with azathioprine or 6-mercaptopurine is protective against HACA formation. However, combination therapy with these agents and infliximab appears to be associated with an increased risk of hepatosplenic T cell lymphoma [179]. Alternative strategies to prevent HACA include three-dose induction therapy at 0, 2 and 6 weeks followed by systematic maintenance therapy every 8 weeks and pretreatment with 200 mg intravenous hydrocortisone. Monotherapy with infliximab is a treatment strategy to minimize the risk of opportunistic infection and hepatosplenic T cell lymphoma. No specific toxicity monitoring is required for infliximab, but clinicians should be vigilant for serious infections including unusual opportunistic infections including fungal infections and tuberculosis. Indications for treatment with infliximab in patients with chronically active ulcerative colitis include: induction and maintenance of remission, mucosal healing and steroid sparing.

Severe ulcerative colitis

Severe ulcerative colitis is defined using the Truelove and Witts criteria (Table 28.2) [6]. Toxic (fulminant) colitis is defined as the sudden extension of mucosal inflammation through all layers of the colonic wall to the serosa and presents clinically as focal visceral tenderness to deep palpation. Megacolon is defined as dilatation of the colon (5–6 cm or more) demonstrated by X-ray examination and presents clinically as abdominal distension, decreased or absent bowel sounds and in some cases decreased stool frequency. Approximately 10% of all patients with ulcerative colitis will develop a severe flare at some point in their disease course, whereas only 1–2% progress to toxic (fulminant) colitis and/or megacolon. The mortality for severe ulcerative colitis is 2%, but remains approximately 30% for toxic (fulminant) colitis.

In patients with severe or toxic colitis, hospitalization is mandatory. The treatment regimen outlined by Truelove and Jewell 35 years ago [87], consisting of intravenous fluids, electrolyte supplements, bowel rest, transfusion if indicated, intravenous antibiotics, intravenous corticosteroids and rectal corticosteroids, remains in use today, although controlled trials have demonstrated that intravenous antibiotics are not of benefit. About 60% of patients treated with this regimen will be symptom free by the end of 5 days, 15% will have significant improvement and 25% will not improve and should be treated with cyclosporin, infliximab or surgery.

Most patients hospitalized with severe ulcerative colitis should continue to receive a normal diet. Two randomized controlled trials have demonstrated that bowel rest does not affect the outcome of severe ulcerative colitis in patients treated with intravenous prednisone [316,317]. Patients with toxic colitis or megacolon should be made NPO because of the potential for eminent surgical intervention. Peripheral or central intravenous nutrition should be instituted if there is evidence of malnutrition. The goal of intravenous nutrition is to replace nutritional deficits rather than for any primary therapeutic benefit.

Factors which have been implicated in the development of toxic megacolon should be avoided, including barium enema, narcotic antidiarrheals (codeine, tincture of opium, loperamide and diphenoxylate), anticholinergic agents, antidepressants and electrolyte imbalance. Patients should be monitored frequently. Abdominal X-ray may be indicated daily in patients with severe colitis and twice daily in patients with megacolon. Frequent physical examination by both an experienced gastroenterologist and surgeon is also of great importance, as is frequent monitoring of the complete blood count, electrolytes and nutritional parameters.

Mesalamine, sulfasalazine, olsalazine and balsalazide should in general be temporarily discontinued in patients hospitalized with severe or toxic ulcerative colitis because of the possibility of a drug-induced exacerbation of colitis which can be indistinguishable from a flare of colitis. Controlled clinical trials have demonstrated that antibiotics have no role in the treatment of severe ulcerative colitis unless a specific infection is suspected. Nevertheless, many authorities continue to advocate broad-spectrum antibiotic therapy with either a combination of metronidazole, an aminoglycoside and a broad-spectrum penicillin or with a third-generation cephalosporin. Intravenous corticosteroid therapy should be initiated with hydrocortisone 300–400 mg per day or methylprednisolone 40–60 mg per day. One controlled trial showed no difference in outcome for patients treated with a continuous intravenous infusion versus bolus injection [86]. For patients who have not been recently treated with steroids, there is some

evidence to suggest that they may respond better to intravenous ACTH administered at a dose of 40 units every 8 h than to conventional corticosteroids.

Patients with severe ulcerative colitis who fail to respond to 5 days of intravenous corticosteroid therapy may be considered for "rescue" therapy with infliximab or intravenous cyclosporin. The data demonstrating safety and efficacy for both therapies are sparse and at present there is insufficient information to recommend one rescue strategy over the other. Infliximab 5 mg kg^{-1} is administered as an intravenous infusion as described above. Patients with known infection or latent tuberculosis should not be treated with infliximab. Cyclosporin is administered at a dose of 2 mg kg^{-1} per day administered as a continuous infusion over 24 h. This dose should be adjusted to maintain a whole blood cyclosporin A concentration (HPLC or monoclonal radioimmunoassay) of approximately 200–250 ng ml^{-1}. An alternative to cyclosporin it oral tacrolimus administered at a starting dose of 0.20 mg kg^{-1} per day and adjusted to maintain a blood level of 5–15 ng ml^{-1}. Patients with known infection, hypocholesterolemia (risk of seizure) or significant renal insufficiency should not be treated with cyclosporin or tacrolimus. Significant abdominal pain is probably a relative indication to treatment with infliximab, cyclosporin or tacrolimus since this may represent transmural inflammation and potentially early perforation. Patients who fail to respond within 7–10 days of initiating rescue therapy should undergo colectomy. It is not recommended that patients who fail to respond to rescue therapy with one agent (infliximab, cyclosporin, tacrolimus) switch to a second rescue therapy due to increased risk of infectious complications from over-immunosuppression. Patients who respond to infliximab should complete their series of three induction doses at 2 and 6 weeks and then continue maintenance dosing every 8 weeks. Patients who respond to intravenous cyclosporin should be discharged on oral cyclosporin at a dose approximately two times the total daily dose that they received intravenously. This may be administered as standard oral cyclosporin (Sandimmune) or as the microemulsion oral formulations of cyclosporin (Neoral or Gengraf). Again, the oral cyclosporin dose should be adjusted to maintain a whole blood concentration of cyclosporin A in the range of approximately 200–250 ng ml^{-1}. Patients who respond to oral tacrolimus should continue it as an outpatient. Oral cyclosporin or tacrolimus should be overlapped for 4–6 months with the slow-acting immune modifier agents azathioprine or 6-mercaptopurine, which are then continued long term for maintenance of remission. Corticosteroids can be tapered over 2–3 months. Prophylaxis for *Pneumocystis carrini* pneumonia with trimethoprim/sulfmethoxazole is recommend while patients are receiving cyclosporin or tacrolimus in combination with prednisone and azathioprine or 6-mercaptopurine.

Conclusion

Initial treatment of mildly to moderately active ulcerative colitis may be sulfasalazine, oral or rectal mesalamine, balsalazide, corticosteroid enemas or oral corticosteroids. Patients with persistent mild to moderate symptoms of active ulcerative colitis in spite of these therapies (treatment-refractory) may require azathioprine/6-mercaptopurine or infliximab. Patients with severely active ulcerative colitis should be treated with intravenous conventional corticosteroids and infliximab, intravenous cyclosporin and oral tacrolimus may be considered in those who do not respond. Remission should be maintained with sulfasalazine, oral or rectal mesalamine, olsalazine or balsalazide and in some cases with azathioprine/6-mercaptopurine or infliximab.

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Chapter 29
Surgical Considerations for Ulcerative Colitis

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Summary

- In patients requiring surgery for ulcerative colitis (UC), physicians involved must be able to decide if the patient is a suitable candidate for ileal pouch anal-anastomosis (IPAA) or whether the patient would be best served with a total proctocolectomy and end ileostomy. A small patient subset may be best served by subtotal colectomy with ileorectal anastomosis.
- In patients undergoing IPAA formation, a consensus must be made on the number of stages required. Only patients who are very well clinically without risk factors for anastomostic leakage should undergo one-stage ileal pouch formation.
- In patients with UC requiring emergency intervention, the standard procedure is a subtotal colectomy with end ileostomy, with the distal stump placed above the fascia. On rare occasions, a Turnbull blow-hole colostomy with diverting ileostomy may be required.
- Physicians, surgeons and gastroenterologists involved in the management of patients who have undergone an IPAA must be aware of both the early and late complications unique to the ileal pouch.
- Salvage surgery for patients requiring repeat intervention because of pouch failure/dysfunction should have this carried out by a surgeon or center with experience in the management of these complex cases.

Introduction

Despite all medical advances and improvements in care, approximately 25–30% of patients with ulcerative colitis (UC) will ultimately require surgery [1,2]. This may be for disease refractory to medical management, medication intolerance, neoplastic changes within the bowel or the development of complications. Close interaction between the patient, gastroenterologist and surgeon is critically important in determining the optimal time for intervention and, if surgery is indicated, then deciding the appropriate procedure. Surgery for ulcerative colitis (UC) may occur in an elective or emergency setting [3]. As a rule, emergency procedures tend to be performed using a standard open approach. A minimally invasive approach may be more suitable for elective or semi-elective cases. Emergency manifestations of UC include fulminant colitis, toxic megacolon, and ongoing hemorrhage. These patients may be critically ill, immunocompromised and on high-dose steroids and/or biological agents. They may have single or multi-organ failure. We would have a low index to intervene at an early stage in patients with co-morbidities such as diabetes mellitus or transplant recipient patients. In this setting, the procedure of choice is typically a subtotal colectomy (STC) with end ileostomy. The remnant rectal stump is exteriorized to the fascia at the lower end of the midline wound [4]. On occasions, the extent of patient compromise may be so extreme that in combination with co-morbidities they may not tolerate the surgical stress associated with colectomy. In this scenario, or with a subset of pregnant patients, one may have to consider a blow-hole colostomy with diverting loop ileostomy [5]. This reduces the operative time. The ileostomy diverts proximal intestinal contents and the blow-hole colostomy acts as a vent for the colon, preventing perforation. However, if there is clinical or intra operative evidence of a free perforation, bowel wall compromise or colonic hemorrhage, then the patient requires a formal STC. Even when procedures for UC occur in the emergency setting in hemodynamically unstable patients, the utmost care should be taken, with meticulous dissection, adherence to hemostasis and quarantining of the gutters and colonic flexures in anticipation of potential colonic content spill. In these patients, no attempt is made to restore intestinal continuity. Once the inflammatory cascade has resolved, their nutritional status returned to baseline and they are free of immunosuppressant

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medication, then a completion proctectomy (CP) with restoration of intestinal continuity may be considered. In the elective setting, the procedure of choice for the majority of patients with UC requiring surgery is a total proctocolectomy (TPC) and IPAA [6]. Whether this is performed in stages and the IPAA is protected with a temporary diverting loop ileostomy depends on the extent of disease, medication requirements and the surgeon's experience [7]. In elderly patients or those with compromised sphincter function, the conventional TPC with end ileostomy may be the best surgical option. Several tertiary centers offer the option of a continent ileostomy [8]. This may be considered for very well-motivated patients who are averse to the concept of a permanent end ileostomy and are not suitable for an IPAA due to sphincter dysfunction or previously removed sphincters or have a failed IPAA and are not suitable for salvage pelvic pouch surgery. In a subset of patients, subtotal colectomy with ileorectal anastomosis may be considered, providing the rectum is compliant with reasonable disease control.

In all patients presenting with severe colitis, it is important to determine if there are any infectious agents, such as *Clostridium difficile*, *Salmonella*, *Campylobacter* or cytomegalovirus (CMV), contributing to the symptoms [9]. In addition, one should be aware that many of these patients are immunocompromised from long-standing disease and may not display the classical manifestations of toxicity. In the postoperative period, despite removal of the diseased colon, they are still prone to develop sepsis secondary to respiratory, urinary and line infections. We are also seeing an increasing number of this patient group affected by methicillin-resistant *Staphylococcus aureus* (MRSA) and other opportunistic infections which we attribute to their inability to mount an adequate immune response. In patients with severe colitis, frequent clinical evaluation and serial abdominal examinations are paramount and will help to determine if the patient is responding to medical management or will require surgical intervention. As a rule of thumb, if the patient fails to respond to intravenous steroids, one is unable to wean to oral steroids or they develop a surgical complication, then intervention is indicated. If despite 3 days of medical therapy the patient still has more than eight bloody bowel motions per day or a stool frequency between three and eight together with a C-reactive protein greater than 45 mg l^{-1}, then it is estimated that 85% will come to surgery [10]. Evidence of peritonitis on clinical examination or pneumoperitoneum on radiological investigation is an indication for immediate surgical intervention. It is important that patients with severe colitis undergo surgical intervention before the onset of single- or multiple-organ failure or develop a perforation, all of which carry a poorer prognosis. In toxic megacolon, it is typically the transverse colon that dilates, which occurs in combination with systemic toxicity. In contrast to Ogilvie's syndrome (pseudo-obstruction of the large bowel), the upper limit of normal on radiographic assessment is 6 cm [11]. Serial X-rays of the abdomen are the imaging modality of choice.

Intractable disease is the most frequent indication for surgical intervention in UC. This includes patients who are symptomatic despite best medical management and patients who have disease remission but are intolerant of the medications due to side effects such as growth retardation in the pediatric population or osteoporosis in adults [11]. In addition, the development of a colorectal malignancy, high-grade dysplasia or a dysplasia-associated lesion or mass (DALM) is an indication for surgery [12]. In these cases, resection is performed with adherence to oncological principles including high ligation of draining vessels which will encompass the accompanying lymphatic field.

The aim of this chapter is to overview our current knowledge with regard to surgical management of UC. It highlights the clinical situations, both elective and emergency, in which one would consider intervention. It discusses the technical aspects of procedures and aims to impart knowledge on practical tips which will help to achieve a successful surgical outcome. It also overviews some of the complications that are inherent to surgery for UC. The care of a patient with UC potentially requiring surgery necessitates close collaboration between the gastroenterologist and surgeon and must be individualized according to the patient involved and their underlying disease.

Elective procedures for ulcerative colitis

Restorative proctocolectomy with ileal pouch–anal anastomosis

Since its introduction by Parks and Nicholls in 1978 [13], restorative proctocolectomy (RP) with ileal pouch-anal anastomosis (IPAA) has become the gold standard of treatment for the majority of patients with UC requiring surgery [6]. It allows restoration of intestinal continuity and maintenance of the normal defecatory pathway, avoiding the need for permanent end ileostomy. Patient satisfaction with the procedure and functional results tends to be very high [14]. There are a number of different techniques used for performing this procedure, which may vary between centers and surgeons. RP with IPAA may be performed via the traditional open approach, using a minimally invasive technique or with what many term a hybrid approach. This includes those who have the colectomy performed laparoscopically and then the pelvic dissection/proctectomy and pouch formation performed through an extended supra-pubic incision. It also includes those who had the IPAA formed using hand-assisted laparoscopic surgery (HALS) [15,16]. Irrespective of the modality chosen, the surgeon must be comfortable

with all the necessary steps that are required for a successful outcome. Ileal pouch surgery itself is associated with a significant learning curve, which in our institute we estimate to be 25 cases using an open approach with stapled pouch–anal anastomosis and considerably higher for patients undergoing a mucosectomy with hand-sewn pouch–anal anastomosis [17]. Traditionally, most surgeons, ourselves included, favored the formation of a temporary diverting ileostomy because of the potential for ensuing pelvic sepsis, pouch failure and need for re-laparotomy in patients who develop a leak [18]. In more recent years, there has been a trend towards omission of an ileostomy in a subset of patients who are clinically well and adequately nourished and in whom intra operative conditions are favorable [7,19]. In our institute, 85–90% of patients still receive a diverting ileostomy which is closed 3 months from the time of the indexed pouch surgery. Prior to ileostomy closure, a gastrograffin enema is obtained and a pouch endoscopy performed to confirm anastomostic integrity and to rule out an occult leak. If there is an anastomostic stricture, this may be dilated, and if the gastrograffin shows an occult leak or anastomotic sinus, then we would delay the ileostomy reversal for an additional 3 months and perform further imaging studies prior to stoma closure.

In the *open approach*, one typically enters the abdomen via a lower midline incision that extends a few centimeters above the umbilicus. To achieve adequate exposure, this midline incision must extend to the symphysis pubis and involves dissection to the level of the pyrimidalis muscle [20]. In very thin or pediatric patients, one may be able to achieve adequate exposure using a Pfannenstiel incision, which gives the added cosmetic advantage of a scar below the belt line. However, great care must be taken when mobilizing the hepatic and splenic flexures using this approach. On entry to the abdomen, the small bowel should be examined from the ligament of Trietz to the ileocecal junction looking for subtle manifestation of Crohn's disease (CD). One should be particularly vigilant if the patient has had a preceding colectomy with a histological diagnosis of indeterminate colitis or a past history of perianal disease which may include a fistula, abscess or anal ulcer. Features of small bowel CD may include classical fat wrapping or small bowel strictures. Other more subtle manifestations include lymphadenopathy, thickening of the mesentery and prominence of the vasa recta. While we would not consider Crohn's colitis an absolute contraindication to ileal pouch formation, the coexistence of small bowel or perianal disease makes it inadvisable [21,22]. In this scenario, if a RP with IPAA was planned, it may be more prudent to perform a STC only and avoid the proctectomy. Whether one then performs an ileorectal anastomosis depends on the extent of terminal ileum and rectal disease. If there is evidence of terminal ileal CD, then the surgeon can be justified in performing a STC with end ileostomy and treating the small bowel disease medically before considering an ileorectal anastomosis. In patients undergoing RP, the abdominal colectomy is performed in the standard fashion and, in the absence of malignancy or dysplasia, vessels are divided close to the colon. Great care must be taken when dealing with the middle colic vessels as they have a propensity to retract. If one is in the unfortunate situation where vascular control is lost, we advise applying pressure with packs to tamponade the hemorrhage. This allows the surgeon a period of grace to regroup, ensure adequate exposure and that all suitable sutures and instruments required are available. Blind ligation will result in inadvertent injuries to surrounding structures. Some surgeons advocate sending a few centimeters of the terminal ileum with the colectomy specimen for histological assessment. There is still considerable debate about the merits of saving or removing the greater omentum. Those who favor its excision with the specimen believe that there is a much smaller incidence of ensuing small bowel obstruction. In this case, the greater omentum is divided distal to the gastroepiploic arcade of the stomach. We prefer to preserve the omentum as it can be used to fill the pelvic dead space in patients undergoing a TPC without intestinal restoration. In female patients undergoing an IPAA, we make an effort to place it anterior to the pouch acting as a buffer with the vagina. There maybe improved fertility with ovarian wrap to reduce fimbrial adhesions. In addition, if there is a sub-clinical leak, the omentum may help with containment, although we have no scientific evidence to prove this. When preserving the greater omentum, it is removed from the transverse colon by dissection in the avascular plane, which is aided by traction and countertraction on the tissues.

Prior to performing *proctectomy*, the ureters should be identified bilaterally. The remaining small bowel is packed within the upper abdomen away from the pelvic field. The rectum is then elevated with the surgeon's left hand and an incision made in the pelvic peritoneal reflection bilaterally. This allows air into the tissue planes, aiding dissection. In benign UC disease, we would divide the inferior mesenteric artery distal to the origin of the left colic as there is no oncological indication for high ligation. One then enters the avascular plane between the presacral fascia and the lamina propria of the rectum (Figure 29.1).

The dissection is then continued to the pelvic floor. Forward traction on the pelvis using deep lighted retractors will aid dissection and visualization. It is very important that one does not breach the presacral fascia posteriorly, which may result in injury to the presacral veins. All surgeons must have a number of techniques in their armamentarium to be able to deal with presacral bleeding if it occurs [23]. If significant, we advise packing the pelvis and completing the proctectomy. Often by this stage the bleeding will have resolved. If it persists, the surgeon must

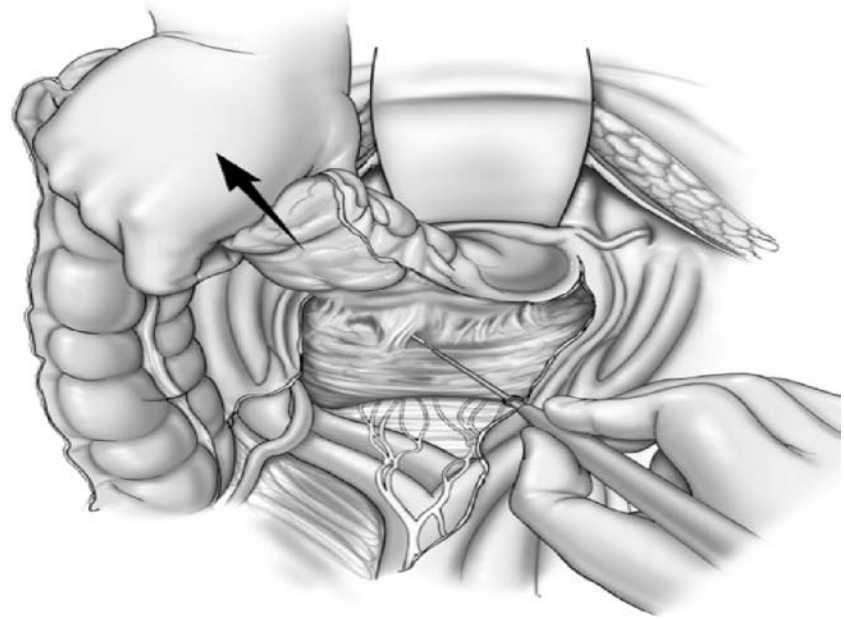

Figure 29.1 Posterior plane of dissection during proctectomy. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

decide if it originates from the presacral venous plexus or the basivertebral veins, as one will vary the hemostatic techniques applied accordingly. If the bleeding can be controlled by pressure applied to the adjacent venous plexus, then it is most likely originating from the presacral veins, which can be controlled by pressure or suture ligation. During suture ligation, one may consider applying small sponge compression (peanut), which will help to keep the operative field dry. When working in the pelvis, all instruments should be extra long. The bleeding may also originate from a basivertebral vein that has torn. These veins have a tendency to retract into the sacral foramina, making hemostasis extremely difficult. In this scenario, one can insert thumbtacs into the bone. On occasions we have used a muscle fragment from the rectus abdominus to provide tamponade. The technique originated from cardiovascular surgery and works on the concept of applying electrocautery through a muscle fragment to achieve hemostasis. In the cases we described, the muscle flap was sutured, which was sufficient to control the hemorrhage [24]. If none of these measures work, then one should make the decision to pack the pelvis. It is important that this decision is made quickly before there is ongoing blood loss that may give rise to disseminated intravascular coagulation. The packs can be left *in situ* for up to 72 h. Many surgeons advocate placing these packs within a bowel isolation bag. This reduces the infection risk and prevents the gauzes from sticking to adjacent tissue, which can give rise to a raw surface and further bleeding when the packs are removed [25].

When the posterior dissection is complete, our attention is turned to the lateral and anterior dissection. In benign disease we would again tend to perform our dissection close to the mesorectum, which reduces the potential for damage to the ureters and nerves lying laterally. On occasions, middle rectal arteries may be found within the lateral ligaments and require ligation or the application of hemostatic clips. Anteriorly, our incision is made on the anterior rectal wall approximately 1 cm above the peritoneal reflection with the ensuing dissection posterior to Denoviller's fascia. This dissection is continued to the lower third of the vagina or the prostate. If one encounters seminal vesicles then the dissection is too anterior and runs the risk of damaging the autonomic plexus, which may result in chronic urinary retention, erectile dysfunction or ejaculatory failure. Once the rectum is fully mobilized to the levators and anorectal junction, a decision must be made on the level of transection. Many surgeons advocate a *nerve-sparing proctectomy*. Here the dissection is made within the mesorectum staying close to the rectal wall. Although one will encounter much more bleeding and no defined tissue planes, advocates believe that it will reduced the potential for damage to the autonomic nerves. However, it is a more difficult dissection and some reports demonstrate no benefit in the extent of erectile dysfunction after a close rectal dissection in comparison with the traditional mesorectal dissection. Age was found to be a more important risk factor for postoperative impotence [26].

All surgeons involved in pouch surgery must be eminently familiar with all technical steps required to *create an ileal pouch* and ensure that it reaches the anal canal without tension. In keeping with any anastomosis, tension at a suture line may result in anastomotic dehiscence. While a diverting loop ileostomy may prevent the need for early re-intervention, the subsequent perianastomosis fibrosis and stricturing result in a poorly functioning

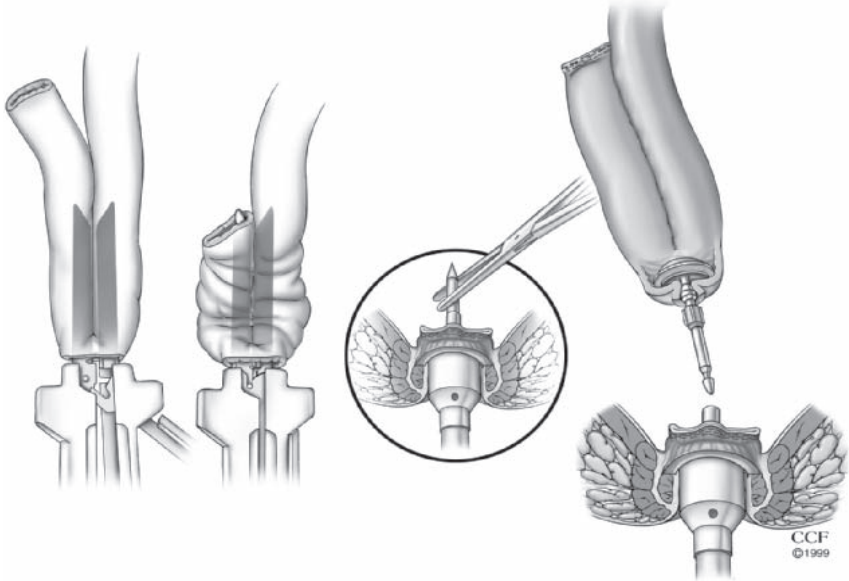

Figure 29.2 Creation of a J-pouch configuration using a GIA stapler and subsequently circular stapler for pouch–anal anastomosis. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

pouch that may require further surgery or a permanent end ileostomy [27]. In our institute, the most common pouch configuration is a J-pouch. This fits very well into the concavity of the sacrum and has been shown to provide excellent functional results. There is still considerable controversy with regard to the optimal technique for pouch–anal anastomosis. Many surgeons advocate a mucosectomy followed by a hand-sewn anastomosis [28]. We generally favor leaving approximately 1–1.5 cm of anal transitional zone (ATZ) above the dentate line and then performing a double-stapled anastomosis. We believe that this is technically easier, associated with fewer complications and better functional results [29,30]. We have previously shown that it is associated with less damage to the internal anal sphincter with improvements in anal canal pressures and subsequent continence compared with mucosectomy [31,32]. The AZT has extensive sensory innervations which are important in discriminating between flatus and stool and this is lost when one performs a mucosectomy [33]. We would perform a mucosectomy and hand-sewn anastomosis if there was an associated rectal malignancy or dysplasia involving the lower two-thirds of the rectum. However, we would be conscious to minimize the duration and extent of anal stretch, which has the potential to reduce significantly anal canal resting pressures with the clinical effect of increased soiling. When UC is associated with cancer, ileal pouch formation may be considered and in appropriately staged patients will not significantly impact on the oncological outcome or long-term pouch function [34]. Anorectal mucosectomy is advised. However, for rectal cancers it is important that the administration of radiotherapy occurs preoperatively because of its potential detrimental effect on pouch function with pouch failure.

Pouch size does matter as a small pouch may be unable to act as a reservoir for stool storage, resulting in the need for frequent defecation. If the pouch is too large, this may cause obstructed defecation. The J-pouch is constructed from the terminal 30–40 cm of small bowel, folded into two 15 cm or two 20 cm segments. A 1.5 cm longitudinal enterotomy is then made at the apex and both limbs of ileum are aligned on their antimesentric borders. A 100 mm linear stapler is then inserted through the apical enterotomy and, after ensuring that there is no small bowel mesentry caught between the anvil and cartridge, the instrument is fired (Figure 29.2). A second application completes the reservoir. The tip of the J-pouch is closed with a linear stapler and oversewn. This can be a potential site for anastomostic leakage in the postoperative period. The afferent limb to the pouch is then occluded and the pouch is irrigated to confirm anastomostic integrity. We also evert the pouch and check the suture lines to ensure that there is no troublesome bleeding. If bleeding occurs, it tends to originate from the mesenteric side of the suture line.

A prolene pursestring suture is applied to the apical enterotomy. The anvil of the circular stapler is then inserted into the pouch and secured with the pursestring suture. The circular stapler is inserted into the anus. In females care must be taken that it is not inserted into the vaginal orifice by mistake, which would result in a disastrous pouch–vaginal communication. The trocar of the circular stapler is then exteriorized just posterior to the staple line of the anorectum and married with the anvil under direct vision. Ensure there is no vaginal tissue anteriorly entrapped into the closed stapler. The stapler is then fired, the doughnuts are checked for integrity and the anastomosis is tested by transanal insufflation with

Figure 29.3 Application of a Babcock to the apex of the intended pouch to ensure adequate reach to the level of the pelvic floor before creating the pouch. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

normal saline or air [35]. At the end of the procedure, we recommend inserting a drain into the pelvis. This will remove any fluid collection and prevent the development of a pre-sacral hematoma, which has the potential to become infected and discharge through a pouch suture line, giving rise to a fistula.

As a guide, if the pouch apex reaches a few centimeters beyond the symphysis pubis then it will comfortably reach the anal canal, permitting a tension-free anastomosis. A useful tip is that before deciding on the pouch configuration, one should apply a Babcock to the apex of the intended pouch, bringing it down to the pelvic floor. A finger is then interested via the anal canal and if the Babcock is palpable then one can be certain that the pouch will reach without tension (Figure 29.3).

Other steps to ensure adequate reach include dividing the ileocolic artery just distal to its origin from the superior mesenteric artery (SMA). In addition, transillumination of the mesentry will also allow the removal of redundant mesenteric tissue while preserving major vessels. Some surgeons advocate preserving the vascular arcade to the right colon while sacrificing the ileocolic artery and distal branches of the SMA. However, this would not be our practice [36]. Other practical tips include mobilizing the mesentery of the small bowel to the level of the third part of the duodenum and scoring the peritoneal reflections transversely [37] (Figure 29.4). If reach is still a significant problem, which may occur in obese patients or tall individuals, then one may consider an S-pouch (Figure 29.5). The efferent limb which is used for the pouch anal anastomosis

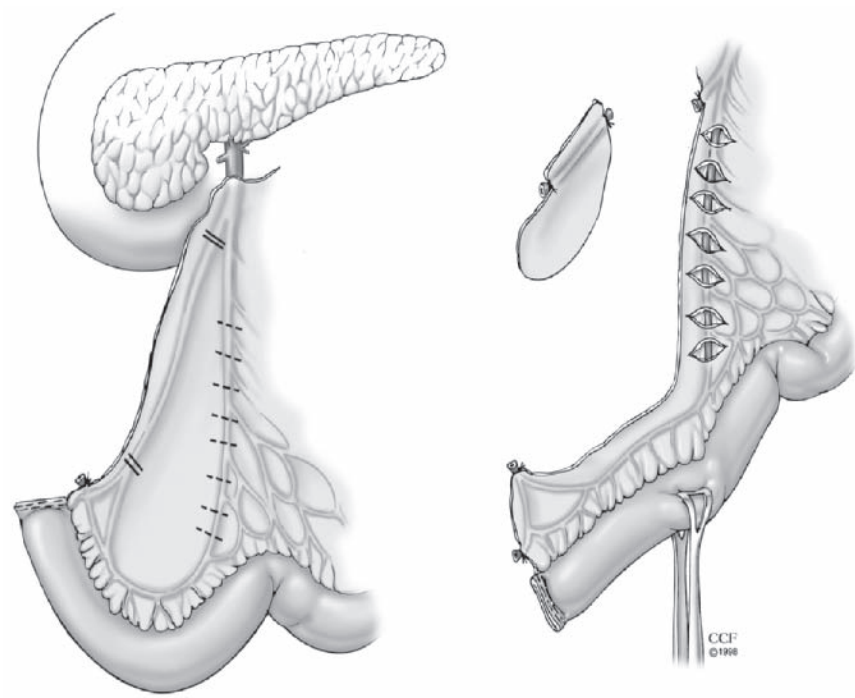

Figure 29.4 Mesenteric lengthening techniques including division of the ileocolic artery and creation of peritoneal windows. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

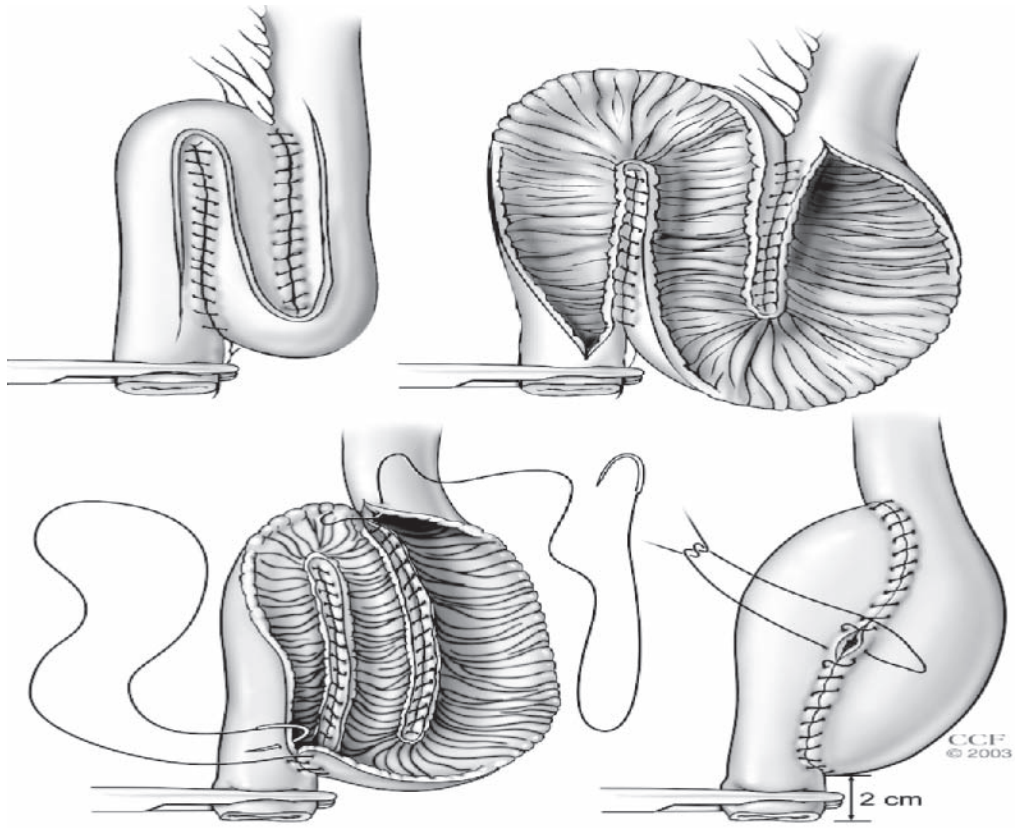

Figure 29.5 Creation of the S-pouch reservoir. The bowel is opened on its antimesenteric border and the adjacent loops sutured to create the pouch. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

should be kept as short as permissible because a long efferent limb may give problems with obstructed defecation that may ultimately require shortening of the efferent limb or pouch revision [38,39]. On very rare occasions, despite all maneuvers, the pouch may not reach. Here we would consider attaching the pouch to the pelvic floor/levator ani muscles and then performing an upstream ileostomy. The intention is to stretch the pouch so that when one returns in 6 months an anastomosis may be feasible.

Nowadays, an increasing number of colorectal surgeries are been performed using *minimally invasive surgery*. Beneficial effects include reduced postoperative pain, earlier return of bowel function and quicker time to discharge. In addition, it is associated with fewer adhesions. This is attributed to less small bowel manipulation and the closed cavity means that the potential for introducing foreign bodies such as swabs and gloves, which are a major stimulus for adhesion formation, is greatly reduced [40,41]. The potential long-term beneficial effects of this include reduced incidence of small bowel obstructions and infertility. Although the reduced incidence of small bowel obstruction is supported by the literature [40], a prospective study would be required to look at the female infertility issue and modality chosen for pouch formation. Laparoscopic pouch formation is complex and should only be performed by surgeons with extensive laparoscopic colorectal experience. A laparoscopic approach to completion proctectomy and IPAA is still feasible even if the patient has had a preceding subtotal colectomy performed using an open approach [42]. In this scenario, we take down the end ileostomy and insert our first port at the ostomy site. Following the creation of a pneumoperitoneum, subsequent ports may then be inserted under direct vision. We then perform a CP and excise the specimen with subsequent ileal pouch creation through a small suprapubic incision. Many surgeons favor a hybrid approach in which they perform the colectomy using a minimally invasive approach and then complete the rectal dissection and pouch formation via an extended Pfannenstiel-type suprapubic incision. Another modification is the use of hand assisted laparoscopic surgery (HALS). With advances in technology and increased surgeon experience, there is an increasing demand for these procedures to be performed using a minimally invasive technique. These procedures are associated with a longer operating time but the patient generally gets all the benefits of a laparoscopic approach. This includes earlier return of gut function, less postoperative pain requirements and reduced time to discharge. The better cosmetic result may be particularly beneficial in young patients who are in their formative social years and

for whom body image is important. Any surgeon contemplating a laparoscopic IPAA must already be comfortable with the steps involved in segmental colon resection and laparoscopic anterior resection. There should be no patient compromise and the surgeon should be doing sufficient numbers.

In our institute, when performing a *laparoscopic IPAA*, we follow a standard technique and protocol. This ensures that all members of the team are familiar with all the steps at various stages of the procedure. Following intubation and ventilation, we insert a nasogastric tube and Foley catheter to decompress the stomach and bladder, respectively. The patient must be taped to the bed or supported in a bean bag as they are very often placed in the extremes of Trendelenburg and reverse Trendelenburg positions to allow the small bowel to fall away from the operative field and to aid mobilization of the hepatic and splenic flexures. Patients are also placed in the modified lithotomy position, which will allow access to the anal canal when performing the pouch anal anastomosis. This also allows the operating surgeon to stand between the patient's legs, which will help with some parts of the procedure. We insert our first port just above the umbilicus using an open Hassons technique. Some surgeons still prefer the veres needle technique. A pneumoperitoneum in created by insufflating with carbon dioxide. We use a 30° lens throughout the procedure. If the laparoscopy is normal, then we insert the other ports also under direct vision. We place a 10–12 mm port through the site of the intended diverting ileostomy in the right flank and place a 12–15 mm port in the supra-pubic region. We also place an additional 5 mm port in the left flank lateral to the inferior epigastric vessels. We start with mobilization of the right colon and favor a medial to lateral dissection. The patient is placed in steep Trendelenburg position with right side up. All the small bowel loops are swept away from the operative field. The ileocolic artery is placed under traction by a grasping forceps on the mesentry adjacent to the ileocecal junction. The duodenum must be visualized and this helps to ensure that one is not inadvertently grasping the superior mesenteric artery. The tissue overlying and inferior to the vessel is scored. The ileocolic vessels may then be ligated between clips or staples or with the use of one of the modern energy devices. In benign disease, this can be taken at a safe distance from the SMA. The distal end of this ileocolic pedicle is then elevated in forceps and the inferior plane developed. This will allow the retoperitoneal structures including the ureter to fall away. It is a very precise dissection which is continued as far laterally as permissible. The lateral dissection should then be reasonably easy given the extent of medial mobilization. Care must be taken that one does not end up in the plane posterior to the kidney. Mobilization of the hepatic flexure is then performed. The right colon mobilization is complete when the terminal ileum can be brought into the intended incision site without tension. This may require further mobilization at the pelvic brim.

Our attention is then turned to the transverse colon. This is aided by placing the patient in the reverse Trendelenburg position. As described for the open approach, the greater omentum may be preserved or removed. We approach the middle colic vessels from the right side and divide them using a laparoscopic energy device. Great care must be taken with this part of the dissection, ensuring that the mesentery of the small bowel is not mistakenly caught within the dissection field. We continue to the splenic flexure. The patient is then placed in the Trendelenburg position with left side up. This allows access to the medial and lateral attachments of the sigmoid colon. Similarly to the open approach, we make an incision in the peritoneal reflection and perform a medial to lateral dissection. The inferior mesenteric artery is placed under traction and a plane of dissection created inferiorly. The vessel can be divided once the left ureter has been identified in the retroperitoneum. Only when visible peristalsis can be seen is one assured that the ureter has been located. In the majority of cases, ureteric injury occurred because the surgeon assumed that some other similar structure was the ureter.

After division of the inferior mesenteric artery, the inferior mesenteric vein will be encountered and is divided. At all times one should anticipate bleeding from a divided vessel or slipped ligature. Prior to dividing any vessel, we position an instrument proximal to the division point in anticipation of loss of vascular control. Any inadvertent bleeding must be localized and can be further controlled with a pre-tied surgical ligature, clips or a laparoscopic stapling device. The lateral attachments can then be divided and mobilization completed to and including the splenic flexure.

The rectal dissection is similar to that for the open approach and is facilitated by traction and countertraction on the relevant tissues. Indeed, the views of the pelvic planes afforded through the laparoscope are excellent. Once the mesorectal tissue has been divided and the rectum mobilized to the level the pelvic floor, a laparoscopic stapler is inserted through the supra-pubic port. The articulation of the stapler helps with positioning. Pressure on the perineum from below may also help. Two fires are usually required. The specimen is then exteriorized through a 5 cm supra-pubic incision which is covered with a wound protector and encorpates the supra-pubic port. The pouch is then created, taking care that the small bowel has not been rotated. All the principles of open pouch design similarly apply to the laparoscopic approach. The diverting ileostomy is then created. We would manage these patients using a fast-track postoperative care pathway including oral intake on the evening of surgery, limited postoperative intravenous fluids, early mobilization and minimizing the use of systemic opioids.

An increasing number of ileal pelvic pouches and accompanying colorectal resections have been performed for UC using a minimal invasive approach. However, good surgical judgment is required and the need to convert to an open approach should not be seen as a failure.

Salvage surgery for pelvic pouch complications

A small subset of patients will require repeat pouch surgery because of pouch complications not amenable to conservative management or local procedures [27]. Any intended surgery should be delayed for at least 6 months from the last abdominal surgery to allow attenuation of the inflammatory process and softening of adhesions. As per all re-do pelvic surgery, we recommend the insertion of ureteric stents. Although stents may not prevent a ureteric injury, they will allow early identification of any defect. In addition, the ability to palpate the ureters allows one to dissect safely through planes that may have extensive fibrosis and scarring. Indigo carmine or methylene blue may be administered intravenously if one suspects a breach in the ureter. All patients are placed in the modified Trendelenburg position with Lloyd-Davies' stirrups. In general, the pouch is mobilized using a combination of an abdominal and transanal approach. The pelvic dissection should begin posteriorly after one has identified a plane between the sacral promontory and mesentry of the pouch, taking care to preserve the blood supply. Some surgeons prefer to place the mesentery anteriorly with the pouch lying in the sacrum which increases the risk of pouch damage in salvage surgery. Deep lighted pelvic retractors are essential when performing the anterior and lateral dissection. These will aid visualization, reducing risk of damage to nervi erigentes which control sexual function. After disconnection of the pouch, it is brought into the wound and all adhesions are divided. In re-do pouch surgery, care must be taken during pouch dissection because in the majority of cases one can use the existing pouch. We recommend that salvage pouch surgery be performed in tertiary centers with extensive experience in the management of these problems.

Continent ileostomy (K-pouch)

Since its conception by Professor Neil Kock in 1969 [43], the continent ileostomy has been performed by only a few enthusiasts, mainly in specialized colorectal units. This is principally because of the technical difficulties with pouch creation, the frequent need for pouch revision and associated complications. The multiple suture lines necessary for construction of the reservoir carry the risk of anastomostic leakage. In addition, the diminishing number of surgeons performing the procedure means that the exposure and potential to overcome the learning curve are significantly reduced for each passing generation. However, there is still a role for the continent ileostomy in a subset of patients with UC. Potential candidates include those who have had their anal sphincters removed, those who suffer from significant sphincter dysfunction or those who have a failed pelvic pouch. The conversion of a pelvic IPAA to a K-pouch involves a significant degree of technical difficulty. Nowadays, the continent ileostomy is mainly performed in tertiary colorectal units [8].

In our institute, we would consider obesity and the previous loss of a significant amount of small bowel as relative contraindications. We would not knowingly create a continent ileostomy in patients with CD. We would consider its construction in older patients. Patients willing to undergo a K-pouch formation are typically very motivated and intolerable to the concept of a permanent ileostomy. Many of those who already have an ileostomy *in situ* report that the ongoing discharge of malodorous contents on to the skin and the need for a constant external appliance impringes significantly on their quality of life. Despite many patients requiring pouch revision, most with a continent ileostomy are extremely satisfied. The K-pouch itself consists of a reservoir constructed out of three loops of bowel with a mechanically created valve to preserve continence until time of intubation.

All surgeons and gastroenterologists involved in the care of patients with UC should have a modicum of knowledge with regard to the *technical aspects of K-pouch construction*. This will allow them to discuss with the patient whether it would be a suitable operation to meet their needs and in those who have had a continent ileostomy it allows them to participate in the patients' postoperative care and long-term follow-up. The patient's intended K-pouch ostomy site must be marked preoperatively by an enterostomal therapist. Given that no external appliance is required, the stoma can be placed very low (below the belt line) and flush with the skin. We enter the abdomen via a lower midline laparotomy incision. If the patient has had previous surgery, great care must be taken to avoid inadvertent enterotomies. The small bowel is examined to ensure that there is no macroscopic evidence of CD, which would be a contraindication to creation of a K-pouch because of the potential for subsequent fistulization and significant loss of small bowel. Techniques used vary between centers. In our institute, we prefer to create the ileal reservoir from three 12–15 cm loops of terminal ileum using a hand-sewn suture technique. An additional, 15–20 cm of ileum is required to create the nipple valve. The bowel loops are aligned in an S-shaped fashion with approximation of the anti-mesenteric borders using a 3/0 absorbable suture. The bowel limbs are then opened using a Bovie. The posterior wall of the pouch is sutured using absorbable material. The valve is created by intussuscepting the efferent limb to provide a 6 cm nipple. Prior to this, the peritoneum of this efferent limb is stripped off the adjacent mesentry, which is also defatted using electrocautery. This promotes adherence of the efferent limb

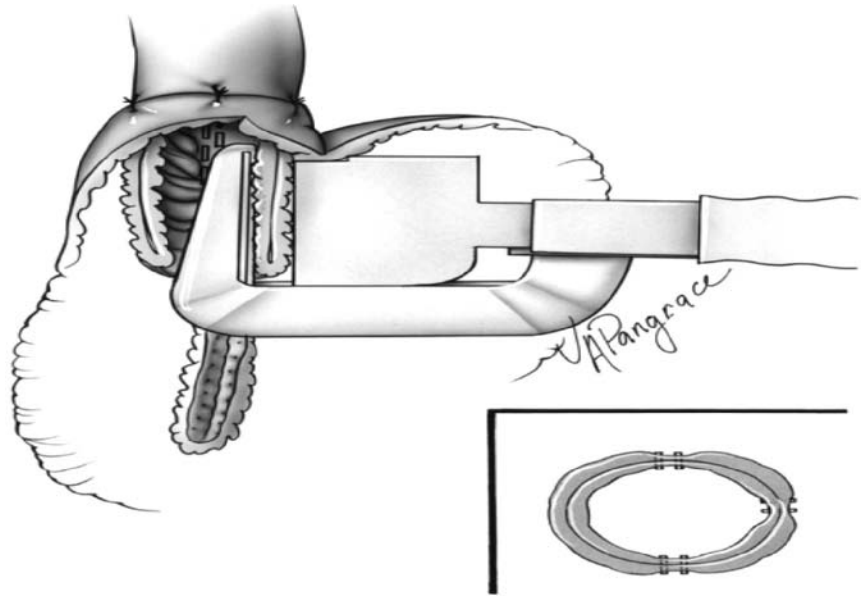

Figure 29.6 Stabilization of the nipple valve during K-pouch construction. Image courtesy of the Art Department, Cleveland Clinic Foundation, Cleveland, OH.

when the pouch is intussuscepted. The critical step is then stabilization of the valve using three or four applications of a transverse stapler (Figure 29.6). The pouch is then closed anteriorly. Many surgeons prefer to create the pouch using a stapled technique. The fundus of the pouch is then sewn on to the base of the exit conduit to strengthen the intussusception.

Following construction, a 28F or 30F polymeric silicone catheter tube is inserted into the pouch and the capacity plus continence is tested. The distance between the abdominal wall orifice and pouch should be kept as short as possible to facilitate ease of intubation. At the end of the procedure, we secure the tube in place using tripod sutures which prevent tube slippage or advancement into the pouch, which may result in its erosion through the reservoir wall. The tube is then allowed to drain by gravity. In the next 24–48 h the enterostomal therapist will cut the stabilizing sutures and apply a faceplate. The catheter is then left *in situ* for 4–6 weeks to facilitate reservoir drainage, thus reducing the potential for pouch distension with subsequent tension on the suture lines. It is important that the patient is actively involved in pouch maintenance. The patient and their family must be educated on how to irrigate the pouch and reposition the catheter if drainage is inadequate. We also encourage them to follow a low-residue diet and to avoid any foods that have the potential to block the pouch.

Long-term complications are mainly related to problems with the nipple valve and several modifications have been added to the original procedure to try and minimize this difficulty. K-pouch complications can be broadly divided into those occurring early in the post-operative period and those occurring later. One should have a high index of suspicion for any problems early in the postoperative course. The presence of a persistent tachycardia, leucocytosis, pyrexia or ileus should alert one to the possibility of leakage/intra-abdominal abscess. A CT scan with oral and pouch contrast will help to delineate if there is extravasation of contrast or an abscess that is amenable to percutaneous drainage. If the patient develops a pouch dehiscence, we would aim to create a proximal ileostomy to divert intestinal contents. *Late complications* including slippage or prolapse of the nipple valve are the most common cause of pouch incontinence. It may be initially preceded by difficulties with pouch intubation due to a change in the angulation of the efferent limb. On occasions, patients may present as an emergency because of failure to intubate. The pouch becomes over-distended and one needs to decompress the reservoir typically using an ileoscope. A catheter is then inserted into the pouch under direct vision and fixed in place. This is left *in situ* until the patient can be scheduled for an appropriate procedure to correct the valve slippage. On one occasion we encountered a patient who lost their medina catheter within the reservoir and this could not be removed despite multiple endoscopic attempts. The patient needed a formal laparotomy to retrieve the catheter and correct the valve slippage.

Thus a significant number of these patients will at some stage require *pouch revision* or reconstruction of a new nipple valve due to slippage. In some instances this can be performed by taking down the ostomy and extending the incision superiorly and inferiorly, allowing mobilization of the pouch. However, in general we prefer to perform a formal laparotomy with abdominal access through the existing scar. Great care must be taken to prevent enterotomies and it may be prudent to enter at a site removed from existing scar tissue. All adhesions relevant to the pouch are divided. The efferent limb is carefully dissected

away from the abdominal wall. One should be able to encircle the entire pouch before taking down the ostomy. The ostomy or its blood supply must not be damaged as this may be used again. If the problem is valve slippage then we open the pouch at the side on which one intends to fix the valve. Provided that there is sufficient length, the existing valve can be further intusscepted and stabilized. If this is not suitable, then a new valve is created by intussuscepting the afferent limb followed by pouch rotation. The proximal edge of the ileum is then anastomosed to the reservoir and the stoma matured. Other continent ileostomy-related problems include parastomal herniation, which may lead to intubation difficulties, pouchitis, fistulization, hemorrhage and detachment of the pouch from the anterior abdominal wall.

Total proctocolectomy with end ileostomy

Total proctocolectomy (TPC) with end ileostomy is still a very reasonable option for a subset of patients with UC. It can be performed in a single stage, is curative and avoids the potential complications associated with pouch formation. This operation should be discussed with all patients. In our practice, this procedure would be more commonly performed in patients more then 60 years of age, especially if they already have compromised sphincter function or extensive co-morbidities. However, we would not consider age as a barrier for reconstructive surgery of the intestinal tract [44]. The key component to achieving a successful outcome in this group is a well-functioning ileostomy. The procedure may be done using an open or minimally invasive approach. The colectomy is performed first followed by the protectomy using the techniques as previously described. The rectal dissection is continued to the level of the pelvic floor and the rectum then disconnected distally with the remaining dissection completed from the perineum. Another option in the open approach is to detach the colon and place a tape with ring attached to the proximal rectum.

Once the specimen is excised, drains inserted, ileostomy created and abdominal wound closed, one's attention is then turned to the *perineal dissection*. This may be performed in the lithotomy or Kraske position. In patients with a significantly increased body mass index (BMI), the Kraske position gives superior views and is very useful for teaching cases. Anal everting sutures are placed in a radial fashion, incorporating a superficial portion of the perineum with the intention of bringing the anal canal into the operative field. They serve the same purpose as the Lone Star retractor. The orifice of the anal canal is then closed with a pursestring suture. Given that we are dealing with benign disease, an intersphincteric dissection is preferred, which reduces the potential for postoperative perineal wound problems. We perform the dissection within the intersphincteric space between the internal and external anal sphincters. We first complete the dissection posteriorly and enter the prescaral space by incising the anococcygeal ligament. The coccyx serves as a landmark posteriorly with all dissection occurring anterior to this. A finger is then inserted into the presacral space and the levator ani divided close to the rectum. The ring with tape attached should be palpable, allowing the rectal specimen to be exteriorized. The anterior dissection is then completed. In females, a finger in the vagina will help to identify the plane of dissection. To close the wound, we release the traction sutures and approximate the levator ani and external sphincter muscles. The skin is then closed with interrupted absorbable sutures.

Given the pelvic dead space, there is an increased potential for hematoma and abscess formation. To reduce this risk we place a drain in the pelvis and exteriorize it through a stab incision in the flank opposite the site chosen for ileostomy formation. It is then placed on continuous suction and should be irrigated at the end of the procedure to prevent any clot formation blocking the drain. Some surgeons prefer to exteriorize the pelvic drain through the buttock. In addition, during the abdominal component of the procedure we aim to obliterate the pelvic dead space using the greater omentum.

Despite all the attention to detail, these patients do have a risk of perineal wound problems, including delayed healing and perineal sinus formation. This risk is further increased for patients with diabetes, increased BMI, obesity and patients who are malnourished [45]. If the patient is diagnosed with an unhealed perianal wound, it is very important to ensure there is not a co-existing enteroperineal fistula. We would not consider any intervention for 12 months. If it is a superficial sinus only, then the treatment includes examination under anesthesia (EUA) and curettage of the sinus tracts, leaving an adequate opening in the skin to allow the deep tissues granulate. If the patient has a large wound with a chronic pelvic cavity, then more extensive surgery may be required. This may involve the mobilization of a gracilis muscle flap to fill the space of the chronic cavity [46]. Other postoperative problems include ongoing perineal pain which has been attributed to a neuroma. If the pain persists and does not respond to conservative measures, then excision of the perineal fat pad may be of benefit. Patients also complain of the unusual symptom of needing to have a bowel motion despite the rectum been absent. This is attributed to intact cerebral pathways and patient reassurance is usual adequate.

Only on very rare occasions would a TPC with end ileostomy be performed in an emergency situation. This may be for a low rectal perforation or in a patient with intractable bleeding. If the bleeding fails to respond to active resuscitation, transfusion of blood products or the patient is hemodynamically unstable, then a colectomy may be indicated. The difficulty arises if the site of bleeding also involves the rectum. Ongoing bleeding in a patient with inflammatory bowel disease should also raise an index of

suspicion for CD as the deep penetrating ulcers that are characteristic of CD are more likely to bleed. In young patients, the dissection may be performed to the midrectum, which leaves the option of a reconstructive procedure at a later stage. In elderly patients, the dissection may be made to the level of the pelvic floor. When performing a proctectomy in the emergency setting, great care is required as there is an increased risk for injury to the ureters and pelvic nerves.

Severe colitis and toxic megacolon

Close interaction between all relevant personnel will ensure that patients with severe colitis who are not responding to best medical management will undergo timely surgical intervention. The indication for surgery may be immediately apparent when the patient presents or more commonly it becomes evident because of a progressive deterioration over 72 h. If the patient fails intravenous steroids, then intravenous cyclosporin may be tried under the direction of the patient's gastroenterologist. If there is some improvement but the patient still languishes in hospital beyond 5 days, then surgical intervention may be indicated. We would also have a lower threshold to intervene in patients with co-morbidities.

The definition of toxic megacolon is a non-obstructive dilation of the colon in association with systemic manifestations. It is typically the transverse colon which dilates and although there is great variation in the definition, most physicians consider the upper limit of normal as 6 cm. With improvements in medical care and increasing awareness of this problem, the number of patients needing surgical intervention for toxic megacolon is reducing. However, a significant number of patients with toxic megacolon who respond to initial medical management will require a colectomy at a future time. Patients should be serially examined to determine if there is any change in their baseline examination including abdominal distension or tenderness. They are also followed with serial X-rays of the abdomen to elicit if there is any caliber change in the colon. A rapid increase in bowel diameter is a sinister development and requires intervention. Although toxic megacolon is more common in patients with fulminant colitis and pancolitis, it may also occur with left-sided disease. If it is the primary presentation of colitis, then it is important to rule out infectious causes. Manifestations of systemic toxicity include the presence of at least two of the following: (1) tachycardia >100 beats per minute, (2) pyrexia >38.6, (3) leucocytosis $>10.5 \times 10^3$ per μl or (4) hypoalbuminemia $<3\,\mathrm{g\,dl^{-1}}$. The presence of steroids may mask intra-abdominal sepsis. In toxic megacolon, the indications for intervention are similar to those applied to severe colitis. However, the addition of the megacolon means that we would have an even lower threshold to intervene if the patient fails to make progress in a timely fashion. A subsequent reduction in stool frequency should not always be regarded as a sign of improvement. Rather, it may reflect progressive paralysis of the bowel with deterioration in the patient's condition [47]. A diverting ileostomy was one of the original surgical options for patients with toxic megacolon. However, the mortality did not change considerably because the diseased, friable colon can still perforate, giving rise to intra-abdominal sepsis and septic shock. Thus in 1951, Crile and Thomas of the Cleveland Clinic proposed the operation of STC and end ileostomy [48], which is still the operation of choice for patients with severe colitis and toxic megacolon requiring emergency surgery (Figure 29.7). Patients should be actively resuscitated. All patients must be marked preoperatively at the intended ileostomy site.

An *emergency subtotal colectomy with end ileostomy* is generally performed with the patient in the supine position. During the colectomy, great care must be taken to avoid

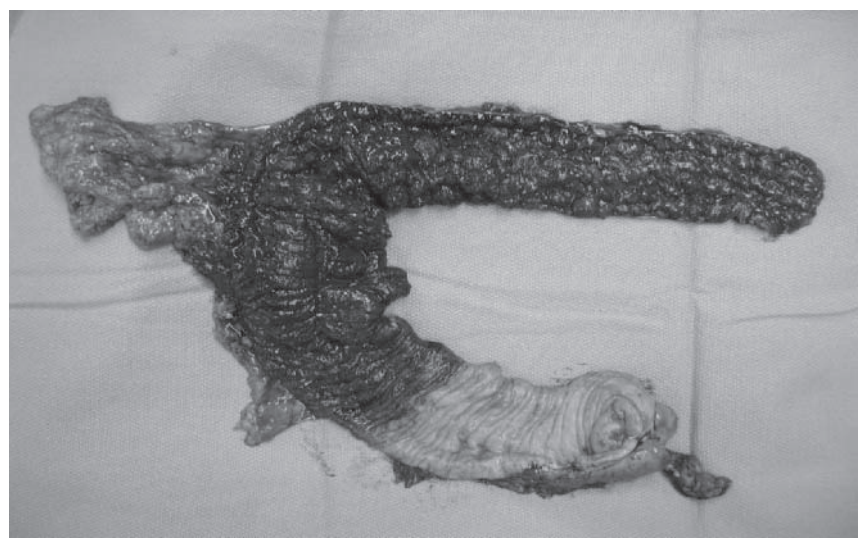

Figure 29.7 Typical ulcerative colitis pattern with distal involvement and proximal sparing.

an iatrogenic perforation, with spillage of fecal contents. If the bowel is significantly distended, then one may perform decompression with a wide-bore needle attached to suction tubing. The abdomen is quarantined to prevent any contamination. The greater omentum is often plastered to the bowel and it is safer to divide it just distal to the gastroepiploic arcade. An energy device such as a LigaSure or harmonic scalpel may assist with hemostasis. At all times, one must stay close to the colon. Many surgeons would advocate approaching the splenic flexure only when the remainder of the colon has been mobilized, as this is the site that carries the greatest risk of perforation. When mobilizing the left side of the colon, a sufficient amount of distal sigmoid must be left *in situ* to allow it to be easily exteriorized to the fascia. It is easier to remove redundant colon rather then being left with a short rectal stump that will not reach the fascia. The inferior mesenteric artery should not be divided. This can be used as a guide to subsequent surgery. We like to divide the distal bowel with a linear stapler and place it into the subcutaneous plane above the fascia. It is then sutured in place. If the stump bursts, then it will give rise to a lower wound abscess and discharge on to the skin rather than causing intra-peritoneal contamination, which would be much more serious. On occasions the distal bowel may be so friable that it will not hold staples or sutures. In this scenario, the sigmoid stump is left protruding beyond the skin and wrapped in a gauze roll. In approximately 7 days the stump can then be amputated at the skin level using local anesthesia or in many cases it may auto-amputate. On occasions the extent of disease may not permit the rectal stump to reach the fascia. This is left *in situ* with an abdominal drain. We also insert a rectal tube to decompress the residual rectum. Once the abdomen has been closed, the ileostomy is fashioned. The majority of patients make a very swift recovery once the diseased colon has been removed.

Even in the emergency setting, it is important that one does not underestimate the importance of a *well-created, functioning stoma*. In many cases the ileostomy may be permanent and in others it may be temporary, albeit it for a considerable period of time. It must allow the patient resume their normal life-style including the ability to return to work following a period of convalescence. When considering surgical intervention in a patient with UC, whether in the elective or emergency setting close coordination with enterostomal therapy is important. Even if one is planning a one-stage procedure without a diverting loop ileostomy, it is always prudent to mark the patient for a stoma prior to any intervention. Preoperative marking allows one to take into account body habitus, where the patient wears their belt, and the ability of the patient to see the stoma. Preoperative stoma marking is one the factors consistently found to result in improved patient satisfaction following the procedure. We prefer to mark our selected sites with India Ink, which provides a permanent tattoo. In general, we place all our stomas through the rectus abdominus muscle, which will reduce the potential for a parastomal hernia. It is important that the abdominal wall opening is of sufficient caliber to allow the ileostomy to be brought to the surface without tension. Often in these cases the mesentery of the bowel is extremely thickened and requires a considerable opening in the rectus sheath to allow the identified bowel loop to be brought to the surface. As a rule, we would prefer to manage a parastomal hernia in contrast to the ill-effect of an ischemic stoma in patients who are already critically ill. On occasions, particularly in patients with a significantly increased BMI, a large fascial opening with a loop end ileostomy may be required.

Blow-hole colostomy with diverting ileostomy

On occasions, a blow-hole colostomy with diverting ileostomy may be indicated. Typically the patient is extremely ill with extensive co-morbidities such that a colectomy carries a significant potential for mortality. We would also consider it in pregnant patients particularly in the presence of an enlarged uterus and dilated ovarian vessels, damage to which may result in life-threatening hemorrhage. The other indications include patients with *Clostridium difficile* colitis and in patients with metastatic malignant disease that develop an obstruction which is not amenable to colonic stenting. In patients with UC and super-imposed *Clostridium difficile* infection the vancomycin may then be directed down the efferent loop of the ileotomy or through the colostomy allowing direct contact with the mucosal colonic surface where it is most effective. A blow-hole colostomy with diverting ileostomy may also be considered in obese patients and those with a high splenic flexure that carries the risk of iatrogenic splenic injury of colonic perforation. One would hope to recognize this patient group prior to surgical intervention. However, on occasions at the time of laparotomy one may encounter extremely diseased, friable bowel which may already have associated sealed perforations. These perforations are most often present in the retroperitoneal portion of the colon and on mobilization give rise to fecal spillage and further exacerbation of the inflammatory/septic state. Turnbull *et al.*, in 1971, recognized this problem which existed in a subset of patients and advised colonic decompression in the form of a skin-level cutaneous colostomy "blow-hole" with a proximal loop ileostomy [5]. This allows patients to recover from the toxic state and definitive surgery may be performed in 6–12 months time once the inflammatory process has settled. Given the improvements in care, medical therapy and time to diagnosis, this operation is only performed infrequently.

The technique of blow-hole colostomy with a proximal loop ileostomy is as previously described [5,47]. If one has

a preoperative indication that this operation may be performed, then we mark the site of the potential transverse colostomy by placing a coin over the skin at the umbilicus and taking a plain abdominal X-ray. This will allow the upper abdominal incision to be directed to the site of maximum colonic distension within the transverse colon. The abdomen is entered via a small lower midline incision. Great care must be taken not to enter the dilated colon inadvertently. If on entering the abdomen one encounters bile, fecal soiling or purulent fluid, suggesting a free perforation, then a full laparotomy with colectomy will be required. If there is no free perforation, then a mobile loop of ileum is identified using the cecum as the landmark. The bloodless fold of Treves will also help to identify the distal ileum. A tape is placed behind the loop, which is then exteriorized at the marked ileostomy site. We place a 3/0 chromic suture distally to indicate the efferent loop and a 3/0 vicryl suture proximally to mark the afferent loop. This helps with the orientation when the ileum is exteriorized and ensures that one does not mature the wrong limb. The exteriorized ileum is placed over a stoma rod. It is important that it is brought out without tension. A small upper abdominal incision incorporating the intended colostomy site is then made. If one is unsure of anatomy a full laparotomy is required. The loop of transverse colon that one is targeting may be covered by omentum, which must be dissected free, taking great care with hemostasis. Sutures are placed between the parietal peritoneum of the abdominal wall and the seromuscular layer of the colon. Needle decompression via the tenae coli is then performed. A vertical incision is made in the colon and the edges are then sutured to the skin edges (Figure 29.8). Great care must be taken as the bowel is extremely edematous and perforates easily. No attempt should be made to try and get posterior to the colon to bring up a diverting loop. This will result is a posterior perforation that will necessitate a full laparotomy. In obese patients, the colostomy may have to be sutured to the abdominal wall fat. Care must be taken to quarantine all affected areas to prevent spillage and wound contamination from bowel contents.

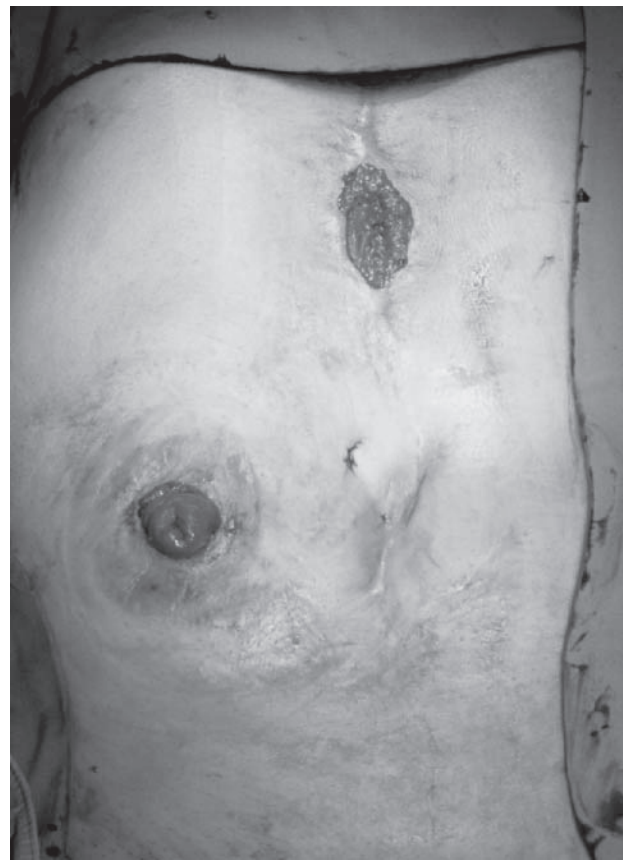

Figure 29.8 Diverting ileostomy with blow-hole colostomy.

Institute results on surgical procedures for ulcerative colitis

In 2002, we reported the function, complications and quality of life after IPAA for patients with indeterminate colitis (IndC) and UC. Follow-up data were available for 1911 patients, of which complete follow-up data were available for 115 patients with a postoperative pathological diagnosis of IndC. Patients with IndC were more likely to develop minor perineal disease and abscesses and have their diagnosis changed to CD. However, the pouch failure rate of 3.4% was equivalent in the two groups. In addition, there was no significant difference in patients' quality of life or satisfaction with IPAA surgery. Over 93% of IndC patients would undergo the same procedure again and 98% would recommend the IPAA to others with IndC. Thus, in addition to UC patients, those with IndC should not be precluded from having IPAA surgery [49].

In 2006 we reported on our institute's long-term outcomes and patients' quality of life after continent ileostomy. There were 330 patients in our prospectively maintained database from 1974 to 2001. Quality of life was evaluated using the continent ileostomy surgery follow-up questionnaire and the Cleveland Global Quality of Life Scale. Comparisons were made between those with the continent ileostomy *in situ* and those who required its removal with the creation of a permanent end ileostomy. Our median follow up was 11 years (range, 1–27 years). The 10 and 20 year pouch survival rates were 87 and 77%, respectively. Patients had on average 3.7 complications and 2.9 pouch revisions during this time. On multivariate analysis the factors that were independent predictors of pouch loss included CD, female gender, fistula development and increased BMI. As expected, the quality of life for patients with a continent ileostomy was significantly higher than that for patients who required pouch excision with conversion to permanent Brooke ileostomy [8].

We have also previously reported our results for salvage pouch surgery. Of 101 patients undergoing laparotomy, ileoanal disconnection and repeat IPAA, 80 were referred from other institutes. Indications for surgery included anastomotic leak ($n = 27$), perineal or pouch–vaginal fistula ($n = 47$), anastomotic stricture ($n = 22$), dysfunction/long efferent limb of S-pouch ($n = 36$) and previous IPAA excision or exclusion ($n = 6$). In 64 cases a "septic" indication was observed. Pathologic features of CD were present in four patients preoperatively and 15 more after repeat ileal pouch-anal anastomosis. After surgery, three patients had no ileostomy and 82 patients had temporary ileostomy closure. Of these, 82% had a functioning pouch, with a median follow-up of 32 months. Two were re-diverted and 13 had the pouch excised. Five-year pouch survival was 74%, higher for UC (79%) than CD (53%; $p = 0.06$). Patients defecated 6.3 ± 2.8 (mean $\pm$ standard deviation) times per day and 2 ± 1.9 per night. About 35% of patients never described urgency. Fecal seepage occurred in 50% during the day and 69% at night. Using the Cleveland Global Quality of Life Score to assess patients' quality of life, health, level of energy and happiness with surgery (each scored from 0 to 10), quality of life was 8.2 ± 1.6 and happiness with surgery was 9 ± 2; 97% would undergo repeat ileal pouch–anal anastomosis again and 99% would recommend it to others. Hence salvage pouch surgery should be considered for patients with IPAA complications or dysfunction that does not respond to conservative measures or local procedures [50].

In 2003, our institute reported on 17 patients who underwent a blow-hole colostomy with ileostomy ($n = 15$) and without ileostomy ($n = 2$). The indication for this procedure was mainly toxic megacolon associated with IBD. We also reported on toxic megacolon associated with pregnancy, *Clostridium difficile* colitis, adult Hirschsprung's disease, pancreatitis with obstructing pseudocyst and palliation for malignant bowel obstruction with metastases. Excluding patients with non-malignant disease, 13 had intestinal reconstruction [51].

Special considerations

Ulcerative colitis during pregnancy

The peak incidence for UC is within the third to fourth decade of life and thus includes a large percentage of female patients of reproductive age. Up to 50% may have an exacerbation during pregnancy or in the postpartum period [52]. Despite best medical management, a number of these patients will require surgical intervention. Optimal treatment of the mother provides the fetus with the best chance of survival. On occasions the diagnosis may occur for the first time during pregnancy. Typically the patient presents with bloody diarrhea. A flexible sigmoidoscopy can be safely performed without any detrimental effects to the fetus or mother [53]. This helps to establish the diagnosis of UC while excluding infectious causes such as *Clostridium difficile* colitis, which is characterized by pseudomembranes on endoscopic examination. The traditionally high maternal mortality rate led to the recommendation of a therapeutic abortion in some instances for those with severe disease. Nowadays the recommendation as a first-line approach is to treat the disease medically. If the patient's symptoms are refractory to medical management or they develop fulminant colitis, then despite risks to mother and fetus surgery is required.

In pregnant patients, the enlarging uterus means that the optimal location for a stoma may be higher than normal. Intra-operatively there should be minimal manipulation of the uterus. Dozois *et al.* reported on five females who underwent an operation for fulminant colitis during pregnancy [54]. Postoperative problems included a superficial wound infection and an intra-abdominal abscess. All patients had a successful pregnancy with no maternal or fetal deaths. Thus in pregnant patients with sever colitis a multi-disciplinary approach with involvement of experienced personnel including gastroenterologist, obstetrician and surgeon will achieve the best outcome.

Ulcerative colitis and dysplasia

Patients with long-standing pancolitis are at an increased risk for the development of dysplasia and colorectal malignancy. The presence of primary sclerosing cholangitis and a positive family history for colorectal cancer are also significant risk factors. Appreciation of this means that patients with UC, in particular the high-risk group, are screened with increased frequency. If a patient with UC is undergoing a proctocolectomy for a diagnosis of cancer or dysplasia, then one must adhere to oncological principles. If the patient is adequately staged and any required radiotherapy is completed preoperatively, then we would consider ileal pouch formation provided the terminal ileum is not affected by radiation enteritis and there is adequate clearance distal to the rectal tumor, allowing preservation of the anal sphincters.

Many surgeons advocate a mucosectomy with handsewn pouch–anal anastomosis in patients with UC who diagnosed with colorectal malignancy or dysplasia. However, our concern is that a muscosectomy is technically difficult and that remnants of residual mucosa may be left *in situ*. In addition, these cells are now located posterior to the pouch and therefore cannot be adequately screened. Thus in the majority of our patients we would perform a stapled anastomosis despite the presence of a colonic malignancy or dysplasia within the upper one-third of the rectum. However, patients must agree to participate in a regular program of follow-up. In patients with a rectal cancer and or dysplasia involving the lower two-thirds of the rectum, we would recommend removal of the ATZ.

Conclusion

All surgeons involved in the care of patients with UC must have a range of surgical procedures in their armamentarium that can be applied depending on the clinical situation. In the emergency setting for severe colitis refractory to medical management, the most common procedure performed is a STC with end ileostomy. We would advise great care in fashioning the ileostomy and exteriorization of the rectal stump. This may also be the optimal procedure in the elective setting for chronically malnourished patients, those on significant immunosuppressant medication including infliximab and patients with extensive co-morbidities. It is formed as part of a staged process with consideration given to completion proctectomy and reservoir creation at a later stage. On rare occasions in extremely sick patients, the option of a diverting ileostomy with blow-hole colostomy may have to be considered. The conventional operation of total protocolectomy with end ileostomy still has a role in elderly patients, those with sphincter dysfunction, and patients wishing to avoid multiple procedures who are content with an end ileostomy. The most common procedure performed for restoration of intestinal continuity is an ileal pouch–anal anastomosis. In a subset of patients who meet well-defined clinical criteria, one may consider a one-stage procedure with omission of the diverting ileostomy. IPAA is associated with a significant learning curve. On occasions in the elective setting one may consider the option of an STC with ileorectal anastomosis. This may apply to female patients of childbearing age in whom the ability to conceive is an important part of their decision making. Thus one wishes to avoid the pelvic dissection that occurs with proctectomy. It may also be considered in obese patients in whom rectal dissection may be challenging. Patients must have a compliant rectum with reasonable control of their rectal disease. We would recommend that any patient requiring a continent ileostomy or salvage pouch surgery for pouch dysfunction attend a tertiary center or surgeon who has had exposure to a significant volume of these complex cases.

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Chapter 30
Clinical Characteristics and Management of Pouchitis and Ileal Pouch Disorders

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Summary
- Pouchitis is a spectrum of disease processes with phenotypes ranging from antibiotic-responsive to antibiotic-dependent and to antibiotic-refractory entities.
- Pouch endoscopy is the most valuable tool for diagnosis and differential diagnosis of pouchitis and ileal pouch disorders.
- *Clostridium difficile* infection may be an emerging issue for pouchitis.
- A combined assessment with clinical presentation, endoscopy, radiography and histology is often needed for the diagnosis of Crohn's disease of the pouch and other complex pouch disorders.
- Although the majority of patients with pouchitis respond favorably to antibiotic therapy, some can develop antibiotic-refractory pouchitis, for which secondary etiology should be excluded.

Introduction

Approximately 30% of patients with ulcerative colitis (UC) eventually require surgery [1]. Ileal pouch–anal anastomosis (IPAA) following total proctocolectomy has become the surgical treatment of choice for the majority of patients with UC who fail medical therapy or develop dysplasia or neoplasia and patients with familial adenomatous polyposis (FAP). Gut continuity is re-established with IPAA. The surgical procedure helps to improve symptoms and health-related quality of life and substantially reduce the risk for dysplasia. However, complications often occur after the surgery. Common long-term inflammatory and functional complications of restorative proctocolectomy are pouchitis, Crohn's disease (CD) of the pouch, cuffitis and irritable pouch syndrome (IPS). Patients should be counseled for the risks for these and other potential complications before the surgery.

Pouchitis

Pouchitis, a nonspecific inflammatory condition at the ileal pouch reservoir, is the most common long-term adverse sequela in patients with IPAA which significantly affects their quality of life [2]. Reported cumulative frequency rates of pouchitis 10–11 years after IPAA surgery range from 23 to 46% [3,4]. The incidence within 12 months after ileostomy take-down was 40% in one study [5]. The discrepancy in the reported cumulative frequencies from different institutions likely results from diagnostic criteria used (e.g. diagnosis made based on symptom assessment alone or based on a combined assessment of symptoms, endoscopy and histology), intensity of follow-up, inclusion or exclusion of other inflammatory and functional disorders of the pouch.

Pouchitis almost exclusively occurs in patients with underlying UC and is rarely seen in patients with FAP [6,7]. Although the etiology and pathogenesis of pouchitis are not entirely clear, the bulk of the evidence points towards an abnormal mucosal immune response (innate and adaptive) to altered microflora in the pouch leading to acute and/or chronic inflammation. [5,8–12] Genetic polymorphisms such as those of IL-1 receptor antagonist [13,14] and NOD2/CARD15 [15] may increase the risk for pouchitis. Reported risk factors for pouchitis include extensive UC [4,16], backwash ileitis [16], pre-proctocolectomy thrombocytosis [17], extra-intestinal manifestations, especially primary sclerosing cholangitis [3,18–20], the presence of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) [21,22], being a non-smoker [23,24] and regular use of non-steroidal anti-inflammatory drugs (NSAIDs) [24].

Patients with pouchitis have a wide range of clinical presentations, endoscopic and histologic features, disease course and prognosis. Increased stool frequency, urgency, tenesmus, incontinence, nocturnal seepage, abdominal

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cramping and pelvic discomfort are the most common presenting symptoms. Patients with severe pouchitis may have fever and dehydration. Occasionally, patients present with predominantly extra-intestinal symptoms such as arthralgia. These symptoms, however, are not specific and can be present in disorders of the pouch other than pouchitis, such as cuffitis, CD of the pouch, proximal small bowel bacterial overgrowth and IPS.

Diagnosis of pouchitis should not be based solely on presenting symptoms. The severity of symptoms often does not correlate with the degree of endoscopic or histologic inflammation of the pouch [25,26]. A combined assessment of symptoms and endoscopic and histologic features are key to making an accurate diagnosis and are necessary to differentiate pouchitis from other inflammatory and non-inflammatory disorders of the pouch. Pouch endoscopy yields valuable information on the severity and extent of mucosal inflammation, presence or absence of concurrent ileitis or cuffitis and presence or absence of anatomic abnormalities such as strictures, sinuses and fistula openings. In addition, pouch endoscopy is an indispensable tool for dysplasia surveillance and it can deliver effective therapy, including stricture dilations. Histopathology of mucosal biopsy has a limited value in the quantification of pouch inflammation. However, histopathology is invaluable for the detection of dysplasia, granulomas, pyloric gland metaplasia, mucosal prolapse and ischemic changes, viral inclusion bodies (for cytomegalovirus infection).

There are no universally accepted diagnostic criteria for pouchitis. The 18-point Pouchitis Disease Activity Index (PDAI) is the most commonly used instrument in published clinical trials and it applies quantitative scores to clinical symptoms and also to endoscopic and histologic inflammation [27].

The natural history of pouchitis is not entirely clear. In a study consisting of 100 consecutive UC patients who had restorative proctocolectomy and IPAA, 32 patients developed episodes of pouchitis and 5 had chronic refractory pouchitis, 2 of whom had pouch failure with pouch resection [28]. Few studies were performed to characterize chronic course of pouchitis. Reported risk factors include pre-colectomy thrombocytosis [17], NSAID use [29] and presence of pANCA [21] or presence of serologic response profile including pANCA, anti-*Saccharomyces cerevisiae* antibodies (ASCA), a CD-related antigen from *Pseudomonas fluorescens* (I2) and the outer membrane porin C (OmpC) of *Escherichia coli* [30]. Chronic refractory pouchitis is one of the most common causes of pouch failure. Patients with initially episodes of pouchitis almost uniformly respond to antibiotic therapy. However, relapse of pouchitis is common. Of the patients with acute pouchitis, 39% have a single acute episode that responds to antibiotic therapy whereas the remaining 61% of patients go on to develop at least one recurrence [19]. Approximately 5–19% patients with acute pouchitis develop refractory or rapidly relapsing symptoms [31–33]. Here is a common scenario: the more frequent episodes of pouchitis a patient has and the more often antibiotic therapy is administered, the less likely the patient is to maintain a favorable response to the treatment.

From various perspectives, pouchitis can be categorized into (1) idiopathic versus secondary, based on etiology, (2) remission versus active, based on disease activity, (3) acute versus chronic, based on disease duration, (4) infrequent episodes versus relapsing versus continuous, based on disease course, and (5) responsive versus refractory, based on response to antibiotic therapy [28]. While the majority of patients with pouchitis have non-identified pathogenetic factors (idiopathic pouchitis), pouchitis in a subpopulation of patients may be associated with specific etiologic causes (secondary pouchitis), such as *Clostridium difficile* [34–36], *Candida albicans* or cytomegalovirus [37,38] infections, NSAID use [39], collagen deposition of the pouch mucosa [40], ischemia, radiation injury and chemotherapy. Pouchitis can occur concomitantly with other local disorders, such as CD of the pouch and cuffitis, or systemic disorders, such as autoimmune thyroiditis, PSC [41] and celiac disease.

Most patients with pouchitis respond favorably to antibiotic therapy, particularly in the initial stages of disease course. This leads to another useful clinical classification based on the response to antibiotic therapy [42]. Analogous to the classification of UC according to the response or dependence on corticosteroids, pouchitis can be classified based on the manner of the patient's response to antibiotics: antibiotic-responsive pouchitis, antibiotic-dependent pouchitis and antibiotic-refractory pouchitis [24,42].

The management and prognosis vary in different types of pouchitis (Table 30.1). For antibiotic-responsive pouchitis, the first-line therapy includes a 2 week course of metronidazole (15–20 mg kg^{-1} per day) or ciprofloxacin (1000 mg per day) [43,44. A randomized trial of ciprofloxacin and metronidazole showed that both agents were effective in reducing PDAI symptoms and endoscopic and histologic inflammation scores, but patients treated with ciprofloxacin experienced significantly greater reductions in the PDAI scores and fewer adverse effects than those treated with metronidazole [44]. Other agents include tetracycline, clarithromycin, amoxicillin/clavulanic acid, doxycycline, rifaximin and budesonide enemas [45].

Patients with antibiotic-dependent pouchitis often require long-term antibiotic or probiotic therapy to keep disease in remission. Probiotics have been used for primary and secondary prophylaxis of pouchitis. As the majority of patients who develop acute pouchitis do so within the first year after IPAA [46], VSL#3 containing viable lyophilized bacteria of four strains of *Lactobacillus*, three

Table 30.1 Classification and treatment of pouchitis.

Classification based on disease course	Classification based on response to antibiotic therapy	Therapeutic options
Acute pouchitis	Antibiotic-responsive pouchitis	2 week single antibiotic agent, metronidazole or ciprofloxacin
Acute relapsing pouchitis	Antibiotic-dependent pouchitis	2–4 week single or dual antibiotic therapy (ciprofloxacin ± metronidazole or rifaximin) for induction followed by probiotic (such as VSL#3) or low-dose antibiotic agents for maintenance
Chronic pouchitis	Antibiotic-refractory pouchitis	4 week dual antibiotics (ciprofloxacin + metronidazole or rifaximin or tinidazole)
		Oral or topical mesalamine agents?
		Immunomodulators?
		Oral or topical corticosteroids?
		Anti-TNFα agents?

Bifidobacterium species and *Streptococcus salivarius* subsp. *thermophilus* was evaluated for the primary prophylaxis of the initial episode of pouchitis. Two of 20 patients (10%) treated with VSL#3 developed pouchitis within 12 months after IPAA, whereas 8 of 20 patients (40%) experienced pouchitis in the placebo group during the same period [5].

For patients with antibiotic-dependent pouchitis, probiotics may be used as maintenance therapy. A randomized trial of VSL#3 at a dose of 6 g per day was conducted for the secondary prophylaxis of relapse of pouchitis, after remission was induced by oral ciprofloxacin (1000 mg per day) and rifaximin (2000 mg per day). During the 9 month trial of 40 patients with relapsing pouchitis, only 15% in the probiotic group relapsed whereas 100% in the placebo group relapsed [47]. A separate randomized trial of VSL#3 in patients with antibiotic-dependent pouchitis showed that 17 of 20 patients (85%) in the VSL#3 group (the majority of patients were from the same institution as the above trial) maintained clinical remission, compared with remission in 1 of 16 patients (6%) in the placebo group [11]. However, in a recent post-market open-label trial of VSL#3 in 31 patients with antibiotic-dependent pouchitis, patients received 2 weeks of treatment with ciprofloxacin followed by VSL#3 [19]. After 8 months, 6 of the 31 patients (19%) were still taking VSL#3 and the remaining 25 patients (81%) had stopped the agent mainly because of the lack of efficacy or development of adverse effects [19]. It is not clear whether VSL#3 is effective in treating active pouchitis.

Antibiotic-refractory pouchitis is often difficult to treat and this type of pouchitis is a common cause of pouch failure. The patients typically do not respond to full-dose, single-agent antibiotic therapy. It is important to investigate contributing causes (in secondary pouchitis) related to failure of antibiotic therapy. Possible causes of refractory disease include NSAID use, concurrent *Clostridium difficile* or cytomegalovirus infection, celiac disease and other autoimmune disorders, cuffitis, CD, pouch ischemia and inflammatory polyps of the pouch [48]. There were no published randomized trials for this category of pouchitis. For patients without obvious causes, treatment options include a prolonged course of combined antibiotic therapy, 5-aminosalicylates, corticosteroids, immunosuppressive agents or even biological therapy. Safe and effective regimens reported in open-label trials including combined ciprofloxacin (1000 mg per day) with rifaximin (2000 mg per day) or metronidazole (1000 mg per day) or tinidazole (1000–1500 mg per day) for 4 weeks [49]. However, maintenance of remission in this group of patients remains challenging [50]. Alternatives to antibiotic therapy, anti-inflammatory agents, immunomodulators and biological therapy have been used to treat pouchitis. These agents include enema formulation of alicaforsen, an antisense inhibitor of intercellular adhesion molecules-1, bismuth carbomer enemas, short-chain fatty acid enemas and glutamine enemas, mesalamine enemas, oral budesonide, 6-mercaptopurine and infliximab.

Inflammatory polyps in the pouch can occasionally occur in patients with chronic pouchitis [51,52]. From a large 2512-case pouch database of patients with UC, 23 patients were found to have large polyps (size $\geq$1 cm) of the ileal pouch or anal transitional zone (ATZ), of whom 21 (91%) had inflammatory polyps with concomitant pouchitis, cuffitis or CD of the pouch; 2 (9%) had dysplastic or malignant polyps. Endoscopic polypectomy with concurrent medical therapy is recommended for the risk for malignancy and potential therapeutic benefits (Plate 30.1) [48].

Cuffitis

Cuffitis is considered a unique form of UC which is a flare of UC in the rectal cuff. Cuffitis is common in patients with IPAA, particularly in those with stapled anastomosis without mucosectomy. One of two anastomotic techniques is

used to construct IPAA: a hand-sewn IPAA with mucosectomy of the ATZ mucosa or a stapled IPAA at the level of the anorectal ring without mucosectomy of the ATZ [53]. The hand-sewn anastomotic technique and mucosectomy may reduce the risk for the development of inflammation (cuffitis) and dysplasia in the ATZ, although small islands of rectal mucosa may be left. In contrast, the preservation of the ATZ during staple IPAA without mucosectomy has increasingly been used to optimize anal canal sensation, eliminate sphincter stretching during the surgery and preserve normal postoperative resting and squeeze pressures. However, in order to allow transanal insertion of the stapler head, surgeons typically leave a 1–2 cm strip of the rectal columnar cuff. This piece of rectal mucosa may be subject to symptomatic inflammation (cuffitis) or dysplasia [53].

Patients with cuffitis often present with diarrhea, urgency, abdominal pain and perianal discomfort. These symptoms are similar to those in pouchitis. Patients with cuffitis may have concurrent anismus, dyschezia and bloating. In addition, patients with cuffitis often present with bloody bowel movements, which may server as a clinical feature distinguishing cuffitis from pouchitis and IPS. However, cuffitis can occur concomitantly in patients with pouchitis (Plate 30.2).

Cuffitis can be treated with topical 5-aminosalicylate agents [54] or topical corticosteroid agents. Duration of the therapy may vary. It appears that development of dysplasia in ATZ may be associated with chronic cuffitis. If that is the case, long-term maintenance therapy with topical mesalamines for chemoprevention warrants investigation. Cuffitis may also be a part of other disease processes, including CD, concurrent pouchitis and sinus or stricture at the pouch outlet. Under these circumstances, cuffitis may not respond to topical mesalamine and/or corticosteroid therapy. Further clinical, endoscopic and radiographic evaluations are warranted. For example, water-soluble contrast enemas, MRI of the pelvis and examination under general anesthesia can be performed to exclude peri-cuff fistulae, sinuses and abscesses associated with refractory cuffitis.

Crohn's disease of the pouch

Although restorative proctocolectomy with IPAA has been performed in patients with Crohn's colitis who did not have small intestinal or perianal diseases [55], the procedure is generally not recommended. However, CD of the pouch can occur in patients with IPAA. Reported cumulative frequencies of CD of the pouch ranged from 2.7 to 13% [56–65] In a large series of 1816 patients who underwent an IPAA with a preoperative diagnosis of UC or indeterminate colitis, 74 (4.1%) had CD based on pre- and postoperative pathology of colon specimens or ileal pouches [56]. In a broad sense, disease activity in the pouch may not be limited to the pouch *per se* and CD in patients with IPAA can occur in any part of the gastrointestinal tract, including the stomach and proximal small bowel.

Clinical phenotype of CD of the pouch can be classified into inflammatory, fibrostenotic or fistulizing phenotypes, modified from the Vienna Classification [65] and recent Montreal Classification [66] for CD in non-pouch patients. The clinical phenotypes may not be static. For example, inflammatory CD can "evolve" into fibrostenotic or fistulizing CD. The clinical phenotypes of CD of the pouch are associated with different risk factors and clinical presentations [67].

The natural history of CD of the pouch is largely unknown. CD of the pouch can occur after IPAA which is intentionally performed in a highly selected group of patients with Crohn's colitis with no previous small intestinal or perianal diseases [55]; CD is also inadvertently found in colectomy specimens of patients with a preoperative diagnosis of UC. *De novo* CD of the pouch, the most common form, may develop weeks or years after IPAA and a reassessment of the proctocolectomy specimens may show no evidence of CD. A recent study showed that the risk factors for CD of the pouch were a long duration of IPAA and active smoking [68]. An additional study also found that each of the three clinical phenotypes of CD of the pouch was associated with different risk factors, suggesting that various etiopathogenetic pathways may be involved [69].

The diagnosis of CD of the pouch should be based on a symptom assessment, endoscopy, histology and radiography. Although symptoms from various disease conditions of the pouch largely overlap, there are some symptoms and signs which would suggest a diagnosis of CD, particularly fibrostenotic and fistulizing CD, such as persistent abdominal pain, nausea, vomiting, weight loss, anemia and fistular drainage. Clinicians may diagnose CD based on the presence of these symptoms together with characteristic endoscopic, histologic and radiographic features. Endoscopic features suggestive of CD of the pouch include the presence of afferent limb ulcers and/or stricture in the setting of ulcerated stricture of the pouch inlet and the presence of ulcers or stricture in other part of the small bowel, in the absence of NSAID use [67,70]. We found that morphologic characteristics of ulcers in the pouch were not reliable for the distinction between CD of the pouch and pouchitis. Clinicians should resist the temptation to take tissue biopsy from the suture line in order to avoid the detection of foreign-body granulomas or pseudogranulomas. It is critical to differentiate NSAID-induced ileitis/pouchitis from CD ileitis and backwash ileitis with diffuse pouchitis. Typically, CD ileitis is characterized by discrete ulcers in the distal neo-terminal ileum (>10 cm beyond the pouch inlet) and ulcerated stricture at the pouch inlet. In contrast, backwash ileitis from diffuse

pouchitis is characterized by the presence of continuous endoscopic and histologic inflammation from the pouch to the distal neo-terminal ileum with widely patent pouch inlet.

It is also important to distinguish surgery-associated stricture, sinus, leak, and fistulizing complications from fibrostenotic or fistulizing CD. However, these distinctions can be difficult to make. In clinical practice, CD of the pouch should be suspected if a patient develops *de novo* fistula 6–12 months after ileostomy take-down in the absence of postoperative leak, abscess and sepsis. Cuffitis that persists after topical therapy with anti-inflammatory agents should raise suspicion for CD-associated cuffitis. Imaging studies such as pelvic MRI often yield anatomic abnormalities outside of the cuff such as fistulae, leaks and even abscesses (Plate 30.3). Histologic evidence of granulomas or pyloric gland metaplasia would also suggest a diagnosis of CD [71].

Reported frequencies of pouch failure from CD leading to pouch excision or permanent diversion range from 25 to 100%, depending on the duration and intensity of follow-up after IPAA, use of medical or endoscopic therapy and threshold of initiating pouch excision or diversion operation [4,56,58–60,62,63,72]. Fistulizing CD appeared to have a poorer prognosis [73]. Patients who are diagnosed with CD of the pouch often require long-term maintenance therapy. However, data on the treatment of CD of the pouch are limited. In a case series of 26 patients with CD of the pouch, 62% had a complete response to infliximab infusion and 23% had a partial response. After a median follow-up of 22 months, 33% had pouch resection, whereas the pouch was functional in the remaining 67% of patients [74]. Fibrostenotic CD can be treated with combined medical [75], endoscopic (e.g. endoscopic balloon dilations of strictures) [76] and surgical (e.g. stricturoplasty) [77] therapy. Appropriate medical and endoscopic therapy may be effective in postponing or avoiding pouch excision or diversion. In a study of 73 cases with CD of the pouch managed with medical and/or endoscopic therapies, pouch failure occurred in 8.0% of patients with inflammatory CD, 5.9% of patients with fibrostenotic CD and 16.1% of fistulizing CD, with a median follow-up of 27 months after diagnosis of CD of the pouch [75].

Irritable pouch syndrome

Irritable pouch syndrome (IPS) is a newly described functional disorder in patients with IPAA, which can be considered a variant of irritable bowel syndrome [78]. Patients with IPS share similar clinical presentations to those with irritable bowel syndrome. The etiology and pathophysiology are not clear. IPS may be attributed to psychosocial factors [24], visceral hypersensitivity [79] and enterochromaffin cell hyperplasia in the pouch mucosa [80]. Patients with IPS have significantly poorer health-related quality of life scores than patients with healthy pouches [2]. Currently, IPS is a diagnosis of exclusion based on the presence of symptoms of increased frequency of bowel movements with a change in stool consistency, abdominal pain or cramping and perianal or pelvic discomfort in the absence of endoscopic and histologic inflammation. These clinical features are similar to those in pouchitis and other disorders of the pouch.

It is important to exclude celiac disease, lactose or fructose intolerance and proximal small bowel bacterial overgrowth since the clinical presentations of these conditions are similar to those of IPS. A subset of patients with IPAA will present with diarrhea, urgency, bloating and abdominal cramps, suggestive of pouchitis. However, pouch endoscopy and histology evaluation will show little or no inflammation. Although these patients may be diagnosed with IPS, they may also report that their symptoms improve or completely resolve after antibiotic therapy. These patients may actually have proximal small bowel bacterial overgrowth. However, confirming the diagnosis can be difficult since there are no validated criteria of hydrogen breath tests or quantitative bacterial culture of small bowel aspirates for this unique patient population. Therefore, the diagnosis is empiric.

Treatment of IPS is empiric and therapeutic agents include oral antidiarrheals, antispasmodics, tricyclic antidepressants and topical belladonna and opium suppositories. Dietary modification can be helpful, but should be individualized. For patients with proximal small bowel bacterial overgrowth, oral antibiotic therapy with agents such as rifaximin, ciprofloxacin and tetracycline are needed.

Conclusion

Pouchitis is the most common long-term adverse sequela of IPAA. Patients with pouchitis can have a wide range of clinical presentations, disease courses and prognoses. Accurate diagnosis and classification of pouchitis are the key for appropriate managements. Treatment of pouchitis is largely antibiotic based. Maintenance of remission in antibiotic-dependent pouchitis and management of antibiotic-refractory pouchitis are challenging. Differential diagnosis should include cuffitis, CD of the pouch, and IPS.

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Chapter 31
Therapeutic Approaches to the Treatment of Crohn's Disease

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Summary
- Identify patients at diagnosis with a poor prognosis.
- Such patients are likely to have two or more of the following at diagnosis: perianal disease, weight loss >5 kg, stricturing behavior, need for steroids at diagnosis, age <40 years.
- These patients are suitable for early immunomodulator (thiopurine or methotrexate) therapy and/or biological therapy.
- Biological (anti-TNF) therapy is best continued once started, because it reduces 12 month hospitalization and surgery rates and because response is rarely regained.
- Failure to consider surgery is a common cause of lack of response to anti-TNF therapy.

Introduction

The general principles for treating active Crohn's disease are to consider the activity, site and behavior of disease, before treatment decisions are made in conjunction with the patient. Nevertheless, although the medical management of Crohn's disease is supported by a better evidence base than for most other disorders, when patients in clinical trials are stratified according to the site or behavior of disease, numbers usually become too small for statistically valid conclusions to be drawn. Consequently, optimum management of many clinical dilemmas remains to be resolved despite evidence-based guidelines on both sides of the Atlantic [1,2]. Only 20% of 64 recommendations on the current management of Crohn's disease were graded A (based on consistent level 1 studies) in the European Consensus [3], indicating a "knowledge gap" in the majority of therapeutic decisions.

The goals of treating Crohn's disease have traditionally been to treat active disease, maintain remission and prevent complications. With appropriate use of conventional and biological medical therapy, nutritional support and timely surgery, the bar can be set higher. The aims should be to achieve and maintain steroid-free remission, reduce hospitalization and surgery, avoid cancer and other complications and reduce mortality. There is debate about whether intestinal mucosal healing is a surrogate marker for modifying disease behavior, since this can be achieved by biological therapy although uncommonly by conventional therapy. These outcomes are not measured in current clinical trials, which analyze "response", "remission" (variously defined) and "steroid-sparing" effects. They are not the outcomes that matter most to patients, who are concerned about the impact of the disease and its treatment on their lives. In a survey of 5636 patients from seven Western European countries, 72% reported that symptoms affected work and 78% leisure activities [4]. Interestingly, 61% said that doctors never asked whether symptoms affected their quality of life and 56% that they did not talk of new treatments.

An alternative explanation for symptoms other than active disease should be considered (such as infection, bacterial overgrowth, bile salt malabsorption, dysmotility, gall stones) and disease activity confirmed [usually by C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)] before starting medical management. Patients should be encouraged to participate actively in therapeutic decisions. No treatment is an option for some patients with mild symptoms. In a systematic review of clinical trials, a mean 18% [95% confidence interval (CI) 14–24%] of patients entered clinical remission when receiving placebo [5]. In contrast, there are others with severe disease who need to be identified for early biological therapy and primary prophylaxis with immunomodulators. There are still others who will benefit most from early surgery (such as those with stricturing ileocecal disease), and this challenges physicians with an increasing therapeutic armory to avoid inappropriate delay in surgical resection to restore quality of life. The appropriate choice depends on

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many factors that are best tailored to the individual patient and are best managed jointly between surgeon and physician. This is most readily achieved by the organizational expedient of running parallel surgical and medical clinics so that immediate opinions can be obtained and strategy agreed for individual patients.

The management of active Crohn's disease needs to be placed in the context of the likely course or pattern of the disease and the risk of relapse, so this chapter discusses these factors before sections on the management of active disease, maintenance of medically-induced remission and common therapeutic dilemmas. Details on specific medications, surgery and management to prevent postoperative relapse are covered separately in other chapters.

Predicting the pattern of Crohn's disease at diagnosis

The approach to managing Crohn's disease in 2010 is changing from reactive to proactive, by designing a therapeutic strategy that specialists hope will modify the pattern of disease. This requires an ability to predict the pattern of disease, which in turn helps select individuals for early biological or immunomodulator therapy. Simple clinical features at diagnosis provide a rough guide and these have the advantage that they have been validated in separate populations. In a single (tertiary) center series of 1123 patients from Paris, factors present at diagnosis and significantly associated with disabling disease over the next 5 years were the initial requirement for steroid use [odds ratio ((OR) 3.1 (95% CI 2.2–4.4)], age <40 years [OR 2.1 (95% CI 1.3–3.6)] and the presence of perianal disease [OR 1.8 (95% CI 1.2–2.8)] [6]. The positive predictive value of disabling disease in patients with two and three predictive factors was 0.91 and 0.93, respectively. Predictive values were 0.84 and 0.91, respectively, when tested prospectively in an independent group of 302 consecutive patients [6]. The problem is that 85% of their patients had "disabling" disease, defined as the need for surgery, immunomodulators or more than two courses of steroids or hospitalization. Nevertheless, these factors have been independently confirmed in both Liege, Belgium, and Olmstead County, MN, USA [7,8]. The Belgian group used a more practical definition of "severe" course of Crohn's disease, meaning a definitive stoma, >50 cm small bowel resection, any colonic resection or complex perianal disease within 5 years of diagnosis; 37% of their 361 patients had such a "severe" course and perianal disease or weight loss >5 kg at diagnosis, and also stricturing behavior and the need for steroids at first presentation were associated with the defined, poor outcome within 5 years. Although intervention in this group of patients has yet to be shown to alter the pattern of "severe" or "disabling" Crohn's disease, there is enough evidence to recommend that an attempt be made to identify such patients. This is not difficult. Patients with perianal disease or a high inflammatory burden at diagnosis (weight loss >5 kg, fever, need for steroids) or stricturing behavior at presentation should be marked out as having a potentially poor prognosis, especially if age <40 years. It is these who appear most likely to benefit from early immunomodulator or biological therapy, perhaps from diagnosis.

Epidemiology of relapse

Approximately half of patients with Crohn's disease have a relapse in the year following an episode of active disease. Patients in remission for at least 1 year have a much lower risk of relapse than those with active disease during the previous year. Patients with moderate or severely active disease needing treatment with steroids are at a high risk of relapse or of steroid dependence in the following year, which reflects the severity of disease rather than the consequence of treatment. Biological markers of active inflammation (such as the CRP), disease location or environmental factors such as smoking are associated with an increased risk of relapse. Although none are yet sufficient to calculate a predictive index for individual patients, there are useful pointers that can be used to guide management.

Clinical relapse rates range from 30 to 60% at 1 year and from 40 to 70% at 2 years among patients receiving placebo in clinical trials of maintenance therapy [9,10]. A population-based study carried out in the county of Copenhagen, Denmark [11] included 373 patients whose diagnosis had been made between 1962 and 1987 and described the outcome of patients in the years following diagnosis. Each year approximately 30% of patients had very active disease, 15% less active disease and 55% were in remission. Approximately 70–80% of patients with active disease during 1 year of follow-up had active disease in the following year; conversely, 80% of patients in remission had no flare in the following year. No other predictive factors of relapse were found. A tendency for disease activity to diminish with time was noted, but the pattern of relapse during the first 3 years correlated well with that observed during subsequent years. These are helpful clinical points when discussing appropriate approaches to induce and maintain steroid-free remission with patients.

Patients with more severe disease requiring steroids may have a different outcome to the overall population of patients with Crohn's disease. In a population-based study in Olmsted County, MN, USA,, the outcome of 173 patients diagnosed between 1970 and 1993 was analyzed 1 year after a course of steroids [12]. Among the 74/173 patients treated with corticosteroids, 32% were in remission ("partial" or complete) without steroids, 28% were steroid dependent and 38% had come to surgery. In contrast, the overall rate of steroid dependence after treatment of active disease in a European study was 18% [13], which may

reflect different thresholds for using corticosteroids between Europe and America. Both of these studies, however, were performed before the introduction of imunomodulators and almost none received immunomodulators.

Reproducible predictive factors of early relapse within the next 6 months are few, but include:

- age $\leq$25 years
- an interval less than 6 months since the previous episode
- colonic involvement.

There are others which have been evaluated in prospective or retrospective studies, including long duration of disease (>5 years since first symptoms) and smoking, especially in young women. A CRP >20 mg l^{-1} and other biological markers of inflammation (such as α_1-glycoprotein >1.3 g l^{-1}, α_2-globulin >9 g l^{-1} and ESR >40 mm h^{-1}) have also been associated with a higher relapse rates. The problem with these markers is that they have a much higher negative predictive value (i.e. a low risk of relapse if all are normal) than positive predictive value.

The potential value of these data for everyday practice is clear even if an intervention study is needed to confirm their clinical relevance. When treating a young (age $\leq$25 years) patient with colonic disease or someone who has recently (<6 months) relapsed, then prophylaxis with immunomodulators is appropriate whether steroids or biological therapy are used to achieve remission. In contrast, intermittent therapy is more appropriate for older patients with infrequent relapse when remission is associated with normal inflammatory markers. Bearing these issues in mind, treatment of active Crohn's disease according to the location and activity of disease can be considered.

Treatment of active Crohn's disease

Mildly active localized ileocecal Crohn's disease

Budesonide 9 mg daily achieves remission in up to 60% over 8–10 weeks [14]. It is superior to both placebo [relative risk (RR) 1.96, 95% CI 1.19–3.23] and mesalazine 4 g per day (RR 1.63, 95% CI 1.23–2.16). Budesonide is significantly less effective than prednisolone (RR 0.86, 95% CI 0.76–0.98), particularly among patients with severe disease (RR 0.52, 95% CI 0.28–0.95) [14]. It is nevertheless preferred, because it is associated with fewer side effects. Corticosteroid-related adverse effects on budesonide are no different to those on placebo and fewer than those on prednisolone (RR 0.64, 95% CI 0.54–0.76) [14]. This matters, especially when considering therapy for patients who are overweight, worried about cosmetic effects or have diabetes, osteopenia or other relative contraindications to systemic steroids.

Mesalazine is generally not recommended, because a meta-analysis has shown that it has only a limited effect compared with placebo [15]. In this meta-analysis there was a significant reduction in the Crohn's Disease Activity Index (CDAI) in patients with active ileocecal Crohn's receiving mesalazine 4 g per day, but this was just 18 points compared with placebo (–63 vs –45, $p = 0.04$) in 615 patients. The clinical significance of this minor reduction is at best termed "uncertain", since even the most liberal interpretation of response to therapy in clinical trials is determined by a reduction in CDAI of 70 points or more [1]. Doses of mesalazine <4 g per day cannot be recommended at all for active Crohn's disease. However, there are those who argue that even if mesalazine has minimal efficacy, it is very well tolerated and that no treatment is not the same as treatment with a placebo. There may, of course, be patients who do respond to mesalazine, but that these are obscured in the heterogeneous nature of the trials to date. This calls for clinical judgment, so the impact of symptoms on a patient's quality of life should be carefully discussed.

It is also difficult to recommend antibiotics (metronidazole, ciprofloxacin), with or without mesalazine or nutritional therapy, for mildly active Crohn's disease in adults. This is because side effects are commonplace or there is difficulty in administration (for nutritional therapy), despite case series or small trials that have shown them to be modestly effective. It is appropriate to discuss these options with patients when discussing the pros and cons of treatment with budesonide, but important to put the relative efficacy and adverse event profiles into perspective.

Moderate or severely active localized ileocecal Crohn's disease

When disease is moderate or severely active, systemic steroids are appropriate as initial therapy. Two major trials established corticosteroids as effective therapy for inducing remission in Crohn's disease. The National Cooperative Crohn's Disease Study randomized 162 patients, achieving 60% remission with 0.5–0.75 mg kg^{-1} per day prednisone (the higher dose for more severe disease) and tapering over 17 weeks, compared with 30% on placebo [number needed to treat (NNT) = 3] [9]. The comparable European Cooperative Crohn's Disease Study on 105 patients achieved 83% remission on 6-methylprednisolone 1 mg kg^{-1} per day compared with 38% on placebo (NNT = 2) over 18 weeks [10]. The dose of prednisolone is adjusted to the therapeutic response over a period of weeks. More rapid reduction is associated with early relapse. A standard tapering strategy is recommended, since this helps to identify patients who relapse rapidly, do not respond or need adjunctive therapy with immunomodulators or inpatient treatment. There are no trials between different regimens and "standard" regimens differ between centers. Although good at inducing remission, steroids are ineffective at maintaining remission and alternative therapy to prevent relapse should be introduced at an early stage. Azathioprine (or mercaptopurine) should be added for those who have relapsed, because it has a steroid-sparing

effect (NNT = 3) and is effective at maintaining remission (see below). Methotrexate should be considered as an appropriate alternative if thiopurines cannot be tolerated, but has specific contraindications, such as pregnancy.

Anti-tumor necrosis factor (TNF) therapy (such as infliximab, adalimumab or certolizumab pegol) should be considered as an alternative for patients with objective evidence of active disease who have previously been steroid refractory, dependent or intolerant, when surgery for localized ileocecal Crohn's disease is considered inappropriate. This does not mean that surgery takes precedence over anti-TNF therapy. Both the indication and timing are joint decisions between patient, physician and surgeon. Risks should be carefully considered and discussed with patients. Anti-TNF therapy offers a conservative option for cases with severe inflammatory activity and it is in these that primary surgery will often be inappropriate. Surgical options should, however, be considered and discussed with the patient as part of an overall management strategy. Although anti-TNF therapy with or without an immunomodulator may be appropriate, restarting steroids with an immunomodulator may be more appropriate for patients who have infrequently relapsing disease. The stage at which anti-TNF therapy is introduced is changing, partly because of mounting evidence that response to early therapy (within 3 years of diagnosis) is better and that this may reduce the risk of hospitalization or surgery [16,17]. Nevertheless, the threshold for surgery for localized ileocecal disease is lower than for disease elsewhere and some experts advocate surgery in preference to anti-TNF therapy for disease in this location. Others advocate resection if medical therapy is not effective within 2–6 weeks. The decision is influenced by the severity of obstructive symptoms, the potential for laparoscopic resection by an experienced colorectal surgeon, the views of the patient and external factors such as the impact on schooling, occupation or other life events.

It may sometimes be difficult to distinguish between active disease and a septic complication, but antibiotics should be reserved for patients with a temperature or focal tenderness or in whom imaging has indicated an abscess. Adding ciprofloxacin and metronidazole to budesonide has shown no advantage over budesonide alone in active Crohn's disease.

Active colonic disease

Initial treatment is best modified when the colon is predominantly affected. Systemic corticosteroids (prednisolone or equivalent) are effective and immunomodulators are appropriate steroid-sparing agents. Given the increased risk of relapse in those with colonic disease, especially in young women or those who smoke, primary prophylaxis with azathioprine or mercaptopurine is usually appropriate at diagnosis. In its current formulation, oral budesonide has no role in therapy of colonic disease, unless it primarily affects the proximal colon (with or without ileal involvement). Other steroids with a colonic release mechanism and low systemic bioavailability (prednisolone metasulfobenzoate, budesonide MMX) are being developed, although their role in colonic Crohn's has yet to be determined.

It is notable that infliximab appears to be twice as effective for isolated Crohn's colitis (OR 1.91, 95% CI 1.01–3.60) as it is for isolated small bowel Crohn's disease (ileitis) and four times more effective in steroid-refractory Crohn's colitis (OR 4.9, 95% CI 2.2–11.0) [18]. This means that patients with severe colonic Crohn's disease requiring hospital admission are candidates for early treatment with biological therapy. In practice, such patients are generally treated with intravenous steroids and antibiotics until the diagnosis is established and infection excluded, before anti-TNF therapy is considered.

Sulfasalazine 4 g daily is effective for active colonic disease [9,10], but cannot be recommended in view of a high incidence of side effects. It is occasionally appropriate in selected patients such as those with an associated arthropathy, although anti-TNF therapy would be more effective. Opinion varies about the value of topical mesalazine as adjunctive therapy in distal colonic Crohn's disease or Crohn's proctitis. It is simple, safe and a therapeutic option, but there has been no controlled trial of topical therapy in Crohn's disease, so there is no evidence base. Metronidazole may also be considered for colonic Crohn's disease, but induces a response and not remission (change in CDAI –97 points for 20 mg kg^{-1} per day, –67 for 10 mg kg^{-1} per day vs –1 for placebo, $p = 0.002$) [19]. It only has a role in selected patients with colonic disease who wish to avoid steroids and biological therapy.

Once again, all medical treatment has to be placed in the historical context of a high prospect of surgery. In 592 patients followed over 13 years, 91% of those with ileocolic disease, 72% with pancolonic and 29% with segmental colonic disease came to surgery [20]. Although surgery should always be considered as an option, it becoming less common with the early introduction of immunomodulators and the option of biological therapy. This has yet to be confirmed objectively, but the indication and timing are important interdisciplinary issues that should be tailored to the individual.

Extensive small bowel disease

The inflammatory burden is greater in extensive (>100 cm) than in localized small bowel disease, so it is generally more severe, with nutritional consequences. Nutritional support with enteral nutritional supplements, best supervised by a dietitian, should be given as an adjunct to other treatment. It may be considered as primary therapy if disease is only mild [21]. Consideration should be given to

micronutrient deficiencies (vitamin B_{12}, folic acid, selenium and other trace elements) with correction as appropriate, in addition to macronutrient deficiency. For patients who have relapsed, anti-TNF therapy with or without azathioprine is an appropriate option if there is objective evidence of moderate or severely active disease. Anti-TNF therapy is best considered at an early stage (potentially as initial therapy), because of the potential to induce mucosal healing, although trials have failed to distinguish between those with extensive and more localized disease. Subgroup analysis of the ACCENT 1 trial showed that hospitalization and abdominal surgery relate to (ileocolonic) mucosal healing [22]. Both were lower in those with mucosal healing, with rates of hospitalization of 4/100 patients compared with 34/100 patients who had no mucosal healing. No patient with mucosal healing at both 10 and 52 weeks was admitted to hospital. Similarly, no patient with mucosal healing had abdominal surgery, compared with 6/100 patients without mucosal healing. There are similar data on the reduction of hospitalization and surgery for adalimumab [16], although data on mucosal healing are awaited. Early introduction of immunomodulators is also appropriate, especially if systemic steroids are used, for their steroid-sparing effect (see below). Resection risks creating a short bowel, but nutritional support prior to multiple stricturoplasty is a valid strategy for managing extensive stricturing small bowel disease, possibly followed by maintenance anti-TNF therapy to reduce the risk of relapse [23]. In a small group of 24 patients randomized to receive infliximab or placebo infusions every 8 weeks, endoscopic recurrence (which is associated with clinical relapse and need for further surgery) in the infliximab group at 12 months was just 9%, compared with 85% in the placebo group, although clinical remission rates were not significantly different [23].

Active esophageal or gastroduodenal disease

Crohn's disease affecting the proximal gut is uncommon, but it is associated with a worse prognosis [24]. Controlled trials are lacking, but there are case series of treatment strategies [25]. It is common practice to add a proton pump inhibitor to conventional therapy to induce of remission and advocate early introduction of immunomodulators, in addition to early introduction of biological therapy, because of the worse prognosis.

Management of medically induced remission

A patient's response to initial therapy should be assessed within several weeks. If treatment is effective, the patient should continue until symptomatic remission is achieved or further improvement ceases. The goal should be steroid-free remission. Maintenance therapy is generally recommended after successful medical treatment of active disease.

The choice of medications to prevent relapse after medically induced remission should take three main factors into account: the anticipated course of the disease (see above), the effectiveness and tolerance of treatments previously used for induction of remission or maintenance and the extent of disease. Other factors such as the presence of biological signs of inflammation and smoking status should also be considered, in addition to constraints (logistic, social or financial) of the treatment. In view of the adverse effect of even light cigarette smoking on the course of Crohn's disease [26], smoking should be discouraged in all patients. Patients in remission should be clinically assessed on a regular basis. Although monitoring of CRP is frequently performed and a CRP $>20\,\mathrm{mg\,l^{-1}}$ during clinical remission is associated with a higher risk of relapse (see above), the consequences for adjusting treatment have not been investigated systematically.

Preventing relapse after first presentation

Mesalazine remains commonplace therapy in Crohn's disease, but there is no consistent evidence that it works. Four meta-analyses on therapeutic trials of mesalazine to maintain remission have been performed, including one that showed no benefit [27]. To this has been added a provocative systematic review that provides evidence that the delivery system may matter [28]. Two early meta-analyses (in 1994) showed a benefit of mesalazine (OR 0.63, 95% CI 0.50–0.79), but not of sulfasalazine (OR 1.08, 95% CI 0.81–1.34) and a 53% reduction in the risk of clinical relapse between 6 and 12 months (OR 0.47, 95% CI 0.33–0.67; $p < 0.001$) [1]. A subsequent meta-analysis (in 1997) was more complete, but included five studies designed for postoperative prevention among the 15 studies analyzed. The Cochrane systematic review on mesalazine for maintenance of remission in Crohn's disease, based on six studies where participants were followed up for 12 months, showed no benefit whatsoever (OR 1.00, 95% CI 0.80–1.24). In contrast (and to add to the confusion), when different formulations were considered, treatment with pH 7-dependent mesalazine (e.g. Asacol) significantly reduced the risk of relapse in patients with either surgically (OR 0.28, 95% CI 0.12–0.65; $p = 0.0032$) or medically induced remission (OR 0.38, 95% CI 0.17–0.85; $p = 0.0113$). However, treatment with controlled-release mesalazine (e.g. Pentasa) or pH 6-dependent mesalazine (e.g. Salofalk) failed to show a significant advantage over placebo [28].

Compare the debatable impact of mesalazine with consistent evidence that azathioprine works. Two meta-analyses have been published, the more recent of which analyzed five clinical trials, including 319 patients [29]. The 1 year remission rate was 67% for azathioprine and 52% for placebo (OR 2.16, 95% CI 1.35–3.47, NNT to prevent one relapse = 7). There was a dose–response effect

(OR 1.20, 95% CI 0.60–2.41 for 1 mg kg^{-1} per day; OR 3.17, 95% CI 1.33–7.59 for 2 mg kg^{-1} per day; and OR 4.13, 95% CI 1.59–10.71 for 2.5 mg kg^{-1} per day). Azathioprine and mercaptopurine have a steroid-sparing effect (OR 5.22, 95% CI 1.06–25.68), but about 25% of patients have to stop treatment due to side effects (OR 4.36, 95% CI 1.63–11.67 compared with placebo). Furthermore, early treatment with mercaptopurine (1.5 mg kg^{-1} per day) after steroid induction in 55 children within 8 weeks of initial diagnosis showed that 91% remained in remission over 18 months, compared with 53% of controls ($p = 0.007$) [30].

So how is this to be interpreted? After first presentation, the risk of relapse should be evaluated as objectively as possible (see above). Some consider that no treatment is an option, particularly those with ileal disease or people who smoke and can be supported to stop smoking. For young patients, especially those with colonic disease at higher risk of relapse or those who have needed steroids to induce remission, a strong case can be made for primary prophylaxis with azathioprine or mercaptopurine. If biological therapy has been used to induce remission, then this is best continued, because steroid-free remission rates are better and once stopped the initial response may not be attained again if it has to be restarted [31,32]. The balance between benefit and risk should be discussed with individual patients. Low doses of mesalazine (<2 g per day) are inappropriate, as are balsalazide and olsalazine. There is no place for continuing steroids.

Preventing relapse of localized ileocecal disease

When Crohn's disease is localized to the ileocecal region, the goal of medical therapy should be symptom- and steroid-free remission, because patients will generally do very well after ileocecal resection. For arbitrary but practical purposes [1], thiopurines (azathioprine or mercaptopurine) are considered appropriate for

- patients who have a severe relapse;
- those who require two or more corticosteroid courses within a calendar year;
- those whose disease relapses as the dose of steroid is reduced below 15 mg;
- those who relapse within 3 months of stopping steroids;
- postoperative prophylaxis of complex (fistulating or extensive) Crohn's disease;
- primary prophylaxis for patients predicted to have a severe course of Crohn's disease at diagnosis (see above). In 2010, it might be argued that these are also the indications for biological therapy.

Azathioprine is usually used before methotrexate, because of longer clinical experience, more controlled data and safety during conception or pregnancy. Some patients who are intolerant of azathioprine may tolerate mercaptopurine. Cessation of treatment after 3.5 years is associated with a higher risk for relapse compared with controls [33], although remission is maintained for at least 18 months in 80% of those who stop thiopurines at this stage. Most believe that it could safely be continued for more than 4 years with appropriate monitoring. Whether azathioprine actually reduces the high probability of surgery in ileocecal Crohn's disease has been questioned. A retrospective analysis showed that despite an increase in the proportion of 565 patients on azathioprine from 13 to 56% between 1983–87 and 1998–2002, the proportion of patients needing surgery remained unchanged (35–34%) [34]. The authors pointed out, however, that those on thiopurines were not the same as those needing surgery. Patients receiving azathioprine or mercaptopurine who relapse should be evaluated for adherence to therapy and have their dose optimized. Change of their maintenance therapy to anti-TNF therapy or methotrexate can be considered, but surgery is always a potential option in localized disease.

An alternative to thiopurines is methotrexate. Some advocate methotrexate before azathioprine, but in an investigator-blind randomized comparison of intramuscular methotrexate 25 mg per week with oral azathioprine 2 mg kg^{-1} per day in 54 patients with steroid-dependent active CD showed little difference between the two. Remission rates at 3 months were 44% (methotrexate) and 33% (azathioprine) ($p = 0.028$) and at 6 months were 56 and 63%, respectively ($p = 0.39$) [35]. There have been two placebo-controlled trials of methotrexate for maintaining medically induced remission in Crohn's disease. The larger study included 76 patients who had achieved remission on intramuscular methotrexate (25 mg per week) [36]. Patients were randomly allocated to continue intramuscular methotrexate (15 mg per week) or placebo. After 40 weeks, remission rates were 65 and 39% ($p = 0.04$), respectively. Among the 36 patients who had a relapse, 22 were then treated with open-label methotrexate 25 mg per week and 55% achieved remission.

As with Crohn's disease in any location, steroids do not maintain remission. Frequent or prolonged treatment with steroids is particularly inappropriate for localized ileocecal disease. No significant difference was found between steroids and placebo when patients were treated for 6, 12 or 24 months [37]. The same largely applies to budesonide [38]. A meta-analysis of placebo-controlled clinical trials evaluating budesonide in ileocolic Crohn's disease for maintenance of medically induced remission included four trials of identical design (380 patients) [39]. Patients were randomized to receive oral budesonide 6 mg, 3 mg or placebo daily for 12 months. The median time to relapse was 268, 170 and 154 days for budesonide 6 mg, budesonide 3 mg and placebo groups, respectively ($p = 0.0072$). However, it is notable that this effect was not readily

discernible in the original trials and that budesonide was not effective at maintaining remission for 12 months.

Preventing relapse of extensive disease

Patients with extensive disease, often in more than one location, have a high risk of relapse [13]. Consequently, immunomodulators with or without biological therapy are appropriate at an early stage, preferably at diagnosis (see above). If biological therapy has been used to induce remission, this is best continued. As with other questions that are relevant to a small number of selected patients, evidence can only be extrapolated from clinical trials designed to address overall benefit in a heterogeneous group of patients with Crohn's disease. Patients with extensive disease, however, are a group in whom the risks of surgery are higher and in whom maintenance therapy with biological agents is more often appropriate.

The evidence for continuing biological therapy is good, but not as good at maintaining steroid-free remission as the industry would have people believe. Two placebo-controlled trials have evaluated the effectiveness of repeated infusions of infliximab for the maintenance of infliximab-induced response in non fistulating Crohn's disease [1]. The largest trial (ACCENT 1) recruited 573 patients [40]. The design was complex. Responders to an initial infusion of 5 mg kg^{-1} ($n = 335$) received infliximab (5 mg kg^{-1}) or a placebo at weeks 2 and 6 and then, every 8 weeks, infusions of placebo, infliximab 5 mg kg^{-1} or infliximab 10 mg kg^{-1}. The primary endpoint was loss of response and the median time to loss of response were 19, 38 and 54 weeks, respectively. The differences between infliximab and placebo were highly significant ($p < 0.001$). Examine, however, the clinical endpoint (steroid-free remission) that matters most to patients. This was a secondary endpoint and rates of steroid-free remission were 9, 24 and 32%, respectively. Although these rates are indeed significant, there is a "therapeutic gap" of 76% in achieving steroid-free remission among initial responders to infliximab after 1 year on 8 weekly infusions of 5 mg kg^{-1}. The same goes for other anti-TNF agents as far as steroid-free remission is concerned. Adalimumab has some of the best data for extended use: in an open-label extension study (ADHERE) of 145 patients (out of a total 467) who were in remission at the end of the 1 year adalimumab maintenance study (CHARM), up to 83% remained in remission 2 years later [41]. For certolizumab pegol, among those with a response to induction therapy at week 6, remission at week 26 was achieved in 48% of patients in the certolizumab group and 29% of those in the placebo group ($p < 0.001$) [17]; 107/141 patients (76%) then retained this response over the next 18 months in an open-label extension phase (PRECiSE 3). However, the trials are not directly comparable and these high continuing response rates refer only to those who responded to treatment in the first place.

Common therapeutic dilemmas

Treatment decisions differ between patients at initial presentation and subsequent relapse, depending on the pattern of relapse and previous response to therapy. Some patients have active disease that persists in spite of appropriate treatment and these are best considered as separate groups, such as those with steroid-refractory, immunomodulator-refractory or anti-TNF therapy-refractory Crohn's disease. Definitions of these groups remain to be agreed for the purpose of clinical trials, but they are clinically relevant when considering treatment options in outpatients.

Treatment of relapse compared with new cases

The initial treatment of relapse best uses the treatment that worked first time, but consideration should be given to other factors. These include the views of the patient (adverse effects, necessary speed of response, convenience, etc.), timing of relapse, concurrent therapy (whether a relapse occurred during treatment with immunomodulators) and adherence with therapy.

Early relapse

Any patient who has an early relapse (within 3–6 months) should be started on an immunomodulator (azathioprine, mercaptopurine or methotrexate) or anti-TNF therapy, because the treatment strategy should think beyond the current relapse and aim to reduce the risk of a further relapse. Opinion is divided whether to use the same treatment to induce remission and taper more slowly or use more potent induction therapy (such as biological therapy). Although active disease should be confirmed as a cause of recurrent symptoms, it is generally unnecessary to re-evaluate the distribution of disease unless this will influence medical or surgical management.

Steroid-dependent Crohn's disease

Patients who relapse as steroids are reduced or within 3 months of stopping steroids present a *prima facie* case for immunomodulators (NNT = 3 to achieve withdrawal of steroids). The question is whether biological therapy (infliximab) should be used as adjunctive therapy. The French GETAID group randomized 113 patients with moderately active, steroid-dependent Crohn's disease randomized to receive three doses of infliximab (5 mg kg^{-1} at 0, 2 and 6 weeks) or placebo, in addition to azathioprine [42]. The relevant primary endpoint was steroid-free remission after 6 months. This was met and induction therapy with infliximab consistently doubled the remission rate at every time point: from 38 to 75% at week 12 ($p < 0.001$), from 29 to 57% at week 24 ($p = 0.003$) and from 22 to 40% at week 52 ($p = 0.04$). The therapeutic bottom line is that induction infliximab followed by AZA doubles steroid-free remission in steroid-dependent patients, but there are

other interesting messages. Half the patients had not previously been on thiopurines. This group responded much better than the thiopurine-refractory (or "failure") group. The 12, 24 and 52 week remission rates in the AZA-naïve group were 83, 63 and 52% compared with 64, 50 and 27%, respectively, in the AZA-MP refractory group. The trouble with thiopurine monotherapy after biological induction for steroid dependence is that during a median 4.5 year follow-up of this cohort, the probability of relapse in the azathioprine-naïve group was $32 \pm 8\%$ at 1 year and $73 \pm 8\%$ at 4 years [43]. An attenuated response on subsequent exposure appears to be true for biological therapy and common experience suggests that it may also be true for steroids, although this cannot be discerned from the original trials. In 2010, infliximab along with azathioprine can be assumed to be better than azathioprine alone for steroid-dependent patients (see combining therapy, below).

Steroid-refractory Crohn's disease

For active CD that is refractory to steroids, local complications (such as an abscess) should be excluded by appropriate imaging and other causes of persistent symptoms considered. If active CD is confirmed, anti-TNF therapy is indicated if a prompt response is needed, septic complications have been excluded and surgery thought inappropriate at that stage. Otherwise, immunomodulators should be added pending surgery if remission is not achieved. The views of the patient should be taken into account. Nutritional support is appropriate as adjunctive therapy, but not as sole therapy.

Relapse while on azathioprine

The first question is whether the dose is optimal (the response to 2.5 mg kg per day is more than three times that at 1 mg kg per day [29]) and whether the patient has been taking the tablets. This is one of the few reasons for measuring thioguanine metabolites of azathioprine, because no detectable metabolites establishes lack of compliance. Higher doses of azathioprine can be used cautiously provided that leucopenia is avoided, but surgery should be considered and biological therapy discussed. Methotrexate is an alternative, but there is no objective evidence that it works in azathioprine failure (as opposed to intolerance). Patients should, of course, be encouraged to stop smoking if (as too often happens) they have not done so.

When to stop azathioprine

Azathioprine (or mercaptopurine) is generally continued for 3–4 years before an attempt is made to withdraw therapy. A controlled study of patients in remission on azathioprine for more than 42 months compared withdrawal (replaced by a placebo) with its continuation. This study showed relapse rates 18 months later to be 21% (withdrawal) and 8% (if continued) [33]. After 3 years, however, 53% of those who stopped azathioprine had relapsed, suggesting a benefit of continuing therapy. The reason why many are minded to stop azathioprine – or at least to try for a drug holiday – is the potential for lymphoma. The risk for patients with inflammatory bowel disease on thiopurines is increased approximately four-fold [44]. This could be a result of the medication, the severity of the underlying disease or a combination of the two. Although this is best discussed with patients, the meta-analysis was unable to demonstrate that the magnitude of risk was related to the duration of therapy. To put it in perspective, the incidence of lymphoma rises with age. Consequently, the number needed to harm (NNH) to cause one lymphoma by treating patients with thiopurines in their third decade (age 20–29 years) is 4357, while the NNH for treating patients in their sixth decade is 1126 [44]. In a large cohort of 13,724 patients with inflammatory bowel disease and complete follow-up over 3 years (2004–07, CESAME study in France), the incidence of lymphoproliferative disease was 2.07 (95% CI 1.25–3.34, $p = 0.006$) compared with the general population [45]; 12/16 (75%) of the incident cases were on azathioprine, compared with 30% of the patient group overall. The optimum duration of azathioprine therapy that balances benefit and risks will thus continue to be debated. Common sense dictates the timing. If a patient in sustained remission is about to take examinations, change jobs, get married or have other reasons for being as confident as possible that remission is maintained, then it is inappropriate to stop therapy. This should be discussed with individual patients. The safety of thiopurines in pregnancy is addressed in another chapter.

When to start anti-TNF therapy

All currently available anti-TNF therapies appear to have similar efficacy and adverse-event profiles, so the choice depends on availability, route of delivery, patient preference, cost and national guidance. In some countries, such as the UK, infliximab is limited to patients with severe active CD (Harvey Bradshaw index >8, Crohn's disease activity index >300) refractory to or intolerant of steroids and immunomodulators for whom surgery is inappropriate. The unanimous European view is that anti-TNF therapy is appropriate for steroid dependence, refractoriness or intolerance and especially after failure of either thiopurines or methotrexate [1]. This matches that in the USA [46]. There is no need to have failed thiopurines and methotrexate before anti-TNF therapy. Re-treatment is necessary [46,47]. All patients given infliximab best receive an immunomodulator, since this may enhance efficacy [48], reduces the development of antibodies to infliximab that in turn may reduce efficacy and may increase side effects. Concomitant immunomodulator therapy appears to add no benefit to treatment with adalimumab or certolizumab pegol [17,31]. A useful acronym used as an aid to prevent inappropriate use of anti-TNF therapy is

STOIC [Sepsis, Tuberculosis, Optic neuritis, Infusion reaction and Cancer (or Cardiac failure, if you prefer)] [49]. Methods for managing risk and potential adverse events related to anti-TNF therapy are discussed in another chapter.

Episodic versus scheduled biological therapy

With the advent of adalimumab [31] and certolizumab pegol [17,46], the question is likely to become academic. Once biological therapy is started with subcutaneous anti-TNF therapy, it is best continued. The levels of evidence for using thiopurines or methotrexate, however, are lower than for biological therapy. The evidence is less robust because it is based on studies performed a decade or two before biotherapy and is a problem of trial design. It does not necessarily mean that biotherapy is better than treatment with immunomodulators for all patients. Compare, for instance, steroid-free remission rates in the ACCENT I trial [40] and that in the GETAID study of induction with infliximab followed by azathioprine [42], discussed above. Nevertheless, evidence derived from trials of infliximab [22] and adalimumab [16] indicates that rates of hospitalization and abdominal surgery are lower in those receiving scheduled anti-TNF therapy [22].

How to identify patients for early biological therapy

This is a fundamentally important question and is addressed, in part, in the section on predicting the pattern of Crohn's disease, above. The truth is that there are no accepted criteria, although it is possible to deduce practical guidance on those patients with the worst prognosis, even if the literature is conflicting. Patients at diagnosis with perianal disease or a high inflammatory burden or stricturing behavior at presentation have a particularly poor prognosis, especially if aged <40 years [6–8]. Those with extensive (100 cm) or proximal (gastroduodenal) small bowel disease have a 3–6-fold higher mortality in the first 5 years after diagnosis [50,51]. Early biological therapy seems appropriate for such patients. Early treatment ("top-down" therapy) with infliximab has been compared with a conventional approach ("step-up" therapy) [52]. A total of 133 patients were randomized to either early combined immunosuppression (three infusions of infliximab at weeks 0, 2 and 6, with azathioprine) or conventional treatment (corticosteroids, followed by, in sequence, azathioprine and infliximab). At week 26, 39 (60%) of 65 patients in the combined immunosuppression group were in remission without corticosteroids and without surgical resection, compared with 23 (36%) of 64 controls (95% CI 7.3–40.8, $p = 0.006$). Corresponding rates at week 52 were 40/65 (62%) and 27/64 (42%) (95% CI 2.4–36.3, $p = 0.028$). Endoscopic mucosal healing was higher using the top-down approach, although the study was too small to show differences in hospitalization or surgery.

Combining immunomodulators and biological therapy

The development of antibodies against biological therapies is common and decreases the degree and duration of response. Adalimumab and certolizumab pegol are less immunogenic than infliximab, but anti-drug antibodies still occur in 5–10% of patients [53,54]. The risk of developing immunogenicity and loss of response to an individual biologic agent, however, must be balanced against potential toxicities of combining biologics with immunomodulators to reduce immunogenicity. An example of toxicity potentially caused by combining therapy is hepatosplenic T-cell lymphoma (HSTL), reported in association with infliximab in young patients with Crohn's disease, most of whom were male [55]. Like the majority of the other 120 reported cases of HSTL in the literature, these patients were on concomitant therapy with azathioprine or mercaptopurine. No cases have yet been reported in patients with rheumatoid arthritis, who generally do not receive azathioprine, nor any in association with methotrexate. The combination of infliximab and methotrexate did not show any advantage in maintaining remission over the course of a year in a randomized trial (COMMIT), but steroid-free remission rates were particularly high (>55% at 12 months) [56]. This may reflect the concomitant use of steroids, as well as infliximab, to induce remission. Nevertheless, there was then a trend towards giving immunomodulators at the time of induction with infliximab, for 6 months, before infliximab was continued alone. Preliminary data from a trial of infliximab and azathioprine, alone or in combination (SONIC), is changing this [48]. Patients with early Crohn's disease <2 years after diagnosis who had relapsed after a course of steroids were recruited. Steroid-free remission was achieved at 26 weeks in just 31% (52/170) patients on azathioprine alone, 44% (75/169) on infliximab alone and in 57% (96/169) on the combination. Consequently, combination therapy appears appropriate for infliximab.

Loss of response to biological therapy

Primary non-response and secondary loss of response should be distinguished. At present, data apply only to infliximab. Primary non-response is uncommon: only 11% of 547 patients with Crohn's disease treated in Leuven [57]. In these cases, the diagnosis should be reassessed, complications (intercurrent infection or abscess) excluded and disease activity confirmed, before surgery or therapeutic approaches other than anti-TNF therapy. In contrast, secondary loss of response is common, with 22% of those in the Leuven cohort needing to stop treatment and almost twice this number needing dose escalation or other intervention to maintain response. For adalimumab, dose

escalation (from every other week to weekly dosing) was necessary within 1 year in 27% in the initial maintenance study (CHARM [31]), but outside clinical trials in the real world it was necessary in 16% of 1335 patients [58]. For secondary loss of response, reasons for symptoms other than active disease should be considered (irritable bowel syndrome, bacterial overgrowth, gall stones, etc.) and complications (stricture or abscess excluded). Surgery should be considered and failure to do so is a common cause of deteriorating response to infliximab. Only after these factors are considered should a switch to alternative anti-TNF therapy be made. The GAIN study randomized 325 patients with secondary loss of response, or adverse reaction to infliximab, to adalimumab (160 mg, then 80 mg after 2 weeks) or placebo [59]. Remission at 4 weeks was 25% and response 38%. Similar results have now been reported for certolizumab pegol when used for infliximab loss of response or intolerance: 62% (334/539) of patients responded within 6 weeks to an induction regimen and, of these, 30% were in remission after 26 weeks [60]. There is, however, a consistent finding that loss of response to one anti-TNF therapy means that the response to a second (or third agent) is less than seen in treatment-naïve patients, even though about 30% respond to a third anti-TNF agent in Crohn's disease. This means that expectations must be managed and a strategy planned. At present there are three options, assuming that the symptoms are due to active Crohn's disease and surgery is inappropriate, which should be carefully considered: natalizumab, stem cell transplantation or a therapeutic trial of novel therapy. Natalizumab, an α_4-integrin antagonist, is licensed in the USA, but unavailable in most of Europe. It is modestly effective as an induction agent, but particularly effective at maintaining remission in those who respond [61]. Of 339 patients who had a response to natalizumab, 61% had a sustained response compared with 28% on placebo maintenance at week 36 ($p < 0.001$). The major problem is increased susceptibility to the potentially (and usually) fatal brain infection progressive multifocal leucoencephalopathy. As a consequence, there is a closed prescribing and distribution (CD-TOUCH) program for natalizumab in the USA. Autologous stem cell transplantation has been the source of case series and is now subject to an international controlled trial (ASTIC) [62]. Perhaps the most useful message is that with a plethora of new therapies evolving, it is appropriate when faced with refractory active disease to call a colleague to reconsider options, including entry into clinical therapeutic trials.

Opportunistic infections and biological therapy

Particular care should be taken to consider opportunistic infections as a complication of anti-TNF therapy. Patients with a fever, cough, systemic symptoms or other unexplained illness should be evaluated for opportunistic infection including tuberculosis or fungal infection, if possible with advice from a specialist in infectious diseases. A European Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease has practical implications for investigation and vaccination before immunomodulators (including biologics) are started [63].

When to stop biological therapy

This is a perennial and difficult question, since there has been no withdrawal trial of any of the anti-TNF agents. Longer term data are, nevertheless, emerging. Among patients in the Leuven cohort (the largest single-center experience [41]), 63% (347/547) had a sustained response on continued infliximab over a median 41 months and in 32% infliximab was stopped, with the patient being in remission. Of these, the great majority remained in remission for up to 28 months, most (75%) on immunomodulators. Emerging data suggest that if the CRP is elevated or there is incomplete mucosal healing, then the chance of relapse is appreciably increased. Consequently, there is no definitive answer at present, but for many patients the answer appears to be to continue anti-TNF therapy "for the foreseeable future". Just how far the future can be seen is, of course, open to debate! Decisions must be made on an individual basis, with potential benefits and risks discussed with the patient. When a withdrawal of anti-TNF therapy is being planned, it seems appropriate to check first whether there is complete mucosal healing at ileocolonoscopy and then gradually to increase the interval between doses, rather than to stop suddenly. This is, however, a practice point rather than evidence-based.

Conclusion

The management of patients with Crohn's disease remains challenging in spite of new therapy. The question that affects clinicians, patients, industry and healthcare funding alike is the timing of different therapies. The pecking order is getting longer, but there is not much order. All therapies have first to be shown to be effective through placebo-controlled or comparative trials of established therapy. It is, however, naïve to believe that all the necessary comparative studies will be performed, since this does not coincide with the interests of industry. Furthermore, it is not at all clear that current endpoints of clinical trials meet the needs of patients. The endpoints that matter are steroid-free remission and reducing hospitalization, surgery and mortality. Clinical judgment is more than ever necessary, since therapeutic goals are not the same as therapeutic indications.

Management of inflammatory bowel disease is about more than drug therapy, dose and timing. With the cost and complexity of biotherapy, inflammatory bowel disease is emerging as a specific subspecialty. When patients

present with persistent symptoms despite a multiplicity of medical approaches, it is best to review the history carefully, record the chronology of different medications and doses, re-evaluate the distribution of disease and activity and discuss this with the patient. A strategy will often emerge, but clinicians should have a low threshold for seeking a second opinion, since this will often confirm the current approach and restore the confidence of a patient frustrated by miserable symptoms. *Ad hoc* trials of treatment without strategic direction usually result in circular motion that makes no progress. Compassion, care and commitment to individual patients are fundamental attributes when navigating a patient through relapses into sustained remission.

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Chapter 32
Surgical Considerations for the Patient with Crohn's Disease/Perianal Crohn's Disease

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Summary
- Surgery should be limited to the management of complications of Crohn's disease.
- Although Crohn's disease often recurs, most patients have excellent quality of life postoperatively.
- When performing a bowel resection, all macroscopic evidence of Crohn's disease should be resected with small margins of normal bowel. The anastomotic type does not appear to affect the risk of recurrence.
- Careful evaluation of the gastrointestinal tract and optimization of patients' health status preoperatively are necessary to ensure excellent postoperative outcome. In addition, if a stoma may be required, the patient should be marked preoperatively.
- Patients with perianal abscesses and fistulae should be evaluated by surgeons.

Introduction

Many new medical therapies have become available that have been shown to be effective for Crohn's disease. Nevertheless, approximately 80% of patients with Crohn's disease will ultimately require surgery [1]. In the case of patients with ulcerative colitis, surgery can cure their disease. The same is not true, however, for Crohn's disease in that it is a panintestinal disease and the disease may recur elsewhere. In Crohn's disease, the goal of surgery is to treat the complications of the disease and improve quality of life for the patients. Because the disease may recur and there is a possibility of the need for further resections and the potential for short bowel syndrome, the surgeon must always be cognizant of both the goals and potential risks of surgery. Avoidance of short-term complications and ensuring that the long-term outcome and quality of life are not impaired should be the goal of the surgeon. However, the need for surgery should not be perceived as a failure of medical therapy. The treatment of Crohn's disease can involve both surgical and medical approaches, and each is required at different points in the course of the disease. Surgeons must also be cognizant of the limitations of surgery and understand that surgery may not be the best therapeutic option, especially for patients with recurrent extensive disease.

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The technical aspects of Crohn's disease may be challenging. The disease may occur at variable sites and the clinical manifestations may vary. The course may be slow and indolent or acute and rapidly progressive. Finally, associated findings such as the presence of an abscess, generalized peritonitis from a free perforation or a cancer may impact on the operative decision making. Hence the surgical approach may be very variable and must often be individualized.

Indications and timing of surgery

Generally, surgery is performed when the disease is refractory to medical therapy or there are complications of the disease. The indications for surgery in Crohn's disease include:
- failure of medical treatment
- bowel obstruction
- intra-abdominal abscess
- fistula
- free perforation
- bleeding
- toxic megacolon
- cancer
- perianal disease

In 40% of cases, the indication for surgery in patients with ileocolonic disease was stricture and in 32%, the indication was abscess or fistula [2]. For patients with colonic disease,

the indication for surgery was failure of medical therapy in 26%, presence of internal fistulae and abscesses in 23%, toxic megacolon in 20% and perianal disease in 19%.

Patients with Crohn's disease typically do not require emergent or urgent surgery; however, an emergency surgery may be required by a small proportion of patients with massive bleeding, toxic megacolon or free perforation. Emergency surgery is always required for the 1–3% of patients with free perforation and the procedure performed will vary depending on the site of the perforation, the extent of the disease and the amount of contamination. The diseased segment is generally resected in this instance. Most often the proximal end is exteriorized as a stoma with reconstruction of the gastrointestinal tract performed in the future once the patient has fully recovered from the operation.

Toxic megacolon, rarely seen now, has always occurred less frequently in Crohn's disease than in ulcerative colitis. Urgent surgery can be required in some patients who have an acute flare-up of their disease and if symptoms worsen or fail to respond after a few days of intense medical therapy. Most of these patients will have pancolonic disease and a subtotal colectomy and ileostomy is the preferred surgical option. A proctectomy can be performed at a later date or alternatively, if there is sparing of the rectum, an ileorectal anastomosis can be performed.

Massive bleeding requiring surgical intervention occurs at a rate of approximately 1%. Preoperative angiography should be performed so the site of the bleeding can be localized. In Crohn's disease patients, the bleeding is thought to originate from one site where a penetrating ulcer has eroded into a vessel, unlike ulcerative colitis where bleeding usually occurs in patients with severe disease and bleeding is not localized to one bleeding vessel but rather is due to severe erosion of the mucosal surface.

Abscesses and fistulae, frequent complications of ileocolonic or terminal ileal Crohn's disease, occur because of micro- or macro-perforations of the intestinal wall. A septic complication should be suspected in patients presenting with a tender mass in the right lower quadrant and fever and symptoms should not be attributed to a disease flare. The cause may be a drainable abscess or a phlegmon with no drainable abscess, in which case antibiotic therapy may be adequate. Computed tomography (CT) scans and other imaging modalities and percutaneous drainage of abscesses through interventional radiology have made urgent surgery for septic complications infrequent. Abscesses can be drained percutaneously, the patient's general status can be optimized and surgery can be performed at a later date. The success rate for percutaneous drainage is approximately 90%, with most of these patients avoiding urgent surgery [3]. Psoas abscesses are the exception. These abscesses fail to settle with percutaneous drainage and surgical intervention is usually required. If the sepsis does resolve with non-operative measures, surgery is indicated since virtually all patients will develop further septic complications in the future. However, depending on the severity of the septic complication and the status of the patient, it may be wise to delay surgery for several months to ensure that both the patient's general status and intra-abdominal condition are optimized.

The etiology of abscesses and fistulae is the same: perforation of the intestinal wall with erosion into a neighboring organ. The most common fistulae are enterocolonic or eneroenteric, often occurring in segments of bowel which are otherwise normal. Other sites of fistulization are those to the bladder, skin, vagina and less commonly involving the stomach and duodenum [4–6]. Approximately one-third of patients have multiple fistula [4]. Fistulae are unlikely to close even with infliximab or other medications. Nevertheless, the presence of a fistula is not an absolute indication for surgery. Many patients will have recurrent episodes of sepsis if surgery is not performed, but in some patients the fistula may be asymptomatic and if so no treatment is warranted. In addition to recurrent episodes of sepsis, patients may become malnourished or have diarrhea if a significant part of the gut is bypassed due to the fistula. Often fistulae occur proximal to a strictured segment of disease or because of severe disease, so surgery may be indicated because of ongoing symptoms due to their Crohn's disease. Patients with ileovesical fistulae may have recurrent urinary infections. Enterocutaneous fistulae are also unlikely to heal but may be low volume or high volume in nature.

The organ involved with the fistula is usually devoid of Crohn's disease and therefore resection of the segment of bowel involved with Crohn's disease may be all that is required. Unless the fistula into the bladder or vagina is large, the opening does not have to be closed. If there is a fistula into another loop of small bowel or colon, management depends on the size and site of the fistula and also the amount of reaction around it. If the fistula is on the antimesenteric side of the unaffected piece of bowel and there is little reaction around it, the edges can be freshened and closed. If not, a short resection of the uninvolved bowel may be required. Only 22% of patients in our series required an ileostomy with those having multiple fistula being more likely to require a defunctioning ileostomy [4]. Preoperative imaging of the entire gastrointestinal tract is especially important if a fistula is suspected so that the surgeon knows the disease status of the rest of the bowel.

Cancer is an uncommon complication of Crohn's disease; however, the incidence of small bowel cancer is increased approximately 10–12-fold over that in the normal population [7]. Bypassed segments of small bowel seem to be particularly at risk for developing cancer. Bypass procedures were at one time performed commonly, but this type of procedure has been abandoned. Cancer risk also appears to be higher in fistulous disease. The risk of cancer in patients with Crohn's colitis is less certain than with ulcerative colitis. It appears, however, that the risk of cancer

in patients with longstanding extensive Crohn's colitis is similar to that of ulcerative colitis. Most strictures in the large bowel generally should be treated surgically because of the concern that there may be an undetected cancer. In addition, the stricture may preclude visualization of the remaining colon.

Bowel stricturing, resulting in obstructive-type symptoms, is a common indication for surgery in small bowel Crohn's disease. In most cases of acute obstruction, the obstruction will settle with conservative measures so an elective procedure can be performed. More frequently, patients present with chronic obstructive symptoms and detailed questioning is required to elicit a history of obstruction because most patients modify their diet to minimize cramping and discomfort after meals. If the narrowing is fibrostenotic in nature it will not respond to medical therapies and surgery is indicated, or if there is a large inflammatory component medication may be effective. Imaging is usually not helpful in distinguishing between a fibrostenotic and inflammatory stricture. C-reactive protein may be increased if there is an inflammatory component. Some authors have suggested that surgery is indicated when two sub-occlusion flare-ups occur over a period of 1 year or if steroid treatment cannot be discontinued at 3 months or doses of prednisone of greater than 15 mg per day are required for more than 3–6 months following an obstructive episode [8].

A common indication for surgery in Crohn's disease is failure of medical therapy, which is nevertheless hard to define. Generally, the goal in the management of Crohn's disease should be to optimize the quality of life of patients. If quality of life is suboptimal because of persistent or worsening of symptoms despite medical treatment or due to drug-related complications, then that constitutes failure of medical management. Steroid dependence is generally considered an indication for surgery. However, various factors must be considered in making the decision to operate, including patient preferences. Some patients may be reluctant to have surgery and will want to try all medical options. Others may opt for surgery if the likelihood of success of medication is low. Currently there is a trial in progress comparing ileocolic resection with infliximab therapy in patients who have failed steroid and immunomodulatory therapy. The results of this trial may be important in addressing some of these questions [9].

Short bowel syndrome is often a concern of patients if they undergo multiple operations. In reality this is a very rare occurrence. There is also little evidence that early or late surgery alters the course of the disease. Nordgren *et al.* reported on their experience with an active surgical approach in 136 patients who were followed a mean of 16.6 years [10]. They concluded that morbidity and mortality are lower with this approach and most patients have good functional outcomes and complete remission of symptoms. A study by Scott and Hughes showed that of 70 patients who underwent ileocolic resection, none regretted having surgery and 77% would have preferred to have had surgery earlier [11].

General considerations

Preoperative evaluation and management

Ideally, the patient's medical status will be optimized and the gastrointestinal tract fully evaluated prior to undertaking surgery. This may not be possible because of the urgency of the condition or status of the underlying disease. However, even with emergency surgery, there are certain measures, such as correction of fluid and electrolyte abnormalities, administration of antibiotic and thromboembolic prophylaxis and stoma marking, which should be done. Thromboembolic prophylaxis is required since inflammatory bowel disease increases the risk of thromboembolic complications [12]. In contrast, the need for a mechanical bowel preparation in patients undergoing colorectal resection has been challenged. A recent meta-analysis of almost 5000 patients showed that the anastomotic leak rate and surgical site infection rate were the same in patients in whom the mechanical bowel preparation was omitted and in those who did receive a mechanical bowel preparation [13].

Preoperatively, the entire gastrointestinal tract should be examined radiologically with a small bowel enema (enteroclysis) and colonoscopy [14]. These examinations are preferred over a gastrointestinal follow-through and barium enema because they are more sensitive in detecting earlier mucosal disease. Although the bowel may be assessed intraoperatively, preoperative information about the extent and site of the disease and the presence of complications (such as a fistula) may be helpful in planning the surgery and also informing the patient. In addition, it may not always be possible intraoperatively to assess the distal colon and rectum and decisions regarding the extent of the resection and whether to do an anastomosis may be difficult to make. In the acute situation, a CT scan may be helpful in detecting abscesses, fistulae and free perforations in addition to identifying the segments of bowel affected with Crohn's disease.

The general status of the patient should be optimized before surgery. There are several considerations. Although there is little evidence to support a course of preoperative total parenteral nutrition (TPN), in some situations it may be worthwhile. Alternately, surgery may be delayed while the nutritional status of the severely malnourished patient is improved or abscesses and inflammatory masses are treated. Percutaneous drainage of intra-abdominal abscesses can help patients to avoid a multiple staged operation and a temporary stoma [3]. If there are associated medical conditions, they should be treated. The patient should be prepared both physically and

psychologically for surgery. Finally, patient education is an important aspect of surgical management. An ileostomy is frequently required in patients with Crohn's disease. It may be permanent or temporary. Preoperative marking of the stoma is essential since how well the stoma functions may have a profound effect on outcome and the patient's acceptance of it [15]. It is particularly important in the emergency situation to mark a stoma site because there may be unexpected findings necessitating the construction of a temporary stoma. When siting a stoma, it should be placed away from scars and creases and in a location where the patient can visualize it adequately when he/she is sitting or lying. If not, the patient may have difficulty changing the appliance. Both stoma placement and siting of incisions are extremely important in patients with Crohn's disease. These patients will often require multiple operations, possibly require stoma revisions in the future and may have significant weight gain or loss in the future. Hence not only must the stoma be placed well initially, but also other sites, say in the left lower quadrant, should be preserved. For this reason, midline incisions are preferred.

In a number of situations, temporary ileostomies may be constructed. If surgery is performed as an emergency because of a free perforation, abscess or obstruction, it may be unwise to perform an anastomosis because of the risk of it not healing. In this situation, the proximal end can be brought out as an ileostomy or colostomy or the anastomosis can be performed with a proximal defunctioning ileostomy. Loop ileostomies are often indicated in patients who have severe perianal disease unresponsive to more conservative surgical procedures or medical therapy. Although it is unusual that it will be possible to close the stoma in the future, it will allow the perianal sepsis to settle before performing a proctectomy. Psychologically, patients may not be willing to have a permanent stoma initially and may be more accepting of a stoma knowing that there is a possibility, albeit remote, of it being temporary. Over 25 years ago, Harper *et al.* advocated performing a split ileostomy so medication could be delivered to the defunctioned colon through the distal limb of the ileostomy [16]. However, that approach has failed to gain acceptance at other centers and there is little evidence to support its use. On the other hand, there has been some success with performing a temporary ileostomy, unroofing of abscesses and superficial fistula tracts in patients with complex perianal disease and then treating them with infliximab [17].

Laparoscopic surgery for Crohn's disease

A laparoscopic approach to surgery for Crohn's disease has become the standard in many situations. Because it is a benign disease, the concerns related to cancer do not apply. In addition, it may result in an improved cosmetic result, which is an important consideration in this often young and single patient population. There tends to be less pain and greater patient satisfaction with laparoscopic procedures. On the other hand, the patient may have multiple adhesions from previous operations or a large inflammatory mass, abscess or fistula or obstructed bowel which may preclude a laparoscopic approach. In addition, hospital stays may be minimally shortened and there is no consistent evidence that patients return to work earlier following laparoscopy [18].

As laparoscopic techniques have become more widely adopted, the indications have widened so it is reasonable to attempt defunctioning stomas in addition to the range of resections including ileocolic resections, segmental resections, subtotal colectomies, proctectomies and total proctocolectomies. Most proponents advocate performing laparoscopic-assisted resections so the bowel is exteriorized through a small incision at one of the port sites, and the mesentery, which is often thickened, is divided extracorporally.

The limiting factors in performing laparoscopic resections are usually adhesions if the patient has had a previous operation or has fistulous disease. In addition, laparoscopic procedures tend to be more difficult than those performed for other benign or malignant diseases because the mesentery is often bulky and friable. The reported conversion rates vary from 5 to 20% [19]. The rates have remained fairly constant because as surgeon experience has increased, the indications have also increased [20]. Thus, while fistulous disease was initially thought to be a contraindication to the laparoscopic approach, now many surgeons will insert a scope and convert early if necessary. However, often the procedure can be performed laparoscopically [20–23]. Regan and Salky reported a conversion rate of 4% in 72 patients having surgery for enteric fistulas [22]. Pokala *et al.* reported a conversion rate of 32.6% in a cohort of 43 patients all of whom had fistulizing Crohn's disease [23]. Tilney *et al.* performed a meta-analysis of 20 studies which included 783 patients who underwent an ileocolic resection [19]. All but one study were retrospective case series. The conversion rate was 6.8%. The complication rates were similar but hospital stay was significantly shorter by 2.7 days. The only randomized controlled trial was reported by Milsom *et al.* [24]. Sixty patients undergoing elective ileocolic resection were randomized to laparoscopic or open procedures. There was no difference in the major complication rates but minor complications occurred significantly more frequently in the open group. Median length of stay was 5 days in the laparoscopic group and 6 days in the open group.

Whether laparoscopic surgery affects the risk of recurrence of Crohn's disease is controversial. Some have suggested that the risk of recurrence may be affected because laparoscopic surgery has been shown to have less effect on the immune system. However, Lowney *et al.* reported on a cohort of 113 patients who had had ileocolic resections and were studied retrospectively [25]. Of these patients, 63 had a laparoscopic resection and 50 had an open procedure. The recurrence rate was 9.5% in the laparoscopic group after a mean follow-up of 62.9 months

versus 24% in the open ileocolic resection group with a longer follow-up of 81.8 months, a difference which was not statistically significant. Our group studied a cohort of patients who completed a randomized controlled trial comparing recurrence rates with two different anastomotic types [26]. A total of 94 patients (55.3%) had their procedure performed open and 76 (44.7%) had an attempted laparoscopic-assisted procedure. Not surprisingly, those in the open group were more likely to have had a previous resections and required additional procedures. In addition, the mean duration of the operation was shorter but the median hospital stay was longer. There was no significant difference in the symptomatic or endoscopic recurrence rates between those having an open or laparoscopic converted versus a laparoscopic procedure.

Other advantages of the laparoscopic approach are related to the decreased size of the incision. Cosmesis and body image were shown to be significantly better in a group of 43 patients having laparoscopic surgery by Dunker *et al.* [27]. Further, wound complications and the long-term risk of small bowel obstruction seem to be decreased [28].

Impact of medical therapies on surgery

Many patients are on infliximab prior to undergoing surgery. Because of the immunosuppressive action of infliximab, there are concerns that its perioperative administration may increase the risk of complications following surgery, particularly with respect to septic complications. Most studies are limited by the fact that the sample sizes have been small, a range of different procedures have been performed and there may be selection biases since patients with more severe disease are probably more likely to receive infliximab and these patients are also at greater risk for developing postoperative complications. Appau *et al.* studied a cohort of patients who had surgery at the Cleveland Clinic [29]. Postoperative outcomes in 60 patients who had a first-time ileocolic resection and received infliximab within 3 months of surgery were compared with a historical control group of 69 patients and also a group of 319 patients who had ileocolic resections during the same period but did not receive infliximab. Those in the infliximab group had a significantly higher rate of postoperative sepsis [odds ratio (OR) 2.32–4.06], abscess (OR 2.44–25] and readmissions (OR 2.4–8.37]. Even after adjusting for confounders such as the presence of an abscess before or after surgery, a significantly increased rate of complications was observed.

Surgery for small bowel Crohn's disease

Crohn's disease may affect any part of the small bowel; however, the terminal ileum is most frequently involved. Alternatively, there may be multiple skip lesions throughout the small bowel. The pattern of disease may also vary, with some patients having primarily inflammatory, fibrostenotic or fistulizing disease. Farmer *et al.*, in a review of 500 patients operated on at the Cleveland Clinic, observed that obstruction was the indication for surgery in 55% and intestinal fistula and abscess in 32% of patients with small bowel disease [2]. The indications in patients with ileocolic disease were similar. Others have reported that patients will continue to manifest with the same patterns of disease after resection [30].

Depending on the site of the disease and indications for surgery, the surgical approach may vary. Resection is performed in most patients with small bowel or ileocolic disease. Although strictureplasty is used in only selected patients, it has been a valuable addition to the surgical armamentarium in Crohn's disease. Bypass procedures (the so-called Eisenhower procedure) were popular in the 1960s but they are rarely performed now because of the high rate of recrudescence of the disease in the short term and the increased risk of small bowel cancer in the long term. At present, the only indication for a bypass procedure would be a gastrojejunostomy for duodenal Crohn's disease. In the unusual situation where the surgeon felt it was unsafe to resect small intestinal disease, a defunctioning ileostomy would be preferable to a bypass procedure. However, this situation is rarely encountered today because of improved imaging techniques and the ability to drain abscesses percutaneously preoperatively.

Bowel resection

For disease involving the terminal ileum, the resection usually encompasses the terminal ileum and cecum since the disease usually extends to or into the cecum. The decision as to whether a primary anastomosis will be performed depends on whether the procedure is performed electively or as an emergency, the status of the patient including whether he/she is on high doses of steroids or immunosuppressive agents, the local conditions of the bowel including whether the bowel is obstructed or whether there is an abscess present and nutritional status. In suboptimal conditions, it may be prudent to bring out the proximal end of the bowel as an ileostomy or to perform an anastomosis and a proximal defunctioning ileostomy with the plan to reanastomose the bowel at a later date.

Although surgery is often successful in treating the complications of the disease and improving patients' quality of life, recurrence of the disease is a frequent occurrence and therefore a major concern. Recurrence rates vary depending on the criteria used to define recurrence [31]. For example, endoscopic recurrence rates varying from 29 to 93% at 1 year have been reported.[32–34]. The reported clinical or symptomatic recurrence rates, which are probably most relevant, range from 6 to 16% per year [35]. In our own study of 76 patients who were followed prospectively, the symptomatic recurrence rate was approximately 12% at 1 year and 47% at 3 years [34]. Thereafter, there was

a decrease in the yearly recurrence rate, which has also been reported by others. It appears that there are various patient factors which may affect the recurrence rate, including the number of previous operations and the indication for surgery [36]. Smokers also appear to have a higher risk of recurrence [37].

Recognizing that recurrence following surgery is a significant problem, surgeons have looked at various maneuvers which might decrease the risk. There are conflicting data regarding the effect of microscopic disease at the resection margin [31]. However, given that Crohn's disease is an intestinal disease, that it is focal in distribution and that histological abnormalities have been demonstrated in segments of bowel which appear to be grossly normal, the significance of microscopic disease at the resection margin is questionable.

The length of the resection margin has also generated conflicting and controversial results. In the 1980s, Krause *et al.* advocated a radical approach of excising 10–30 cm of normal bowel proximal and distal to the affected area [38]. This was based on a retrospective study with follow-up ranging from 7 to 19 years where recurrence rates of 29 and 84% were reported in patients having radical or limited surgery, respectively. However, the two approaches were performed at different hospitals, so the possibility of selection biases is real. Fazio *et al.* published the results of a randomized controlled trial in which 152 patients were randomized to one of two groups, with proximal resection margins of either 2 or 12 cm in length [39]. After a mean follow-up of 56 months, the recurrence rate (as defined by the need for a further resection) was 25% in the limited resection group compared with 18% in the extended resection group, a difference which was not statistically significant. Hence the approach accepted by most surgeons is to resect the bowel which is grossly involved plus a margin of several centimeters of normal bowel. Frozen sections are usually unnecessary.

Recently, there has been interest in the effect of the surgical anastomosis on postoperative recurrence rates. There are several observations which support the hypothesis that the anastomosis may play a role in the risk of recurrence. First, the risk of recurrent disease is extremely low in patients with permanent end ileostomies [40]. Second, approximately 90% of recurrences occur in the preanastomotic segment of the bowel [41]. Third, recurrence of disease occurs rarely in individuals where the anastomosis is defunctioned but early changes of disease occur rapidly if feces are infused into the bowel [42]. The mechanism is unknown but it has been postulated that a narrow anastomosis may lead to fecal stasis and increase the likelihood of recurrence of disease. Thus, a wide anastomosis such as a side-to-side anastomosis may lead to a decreased risk of recurrence compared with an end-to-end anastomosis. Several observational studies, however, have shown conflicting results [43–47]. Our group performed a multicenter trial in which 139 patients having an ileocolic resection were randomized to a wide side-to-side stapled anastomosis or a sutured end-to-end anastomosis [48]. Colonoscopy was performed at 12 months. After a mean follow-up of 11.9 months, there was no difference in the endoscopic and symptomatic recurrence rates. One other underpowered trial compared end-to-end with end-to-side anastomosis [49]. After a mean follow-up of 47 months, the recurrence rates in the two groups were similar at 23% and 31%, respectively. Of interest in this study is that recurrences in the end-to-side group occurred in the pre-anastomotic area rather than the blind end of the small bowel, giving credence to the suggestion that the anastomosis may be important. A second trial reported by Ikeuchi *et al.* randomized patients to stapled or hand-sewn anastomosis [50]. In this study, there were variable types of anastomoses performed (i.e. ileoileal, ileocolic, colocolic and ileorectal) and also anastomotic configurations (functional end-to-end, circular stapled, hand-sewn end-to-end). Some patients had multiple anastomoses. Outcome was need for reoperation for recurrent perianastomotic disease. Recurrence overall was lower in the stapled group than the hand-sewn group (18.9 vs 37.8%, respectively). In the subgroup of patients having ileocolic resections, the recurrence rates were 1/12 (9.1%) in the stapled group compared with 6/21 (28.6%) in the hand-sewn group. A concern with this trial is that it is unclear whether patients were randomized or simply randomly allocated.

Hence currently the standard is to resect all of the gross macroscopic disease with a several centimeter margin of normal bowel. Frozen examination of the margins is unnecessary. The type of anastomosis can be performed according to surgeon preference.

Strictureplasty

Strictureplasty was first advocated in the 1980s for the treatment of fibrotic strictures in Crohn's disease [51]. Although resection of the diseased segment is still the preferred surgical option for most patients, strictureplasties have been used with increasing frequency, especially in patients who have multiple skip lesions or have had multiple resections in the past. As a consequence, the greatest experience has been with strictureplasties performed in the small bowel. However, they may also be performed for strictures involving a previous ileocolic anastomoses in addition to those in the duodenum and colon. Strictureplasty is less applicable to strictures in the colon since there is usually involvement of the rest of the colon which requires resection. Further, one must always be cautious that a stricture in the colon is not cancerous.

There are several types of strictureplasties. The most common types are the so-called Heineke–Mickulitz, which is performed for short strictures, and the Finney, used for longer strictures. In performing a strictureplasty, a

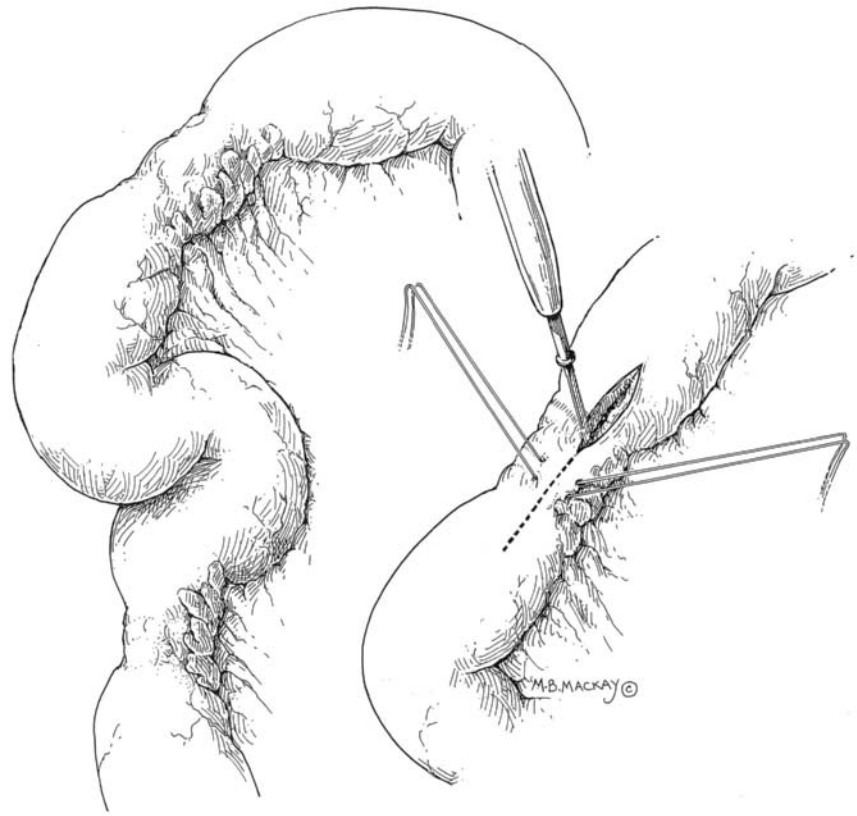


Figure 32.1 A segment of small bowel showing two short strictures due to Crohn's disease. Short strictures due to small bowel Crohn's disease are amenable to strictureplasty. To perform a Heineke–Mikulicz strictureplasty, two stay sutures are inserted. Then a longitudinal incision is made along the antimesenteric border of the small bowel using cautery over the length of the stricture. Reproduced by permission of Margot B. Mackay.

longitudinal incision is made on the antimesenteric side of the bowel over the length of the stricture (Figure 32.1). The base of the strictureplasty should be biopsied to ensure that it is not a malignant stricture. In the Heineke–Mickulitz strictureplasty, the enterotomy is closed transversely (Figure 32.2). Hence this type of strictureplasty is generally reserved for strictures less than 6–8 cm in length. A side-to-side anastomosis is performed in a Finney strictureplasty (Figure 32.3). Usually the bowel is closed in one layer using a continuous absorbable

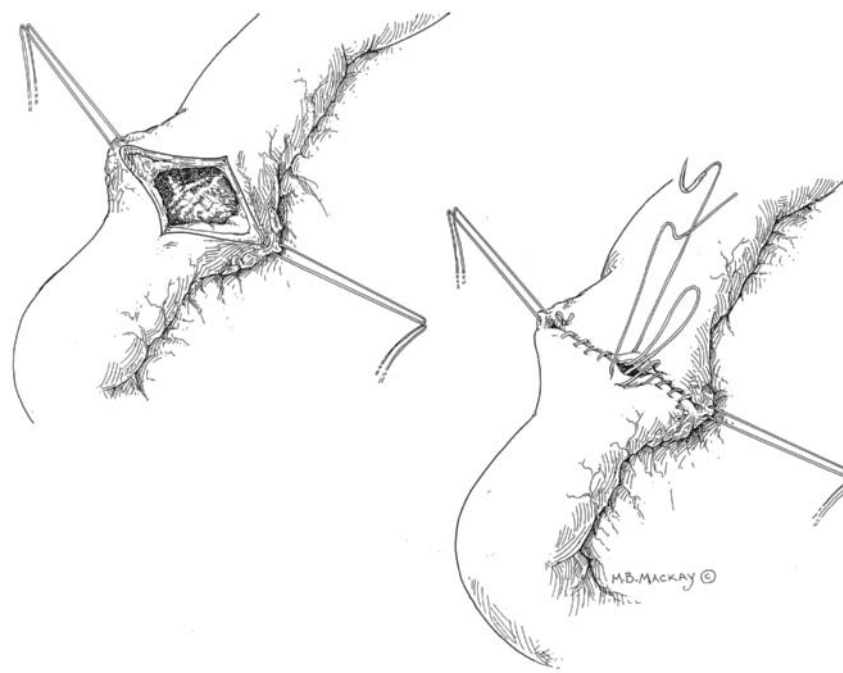


Figure 32.2 To perform a Heineke–Mikulicz pylorplasty, the longitudinal incision is closed transversely using a one layer running inverting suture. Reproduced by permission of Margot B. Mackay.

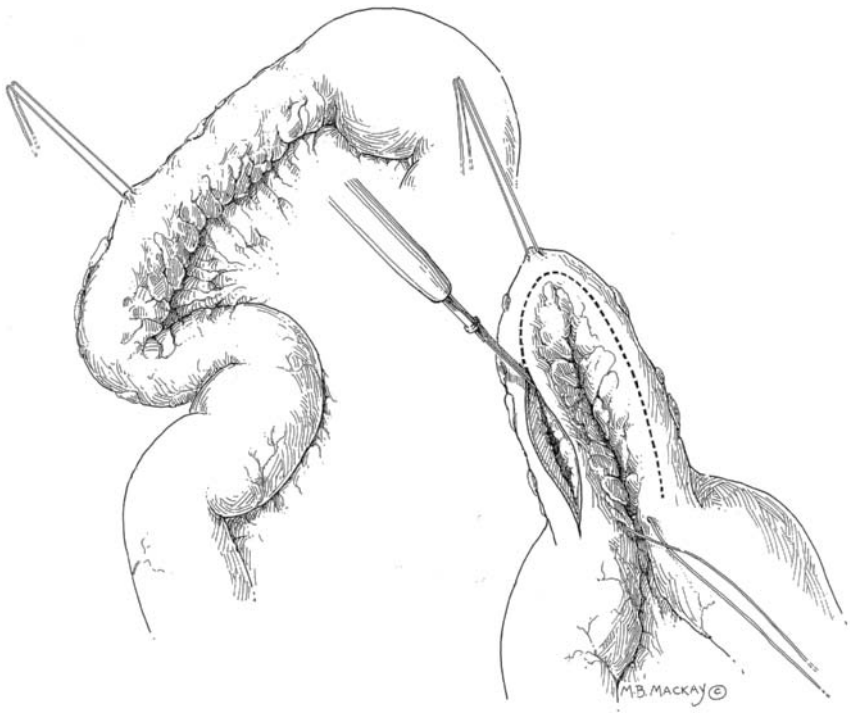


Figure 32.3 Long Crohn's disease strictures may not be amenable to a Heineke–Mikulicz strictureplasty so instead a Finney strictureplasty is preferred. An enterotomy is made over the length of the stricture along the antimesenteric border of the bowel. Reproduced by permission of Margot B. Mackay.

suture. Because the bowel is usually thickened and fibrotic, there is a risk of fracture if a stapler is used (Figure 32.4).

Michelassi *et al.* described a side-to-side isoperistaltic strictureplasty for management of a long segment of disease or multiple strictures in the midsmall bowel [52]. The bowel is divided and a side-to-side anastomosis is performed, thus avoiding a resection, a blind loop or a bypassed segment. Another report from Poggioli *et al.* described a strictureplasty where a side-to-side anastomosis is performed between the diseased terminal ileum and the right colon [53]. This technique had been used in only five patients and therefore the utility of this technique is also yet to be determined.

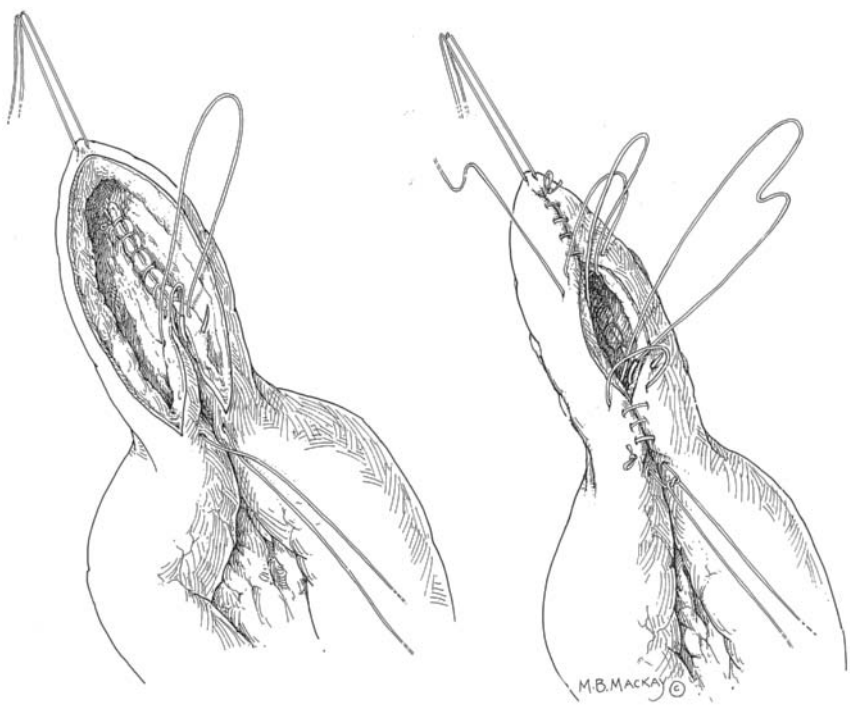


Figure 32.4 To complete the Finney strictureplasty, the bowel is folded on itself and again closed with a running suture beginning with the posterior aspect of the anastomosis and completing it anteriorly. Thus, a side-to-side anastomosis is performed. Reproduced by permission of Margot B. Mackay.

Despite the concerns of anastomosing diseased bowel, the short-term complication rate following strictureplasty is low, with reported complication rates ranging from 1 to 14% [54]. In our own series of 43 patients in whom 154 strictureplasties were performed between 1985 and 1994, there was only one confirmed leak and one other suspected leak [55]. Yamamoto *et al.* performed a systematic review of the literature which included 3250 strictureplasties (81% Heineke–Mikulicz, 10% Finney and 5% isoperistaltic strictureplasties) in 1112 patients [54]. Over 90% of the strictureplasties were performed for strictures in the jejunum or ileum. Septic complications occurred in 4% of patients. Only 3% of patients developed a stenosis at the strictureplasty site.

The other important variable is the long-term outcome. Fearnhead *et al.* reported on the experience at Oxford with 479 strictureplasties performed in 100 patients [56]. The reoperative rate was 52% at a mean follow-up of 40.2 months. In our own series, none of the variables including type of strictureplasty, number of previous operations, site of the stricture and whether a resection was performed in conjunction with the strictureplasty had an effect on the long-term outcome [55]. Yamamoto *et al.* [54] found that young age at surgery (<37 years) was a poor prognostic variable, as did Fearnhead *et al.* [56]. Tichansky *et al.* reviewed 15 series containing 506 patients in whom 1825 strictureplasties were performed [57]. They found a lower reoperative rate in those patients who had Finney rather than Heineke–Mikulicz strictureplasties and in whom the disease was not active and there was no preoperative weight loss.

The world experience with the side-to-side strictureplasty was recently reviewed by Michelassi *et al.* [58]. This retrospective review included 184 patients who had surgery at six centers around the world. The experience ranged from 6 to 70 procedures at each of the institutions. In those with the greatest experience, the procedure was performed as the first operation in a large proportion of patients, whereas at those with less experience, the procedure tended to be reserved for patients who had had previous procedures. Over 90% of the procedures were performed to treat jejunal or ileal disease. In addition, 21–65% of patients had an additional resection and 42–83% of patients had other strictureplasties performed in addition to the isoperistaltic strictureplasty. The average length of the isoperistaltic strictureplasty ranged from 20 to 51 cm. The complication rates ranged form 5.7 to 20.8%. Seven patients (3.8%) developed an anastomotic leak. The long-term follow-up of patients in their series was good. Of 184 patients, 48 (26%) required reoperation at a mean time of 35 months.

Given that strictureplasty can be performed safely and that a conservative approach to Crohn's disease is advocated, strictureplasty has an important role in the surgical management of patients with Crohn's disease. However, at the present time, its use is generally limited to those patients who have multiple skip lesions or who have had multiple resections previously. It is contraindicated in patients where there are abscesses or fistulizing disease. In the future, however, further evaluation of this procedure compared with resection is warranted, especially with respect to long-term outcome. Another question which remains unanswered is whether these patients should receive maintenance therapy. There are no data from randomized controlled trials and opinion seems to be divided on this question. However, since most of these patients have extensive disease, it has been our practice recently to advise prophylaxis with an immunosuppressive agent such as azathioprine.

Surgery for gastroduodenal disease

Gastroduodenal disease is rarely seen in isolation. Yamamoto *et al.* reported that gastroduodenal disease occurred in association with disease elsewhere in 96% of patients [59]. The most common and significant complication of gastroduodenal Crohn's disease is stricture formation. Most patients with a stricture will not respond to medical therapy and will require surgery. Whereas in the past the preferred option for gastroduodenal disease was a bypass procedure (usually gastrojejunostomy), strictureplasty is the preferred option now where it is technically possible. The advantage of strictureplasty is that the pylorus is preserved and hopefully there is a slower transit time and less diarrhea. This is an important consideration in this patient population who frequently have had resections of other parts of their small bowel or colon. Because surgery for gastroduodenal Crohn's disease is performed infrequently, the reported series are small. Yamamoto *et al.* reported the results for 10 patients who had a strictureplasty for duodenal obstruction [59]. For four patients, the strictureplasty included a pyloroplasty. Eight patients had a good result: one patient required a Roux EN Y duodenojejunostomy because of anastomotic breakdown and one required a gastrojejunostomy due to persistent symptoms of obstruction.

When strictureplasty is not possible, gastrojejunostomy is the procedure of choice [56,60,61]. Because of the risk of marginal ulceration with long-term follow-up, vagotomy has been advocated. With the availability of proton pump inhibitors and H2 blockers, vagotomy may not be necessary but there are no data to make recommendations for or against the addition of vagotomy.

Fistulae to the duodenum most commonly occur secondary to Crohn's disease elsewhere [62]. Fistulae arising from the colon or from a previous ileocolonic anastomosis are the commonest sites due to the proximity of these structures to the duodenum. Because the duodenum is usually not involved with disease, the duodenum

and colon/ileum may be separated and the fistula closed primarily. There is often associated induration, so it is important to mobilize the duodenum widely and excise the surrounding tissue before attempting closure. Results are excellent in most patients.

Surgery for large bowel disease

Both the manifestations of and the indications for surgery in large bowel disease differ from those in small bowel disease. Unlike small bowel disease, failure of medical treatment is the most common indication for surgery. The pattern of involvement in colonic disease may be variable, with some patients having predominantly right-sided involvement, others having colonic involvement with sparing of the rectum and others having pancolitis. Furthermore, the disease may be complicated by the presence of perianal disease.

Most patients requiring surgery for colonic disease will require a resection. If there is sparing of the rectum and no or minimal perianal disease, then a colectomy and ileorectal or ileosigmoid anastomosis can be performed. Proctocolectomy and ileostomy will be required for patients with pancolitis or those with severe perianal disease. The obvious advantage of performing an anastomosis is that a stoma is avoided. However, the reported recurrence rates are significantly higher in those patients in whom a colectomy and anastomosis are performed. Andrews *et al.* reported recurrence rates of 46 and 60% at 5 and 10 years, respectively, in patients who had ileorectal anastomoses compared with rates of 10 and 21% in those who had a proctocolectomy and ileostomy [63].

Despite the higher recurrence rates, colectomy and ileorectal anastomosis has an important role in the management of patients with Crohn's disease since many patients are young and would prefer to avoid having an ileostomy. However, patients must be carefully selected. Patients who have significant perianal disease or severe rectal disease are not candidates. Longo *et al.* reviewed 131 patients who underwent colectomy and ileorectal anastomosis at the Cleveland Clinic and found that the presence of small bowel disease preoperatively was the only predictive factor of need for further surgery [64]. The age at surgery, duration of disease, steroid use, presence of proctitis and perianal disease did not affect outcome. However, it is likely that this was a highly selected group of patients and those with significant rectal or perianal disease would not have been included. From the results of reported series, it can be anticipated that approximately 50–65% of patients will develop recurrence of their disease. In some patients, the recurrence may be confined to the small bowel, so a further resection and anastomosis may be possible. The Cleveland Clinic reported that 86% of their patients had a functioning ileorectal anastomosis at 5 years and 48% at 10 years [64]. Similarly, Buchmann *et al.* [65] reported that 70% of their 105 patients had a functioning ileorectal anastomosis at 7 years and Ambrose *et al.* reported that 66% of their patients had a functioning ileorectal anastomosis at 9.5 years [66].

Proctocolectomy is the procedure of choice for those patients with pancolitis or extensive perianal disease. In those patients with perianal disease with associated sepsis, it may be prudent to perform a subtotal colectomy and ileostomy and subsequently perform the proctectomy when the sepsis has settled. This may minimize the risk of an unhealed perineal wound. The major complication of this operation is the risk of an unhealed perineal wound which has been reported to occur in up to 20% of patients. Pelvic nerve injury is an unusual but important complication. As stated previously, the recurrence rates following proctocolectomy and ileostomy are lower than with colectomy and ileorectal anastomosis.

At the time of surgery, measures to decrease the likelihood for sepsis should be employed. As stated previously, a staged procedure may be helpful. Abscesses should be drained preoperatively. Tapering of steroids and improving the general status of the patient with parenteral nutrition may be helpful. An intersphincteric dissection of the anorectum along anatomic planes and meticulous hemostasis are also important in preventing the perineal wound problems that are frequent complications after proctectomy in patients with Crohn's disease.

Another controversy that exists is whether there is a role for segmental resection in Crohn's colitis. Segmental colonic disease occurs uncommonly, so most reported series are small and it is difficult to draw conclusions and treatment may have to be individualized.

Ileal pouch procedure

The ileal pouch anal anastomosis has become the surgical procedure of choice for most patients with ulcerative colitis. When it was first introduced, there was a high complication rate, but today it can be performed safely with relatively few complications, low failure rates and good functional results. However, it has generally been performed only in patients with ulcerative colitis and familial adenomatous polyposis. Crohn's disease has been considered a contraindication because of the risk of small bowel and perianal involvement. Despite careful selection of patients, between 2.7 and 13% of patients having a pouch may ultimately be found to have Crohn's disease [67]. Failure rates of 30–50% have been reported in small series of these patients in addition to poorer functional results [68].

In contrast, Panis *et al.* performed ileal pouches in 31 patients with Crohn's disease who had no evidence of associated anoperineal or small bowel disease and suggested that the procedure can be performed safely in patients with Crohn's disease [69]. The complication rate

in this group was similar to that in 71 patients with ulcerative colitis who underwent IPAA in a similar time period. Amongst the 31 Crohn's disease patients, 19% developed septic complications including three pouch–perineal fistulae, one pouch–vaginal fistula and one extrasphincteric abscess. Two patients developed Crohn's disease of the reservoir. Two of the five patients required pouch excision. At follow-up at 5 years, functional results were similar in both groups of patients. Although this report suggests that selected Crohn's disease patients may have a satisfactory outcome with a pouch procedure, one must be cautious in the interpretation of these results. Since perianal disease frequently complicates Crohn's colitis, these patients are a highly selected group or alternatively may have indeterminate colitis. Others have reported satisfactory results in patients with indeterminant colitis [70].

Generally, Crohn's disease remains a contraindication to performing a pouch procedure. However, in patients where there is uncertainty about the diagnosis, this procedure may be considered. Patients must be carefully selected and fully informed that their risk of complications and failure may be higher. In addition, maintenance medical therapy should be considered if the diagnosis of Crohn's disease is suspected. However, while there is theoretical appeal to this strategy, at present there is little evidence to support this approach. Another option would be to perform a subtotal colectomy and ileostomy and delay construction of the pouch for several years, possibly allowing delineation of the disease pattern before embarking on a pouch procedure. However, even with this approach, one must recognize that recurrence of Crohn's disease may not occur for many years. For patients who subsequently develop Crohn's disease complications with a pouch, there is some evidence that treatment with infliximab may be of benefit and decrease the rate of pouch failure [67,71].

Perianal disease

There is great variation in the reported frequency of perianal lesions. These discrepancies are likely due to differences in the intensity of the search made for anal lesions and in the definition of what constitutes perianal Crohn's disease. In addition, most reviews have been performed retrospectively. Rates ranging from 32 to 80% have been reported [72–75]. While the rates vary from series to series, there is consistency in reporting a higher frequency of perianal lesions with colonic or rectal disease.

There is also a wide range of perianal manifestations. Buchmann and Alexander-Williams classified perianal disease into three categories: skin lesions, anal canal lesions and fistulae [76]. Skin lesions include maceration, erosion, ulceration, superficial abscess formation and skin tags. These lesions are usually due to diarrhea and local irritation resulting in maceration and subsequent ulceration and subcutaneous abscess formation. Non-operative management only is required.

Anal canal lesions include fissures, ulcers and stenosis of the anal canal. Fissures tend to be broad based and deep with undermining of the edges. There may be associated large skin tags and a cyanotic hue to the surrounding skin. They may be multiple and placed eccentrically around the anal canal. Fissures or ulcers such as these are often associated with rectal disease. Medical therapy aimed at both the perianal and gastrointestinal disease is indicated. Anal surgery should be avoided. Occasionally, patients with Crohn's disease develop what appears to be an idiopathic fissure without evidence of the classic features of a Crohn's fissure or any associated rectal disease. These fissures may be difficult to treat but, even in this situation, surgery should be avoided. If the patient is having frequent bowel movements, they should be controlled with management of the proximal disease or, in the absence of disease, anti-diarrheal agents. Local therapies including nitroglycerine or diltiazem ointment may be used. However, sphincterotomy and anal dilatation should usually be avoided because of the concerns about non-healing of the wound and subsequent problems with continence.

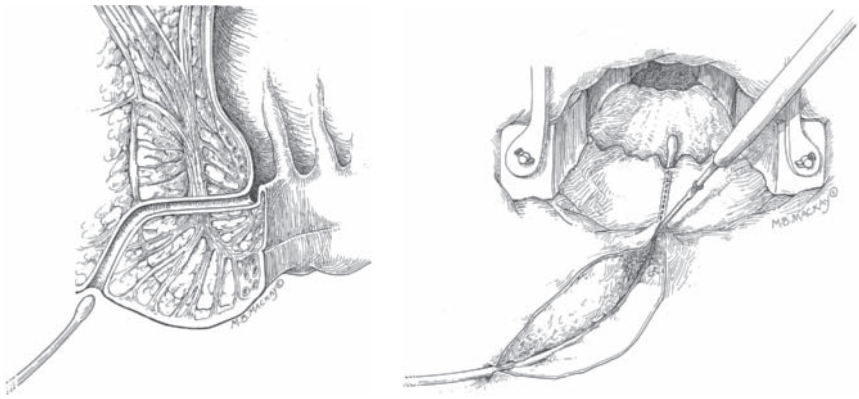
In the third category, abscesses and fistulae often require both a combined medical and surgical approach. Abscesses always require drainage. They should be suspected in patients who have perianal disease and who complain of pain in previously asymptomatic fissures and fistulae. In these patients, one should not hesitate to perform an examination under anesthesia. This may be helpful in assessing the extent of disease and determining whether there is an abscess. Treatment should consist of incision, unroofing and drainage of abscesses. Primary fistulotomy should usually be avoided. There is no role for treating abscesses with antibiotics alone, although combination metronidazole and ciprofloxacin therapy may be a useful adjunct to surgical drainage, especially if there is cellulitis [77]. Although both the diagnosis can be made and the patient treated by performing an examination under anesthesia, a transanal ultrasound or MRI may be helpful in some patients where the abscess is not obvious or complex disease is suspected [78–83].

Fistulae

Fistulae tend to be the most difficult perianal lesions to treat. Often, both medical and surgical modalities must be employed. Initial treatment will depend on the symptoms, the complexity of the fistula and whether there is associated rectal disease.

Simple fistulae

Simple fistulae are generally those that are low lying with only one external opening. They are usually seen in patients without rectal involvement. Even in Crohn's disease, they make up the majority of fistulae. Although



(a) (b)

Figure 32.5 A typical transphincteric fistula arising from a gland at the dentate line, traversing both the internal and external sphincters and ischiorectal fossa with an external opening in the perianal skin is shown. A probe is passed through the external opening to the internal opening. Then the tract is laid open by dividing the overlying tissue In this patient, a part of the internal and external sphincters is divided. The bed of the tract may be curetted but it is unnecessary and usually unwise to excise the tract. The wound is packed and allowed to heal secondarily. Reproduced by permission of Margot B. Mackay.

simple fistulae are often treated with repeated courses of antibiotics when the patient becomes symptomatic, these fistulae are usually amenable to fistulotomy and the fistula can be eradicated without risk of incontinence or delayed wound healing (Figure 32.5). Several series have reported excellent results, with healing rates of 70–100% and low rates of recurrence [84–87]. It is important that the extent of the fistulae and the presence of associated sepsis be properly evaluated by means of an examination under anesthesia and the rectum be evaluated by means of an endoscopic examination before undertaking fistulotomy.

Complex fistulae

Although simple fistulae can usually be treated definitively by means of surgical intervention, complex fistulae or fistulae occurring in the presence of active rectal disease must be approached differently. These include fistulae with multiple external openings or tracts and those that are high. It is unusual that they can be eradicated surgically without leading to significant morbidity. Medical modalities are generally of little value except to control sepsis with the exception of anti-tumor necrosis factor (TNF) agents [88].

Before undertaking any form of therapy, an examination with the patient under anesthesia should be undertaken to evaluate carefully the extent of disease and the presence of associated sepsis. Undetected abscesses may be drained. Some patients with multiple fistula may have less complex disease than suspected, with all the tracts emanating from one internal opening. In these cases, the fistula may be treated definitively with excellent results. If the fistula is complex, the tracts should be identified and unroofed and curetted of all infected granulation tissue. This tissue should be sent for histological assessment to rule out the rare association of cancer in Crohn's disease fistulae. Although these measures may not necessarily allow for complete healing of the fistula, they may lead to partial healing of the tracts and significantly decreased drainage. Drains and setons can be inserted on a long-term basis to allow drainage and prevent reaccumulation of pus.

With the introduction of infliximab, a combined surgical and medical approach to the treatment of complex fistulae has been shown to be effective [17,89,90]. The surgical aspect of treatment includes drainage of all sepsis and insertion of setons. Then infliximab and medical maintenance therapy is instituted. Results have shown healing in approximately 50% of patients. In a fairly large series of 226 patients, Gaertner *et al.* reported healing in 60% of patients with perianal fistulae who had various operative procedures performed including fistulotomy, fibrin glue, advancement flaps and insertion of a seton [90]. The addition of infliximab did not improve healing in this cohort of patients, although healing occurred in only 43% of patients who had a seton inserted only compared with 62% of patients who had a combined medical–surgical approach. It has also been shown that although the fistula appears to be healed clinically, ultrasonography has shown that the tract remains and therefore continuation of the infliximab is required [91]. Nevertheless, even if the fistulae are not eradicated, the sepsis may be controlled so that quality of life is satisfactory [92].

Patients with high fistulae without associated rectal disease and complex tracts may have significant continence problems if a fistulotomy is performed. In these patients, a long-term seton may be inserted or, alternatively, there have been reports of transposing the internal opening of the fistula tract distally to simplify definitive surgical therapy or performing a flap advancement procedure. Although the results of these techniques are encouraging, the number of patients in each series is small [93–95]. There is also some evidence that the use of fibrin glue or fibrin plugs inserted into the tracts may be of benefit, but again the reported experience is limited. O'Connor *et al.* reported an 83% success rate in closing Crohn's fistulae with a fibrin plug [96], and Lindsey *et al.* reported a 69% success rate in healing complex fistula with fibrin glue [97].

Should medical measures fail, be refused by a patient or be contraindicated, other measures may be required. Construction of a loop or split ileostomy to divert the fecal stream may be of benefit in some patients [16,98,99]. Although initial improvement in the local perianal disease usually occurs, the ileostomy does not produce a change in the natural history of the disease. Relapse is common and it is often not possible to restore intestinal continuity. In our series of 12 patients treated with a diverting ileostomy for proctitis or anorectal sepsis, all had temporary remission of their disease [98]. Five required proctocolectomy because of exacerbation of their disease. None has had successful closure of the ileostomy. Similar experiences have been reported by others. In another series reported by Zelas and Jagelman, 22 patients underwent ileostomy for anorectal disease and 6 remained well for 3–5 years [99]. Harper *et al.* reported that 21 of 29 patients (72%) with anorectal Crohn's disease treated with split ileostomy had early improvement [16]. However, only 6 patients had intestinal continuity restored, 8 underwent a proctocolectomy and 15 remained diverted. Despite this, there may be some merit in constructing a diverting ileostomy. First, the general status, including the nutritional status, of the patient often improves and the perianal sepsis resolves to some extent. Therefore, at least in theory, a subsequent proctectomy or other definitive procedure can be performed with less morbidity. Second, some patients may be loathe to have definitive surgery as an initial procedure and a loop or split ileostomy allows them to adjust psychologically to a stoma without committing themselves to a permanent stoma.

Proctectomy may be necessary in patients who are refractory to other medical and surgical measures. It is unusual for proctectomy to be required to treat perianal disease alone. Patients almost always have associated severe rectal involvement. Before performing a proctectomy, it is important that the patient be in optimal condition because this operation is associated with a relatively high morbidity, particularly perineal wound problems. Thus, preoperative measures to decrease local sepsis and improve healing should be undertaken. To decrease local sepsis, a staged procedure may be planned. This may mean a subtotal colectomy or defunctioning ileostomy initially.

Rectovaginal fistulae

The presence of a rectovaginal fistula is often an ominous sign indicating severe rectal disease. Thus, in most instances, proctectomy or proximal diversion is necessary. However, in very selected patients, local repair of the fistula may be undertaken. Medical treatment alone is usually unsuccessful in the treatment of these fistulae because they are short tracts which epithelialize. Similarly, spontaneous closure virtually never occurs. Medical treatment may have a role in inducing a remission of the rectal disease so that a local repair can be undertaken

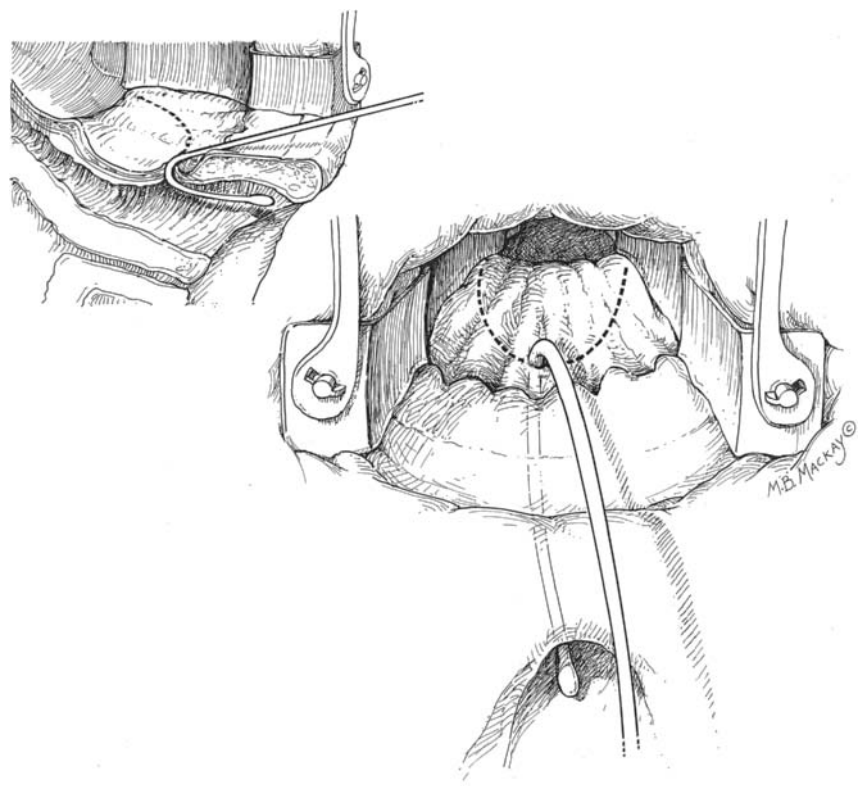


Figure 32.6 Illustration of a rectovaginal fistula. It arises from the dentate line and passes through the rectovaginal septum. Note how thick the rectovaginal septum is and hence most rectovaginal fistula tracts are usually completely epithelialized and will not heal spontaneously or with medical therapy. Reproduced by permission of Margot B. Mackay.

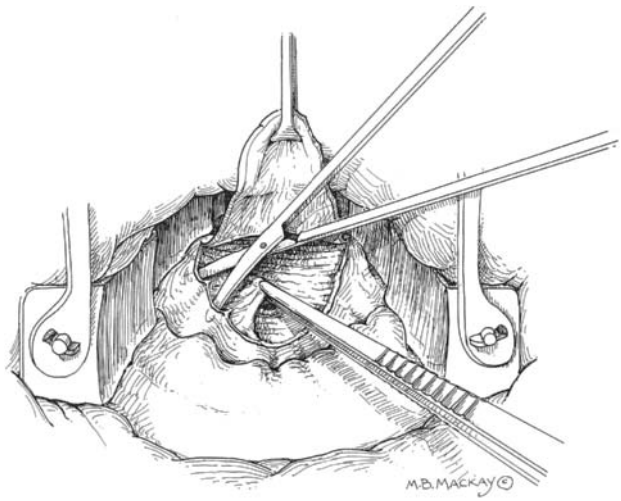


Figure 32.7 Low rectal fistulas in patients who do not have rectal mucosal disease can be closed using a flap advancement procedure. A flap of mucosa and internal sphincter is raised beginning just below the opening of the fistula and extending until the flap can be brought down beyond the fistulous opening without tension. The flap should be rhomboid in shape so blood supply is adequate. Reproduced by permission of Margot B. Mackay.

or improving the consistency of stool so there is less discharge through the fistula opening.

The choice of treatment depends on two factors: patient symptoms and the disease status of the rectum. If the fistula is small and low lying, the patient may experience relatively minor symptoms and no treatment other than medical management of any rectal disease is indicated, although spontaneous closure of the fistula for long periods would be unusual. However, if the patient has persistent fecal or purulent discharge from the vagina or has gross incontinence, treatment is indicated.

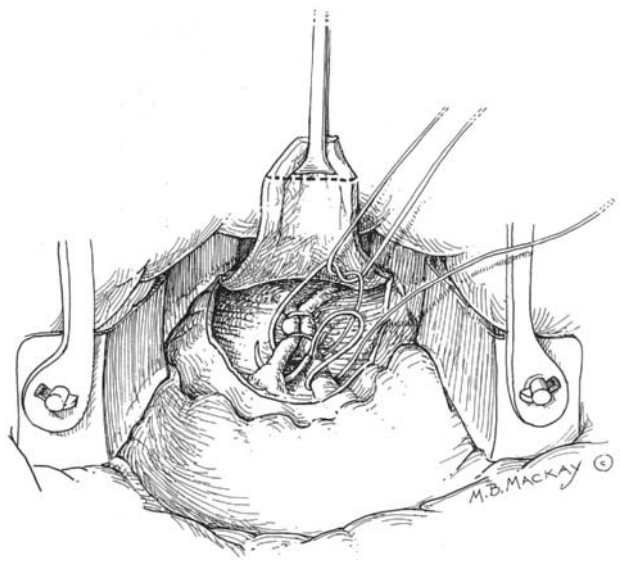


Figure 32.8 Once the flap of tissue has been elevated, the fistula tract is curetted, then closed with several interrupted sutures. Closure of the fistula on the vaginal side is unnecessary. Reproduced by permission of Margot B. Mackay.

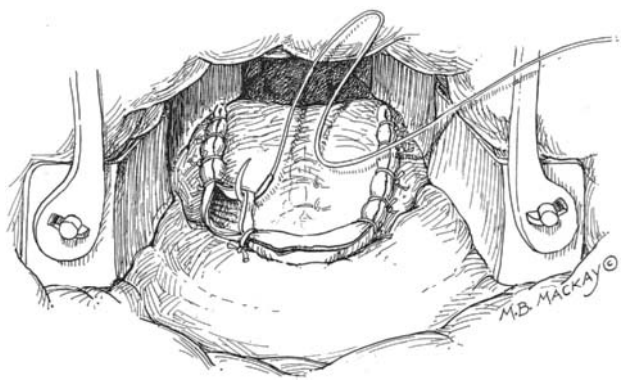


Figure 32.9 The flap is brought down beyond the fistula opening and sutured so the fistula is covered. Reproduced by permission of Margot B. Mackay.

A local repair of the fistula should be undertaken only when the disease is in remission and the rectal tissue is healthy (Figures 32.6–32.9). In the Cleveland Clinic experience, 40% of patients were amenable to local repair of the rectovaginal fistula [100]. In these patients, repair was successful in 54% of women after one attempt and 68% overall. The type of repair performed largely depends on the preference of the individual surgeon. Our preference, where possible, is to perform a mucosal flap advancement via the rectum. Meticulous surgical technique is mandatory. We also recommend temporary fecal diversion with a loop ileostomy in most patients, although in the Cleveland Clinic series, protection with a stoma did not affect outcome.

Conclusion

Surgery plays an important role in the management of Crohn's disease and likely will continue to do so until the etiology of Crohn's disease is elucidated and more specific medical therapies are available. In most instances, surgery leads to an improvement in quality of life and allows patients to regain normal physical wellbeing without experiencing the side effects and dysutility of taking medication. Hence the need for surgery should not be considered a failure of management. Instead, there is a role for both medical and surgical therapy. Because of the variable patterns of disease seen in Crohn's disease and the individual concerns of patients, treatment may have to be individualized. Optimally, care should be given with a team approach including gastroenterologists, surgeons and para-medical personnel such as nurses, enterostomal therapists and psychiatrists. In addition, care should be provided as a continuum. In order to achieve excellent results, however, patients require careful preoperative

evaluation and management and surgeons must be familiar with the various patterns of disease and the complications that they may encounter.

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Chapter 33
Diagnostic and Therapeutic Approaches to Postoperative Recurrence in Crohn's Disease

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Summary

- More than half of Crohn's disease patients require surgery during the course of their disease and recurrence proximal to the anastomosis almost invariably occurs after surgically induced remission. Although endoscopic recurrence does not necessarily imply that patients have symptoms, the high need for repeated surgery indicates that lesions gradually developing after surgical remission lead to fibrostenosis or other complications.
- Risk factors for early recurrence have been incompletely defined but it is more and more obvious that active smoking, perforating disease phenotype and predominant ileal disease accelerate postoperative relapse.
- Despite multiple clinical trials, a clear medical strategy to prevent disease recurrence has not been identified. Aminosalicylates show only very modest reductions of disease recurrence in patients with ileal disease. Systemic glucocorticosteroids and budesonide have failed in this indication.
- The nitroimidazole antibiotics metronidazole and ornidazole are efficacious at retarding postoperative recurrence but face problems of compliance due to side effects. The immunosuppressive purine analogues 6-mercaptopurine and azathioprine have proven efficacy but benefits are modest. Biological agents such as anti-TNF antibodies have not been studied in this respect, although one trial with interleukin-10 failed.
- Because of the modest benefit of medical prophylaxis, treatment algorithms stratifying patients for the risk of recurrence and incorporating endoscopic follow up should be developed.

Introduction

Patients with Crohn's disease have a 50–70% chance of facing surgery anywhere in the course of their disease [1]. Most of these operations will involve resections of the terminal ileum and cecum because of Crohn's disease complications, particularly fibrostenosis, internal fistulae and abscesses. The ileum is reconnected to the colon with an end-to-side or and end-to-end anastomosis. Traditionally, the entire macroscopically involved segment was resected with 5–10 cm margins in normal bowel segments. This curative resection is aimed at inducing full surgical remission. Even if surgical remission is generally achieved, early disease recurrence at the site of the anastomosis appears to be the rule. Detection of these lesions can be achieved with ileo-colonoscopy or with barium contrast X-rays, although endoscopy appears to be more accurate for limited lesions [2]. In the long term, recurrent lesions lead to recurrence of clinical symptoms and repeat surgery for uncontrolled inflammatory lesions or fibrostenosis, but clinical recurrence rates increase at a slower pace compared with radiologic or endoscopic postoperative lesions. Because of the need for repeated ileal resections in many patients, surgeons no longer perform radical resections, which increase the risk of short bowel syndrome upon repeated resections. Alternative surgical strategies to avoid extensive resection have been developed. Strictureplasty is now widely used and has proven to be as efficacious as extensive resection even if it is performed in diffusely inflamed segments. Bypass operations to divert the fecal stream from inflamed or stenosed segments have been generally abandoned because they frequently created blind loops with subsequent bacterial overgrowth. Despite progress in surgical techniques, medical prophylaxis of postoperative Crohn's disease has also been studied in numerous trials. This chapter reviews the different aspects of prophylactic medical therapy, focusing on the evidence of efficacy for the different agents, on the need to define

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clear outcomes and on identifying patients at high risk for early recurrence. Finally, an attempt is made to develop a risk- and endoscopy-based algorithm for medical prophylaxis of postoperative recurrence of Crohn's disease.

Postoperative recurrence of ileal Crohn's disease

The definition of postoperative disease recurrence can be based on clinical, endoscopic, radiologic or surgical criteria. The endoscopic definition is probably the most stringent and controlled, whereas clinical recurrence is most relevant to the patient but criteria are ill defined. Endoscopic recurrence occurs very early after ileo-colonic anastomosis. Rutgeerts *et al.* [3] and Olaison *et al.* [4] reported endoscopic recurrence in 73–93% of patients after 1 year and in 85–100% of patients after 3 years. However, already after 3 months endoscopic recurrence was found in up to 30% of patients. Clinical recurrence is slower with an estimated 20–30% of patients after 1 year and a cumulative rate of 10% of patients per year post-surgery [4,5]. Fortunately, the need for repeated surgery or endoscopic dilation of the anastomosis is much lower with figures between 15–45% after 3 years and 26–65% after 10 years [6].

The disease behavior of Crohn's disease is not influenced by surgical resection. Patients with limited mucosal inflammation and a tendency to slowly develop fibrostenosis will continue to show this disease phenotype. Also, the extent of ileal disease as visualized on radiology does not change after ileo-colonic resection [7].

An important factor in the pathogenesis of recurrent ileal Crohn's disease is the fecal stream. Postoperative lesions can develop within 2 weeks after surgery with ileo-colonic anastomosis. However, fecal stream diversion with loop ileostomy proximal to the anastomosis invariably prevents this disease recurrence. Restoration of the fecal stream after closing the ileostomy induces new lesions within weeks and it is clear that bacterial components are needed for this phenomenon [8,9]. These observations have raised the hypothesis that stasis of endogenous bacterial flora is involved in disease recurrence and have triggered clinical trials using both antibiotics and probiotics in medical prevention strategies. The time shift between endoscopic, clinical and surgical remission indicates that there is a gradual progression from early superficial lesions, to mucosal inflammation and transmural disease, extensive inflammation in the neo-terminal ileum with clinical symptom recurrence and development of complications such as stenosis and fistulae necessitating repeated surgery.

Assessment of postoperative recurrence

Although endoscopic lesions precede clinical symptom recurrence after surgery, a clear correlation between the mucosal appearance of the neo-terminal ileum and the further clinical disease course has been demonstrated [3]. This observation has introduced the use of an endoscopic endpoint as the gold standard in most trials aimed at preventing postoperative disease recurrence. Rutgeerts *et al.* [3] proposed a scoring system to assess the severity of the postoperative lesions that has been implemented in most studies (Table 33.1). The incremental score varies from i0 to i4 ("i" for ileal) and integrates both the severity of the lesions and the extent from the anastomosis. The need for an indirect assessment of clinical recurrence such as endoscopy stems from the time delay between surgery and symptoms and from the limited value of clinical activity scores in the postoperative setting. In the first months after surgery diarrhea and abdominal cramps can be due to the postoperative state and to bile malabsorption. After 6 months the bowel has adapted to these changes and an increase in symptoms becomes relevant. Viscido *et al.* have demonstrated that the positive and negative predictive values of the Crohn's disease activity index using 150 points as a cut-off were only 71 and 65%, respectively, versus endoscopy as the gold standard [10]. However, bearing these shortcomings in mind, preventing clinical disease recurrence and the need for repeated surgery remain the ultimate goal in medical prophylaxis. More recent technical developments in endoscopy, such as capsule endoscopy, have not yielded reliable assessment tools of postoperative recurrence. A French collaborative trial by the Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Intestinales (GETAID) has shown that the diagnostic accuracy of capsule endoscopy to assess mucosal lesions is inferior to that of traditional ileo-colonoscopy. Somewhat contradictory, capsule endoscopy is missing more severe mucosal lesions at the ileo-colonic anastomosis [11]. The reason for this is not entirely clear, but could be an accelerated transit through relatively stenotic segments.

Table 33.1 Endoscopic severity score for postoperative Crohn's disease [3].

Score	Severity
i0	Absence of any lesions at the site of anastomosis and in the neo-terminal ileum
i1	<5 aphthous ulcers (<5 mm)
i2	>5 aphthous ulcers with normal mucosa between the lesions or lesion confined to the ileo-colonic anastomosis (<1 cm)
i3	Diffuse aphthous ileitis with diffusely inflamed mucosa
i4	Diffuse ileitis with large ulcers, nodularity and/or narrowing

Risk stratification for early postoperative recurrence

Location and disease phenotype

The anatomical site involved in the bowel resection is an important factor in disease recurrence. Recurrence rates are highest for ileo-colonic anastomoses in patients operated on for ileal or ileo-colonic disease (Table 33.2). Colo-colonic anastomoses have a lower recurrence rate. Surgical disease recurrence for ileo-colonic anastomosis is between 25 and 60% at 5 years and between 49 and 91% at 15 years. In contrast, re-operation rates for colo-colonic anastomoses vary from 8.5 to 42% at 5 years and from 2 to 40% at 14 years. A recent preliminary report from a Canadian collaborative trial indicates that a larger caliber end-to-side ileo-colonic anastomosis does not offer the advantage of the more commonly used stapled end-to-end anastomosis [12]. Interestingly, recurrence at the ileo-colonic anastomosis also occurred when the ileum was not involved prior to surgery. Narrowing of the lumen at the site of the anastomosis appears to predict recurrent lesions in any case and is lowest in the intestine proximal to an ileostomy. Recurrence rates in the small intestine proximal to ileo-rectal anastomoses appears to follow the pattern of colo-colonic anastomoses, but spreading of the disease into the rectum with local complications often occurs.

Disease behavior prior to surgery was also found to be an important determinant of disease recurrence by studies from Leeds [13] and Mount Sinai, New York [14]. In the Mount Sinai study, perforating and non-perforating disease behavior as an indication for bowel resection was found to be the discriminating factor. Perforating disease was associated with higher postoperative disease recurrence rates. The definition of perforating disease included acute free perforation with overt peritonitis, concealed perforation with abscess formation and chronic perforating disease with intestinal fistulae. Non-perforating surgical indications involved a broad spectrum of intestinal obstruction, intractable inflammatory disease, hemorrhage and toxic dilation. In this retrospective cohort study, time to re-operation was 4.7 years in the perforating group and 8.8 years in the non-perforating group. Indications for first repeat surgery were again perforating disease in 64% of patients with ileitis and with initial perforating disease and in 77% of patients with ileocolitis. In 81% of patients with perforating disease at the time of the second resection, a third resection for perforating disease was necessary. It should be mentioned, however, that not all studies have found the same strong association between perforating disease and the risk or recurrence [15,16].

Table 33.2 Risk factors for early postoperative disease recurrence.

Established risk factors
- Ileo-colonic anastomosis
- Perforating disease
- Smoking (particularly in women)

Possible risk factors
- Young age
- Short disease duration

Smoking habits

As a general rule, active smoking is associated with poor outcome in Crohn's disease. Postoperative disease recurrence appears to be no exception. Almost all studies report active smoking as a risk factor for early recurrence. Cottone *et al.* reported in 1994 that 6 years after surgery 60% of non-smokers [95% confidence interval (CI) 43–72%], 41% (95% CI 11–70%) of ex-smokers and 27% (95% CI 17–37%) of active smokers were free of clinical recurrence [17]. Sutherland *et al.* demonstrated a similar difference for postoperative recurrence [18]. Repeat surgery was performed in 20% of non-smokers and in 36% of smokers. At 10 years the figures rose to 41 and 70%, respectively. In this study, female smokers with small bowel disease were at highest risk for recurrence, an observation that has been confirmed in non-surgical series of refractory Crohn's disease. An interventional study by the French GETAID group demonstrated that smoking cessation resulted in a less aggressive disease course [19]. The same French study group also reported that long-term immunosuppression also antagonizes the deleterious effect of smoking [20]. Several more recent observations confirmed smoking as a risk factor for early postoperative Crohn's disease recurrence [21–24]. However, one study from the University of Pennsylvania did not identify smoking as a risk factor for postoperative recurrence [25].

Other factors

Determinants of early postoperative recurrence supported by less evidence include age at surgery and time to onset of the disease. de Dombal *et al.* reported in 1971 that patients operated on early after initial diagnosis had higher postoperative recurrence rates, probably reflecting a more aggressive disease course [13]. These data were confirmed by the Mount Sinai group in 1983 [26], but not in other series [27,28]. Also, more recently, data from Lautenbach *et al.* clearly contradicted the correlation between short disease duration and postoperative recurrence risk using multivariate analysis [25]. In their retrospective analysis, a long disease history predicted early surgical recurrence. The authors hypothesized that the time of initial diagnosis may not accurately reflect disease onset and that patients with longer disease history may be those who are reluctant to undergo surgery and therefore have more severe disease at the time of resection.

In general, pathology reports of the severity of inflammation in the resection specimen have not been a reliable predictor of early postoperative recurrence. However, Ferrante *et al.* found inflammatory lesions in the myenteric plexus ("plexitis") in the ileal resection margins and in the

absence of mucosal lesions to be highly associated with early endoscopic recurrence (3 months and 1 year) in patients with ileo-colonic resection who had not been treated for 1 year [29]. Further validation of this interesting finding is needed.

Endpoints in clinical trials

Clinical trials investigating the role of medical therapy in postoperative Crohn's disease prophylaxis have used variable endpoints, including mucosal lesions and clinical and surgical recurrence. These discrepancies hinder the direct comparison between the respective trials. The implementation of early endoscopic lesions at the site of the anastomosis as an important endpoint in postoperative recurrence trials is based in the concept that prolonged mucosal healing prevents Crohn's disease complications in the long term. Although the inflammatory infiltrate in early recurrent lesions is different form that of long-standing disease, there is probably a continuum of early mucosal lesions to transmural disease with fibrosis and perforation. Therefore, early recurrent lesions are also important for predicting the postoperative disease course. As mentioned before, the most efficacious surgical procedure to prevent postoperative recurrence is fecal diversion with ileostomy proximal to the anastomosis. Histologic evidence of the disease is found as early as 1 week after closing the ileostomy and most of these patients go on to develop endoscopic lesions after 3–6 months [9].

Clinical trials incorporating endoscopic lesions as an outcome parameter have demonstrated conflicting results. Standard agents for luminal Crohn's disease such as aminosalicylates and corticosteroids have generally failed in this respect. Probiotics also have not proven to prevent the recurrence of mucosal lesions. Antibiotics and azathioprine appear to protect more efficiently against endoscopic recurrence and this effect extends to 1 year and longer.

Even if early endoscopic lesions are probably closely associated with the ensuing postoperative clinical disease course, prophylactic treatment is eventually aimed at reducing clinical symptom recurrence and repeated surgical resections. We illustrated before that endoscopic lesions precede clinical and surgical recurrence by several years in most patients and this complicates the design of clinical trials on prophylactic therapy. Ideally, clinical endpoints should be fixed at late time points (3 years or more) to power trials for detecting relevant differences between treatment groups.

Aminosalicylates: sulfasalazine and mesalamine

Arguably the largest number of clinical trials have focused on the use of aminosalicylates in the prevention of postoperative recurrence. These agents are considered very safe and mesalamine is well tolerated in the long term. However, mesalamine is expensive if given long term at appropriate doses and recently the benefit of aminosalicylates in the maintenance therapy of Crohn's disease has been challenged.

Three studies have looked at the effect of sulfasalazine initiated early after surgery [30–32]. The study of Bergman and Krause [30] and of Wenckert *et al.* [31] showed no benefit over placebo and only the study by Ewe *et al.* [32] found a reduction in recurrence rates in patients on active treatment. Surgical and radiologic recurrence was 16% in the sulfasalazine group at 1 year versus 28% in the placebo group ($p < 0.01$). However, at 3 years recurrence rates were identical at 38% in both groups [32].

Studies exploring the prophylactic effect of mesalamine [5-aminosalicylic acid (5-ASA)] have faced problems of heterogeneous trial design. Dosing regimens, the interval between surgery and start of treatment (from less than 10 days to 8 weeks) and the duration of follow-up have been highly variable.

An open-label comparison study by Caprilli *et al.* demonstrated an important benefit for 5-ASA (Asacol 2.4 g). About 18% of patients receiving Asacol and 41% of patients on placebo had a clinical recurrence after 2 years [33]. This study also reported a benefit of 5-ASA on endoscopic recurrence rates but obviously was non-controlled and open to observation bias. More recently, Caprilli *et al.* reported on the results of a randomized controlled trial prospectively comparing two doses of 5-ASA [34]. A total of 206 patients were randomized and 186 were available for clinical assessment after 12 months. Clinical recurrence was present in 12% in the Asacol 4.0 g group and in 14% of the patients in the 2.4 g group [not significant (NS)]. Also, there was no difference in severe endoscopic recurrence. The placebo-controlled trials investigating the role of 5-ASA in the prophylaxis of postoperative Crohn's disease are summarized in Table 33.3. Only the Canadian study by McLeod *et al.* in 1995 showed a significant benefit for 5-ASA [36]. In four subsequent studies, it was not superior to placebo [35,37–39]. In the study by McLeod *et al.*, the 3 year clinical recurrence rate was 31% (27 of 87) with 1.5 g 5-ASA b.i.d. (Salofalk) versus 41% (31 of 76) in the control group. Again, in this trial 5-ASA was initiated as long as 8 weeks from surgery, a time-point at which numerous patients already have endoscopic lesions. A large German–Austrian multicenter trial with a high dose of 4 g mesalamine (Pentasa) or placebo initiated within 10 days from surgery failed to show a significant benefit for 5-ASA at 12 months [40]. Subgroup analysis showed a benefit for patients operated on for isolated ileal disease, with no colonic resection, but the numbers needed to treat (NNTs) were high (NNT = 8–13). This means that at least eight patients have to be treated long-term with 5-ASA at considerable cost to prevent one postoperative recurrence. Two trials using mesalamine as an "active" comparator for purine

Table 33.3 Efficacy of aminosalicylates in the prevention of clinical and/or endoscopic recurrence of Crohn's disease: results of placebo-controlled trials.

Trial reference	Dose (g)	Start 5-ASA after surgery	Number	Duration (months)	Clinical recurrence (%)	
					Placebo	5-ASA
McLeod 1995 [36]	3	8 weeks	163	72	41	31*
Brignola 1995 [35]	3	4 weeks	77	12	26	18
Sutherland 1997 [37]	3		66	12	23	10
Lochs 2000 [40]	3	10 days	318	18	31	24
Hanauer 2004 [39]	3	14 days	84	24	69	59
					Endoscopic recurrence	
					Placebo	5-ASA
Brignola 1995 [35]	3	4 weeks	77	12	56 (≥i3)†	24
Hanauer 2004 [39]	3	14 days	84	24	98 (≥i2)	86

*$p < 0.05$; †$p < 0.01$.

analogues in the setting of postoperative recurrence prophylaxis have been reported. Ardizzone *et al.* found clinical relapse in 30% of patients treated with mesalamine 3 g and 20% with azathioprine 2.5 mg kg^{-1} per day for 2 years [38]. These low clinical recurrence rates contrast with the data reported by Hanauer *et al.* from a 2 year randomized trial [39]. They found clinical recurrence at 2 years in 61 and 70% of patients treated with mesalamine (3 g) and placebo, respectively.

A meta-analysis by Camma *et al.* [41] showed a pooled risk reduction of 13% when summarizing all trials with mesalamine. However, this meta-analysis did not incorporate the recent negative trials by Lochs *et al.* [40], Ardizzone *et al.* [38] and Hanauer *et al.* [39]. A second meta-analysis by Camma *et al.* [41] incorporated the large trial by Lochs *et al.* but not the antimetabolite trials by Ardizzone *et al.* and Hanauer *et al.* In this meta-analysis, clinical disease recurrence prevention for patients with isolated ileal disease was 15% (NNT = 5.5).

Mesalamine has been shown to suppress early endoscopic lesions at early time points after surgery, but this effect is lost later on in the course of the disease. An Italian placebo-controlled trial with Pentasa 3 g daily initiated 1 month after surgery demonstrated a reduced rate of endoscopic and radiologic recurrence at 1 year, but there was no difference in clinical recurrence [42]. The recurrence rate was 24% in the Pentasa group versus 56% in the placebo group ($p < 0.004$), a difference of 32% (95% CI 22–52). In another trial, Florent *et al.* compared mesalamine (Claversal, eudragit coated) 1.5 g b.i.d. for 12 weeks and started within 15 days after surgery to placebo [43]. Endoscopic relapse was found in 50% of mesalamine-treated patients and in 63% of patients on placebo. This difference was not statistically significant. Also, Fiasse *et al.* [44] failed to demonstrate an advantage of mesalamine over placebo in endoscopic recurrence after 1 year of treatment. This study has only been published in abstract form and the main shortcoming is that mesalamine treatment was only started 3 months after surgery. At that time, most patients had probably already developed early lesions.

It has been proposed that adequate mucosal 5-ASA concentrations at the site of the anastomosis are needed in the prevention of recurrent lesions. An Italian study in 25 patients found that 3 years after surgery patients with higher tissue concentrations had a lower risk of recurrence [45]. However, this was indirectly contradicted by a more recent trial from the Italian collaborative GISC group. In this trial, 2.4 g of mesalamine (Asacol) started 2 weeks after surgery did not offer a significant advantage over 4.0 g in the endoscopic and clinical recurrence rates at 12 months [34]. Taken together, mesalamine at doses of 2.4–4 g has a small benefit in preventing clinical recurrence at the site of ileocolonic anastomosis in patients with isolated ileal disease and NNTs are estimated to range between 5.5 and 8.

Budesonide

A collaborative double-blind placebo-controlled European trial included 129 patients to be randomized to budesonide 6 mg/ per day or placebo within 2 weeks from surgery [46]. The majority of patients were operated on for fibrostenotic disease. Endoscopic and clinical recurrence rates were not different between both groups at 3 and 12 months. A sub-analysis showed a significant reduction in endoscopic lesions with budesonide (12 months: 32 vs 65% for placebo, $p < 0.05$), but only in patients operated on for intractable luminal disease, not in patients with fibrostenosis as the indication for surgery. Based on this trial, there is currently no evidence to support the use of budesonide in this indication.

Purine analogues

Immunosuppression is a highly efficacious maintenance strategy for luminal Crohn's disease inducing mucosal

healing [47] and it is logical to assume that the recurrent lesions developing in the postoperative setting are caused by uncontrolled immune activation and would respond to immunosuppressive agents. Adler and Korelitz have provided preliminary data supporting a role for 6-mercaptopurine (6-MP) in the prevention of postoperative recurrence [48]. Based on these findings, a larger multicenter trial was initiated comparing 6-MP 50 mg versus 5-ASA (Pentasa) 3 g and placebo (38). Endpoints were endoscopic and clinical recurrence. The trial enrolled 131 patients in five centers and they were randomized to any of the three groups in a double-blind, double-dummy design. It was first reported in abstract form in 1998, but appeared as a full paper only in 2004. Patients were assessed for clinical, radiologic and endoscopic recurrence at regular intervals throughout 24 months. Dropout rates were considerable in the course of the trial but evenly distributed over the three groups. Endoscopic recurrence (defined as an i2 score of 2 or more) was demonstrated in 43% of patients on 6-MP [$p < 0.05$ vs placebo, 63% with Pentasa (NS) and 64% with placebo]. 6-MP was more efficacious than placebo or mesalamine at preventing severe endoscopic relapse (score >i2). A reduction in the clinical recurrence rate was not achieved, however, with 6-MP. The benefit of 6-MP was lower than anticipated (22% risk reduction versus placebo for endoscopic recurrence). The dose of 6-MP used may have been sub-optimal and the time to onset of action may have interfered with the prevention of early recurrent lesions. As a back-to-back paper in the same issue of *Gastroenterology*, Ardizzone *et al.* in Milan reported a prospective, controlled, open-label trial comparing azathioprine (2 mg kg^{-1} body weight) or mesealazine (3 g per day) for 2 years in patients after conservative surgery (only resecting grossly inflamed and stenotic segments) [38]. At the end of 2 years, no difference was found for clinical relapse [30% with placebo, 20% with azathioprine, odds ratio (OR) 2.04, 95% CI 0.89–4.67]. Azathioprine was only superior to mesalamine in a subgroup of patients with previous intestinal resection [38]. Preliminary results from a double-blind, placebo-controlled, single-center trial comparing azathioprine (100 mg for ≤60 kg body weight, 150 mg >60 kg) versus placebo for 12 months. Eighty-one patients were included within 14 days from surgery [48]. All patients received metronidazole 3 × 250 mg daily for the first 3 months after surgery. At 1 year, significantly fewer patients had endoscopic recurrence (defined as a Rutgeerts score of i2 or more) 44% versus 69% ($p < 0.05$) in the azathioprine group. At this time, very few clinical recurrences had occurred. The proportion of patients with no endoscopic lesions at 1 year was also significantly higher in the group treated with azathioprine (22 vs 3.2%, $p < 0.03$).

The combined evidence indicates a modest effect from azathioprine in this setting, but clearly this comes with the price of side effects resulting in discontinuation rates up to 30%. Antimetabolite (azathioprine and 6-MP) should be considered in adequate dose in patients with high risk of relapse (previous resection, internal fistulizing disease).

Nitroimidazole antibiotics

The rationale to use antibiotics in the prevention of postsurgical relapse can be deduced from the putative role of bacterial stasis in the pathogenesis of early recurrent lesions and experiments with re-infusion of ileal contents [8,9]. A first double-blind placebo-controlled trial explored the effect of metronidazole 20 mg kg^{-1} body weight during 3 months and started within 1 week from surgery in 51 patients [50]. Only patients with a new ileo-colonic anastomosis after ileal and segmental colonic resection were eligible. The total endoscopic recurrence rates after 3 months were not significantly decreased in the metronidazole group (52 vs 75%). However, severe endoscopic lesions were lower in the active treatment group (13 vs 43%, $p < 0.02$). Clinical recurrence was only suppressed at the 1 year time point, not at year 2 or 3.

A more recent trial using ornidazole 500 mg b.i.d. or placebo for a total of 1 year after surgery and initiated within 1 week demonstrated significant reduction of endoscopic relapse rates at 3 months and 1 year [51]. Severe lesions (≥i2) were detected in 74% of patients in the placebo group versus 41% in the ornidazole group ($p < 0.02$). As in the metronidazole trial, clinical recurrence was only significantly suppressed at 1 year in the ornidazole-treated patients (8 vs 37%). The principal drawback of nitroimidazoles in the setting of postoperative recurrence prevention are the side effects associated with long-term use of these antibiotics. Gastrointestinal intolerance with nausea, metallic taste and peripheral neuropathy preclude long-term administration and they can serve more as an induction agent to bridge the gap to the effect of immunosuppressives.

Probiotics

Several trials have studied the role of probiotics in preventing postoperative recurrence of Crohn's disease. A preliminary report from the Bologna group using VSL#3 for 9 months and preceded by rifaximin 1.8 g for the first 3 months suggested that probiotics had therapeutic potential as compared with mesalamine, but a full report has not been published yet [52]. In contrast, three trials using *Lactobacillus* species were clearly negative. Prantera *et al.* found no difference between placebo and *Lactobacillus* GG 6×10^9 colony-forming units twice daily given for 12 months and started within 10 days from surgery [53]. Forty-five patients entered the trial and 37 were available for assessment at 12 months. Patients with high

recurrence risk were excluded from the trial. Nine out of 15 (60%) patients in the probiotics and 6/17 (35%, NS) in the placebo group had endoscopic recurrence. Clinical recurrence was present in 17% of probiotics-treated and in 10.5% of placebo-treated patients. Two recently reported trials evaluated the effect of *Lactobacillus johnsonii* LA1. The French GETAID group included 97 patients with ileal resection in a double-blind trial comparing *Lactobacillus johnsonii* LA1 with placebo with endoscopic recurrence at 6 months (Rutgeerts score >1) as the primary endpoint [54]. Thirty out of 47 (63%) of placebo-treated and 21/47 (49%, NS) of probiotics-treated patients achieved the primary endpoint of endoscopic recurrence and the distribution within the recurrence score (i0–i4) was also similar. A coincidental but independent trial from Belgium confirmed these negative results [55]. This trial in 70 patients was also placebo controlled and showed no difference for endoscopic recurrence. A similar proportion of patients in the two groups had mild-to moderate (i1 + i2) and severe recurrence (i3 + i4) (mild to moderate recurrence 45 vs 39%; severe recurrence 28 vs 33%). Also, histologic scores were not different between the two groups. Finally, an Israeli prospective placebo-controlled trial in 30 patients randomized 2:1 to receive a "synbiotic 2000" cocktail (with four strains of probiotics and four prebiotics) or placebo once daily found no differences in endoscopic or clinical recurrence at 2 years or at any time point before [56]. Taken together, no clear evidence can be found for a role of probiotics in prophylaxis of postoperative recurrence. VSL#3 contains a cocktail of probiotic strains and in the trial patients received antibiotics for 3 months, which may have contributed to the apparent efficacy. However, given the negative reports in all other trials using probiotics, controlled data with VSL#3 are clearly needed.

Biological agents

Although biological agents, such as the anti-tumor necrosis factor (TNF) antibody infliximab, are now widely used in treatment of refractory Crohn's disease, no data are available on their efficacy to prevent post-surgical relapse. Arguments to justify a trial with infliximab are that it is highly efficacious in luminal Crohn's disease and that it induces mucosal healing in both the ileum and the colon [57,58]. However, given the considerable cost associated and safety issues with biological treatment, it can be predicted that these agents, if proven to be efficacious, will only be used in patients with a high risk profile for early relapse.

A small multi-center trial investigated the role of the anti-inflammatory cytokine interleukin-10 in the prevention of post-surgical relapse. As in other studies with interleukin-10 in Crohn's disease, the trial did not show a benefit of this cytokine over placebo for endoscopic relapse [59].

Algorithm for management of clinical recurrence of Crohn's disease after curative resection

Given the observation that probably 30–50% of patients will never develop early recurrent lesions after curative surgical intervention and the modest benefit shown in the trials with medical prophylaxis, systematic treatment immediately after surgery is not indicated. Risk stratification based on the known disease behavior prior to surgery and the smoking status combined with the findings of an early ileo-colonoscopy 6 months from the operation, is the key determinant in the medical treatment of these patients. Figure 33.1 proposes a treatment algorithm to which we currently adhere, based on the available evidence.

Strategies for the future: where to go from here?

The early recurrent lesions in the neo-terminal ileum developing within months after surgical remission offer a good model to study the natural history of Crohn's disease. The mechanisms driving the uncontrolled inflammation and the progression towards established transmural disease should be further explored in patients with post-surgical relapse. In this respect, the recent finding that inflammation of the neural plexus at the ileal resection margin predicts endoscopic recurrence should be further studied. Also, the role of agents, such as biological therapy, which have proven to alter the course of the disease and prevent complications, should be studied in this setting.

The total body of evidence about the medical prophylaxis of post-surgical relapse in Crohn's disease indicates that our clinical practice needs critical appraisal. 5-ASA treatment in all patients does not seem to be a rational strategy given the data currently available. Immunosuppressive therapy with short-term antibiotics seems promising but more data are needed to define better if all patients would benefit from this treatment regime. Trials with anti-TNF agents would also be most valuable, since the chimeric anti-TNF monoclonal antibody infliximab has proven to prevent surgeries and to induce durable mucosal healing outside of the postoperative setting. Taken as a whole, however, the trials show that we are not successful in preventing the occurrence of new lesions. Medical prophylaxis only postpones their occurrence and in many of the trials the benefit of active treatment is lost after 2–3 years. Therefore, future studies should be aimed at

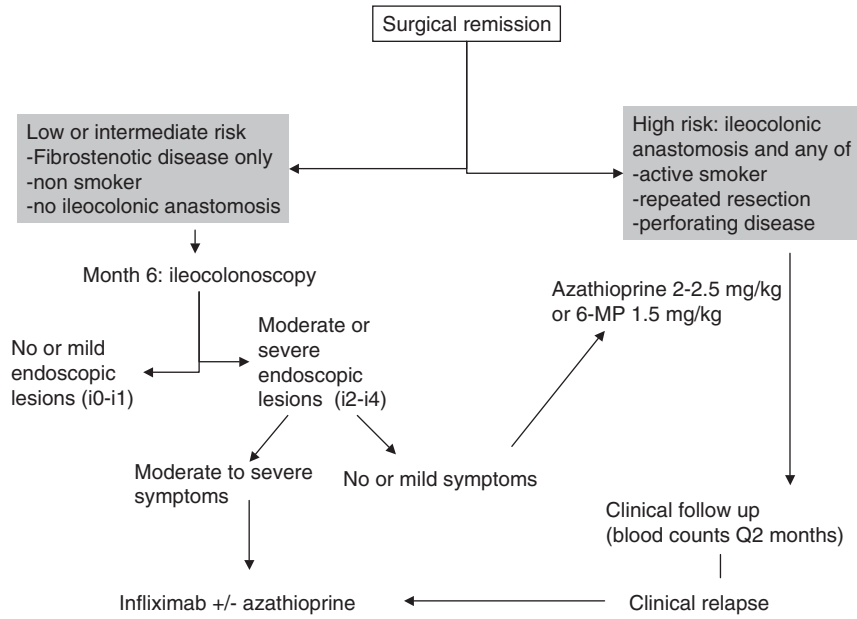

Figure 33.1 Treatment algorithm for the prophylaxis and treatment of early Crohn's disease after curative resection based on risk stratification and endoscopic surveillance.

defining better the patients for whom the benefit of early systematic prophylaxis will outweigh the toxicity and cost of treatment.

New developments: medical prophylaxis

The value of azathioprine in preventing postoperative endoscopic recurrence of Crohn's disease has been confirmed in a recently reported trial [60]. Patients with ileocolonic resection for Crohn's disease ($n = 81$) and an increased risk of postoperative recurrence were randomized to receive metronidazole for 3 months plus placebo or azathioprine for 12 months. Azathioprine or placebo tablets were adjusted to body weight and patients received between 1.8 and 2.5 mg kg^{-1} initially. At the primary endpoint 12 months post-surgery, fewer patients treated with metronidazole short term and azathioprine long term had a significant endoscopic relapse (44 vs 69%, $p < 0.05$). Also, when dropouts were taken into account (19/80), fewer patients in the AZA group had an endoscopic recurrence. Only 10 out of 80 patients experienced a clinical recurrence and this low number precluded any conclusion on the effect of AZA in combination with metronidazole on symptomatic relapse. Another uncontrolled prospective cohort study in Barcelona, Spain, found that endoscopic recurrence confined to the ileo-colonic anastomosis in patients treated with AZA is unlikely to result in clinical relapse [61]. The combined evidence supports a limited but consistent benefit of purine analogues to prevent endoscopic and clinical relapse [33–36]. In a small placebo-controlled trial, the anti TNF monoclonal antibody infliximab (IFX) at a dose of 5 mg kg^{-1} i.v. drastically reduced the incidence of endoscopic postoperative recurrence [IFX 1/11 9%, placebo 11/13 (85%), $p < 0.001$] [62]. However, larger trials are needed to define the benefit-to-risk ratio of anti-TNF therapy in the setting of postoperative prophylaxis.

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Chapter 34
Molecular Alterations Associated with Colitis-associated Colon Carcinogenesis

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Summary
- Patients with longstanding ulcerative colitis and Crohn's colitis are at increased risk of colorectal neoplasia
- Chronic inflammation is considered to be the driving force that predisposes to developing colorectal neoplasia in IBD.
- The same molecular pathways that contribute to sporadic colon carcinogenesis also occur with colitis-associated colorectal cancer, albeit with different frequency and timing.
- Some molecular markers, such as aneuploidy, correlate with colon cancer risk, but so far no marker has been integrated into clinical practice for predicting cancer risk.
- Newer markers and approaches hold promise for helping to identify IBD patients at high risk of developing colorectal cancer.

Introduction

Patients with longstanding inflammatory bowel disease (IBD) are at increased risk of developing neoplastic and preneoplastic lesions in the colon. Whereas the lifetime risk of colorectal cancer (CRC) is almost 6% in average-risk individuals [1], the rates for developing CRC in patients with IBD have historically been much higher. A meta-analysis of CRC risk in ulcerative colitis (UC) patients indicated rates of 1.6% after 10 years of disease, 8.3% after 20 years and 18.4% after 30 years [2]. More recent data suggest somewhat lower rates in UC patients [3] and an approximate 3% rate of CRC after 10 years in patients with Crohn's disease (CD) [4]. However, given that IBD often develops in young adulthood, IBD patients are among the highest risk groups for developing CRC.

This chapter addresses the molecular alterations associated with colitis-associated colon carcinogenesis (CAC). Although none of these alterations has yet been translated into clinical practice, with continued research there is potential for molecular diagnostics one day to enhance the management of patients with longstanding IBD. It is important to note that most of our current knowledge of molecular pathogenesis in CAC comes from studies of patients with UC and not CD. Although studies demonstrate that CRC in CD is similar to that of UC of comparable duration and anatomic extent [4], it is unclear if different molecular alterations are involved in colon carcinogenesis between UC and CD.

Causation: genes versus environment

It is not known how much of the cancer predisposition in IBD is genetic as opposed to environmental. A potential role for hereditable factors is suggested by the observation that IBD patients who have a family history of CRC have an approximately two-fold greater risk of developing CRC than IBD patients with no such family history [5]. A similar degree of familial risk for CRC has been observed in cotton top tamarins – animals that develop CRC in a setting of chronic inflammation [6]. It is not yet known which hereditable genes, if any, contribute to increased CRC risk in IBD.

Because a hereditary cause of CAC has not yet been discovered, it is currently believed that environmental factors contribute more to colon cancer risk in the IBD patient. Perhaps the most obvious clinical difference in the development of sporadic versus CACs is the setting of chronic inflammation in which IBD-associated carcinomas arise. CAC rates correlate with the duration, the extent and the severity of colonic inflammation [7] and it is therefore believed that chronic inflammation plays a significant role. Factors associated with inflammation, such as oxidative stress, are logically assumed to contribute to the molecular alterations seen in IBD tissues [7].

If inflammation is what predisposes to CAC, then it follows that reversing inflammation should lower CRC

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risk. After all, aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) are known to decrease the risk of sporadic CRCs and NSAIDs can significantly regress adenomatous polyps in the rectum of patients with the high-risk condition, familial adenomatous polyposis. In IBD patients, the most commonly used anti-inflammatory agents are 5-aminosalicylic acid (mesalamine; mesalazine), steroids and the purine immunomodulators (6-mercaptopurine and azathioprine). Importantly, many studies, but not all, have demonstrated that 5-aminosalicylic acid lowers the risk of CRC in IBD patients and higher doses have been correlated with lower risk [8]. Some studies also have shown that steroids lower CRC risk [9]. By contrast, however, existing data suggest that the purine analogs seem to have no effect for preventing CAC [10]. Hence it appears that not all anti-inflammatory medicines have the same ability to prevent CAC, so one cannot simply view the suppression of inflammation as a general phenomenon. Instead, just as each anti-inflammatory medication has its own mechanism of action to reduce inflammation, the chemopreventive activities of these drugs are likely to affect different carcinogenic pathways. Although the chemopreventive mechanisms underlying the effects of these anti-inflammatory agents are still unknown, they may, in part, be related to the inhibition of cyclooxygenase-2 (COX-2). Although expressed in normal colonic mucosa, *COX-2* is a gene that is induced in premalignant and malignant lesions of the colon, including those from IBD patients [11]. Mesalamine, in fact, may have several modes of action to prevent CRC in IBD by preventing genomic instability [8].

It is also plausible to assume that factors other than inflammation can lead to cancer. In animal models of CAC, the lumenal bacterial flora appears to contribute to carcinogenesis. For example, *TGFβ1*-deficient mice on an immunodeficient background develop colon adenocarcinoma in association with inflammation. When raised under germ-free conditions, neoplasia does not develop in these mice, but when the animals are re-colonized with enteric flora, neoplasms occur in association with colitis [12]. In this model, colitis is required, but not sufficient, for cancer formation; a genetic predisposition to cancer appears to contribute. A more recent observation indicates that Toll-like receptor-4 (TLR4) seems to participate in colon carcinogenesis in the setting of inflammation [13]. Since TLR4 is an important cell surface receptor that recognizes bacterial products (e.g. lipopolysaccharide), this provides an intriguing connection between bacterial products and CAC. There are several other animal models where CRC arises in the setting of chronic inflammation and in which bacterial colonization seems to play a role [7]. In humans, the role of bacteria in promoting CRC (whether sporadic or colitis-associated) has received little attention to date, partly because it has been difficult to study.

In addition to inflammation and bacterial flora, it is possible that diet and/or nutritional factors might play a role in CAC. Although never demonstrated in a statistically significant fashion, folate deficiency has been suggested to be a risk factor for CRC in UC patients [9]. In a rat model of colon cancer, folate deficiency induced progressive DNA strand breaks within exons 5–8 of the *p53* gene (but did not affect the *APC* gene) and folate supplementation increased the steady-state levels of *p53* transcript [14]. Likewise, folate levels in serum and colonic tissue tended to be lower in UC patients who had demonstrated abnormalities in DNA mismatch repair genes compared with those who did not. In one patient, folate supplementation for 6 months resulted in the resolution of some of these gene abnormalities [15]. Therefore, although environmental factors such as medications, bacteria and nutritional status might interact with certain genes responsible for colon carcinogenesis, more work is needed in this area.

Dysplasia as the precursor to CRC

Sporadic CRCs typically arise from precursor dysplastic lesions called adenomatous polyps, which are usually easily visible, polypoid outgrowths that occur in one or very few distinct areas of the colonic mucosa. By contrast, the macroscopic appearance of precursor dysplasias in IBD can be polypoid or flat. Flat dysplasias create particular clinical challenges with regard to CRC screening of IBD patients since they can be easily missed on colonoscopy. Also, unlike sporadic colonic neoplasia, where only one or two dysplastic lesions arise in very focal areas of the colon, in colitic mucosa it is not unusual for dysplasia or cancer to be multifocal, reflecting a broader "field change". This is best exemplified by careful mapping studies that used DNA aneuploidy as a molecular marker to gain insights into temporal and topographical molecular changes in patients with UC. On the basis of DNA indices, individual cell populations were observed in the same locations of the colon on repeated examinations and became more widely distributed over time, occupying larger areas of the mucosa [16]. Moreover, within an aneuploid area, additional subclones of aneuploid cells emerged from their predecessors [16]. Indeed, whereas only a handful of different aneuploid cell populations have been detected in aneuploid areas without dysplasia, up to 46 different aneuploid populations have been found in areas of aneuploidy that show dysplastic changes [16]. This indicates that substantial genomic alterations can occur in colonic mucosa without disturbing morphology. The fact that genetically abnormal cells have been observed in histologically non-dysplastic mucosa adjacent to or even remote from, dysplasia suggests that the dysplastic cells arise from the pre-existing mutant clones [16].

The model presented in Figure 34.1 suggests that just as sporadic CRC seems to arise as a progression from early adenomas to late (advanced) adenomas, colitic mucosa

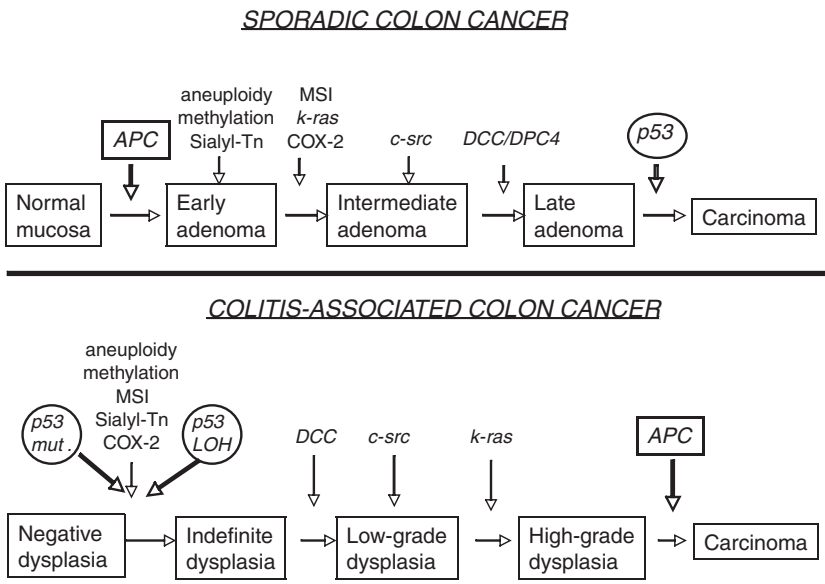

Figure 34.1 Comparison of molecular alterations in sporadic colon cancer and colitis-associated colon cancer. Reproduced with permission from Itzkowitz S, Harpaz N. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology* 2004; **126**:1634–48.

progresses in a systematic fashion, from no dysplasia to indefinite dysplasia, followed by low-grade dysplasia (LGD), high-grade dysplasia (HGD) and then carcinoma. This is a useful paradigm that facilitates the study of cancer risk markers in IBD. However, it should not be taken at face value, because we know that the natural history of dysplasia in IBD is often unpredictable. For example, LGD may progress to cancer without demonstrating HGD and cancers can arise in colitic colons without any apparent prior dysplasia [9]. Indeed, a recently described subset of CACs termed low-grade tubuloglandular carcinomas can arise directly from low-grade dysplasia [17]. These caveats should be kept in mind when interpreting the results of studies describing the predictive value of dysplasia or molecular markers in IBD.

Molecular pathways of sporadic versus colitis-associated carcinogenesis

To place the molecular pathogenesis of colitis-associated neoplasia in proper perspective, it is important to appreciate the molecular events involved in the development of sporadic colorectal neoplasia. The following sections discuss the major pathways and genes involved in sporadic CRC carcinogenesis and address the differences in these pathways that are found in CAC.

Sporadic CRCs arise as a result of genomic instability, of which there are two major types: *chromosomal instability* (CIN) and *microsatellite instability* (MSI). CIN refers to alterations, either gains or losses, in chromosomal content. The MSI pathway, on the other hand, involves the loss of the gene functions that normally repair DNA base pair mismatches that occur during the normal process of DNA replication in dividing cells. Emerging evidence suggests that the two major pathways of CIN and MSI apply to both sporadic CRC and CAC with roughly the same frequency, with CIN accounting for approximately 80–85% of cancers and MSI 10–15% (Table 34.1) [18].

Chromosomal instability (CIN)

The majority of sporadic CRCs arise via the CIN pathway. Anomalous chromosomal segregation in this pathway results in abnormal DNA content (aneuploidy). Frequently, this involves the loss of chromosomal material (loss of heterozygosity or LOH), which contributes to the loss of key tumor suppressor genes, such as *APC* and *p53*. In addition to undergoing LOH, these genes can also be rendered non-functional by mutations of the genes themselves. In the development of sporadic CRC, loss of *APC* function is typically an early event. For this reason, the *APC* gene has been considered the "gatekeeper" of the colon. If the APC protein is mutated or lost, this allows β-catenin to gain access to the cell nucleus, where it complexes with specific transcription factors to turn on genes which in turn contribute to adenoma formation by altering critical cellular functions such as proliferation, apoptosis (programmed cell death) and cell–cell adhesion. Once sporadic adenomas form, other changes in genetic regulation occur, such as induction of *k-ras* oncogene and loss of function of tumor suppressor genes on chromosome 18q in the region of the *DCC* (deleted in colon cancer) and *DPC4* genes (Figure 34.1). Loss of *p53* gene function occurs late and is believed to be the defining event that drives the adenoma to carcinoma.

CIN is also the major pathway of CAC [19]. Curiously, however, the timing and frequency of the common genomic CIN alterations in patients with IBD appear to differ from those of sporadic neoplasms (Figure 34.1). For example, the loss of *APC* function is much less frequent and occurs later in the colitis-associated dysplasia–carcinoma

Table 34.1 Genetic alterations in colitis-associated and sporadic colorectal cancer.

	Colitis-associated CRC (%)	Sporadic CRC (%)
Chromosomal instability		
Overall CIN	85	85
APC-related alterations:		
5q loss	56	26
APC LOH	0–33	31
APC mutation	6	74
β-Catenin LOH	7	34
p53-related alterations:		
17p loss	44	57
p53 LOH	47–85	50
p53 mutation	33–100	75–80
Chromosome 18q genes:		
18q loss	78	69
DCC LOH	54	39
DPC4 mutation	0	–
k-ras mutation:	8–24	40–50
E-Cadherin:		
LOH	0	17
Protein decrease	43	37
CDH1 methylation	57	36
Microsatellite instability		
Overall MSI-positive:	15–40	15–20
HMLH1 methylation*	46	76
TGFβRII mutation*	17	81
Hypermethylation		
P16INK4a	100	40
P14ARF	50	28–33
HPP1	50	84
EYA4	87	83

*In MSI tumors only.

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sequence [20,21]. Conversely, in colitis patients, *p53* mutations occur early and are often detected in mucosa that is non-dysplastic or indefinite for dysplasia [22]. In fact, one study found a high frequency of *p53* mutations in inflamed tissue compared with uninflamed tissues in UC patients and suggested that reactive oxygen species, common by-products of inflammation, contributed to these *p53* mutations, which presumably then set the stage to develop CRC [23]. Others have observed increased p53 expression in actively inflamed UC tissues compared with mucosa in histological remission, not only in patients with longstanding UC, but even in those with shorter disease duration [24].

A possible mechanism to explain the CIN associated with UC is telomere shortening. Telomeres are the protective ends of chromosomes that shorten with age and inflammation. Shorter telomeres become sticky, predisposing chromosomal ends to fuse together indiscriminately, thereby forming bridges that subsequently cause the chromosomal arms to break. In biopsies of non-dysplastic mucosa from UC patients with dysplasia or cancer, chromosomal losses were greater and telomeres were shorter than in similar biopsies from UC patients without neoplasia or from non-UC controls [25].

Aneuploidy

Abnormal DNA content (aneuploidy) occurs as a consequence of chromosomal instability. Of all the molecular markers studied in IBD colon carcinogenesis, aneuploidy is the best studied, with observations dating back two decades. Usually measured by flow cytometry on fresh biopsies, aneuploidy occurs in approximately 14–33% of patients with longstanding UC [26–28] and its presence has been associated with longer duration of colitis [29,30]. Aneuploidy correlates directly with dysplasia; approximately 20–50% of dysplastic lesions and 50–90% of cancers demonstrate aneuploidy [31–34]. Some studies suggest that aneuploidy is more frequent in HGD than LGD lesions [34,35], whereas others have not confirmed this finding [29,31,36]. Importantly, up to 35% of histologically non-dysplastic mucosal biopsies already demonstrate aneuploidy [29,31–33,36]. Thus, aneuploidy is a relatively early event. Curiously, there are examples where a diploid colon cancer occurs in a background of aneuploid mucosa [29]. Moreover, despite intensive repeated surveillance biopsies, colon cancers may arise without preceding dysplasia or aneuploidy [37]. Thus, although aneuploidy as a surrogate measure of CIN appears to be a useful marker of the high-risk colon, aneuploidy may not be universally present, or required, for progression to the malignant phenotype. This suggests that other pathways (MSI and methylator pathways) also contribute.

Loss of tumor suppressor genes

Tumor suppressor genes are normal cellular genes that control cell proliferation, death and differentiation. Loss of function of both copies of tumor suppressor genes impairs their function and predisposes to cancer. Inactivation occurs typically by two separate mechanisms: allelic deletion (LOH) of one allele and mutation of the other. The classical tumor suppressor genes that are important for sporadic colon carcinogenesis, *APC*, *p53* and *DCC*/*DPC4*, have also been implicated in CAC.

APC tumor suppressor gene

As noted above, in UC, *APC* mutations are rare and occur late in the dysplasia–carcinoma progression (Figure 34.1). In fact, mutations in *APC* are rarely, if ever, encountered in colitic mucosa that is negative or indefinite for dysplasia [20] and fewer than 14% of tissues manifesting LGD harbor *APC* mutations [20,38]. Even in UC cancers, fewer than 14% demonstrate *APC* mutations [20,21,39]. The frequency of *APC* mutations in HGD lesions has been reported to be as high as 50–100% [20,38], but this is based on very few cases. With respect to allelic deletion of *APC*, two studies observed a 33% rate of *APC* LOH [40,41], whereas a third

study found no *APC* LOH [39]. Although the APC protein was reported to be abnormally expressed in 76% of UC cancers by immunohistochemistry [42], the data overall suggest that the *APC* gene is rarely involved in CAC.

P53 tumor suppressor gene

The normal p53 protein is considered an important guardian of the genome and acts to prevent clonal expansion of mutant cells by not allowing cells that have acquired damaged DNA to progress through the cell cycle. Allelic deletion of *p53* occurs in approximately 47–85% of UC-associated CRC [31,41]. Burmer *et al*. found that *p53* LOH correlated with malignant progression, finding this molecular change in 6% of biopsies without dysplasia, 9% with indefinite dysplasia, 33% with LGD, 63% with HGD and 85% with cancer [31]. They also noted that *p53* LOH was restricted to biopsies that were aneuploid, suggesting that aneuploidy precedes *p53* LOH. Further studies from these investigators indicated that *p53* mutations were distributed more extensively than *p53* LOH in carefully mapped colectomy specimens. They also found that mutation, but not LOH of *p53*, was present in diploid, non-dysplastic mucosa, suggesting that *p53* mutation was an early molecular change that occurred prior to aneuploidy, which in turn preceded *p53* LOH [22] (Figure 34.1). Other investigators found a more variable association between *p53* mutation and aneuploidy but confirmed that *p53* mutations occurred in 19% of biopsies without dysplasia, with a steady increase in frequency among biopsies that showed progressive degrees of dysplasia [36]. In fact, using tissues from UC patients who did not have cancer, a high frequency of *p53* mutations was observed in inflamed mucosa, suggesting that chronic inflammation itself may predispose to these early mutations [23]. Additional evidence from immunohistochemical studies links altered *p53* expression with dysplasia in UC; with this technique, biopsies that are negative for dysplasia do not often demonstrate *p53* staining [43,44]. Thus, considerable evidence implicates *p53* as playing an instrumental role in UC carcinogenesis, apparently at an early stage.

Tumor suppressor genes on 18q (*DCC*/*DPC4*]

Allelic losses of 18q, in particular 18q21.1, are common in both sporadic and colitis-associated CRC. Allelic loss of 18q has been reported to occur in 78% of UC-associated CRC compared with 69% of sporadic CRC [45]. Moreover, 3/5 (60%) dysplastic lesions and 1/5 (20%) non-dysplastic samples manifested 18q allelic deletion [19]. Candidate tumor suppressor genes that reside in this location include *DCC* and *DPC4*. LOH at *DCC* has been reported to occur in 54% of CAC compared with 39% of sporadic CRC [40]. One study of 10 CACs found 18q LOH in three cases, one of which also demonstrated mutation in *DPC4* [46]. Others were unable to detect any *DPC4* mutations among 10 colitis-associated CRCs [47].

Activation of proto-oncogenes

Proto-oncogenes are normal cellular genes that, when activated by mutation of one allele, can disrupt normal cell growth and differentiation and enhance the progression to neoplastic transformation. With regard to colitis-associated CRC, two proto-oncogenes have received the most attention: *K-ras* and *c-src*.

K-ras oncogene

Typically *k-ras* mutations are not found in UC mucosa that is negative for dysplasia. In fact, most studies also indicate that *k-ras* mutations are rare even in LGD [36,39], although one study, using a highly sensitive assay, detected *k-ras* mutations in 36 and 14% of lesions that were indefinite for dysplasia and LGD, respectively [20]. When *k-ras* mutations occur, they tend to be found in HGD or cancerous lesions. Early studies suggested that the 8–24% frequency of *k-ras* mutations in UC cancers was lower than the approximately 40–50% rate in sporadic CRC [39,44], but more recent studies suggest that 40–50% of UC-associated CRC demonstrate *k-ras* mutations [20,36].

Src oncogene

The cellular oncogene *c-src* is a tyrosine kinase that is associated with malignant transformation in a variety of tumors. Elevated *c-src* levels have been reported in sporadic colon adenomas and carcinomas. In UC patients, *c-src* activity was low in areas of inflammation, but demonstrated a progressive increase in activity in LGD, HGD, DALM and cancer lesions [48].

Microsatellite instability

Whereas tumors that arise via the CIN/tumor suppressor gene pathway are typically microsatellite stable (MSS), approximately 15% of sporadic CRCs arise through the microsatellite instability (MSI) pathway. Several DNA mismatch repair (MMR) genes cooperate to repair these mismatches. Two of these genes, *hMLH1* and *hMSH2*, are most commonly affected by loss of function in CRC, but mutation or loss of other members of the DNA MMR system can also result in DNA replication errors throughout the genome. This replication error phenomenon is what is detected as the MSI phenotype. The MSI phenotype is detected in tissues using a panel of markers that recognize microsatellite sequences in various parts of the genome. Some colon cancers manifest high degrees of MSI (so-called MSI-H) whereas others demonstrate low levels of MSI (MSI-L), depending on the number of markers showing instability using a standardized marker panel [49].

Microsatellite instability is not found in normal colonic mucosa from healthy controls or from patients with other types of benign inflammatory colitis [50,51]. MSI is also

fairly rare in colonic mucosa from patients with Crohn's colitis [52]. However, as many as 15–40% of patients with UC demonstrate MSI in cancer tissues (Table 34.1). In some studies, UC-associated cancers were more likely to express MSI-L rather than MSI-H [39,53,54], but other studies indicate that the two types of instability can occur with similar frequency [55,56]. Some of this difference may relate to the fact that not all studies applied the NIH consensus microsatellite marker panel. Curiously, MSI has been detected as an early event, occurring in non-dysplastic mucosa even from patients with disease of rather short duration [51,55]. The DNA mismatch repair system is important for repairing frameshift mutations that can be induced by oxidative stress that accompanies chronic inflammation [57]. Cells that are deficient in MMR, but even cells that are MMR proficient accumulate frameshift mutations, suggesting that oxidative stress acts as a mutagen [8]. Also, oxidative stress can functionally impair the protein components of the MMR system without necessarily causing genetic mutations [8]. This may contribute to the MSI-L phenotype seen in both non-neoplastic and neoplastic mucosa of IBD patients. The induction of chronic inflammation by DSS results in more frequent development of LGD and HGD in *Msh2* knockout mice compared with wild-type controls and colonic mucosa remote from dysplastic lesions in the DNA mismatch repair deficient animals also demonstrates MSI [58]. In sporadic CRC, hypermethylation of *hMLH1* is an important mechanism for silencing the function of this gene, resulting in MSI-H tumors. Likewise, in UC-associated neoplasms, hypermethylation of *hMLH1* was detected in almost half of MSI-H cancers and dysplasias [56].

Impairment of *TGFβRII*

TGFβ1 receptor mutations permit colonic cells to escape growth control. In particular, the type II TGFβ1 receptor gene (*TGFβRII*) has two microsatellites within its coding region that predispose this gene to replication errors in cells that have abnormal DNA mismatch repair. Thus, as a target gene for MSI, *TGFβRII* instability has been demonstrated in as many as 81% of MSI sporadic CRC. In contrast, among 18 UC neoplasms that demonstrated MSI, only three (17%) demonstrated instability of *TGFβRII* [59], indicating that this same phenomenon occurs in CAC but with lesser frequency.

Promoter methylation

In addition to CIN and MSI, epigenetic mechanisms, especially methylation, can also contribute to altered gene expression in colon carcinogenesis and is an area of active investigation. The CpG island methylator phenotype (CIMP) occurs when cytosines in the promoter region of genes become extensively methylated. This is associated with promoter silencing and hence loss of gene expression. Many genes involved in cell cycle control, cell adhesion and DNA repair can be methylated in colon cancer. So-called type A methylation, which involves, for example, the estrogen receptor (*ER*), occurs as a function of age and is found in both normal colon and colon cancer. Type C methylation, however, is cancer-associated, leading to pathogenic silencing of genes such as *hMLH1*, *MGMT*, *p16*, *p14* and *HPP1/TPEF.*

In general, there is little overlap between CIN and MSI; tumors manifest either one phenotype or the other. However, there can be overlap between CIMP phenotype and MSI. For example, the MSI phenotype can occur when a DNA MMR gene is mutated (for example, hereditary nonpolyposis colorectal cancer) or if the gene is methylated (as in sporadic colon cancer).

The methylation of CpG islands in several genes seems to precede and be more widespread than dysplasia [60]. In colitis-associated neoplasms, *hMLH1* hypermethylation was observed in 6/13 (46%) MSI-H, 1/6 (16%) MSI-L and 4/27 (15%) MSS specimens, implicating this epigenetic change as a cause of microsatellite instability [56]. The cell cycle inhibitor $p16^{INK4a}$, loss of which has been implicated in sporadic CRC, is commonly hypermethylated in UC neoplasms [61]. Approximately 10% of biopsies without dysplasia already demonstrate *p16* promoter hypermethylation, the rate increasing with higher grades of dysplasia and reaching 100% in cancer specimens. $p14^{ARF}$ is an indirect regulator of *p53* and it resides at the same locus as $p16^{INK4\alpha}$. Loss of $p14^{ARF}$ function by promoter hypermethylation has been reported in 50% of adenocarcinomas, 33% of dysplastic lesions and even in 60% of non-dysplastic mucosal samples in patients with UC [62]. Another gene, *HPP1*, recently implicated in the hyperplastic polyp-serrated adenoma–carcinoma pathway of colorectal cancer, undergoes methylation silencing in 50% and 40% of CACs and dysplasias, respectively [63].

E-cadherin (*CDH1*), a member of the calcium-dependent cell adhesion molecule family, plays an important role in cell–cell contacts, thereby functioning as a tumor suppressor gene. Loss of E-cadherin function has been implicated in various cancers, including diffuse gastric cancer, breast cancer and prostate cancer. Loss of E-cadherin expression has been reported in approximately 57% of CAC, due to hypermethylation of the E-cadherin promoter rather than allelic loss [64,65]. In fact, *CDH1* promoter hypermethylation was detected in 13/14 (93%) colonoscopies where dysplasia was present, compared with only 1/17 (6%) of colonoscopies without detectable dysplasia, and it is typically the dysplastic lesion itself that manifests reduced E-cadherin protein expression [66].

Methylation of the Eyes Absent 4 (*EYA4*) gene, a transcription activator involved in apoptosis, appears to be a promising new marker demonstrating hypermethylation in 83 and 67% of cancerous and dysplastic UC tissues, respectively, but not at all in inflamed mucosa [67].

Potential clinical application of molecular markers

Markers of cancer progression

Histologic evidence of dysplasia on colonic biopsies is the gold-standard marker for determining cancer risk and deciding upon clinical management. However, there are many limitations of dysplasia, such as variations in pathological interpretation, focality of dysplasia making random biopsy detection often difficult, and the fact that cancers can arise without any apparent preceding detectable dysplasia. This has raised the question of whether newer molecular markers could be complementary to dysplasia for assigning cancer risk.

To date, most of our knowledge about the types of molecular alterations in colitis-associated neoplasia has come from studies that have taken a particular marker of interest and analyzed its expression in pathological lesions at a single time point from several patients representing the pathological spectrum of no dysplasia, indefinite dysplasia, LGD, HGD and cancer. In this type of horizontal, cross-sectional study design, any genetic alteration that demonstrates a preferential or increased expression in neoplastic (dysplasia and/or cancer) tissues is considered potentially useful. Many such markers, such as those indicated in Figure 34.1, have been evaluated in this way. In many of these studies, when a marker is detected in non-dysplastic tissue from a patient who also has dysplasia or cancer elsewhere in the colon, the marker is considered to be expressed "earlier" than dysplasia. Although this conclusion might be valid, it is not biologically or clinically accurate to assign a chronological sequence to marker expression when the tissue derives from one time point. It would be more clinically relevant to identify markers that are expressed chronologically before dysplasia or cancer in order better to stratify CRC risk and alter surveillance strategies.

Molecular markers of future cancer risk

An advantage to studying patients with IBD is that they typically undergo periodic surveillance colonoscopies with repeated tissue sampling. IBD is one of the few clinical settings in which multiple repetitive sampling of colonic tissue is routinely performed. This provides a unique opportunity to study chronologically histologic and molecular changes. To date, few studies describing a promising new marker in IBD tissues have used a chronological study design. The reasons for this are multifactorial, but include difficulty in obtaining these tissues (which are often more plentiful in tertiary referral centers), interest of the investigator to study the marker chronologically and definition of what a "marker-positive" patient is.

To date, only four molecular markers, aneuploidy, p53, MSI and the mucin-associated sialyl-Tn (STn) antigen, have been evaluated in a chronological framework and each has been demonstrated to be a harbinger of subsequent risk of developing dysplasia or cancer. Longitudinal, prospective studies have demonstrated that aneuploidy is a marker of subsequent progression to neoplasia in patients with longstanding UC who have not yet demonstrated dysplasia. Among 25 high-risk UC patients without dysplasia, all 5 who showed aneuploidy progressed to dysplasia within 1–2.5 years, whereas 19/20 (95%) without aneuploidy did not progress to either dysplasia or aneuploidy over a 2–9 year period [16]. Likewise, among 34 UC patients without dysplasia, 3/4 (75%) patients with aneuploidy progressed to LGD, whereas only 2/30 (7%) without aneuploidy progressed to LGD over a 10 year period [27]. These and other studies indicate that when aneuploidy is detected, it usually is found either before or at the same time as dysplasia. In the largest study with the longest follow-up, all of the 10 patients who developed HGD or cancer had aneuploidy detected either before or simultaneously with these histologically more advanced neoplastic lesions, whereas 5/12 (42%) patients with LGD had aneuploidy detected after LGD [28]. Aneuploidy following dysplasia has also been reported by others [26]. Hence it appears that finding aneuploidy may be a useful marker of cancer risk, but not finding it does not offer reassurance.

Little is known about whether p53 expression in surveillance biopsies predicts the future development of dysplasia or cancer. One study suggested that abnormal p53 immunostaining may precede LGD, HGD and cancer by 8, 26 and 38 months, respectively [68]. Other reports in a handful of patients also suggest that p53 expression may precede cancer by 2–4 years [69,70].

Another study retrospectively analyzed biopsy specimens for MSI and *k-ras* mutations prior to surgical resection [53]. The presence of MSI in the biopsies did not predict the presence of MSI in the resection specimens. There were three cases in which *k-ras* mutations were detected in dysplastic pre-surgical biopsies and two of them demonstrated the identical mutation in the resection specimen. MSI-H was reported to be present in chronic colitis tissue 2–12 years prior to the development of MSI-H CAC in four patients [71].

STn is a mucin-associated carbohydrate antigen whose expression has been studied in both cross-sectional and longitudinal study designs and it fulfills the criteria of being more widespread and chronologically earlier than dysplasia [72,73]. In fact, STn expression was noted as early as 2–9 years before the first detection of dysplasia. Importantly, STn expression does not just overlap with dysplasia, but is complementary to it. Moreover, STn expression is also independent of aneuploidy, thereby adding information beyond what dysplasia or aneuploidy provide [73].

There is no consensus as to how, or even whether, these markers of cancer risk should be incorporated into

clinical management of patients with longstanding IBD. Given our current knowledge, no one is likely to recommend colectomy to a patient solely on the basis of marker positivity without some evidence of dysplasia, even if the patient's tissue demonstrated marker positivity on several colonoscopies. Perhaps more intensive surveillance should be offered to such patients. These issues should be considered as more research is conducted in this field.

The problem of polypoid dysplasia in IBD

As mentioned before, a particular dilemma for clinicians is the finding of dysplasia in a polypoid lesion of a patient with longstanding IBD. Sporadic adenomas can be removed endoscopically, with continued surveillance by periodic colonoscopies. However, if it is a more ominous dysplasia-associated lesion or mass (DALM), this usually prompts a recommendation for total proctocolectomy because of the very high synchronous and metachronous rate of CRC. Recent studies suggest that if the polypoid lesion can be completely removed by endoscopic polypectomy and if numerous biopsies of mucosa adjacent to the polyp base and throughout the rest of colon are negative for dysplasia, the polypectomy alone is sufficient and colectomy can be deferred while the patient continues to undergo surveillance. A study using global gene expression profiles suggested that an artificial neural network may be able to distinguish between a sporadic adenoma and a polypoid dysplastic lesion in a UC patient [74]. Whether this technology will be applied for future molecular diagnostics in IBD remains to be seen.

New molecular screening approaches

Most efforts to date have understandably focused on studying tissues from IBD patients to identify molecular markers that might be helpful for understanding cancer pathogenesis or possibly assigning risk. A more comprehensive technique applied to tissues is molecular profiling using microarrays which, in a recent study, identified 699 transcripts that differed between benign mucosa and HGD and 242 transcripts that differed between benign mucosa and CAC [75]. Additional studies are needed to understand their relevance to the biology of neoplasia in UC.

Another approach worth considering is to examine the stool of patients with IBD for molecular alterations. This technology, which uses markers associated with the more common molecular alterations associated with CIN, MSI and abnormal apoptosis, has already been shown to have reasonable sensitivity and rather high specificity for sporadic CRC and adenomas [76]. Since the DNA shed into stool should theoretically provide a more comprehensive sampling of abnormal cells than random pinch biopsies, stool DNA testing could significantly contribute to the management of patients with longstanding IBD who are at risk for developing CRC. Molecular markers have also been studied in colonic lavage fluid taken at the time of colonoscopy. One such study analyzed *p53* and *k-ras* mutations in colonic effluent and noted mutations in either gene in up to 19% of UC patients, particularly those with longer disease duration [77]. In addition, 15% of patients with Crohn's colitis, but not ileitis, had a positive mutation. Mutations were also found in 2% of non-inflammatory controls and in only 50% of sporadic colon cancer patients. In several patients, the molecular alteration could not be confirmed on subsequent lavage samples and only one patient who repeatedly had a *p53* mutation in the fluid had the same mutation discovered in biopsy tissues. The appealing concept behind stool- or lavage-based molecular diagnostics is the potential to sample a much larger surface area of the colon than the multiple random pinch biopsies currently performed. As this technology becomes further developed and newer, more specific molecular markers become available, this approach may assume greater importance.

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Chapter 35
Cancer Surveillance in Inflammatory Bowel Disease

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Summary
- Although the overall risk of colorectal cancer in ulcerative colitis is less than previously reported, it remains too high to ignore in certain subgroups.
- Endoscopic surveillance provides a reasonably effective means to minimize cancer-related mortality in patients who wish to retain their colons.
- Stratifying patients according to individual risk is an important dimension of surveillance strategies.
- Improved endoscopic techniques increase the diagnostic yield of detecting dysplasia.
- Further study is required to examine the utility of random mucosal biopsies as an adjunct to dye spraying or endoscopic magnification as a surveillance tool.

Introduction

An increased risk of colorectal cancer in inflammatory bowel disease has been well recognized for several decades. As a result, death from this potential complication ranks highly among anxieties reported by individuals affected by the condition. Published information regarding this risk and the way in which it can be clinically managed is more comprehensive in ulcerative colitis than Crohn's disease. Accordingly, this review focuses on the association of colorectal cancer in ulcerative colitis, especially with regard to the evidence on which contemporary clinical practice is based. The difficulties of applying what limited data are available for managing the risk in Crohn's disease are highlighted.

Risk of colorectal cancer in ulcerative colitis

To minimize mortality from colorectal carcinoma in ulcerative colitis, various strategies have been proposed, including prophylactic surgery, chemoprevention and endoscopic surveillance. To date, no definitive study has been conducted to determine whether any of these options are more beneficial or cost-effective than a policy of clinical supervision only. Instead, recommended clinical practice has evolved on the basis of reported experience among patients subjected to these options and from the results of indirect modeling studies.

To a large extent, the value of any preventive measures against colorectal carcinoma depends on the magnitude of risk that applies in ulcerative colitis. Even an effective cancer-prevention strategy is unlikely to be cost-effective if the overall clinical risk is low. Historically, the risk of colorectal cancer complicating ulcerative colitis was regarded as sufficiently high for prophylactic proctocolectomy to be advocated. This was based on the results of early studies which showed a cumulative probability of developing colorectal cancer to approximate 60% after 40 years' duration of ulcerative colitis [1]. In recent years, however, the reported experience has been very different. According to two population-based cohort studies, no increased risk of colorectal cancer was observed among patients with ulcerative colitis compared with what was expected in the general population [2,3].

The disparity in these estimates is related to several factors, including differences in study design, geographic differences in colorectal cancer risk, secular changes in colorectal cancer risk over time and even in the definition of "incidence" [1]. Ulcerative colitis is a heterogeneous condition in relation to disease extent, duration and severity. All these factors influence the subsequent chance of developing of colorectal carcinoma. The proportion of patients with chronic, active pancolitis or coexisting associations such as primary sclerosing cholangitis or family history of bowel cancer impacts on estimates of cancer risk. Patients attending tertiary referral centers generally

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experience more complicated disease and a correspondingly higher risk of colorectal cancer than those included in population-based surveys.

Changing clinical practice may also contribute to the reported differences in cancer incidence among patients with colitis. For example, surgical therapy for active colitis eliminates any future risk of bowel cancer and a vigorous surgical policy early in the disease process may account for the relatively low cancer incidence reported in some series [2]. Maintenance medical therapy and endoscopic surveillance may also reduce the possibility of colorectal cancer among patients with an intact colon. Although these interventions are frequently practiced nowadays, early studies evaluating cancer rates in colitis did not generally include patients subjected to these measures.

In order to overcome these confounding factors, a meta-analysis was undertaken in 2001 in which the results of 116 studies involving 54,478 patients with ulcerative colitis were pooled from various referral-based centers, regional hospitals and population surveys [4]. According to this analysis, the overall prevalence of colorectal cancer in ulcerative colitis was 3.7%. The overall cancer incidence derived from 41 studies which reported disease duration was 0.3%. The annual colorectal cancer incidence increased from 0.2% in the first decade to 0.7% in the second decade and 1.2% in the third decade.

The risk of colorectal carcinoma among patients with ulcerative colitis has also been expressed in relation to what is expected in the general population. According to a Swedish population-based study, a 4–6-fold increase in cancer risk has been reported [5]. In comparison, a population study from Manitoba found the relative risk of colon cancer to be only 2.75 and 1.90 for rectal cancer [6]. A population-based cohort study of 1160 patients with ulcerative colitis diagnosed in Copenhagen County, Denmark, found 13 colorectal cancers compared with 12.42 expected in the general population [standardized mortality ratio (SMR) 1.05, 95% confidence interval (CI) 0.56–1.79] [2]. This result was similar to that of another population-based cohort study from Olmsted County, MN, USA, which found 6 cases of cancer in patients with ulcerative colitis compared with 5.38 expected [standardized incidence ratio (SIR) 1.1, 95% CI 0.4–2.4] [3].

Risk factors for colorectal cancer in colitis

In spite of the encouraging improvement in overall cancer rates reported in recent years, preventive strategies still need to be considered for subgroups of patients who remain at significantly increased risk for its development.

Duration of IBD

There is little doubt that disease duration represents a major risk factor for colorectal cancer in ulcerative colitis. Cancer occurrence is exceptional among patients with disease duration less than 10 years, unless there is coexisting primary sclerosing cholangitis. In a review of 19 studies which reported the incidence of colorectal cancer at 10 yearly intervals, the cumulative probability of colorectal cancer was 8.3% at 20 years and 18.4% at 30 years [4]. A subsequent study from St Mark's Hospital involving 600 patients with extensive colitis followed for 5932 person-years showed the cumulative incidence of colorectal cancer was 2.5% at 20 years, 7.6% after 30 years of disease and 10.8% after 40 years disease duration [7]. A population-based study from Uppsala, Sweden, found that the relative risk of colorectal cancer among patients with ulcerative colitis increased from 3-fold in the first decade to 17-fold during the third decade [5].

Extent of colitis

Patients with pancolitis are at much higher risk of developing colorectal cancer than those with limited extent of disease. When studies containing information about disease extent were reviewed in the previously mentioned meta-analysis, the prevalence of colorectal cancer was 5.4% among those with extensive disease compared with 3.7% overall for any patient with ulcerative colitis [4]. According to the population study from Uppsala, the risk of colorectal cancer among patients with proctitis was not increased, whereas those with left-sided disease had a 2.8-fold greater risk. In comparison, patients with inflammation extending proximal to the splenic flexure had a 14.8-fold increased risk of colorectal cancer [5]. Although the population-based study from Olmsted County found no difference in overall relative risk of colorectal cancer among patients with ulcerative colitis, a subgroup with extensive disease was found to have a 2.4-fold increased risk [3].

Primary sclerosing cholangitis

During the past decade, evidence implicating primary sclerosing cholangitis as an independent risk factor for the development of colorectal cancer in patients with coexisting ulcerative colitis has accumulated. A population-based cohort consisting of 125 patients with verified primary sclerosing cholangitis and ulcerative colitis found the cumulative risk for colorectal cancer was 33 and 40% at 20 and 30 years, respectively, after the diagnosis of ulcerative colitis [8]. Features of neoplasia in these cases include a short interval between the onset of primary sclerosing cholangitis and the development of cancer, a predominately right-sided location and an overall poor prognosis.

The factors responsible for this association are unknown. Because patients with primary sclerosing cholangitis and ulcerative colitis often have a milder form of colonic inflammation, disease activity is unlikely to be causal. It has been suggested that patients with primary

sclerosing cholangitis may have unrecognized subclinical colitis prior to its detection and that increasing disease duration may be contributing to the risk of malignancy. Alternatively, the carcinogenic effects of secondary bile acids may potentially lead to cancer formation. To date, evidence concerning the use of ursodeoxycholic acid as a preventative measure against the development of neoplasia in ulcerative colitis is conflicting.

Family history of bowel cancer

A two-fold increased risk for bowel cancer was observed in a large Swedish population-based cohort study among patients with ulcerative colitis and a family history of sporadic colorectal cancer [9]. According to a case–control study conducted in the United Kingdom in which 102 cases of colorectal cancer in ulcerative colitis were compared with matched controls, a family history of sporadic colorectal cancer in any relative increased the risk five-fold [10].

Severity of inflammation

Recent evidence suggests that the activity of mucosal inflammation in ulcerative colitis may be an important determinant for the subsequent development of bowel cancer. Among patients with ulcerative colitis, the endoscopic and histologic features of 68 cases with cancer or precancer were compared with 136 cases without evidence of neoplasia. Multivariate analysis detected a significant association between histologic scores of severity and the risk of neoplasia [odds ratio (OR) 4.7, 95% CI 2.1–10.5]. This study also suggested that patients with inactive colitis were possibly at a reduced risk of developing neoplasia [11]. Other endoscopic features such as pseudopolyps and strictures which reflect longstanding, severe inflammation were also associated with a higher risk of neoplasia.

Age at diagnosis of IBD

It remains unclear if patients with an early age of disease onset are at increased risk for colorectal cancer. The development of colorectal cancer at a young age in the general population is fairly uncommon and cases observed in ulcerative colitis disproportionately increase the relative risk. Moreover, children with ulcerative colitis have longer disease duration and the degree of mucosal inflammation is generally more severe and extensive. Still, a population study which corrected for disease duration found the risk of colorectal cancer to be much higher among patients diagnosed with colitis before the age of 15 years compared with those diagnosed at a later stage [5]. In Eaden *et al.*'s meta-analysis [4], 12 studies were reviewed in which colitis was reported to have developed in childhood. The cumulative probabilities of any child developing cancer were estimated to be 5.5% (95% CI 2.5–12.3) at 10 years, 10.8% (95% CI 4.8–23.1) at 20 years and 15.7% (95% CI 7.2–32.6) at 30 years. These rates were higher than the corresponding calculations for adults. In contrast, data from St Mark's Hospital found that the median age of disease onset among patients who developed colorectal cancer was greater than that among those not developing malignancy, suggesting that an early age at onset may not be an independent risk factor [7].

Clinicopathological characteristics of cancer in colitis

Colorectal adenocarcinoma complicating ulcerative colitis is consequential to the effects of underlying mucosal inflammation and genetic predisposition. Macroscopically, tumors are flat, villiform, nodular, polypoidal or stricturing and histologically feature mucinous, high grade and anaplastic change. In comparison with sporadic colorectal cancer, they are confined to areas of the colon affected by inflammation, are commonly multifocal, occur at an earlier age and probably have a poorer prognosis [12–14]. The lesions are characteristically preceded by mucosal dysplasia and, once malignant transformation occurs, dysplastic change often coexists in the immediate vicinity or elsewhere in the colon [13].

Dysplasia represents the histologic manifestation of widespread genomic instability caused by the effects of persistent inflammation [15]. The underlying genetic changes responsible for neoplastic transformation in inflammatory bowel disease are unknown, although similar carcinogenic pathways are seen to sporadic colorectal cancer, including chromosomal instability, microsatellite instability and hypermethylation. However, the timing and frequency of these changes appear to be different in colitis.

Dysplasia may develop in any area of the bowel affected by chronic mucosal inflammation and, depending on the stage of development, may be invisible or visible. Although its natural history has not been defined, dysplasia is presumed to commence as an invisible focal histologic abnormality which eventually enlarges to become a visibly raised lesion. This process may be confined to one site or occur synchronously throughout the bowel. Elevated lesions become visible as a discolored, slightly irregular deformity before progressing into a raised, villiform plaque or nodule, eventually becoming polypoidal or ulcerated. In contrast to sporadic adenomas in which dysplastic cells are confined to the raised lesion, the term DALM (so-called dysplastic associated lesion or mass) is used to describe a visible dysplastic lesion occurring within a background of dysplastic mucosa in ulcerative colitis. Such lesions are highly significant because they are commonly associated with invasive carcinoma [16].

Occasionally, colorectal cancer develops in patients with ulcerative colitis from a coincidental sporadic adenoma. In these cases, the lesion is solitary, located in any part of

the colon and share similar clinicopathological properties to those developing in the non-colitic general population. Notably, these cases are not accompanied by mucosal dysplasia elsewhere in the colon. Such cases are clearly evident if the tumor is located in mucosa that is unaffected by colitis, but more difficult to ascertain when it arises in an inflamed area of the bowel. The incidence of adenomatous polyps in ulcerative colitis is reportedly lower than in the general population [17].

The time taken for a dysplastic focus to become malignant in colitis is uncertain, but is probably shorter than for sporadic colorectal carcinoma. Dysplasia runs a parallel course to colorectal carcinoma in colitis, although at an earlier stage. According to two studies, the onset of dysplasia occurs at a mean of 16.6–17.7 years after the first symptoms of ulcerative colitis [18,19]. In a review of 10 dysplasia studies reported up to 1994, Bernstein *et al.* found that dysplasia or cancer was observed in 12% of patients at the time of presentation for initial colonoscopic screening [20]. Experience from St Mark's Hospital found the cumulative incidence of colorectal neoplasia in ulcerative colitis was 1.5% at 10 years, 7.7% at 20 years, 15.8% at 30 years, 22.7% at 40 years and 27.5% at 45 years [7].

Histologically, the criteria for diagnosing dysplasia include cytological abnormalities of increased epithelial height, variation in the size and shape of nuclei and increased nuclear/cytoplasmic ratio and altered nuclear polarity. Increased mucosal depth, budding of the glands and villous change may be also present. It can be very difficult to distinguish dysplasia when active inflammation is present, since regenerative hyperplasia can produce cytological changes in the epithelial cells which resemble neoplasia. A greater degree of inflammation, crypt abscess formation, epithelial destruction and ulceration is usually associated with regenerative hyperplasia and there is no stratification with variation in the size and shape of nuclei.

Based on recommendations made by the Inflammatory Bowel Disease Dysplasia Morphology Study Group, the severity of dysplasia is classified as being "indefinite", "low grade" or "high grade" [15]. The term "indefinite dysplasia" refers to cases where there is marked atypia of epithelial cells, raising the suspicion of dysplasia, but in which the degree of change is insufficient to establish a definite diagnosis. Indefinite dysplasia may be diagnosed in the presence of acute inflammation, when interpretation of the mucosal changes is difficult to make due to epithelial injury. The main feature used to differentiate low-grade from high-grade dysplasia is the distribution of nuclei within the cells. In low-grade dysplasia, the nuclei are confined to the basal half of the cells. In high-grade dysplasia, there is a loss of cellular polarity, with nuclei scattered haphazardly between the basal and apical regions of the cells.

What can be done about the risk of cancer in colitis?

The various options that are available to deal with the cancer risk in ulcerative colitis include prophylactic proctocolectomy, clinical supervision, chemoprophylaxis or endoscopic surveillance. In practice, these options may be employed alone or in combination, depending on the stage of disease, the individual's level of risk for cancer, the availability of health-care resources and a clear appreciation of the benefits and limitations of each strategy.

Surgery – prophylactic proctocolectomy

The most effective means to abolish the risk of death from colorectal carcinoma in colitis is to remove the entire colon prior to its development. Previously, this approach was advocated in selected patients for whom the lifetime risk of cancer was thought to be very high. Originally, this required a total proctocolectomy and permanent ileostomy, but with improved surgical techniques continence can be preserved by the creation of an ileal pouch–anal anastomosis. In spite of its advantages, this operation remains a major undertaking and is associated with several potential problems. Early postoperative morbidity involving small bowel obstruction, anastomotic stricture and pouch leakage with pelvic abscess occurs in up to 25% of cases. After 10 years, the cumulative probability of developing pouchitis is 24–46% [21] and 10% develop pouch failure, which may necessitate conversion to permanent ileostomy. Even in those who retain their pouch, quality of life is reduced with regard to ongoing bowel symptoms, sexual dysfunction and infertility. For all these reasons, restorative proctocolectomy is only justified as a cancer preventive measure when the risk of malignancy is confirmed as being significantly increased.

Clinical supervision

Rather than undergo preventive measures to reduce the risk of colorectal carcinoma, some patients with colitis elect to continue usual clinical management where symptoms are only investigated if and when they arise. This option is reasonable for patients whose risk of bowel cancer is not increased, such as those with disease duration less than 8 years, or as a long-term approach in patients with inflammation confined to the rectum. However, it is potentially hazardous for those at higher risk because symptomatic cancers are usually diagnosed at an advanced stage.

Chemoprophylaxis

During the past decade, maintenance aminosalicylates have been advocated to reduce the risk of bowel cancer in ulcerative colitis. Originally, the continued use of these drugs was cited as a possible reason for the low incidence of colorectal cancer reported at some centers.

Subsequently, a case–control study found that the regular use of aminosalicylates reduced the colorectal cancer risk in ulcerative colitis by 75% [10]. Among patients with ulcerative colitis exposed to more than 1.2 g aminosalicylate therapy each day, Rubin *et al.* reported a 72% risk reduction in the odds of dysplasia and carcinoma (OR 0.28, 95% CI 0.09–0.85) [22]. A meta-analysis which combined the results of nine case–control or cohort studies found that mesalamine chemoprevention reduced the risk of cancer in colitis by 54%. When the endpoints of cancer and dysplasia were combined, treatment provided a 51% risk reduction [23]. These findings contrasted with the results of a large population based study in Manitoba, which found no difference in cancer incidence among patients with inflammatory bowel disease exposed to aminosalicylates [24].

Endoscopic surveillance

Since the introduction of colonoscopy in routine clinical practice, endoscopic surveillance has been recommended as a means to minimize cancer-related mortality among patients with chronic ulcerative colitis who wish to retain their colons. The objective is to identify patients at significantly high risk of imminent or established malignancy so that preventive or curative intervention can be undertaken. The practice constitutes a formal process in which colonosocopic examination is conducted in selected individuals, at predefined intervals and in accordance with a recommended endoscopic protocol. It is to be distinguished from *ad hoc* cancer screening in which colonoscopic investigations are performed periodically, usually in relation to a change in bowel symptoms.

Inclusion criteria

Before endoscopic surveillance is even contemplated, all patients with ulcerative colitis are advised to undergo a screening colonoscopy approximately 8–10 years after disease onset. Because the risk of colorectal cancer occurs earlier in patients with coexisting primary sclerosing cholangitis, screening should be initiated shortly after its diagnosis in those with colitis. The purpose of a cancer screening investigation is to identify which patients have already developed neoplasia (dysplasia or carcinoma) and to reassess disease extent. A high proportion of patients developing cancer in ulcerative colitis already have neoplastic changes on initial screening [20,25].

The importance of reassessing disease extent is underscored by the observation that this feature can change throughout the course of disease. Patients are classified as having "extensive" colitis if there is macroscopic or microscopic evidence of inflammation existing proximal to the splenic flexure, irrespective of when this was diagnosed.

Guidelines reflecting contemporary expert opinion regarding details of endoscopic surveillance have been published [26–28]. There is general agreement that patients who are highest risk of developing colorectal cancer, such as those with extensive colitis, primary sclerosing cholangitis or family history of bowel cancer, should be offered endoscopic surveillance. Most guidelines recommend colonoscopic surveillance at 1–3 yearly intervals. Decreasing the time between colonoscopies may reduce the risk of interval cancers. In view of a perceived increase in the risk of cancer after 20 years, a greater level of surveillance has been advised after this time. However, a report from St Mark's Hospital found a constant cancer incidence up to 40 years of disease duration, thereby disputing a need to intensify surveillance with increasing time [7].

The inclusion of patients at lower risk for colorectal cancer, such as those with distal colitis, is controversial because it reduces cost-effectiveness. The risk of cancer from left-sided colitis may eventually equal that of total colitis, but possibly occurs at later time [29]. Guidelines regarding cancer surveillance in patients with distal colitis vary in terms of its commencement, ongoing frequency and the option of using flexible sigmoidoscopy as an adjunct to colonoscopy.

Endoscopic protocol

The cornerstone to colonoscopic surveillance is the premise that most cases of cancer are preceded by epithelial dysplasia, which, if detected, signifies a high risk of malignant transformation. Until recently, the recommended endoscopic protocol was based on the belief that most cases of dysplasia were invisible to standard diagnostic instruments. In order to detect dysplasia, extensive mucosal sampling from flat mucosa in each segment of the colon was advocated, in addition to targeted biopsies from raised suspicious lesions. If dysplasia was diagnosed and confirmed by a separate pathologist, surgical intervention was recommended.

Published results of various surveillance programs using this protocol indicate that many patients were able to avoid cancer successfully by undergoing preventive surgery once dysplasia was detected endoscopically [7,18,25,30–37]. However, advanced cancer or death from malignancy was not totally prevented by this strategy and its cost-effectiveness has not been conclusively established (Table 35.1). Overall, this experience has highlighted that dysplasia is a helpful, but imperfect marker for predicting malignancy in colitis. The main limitations of dysplasia include difficulties with accurately diagnosing the entity, problems detecting its presence when it is invisible and management decisions once dysplasia is found.

The histologic diagnosis and classification of dysplasia is qualitative and subject to considerable inter-and intra-observer variation, even among expert gastrointestinal pathologists. Generally, there is concordance among pathologists in excluding dysplasia when it is absent and in recognizing high-grade dysplasia. However, there is

Table 35.1 Summary of reported endoscopic surveillance programs.

Study	Total patients	Cancers	Advanced cancers
Rutter [7]	600	30*	13
Lynch [18]	160	1	1
Nugent [25]	213	10	6
Rosenstock [30]	248	7	3
Lofberg [31]	72	2	0
Leidenius [32]	66	0	0
Jonsson [33]	131	2	1
Rozen [34]	154	4	0
Hata [35]	217	5	0
Lindberg [36]	143	7	1
Lashner [37]	99	8	6

*Overall 5 year survival rate 73%.

less agreement regarding the diagnosis of low-grade dysplasia or indefinite dysplasia [38].

Dysplasia that is invisible to standard endoscopic instruments may still escape detection by random mucosal biopsies. One study using jumbo biopsy forceps found that 64 biopsies need to be taken to ensure a 95% chance of detecting the highest degree of histologic abnormality [39]. Even when this approach is conducted, the proportion of total colonic mucosa sampled is very small and unsuspected foci of dysplasia may remain undetected. Conversely, some patients with cancer in colitis do not have evidence of dysplasia elsewhere in the colon [40]. Regardless of how many random biopsies are obtained, it is unrealistic to expect that dysplasia can be found in all cases of malignancy unless the exact site is suspected or fortuitously sampled.

In the past decade, there has been an increasing recognition that most cases of dysplasia in ulcerative colitis are actually visible, even when standard endoscopic instruments are used [41]. When new endoscopic techniques such as chromoendoscopy are performed, the detection of raised lesions is enhanced. By obtaining targeted biopsies from visible lesions, the diagnostic yield for detecting dysplasia is improved. In a study of 100 patients with chronic extensive ulcerative colitis undergoing cancer surveillance, dysplasia was detected in 0 from 2904 random biopsies and 9 from 157 targeted biopsies [42]. Similar findings were reported in an endoscopic study in which biopsies were obtained from targeted lesions identified by chromoscopy and evaluated for neoplasia by confocal endomicroscopy [43]. These data suggest that future guidelines concerning cancer surveillance in colitis need to incorporate careful endoscopic examination using dye spraying or magnified imaging to facilitate the diagnosis of dysplasia. The role of adjunctive mucosal biopsies must be re-examined in this context, although there is currently insufficient evidence to discard the practice altogether.

What to do once dysplasia is detected in flat mucosa

If high-grade dysplasia is diagnosed in flat mucosa and confirmed by a separate pathologist, the risk of concurrent or imminent cancer is sufficiently high to warrant total proctocolectomy. Several studies have shown that high-grade dysplasia does not disappear and is already associated with established cancer in a high proportion of cases [25,30]. In one review of 10 dysplasia studies, high-grade dysplasia was associated with an established cancer in 42% [20]. In the remainder, definite dysplasia is usually detected in colectomy specimens when surgery was undertaken.

The significance of low-grade dysplasia in flat mucosa is controversial. Data from major tertiary referral centers indicate that this finding confers a reasonably high risk of progressing eventually to high-grade dysplasia or cancer that surgical intervention is warranted [7,44]. Unexpected advanced neoplasia occurred in 23.5% of patients reviewed at the Mount Sinai Hospital who underwent colectomy for flat low-grade dysplasia and the rate of neoplastic progression was 53% at 5 years [44]. Among 46 patients who were diagnosed with low-grade dysplasia during endoscopic surveillance at St Mark's Hospital, 19.4% developed colorectal cancer and 39.1% developed either high-grade dysplasia or carcinoma [7]. In contrast, expectant follow-up for several years of low-grade dysplasia in flat mucosa from Leeds and Olmsted County, MN, USA found little evidence of neoplastic progression [45,46].

A meta-analysis reviewing 20 surveillance studies involving 508 cases of low-grade dysplasia in flat mucosa or DALMs were associated with a cancer incidence of 14 of 1000 person-years duration and the incidence of any advanced lesion was 30 per 1000 person-years duration. Put another way, when low-grade dysplasia is detected on surveillance, there is a 9-fold risk of developing cancer and a 12-fold increase of developing any advanced lesion [47]. Overall, these data support a policy for recommending surgery if low-grade dysplasia is diagnosed in flat mucosa, especially if it is found in more than one location. In contrast, it seems reasonable to follow up patients with intensive surveillance if unifocal low-grade dysplasia in flat mucosa is found.

If a biopsy is diagnosed as being indefinite for dysplasia by two gastrointestinal pathologists, a follow-up colonoscopy with multiple biopsies is indicated at a shorter interval than normal. Often, the basis for diagnosing indefinite dysplasia is because of the confounding effects of inflammatory or regenerative change. In these circumstances, a short course of intensive anti-inflammatory therapy may be undertaken before the next follow-up endoscopy. A biopsy reported as indefinite for dysplasia, but with high suspicion, requires a further colonoscopic examination within 3–6 months. If an indefinite for

dysplasia biopsy is thought to be of low suspicion, then repeat biopsies should occur within 6–12 months.

Dysplasia in raised lesions

Raised lesions confined to non-inflamed areas of the colon are sporadic adenomas that can be managed endoscopically in the same way as in the general population. When a mass lesion arises in an area of inflamed bowel, the distinction cannot be made with certainty. In both cases, a high malignant potential persists unless the entire dysplastic area is expunged by surgery or endoscopic resection. Recent studies indicate that it is reasonable to remove endoscopically a well-circumscribed elevated dysplastic lesion provided that there is complete resection of the tumor and no dysplasia is found anywhere else in the colon [48]. In contrast, if dysplasia is detected elsewhere or if the lesion cannot be entirely removed endoscopically, surgical intervention is required. Because of the multifocal nature of dysplasia in colitis, intensive endoscopic follow-up with dye spraying and extensive mucosal sampling is mandatory if dysplastic lesions are to be managed by endoscopic resection alone.

The endoscopic distinction between inflammatory polyps and dysplastic lesions can also be difficult. When multiple inflammatory polyps are found at endoscopy, it is not feasible to biopsy every one and a truly dysplastic lesion may escape detection. The introduction of chromoscopy combined with confocal endomicroscopy may provide an important means by which neoplastic lesions can be accurately identified and selectively biopsied [43].

If dysplasia is detected, but cannot be fully removed endoscopically, patients are advised to undergo restorative proctocolectomy, preferably with ileal pouch–anal anastomosis. Recent studies have explored the possibility of limited colonic resections in patients who were found to have localized neoplasia. Preliminary evidence suggests this may be undertaken safely in selected patients, although long-term follow up is lacking [49]. On the other hand, neoplastic change may be multifocal and previous studies have demonstrated a substantial risk of developing rectal cancer among patients who had undergone subtotal colectomy and ileorectal anastomosis for ulcerative colitis [13]. Based on these observations, the practice of segmental resection for neoplasia in colitis should be reserved on a case by case basis, with patients being fully informed that future malignancy may occur in the remaining bowel, even in spite of intensive ongoing surveillance.

Efficacy and cost-effectiveness of surveillance programs

Various indirect methods have attempted to evaluate the efficacy of surveillance programs in ulcerative colitis. According to one decision analysis, the greatest gain in life expectancy was associated with prophylactic surgery, and colonoscopic surveillance was more effective than a policy of no surveillance [50]. In contrast, a separate medical decision analysis linking outcomes to cumulative cancer risk demonstrated no overall benefit of surveillance [51]. A population-based case–control study revealed a statistically insignificant trend in favor of colonoscopic surveillance among 4664 patients with ulcerative colitis. The proportion of patients dying from colorectal cancer who had undergone at least one surveillance colonoscopy was 2/40 compared with 18/102 who had not. This difference was not statistically significant (RR 0.29; 95% CI 0.06–1.31), but a more pronounced protective effect was observed among patients who underwent two or more colonoscopies [52]

A Cochrane review of strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease examined three case–control studies and concluded that there is no clear evidence that surveillance colonoscopy prolongs survival in patients with extensive colitis [15,37,52]. This was not surprising given the fact that none of the studies reviewed were true prospective, controlled assessments of endoscopic surveillance among patients who had previously tested negative to a screening colonoscopy. The review did find evidence that cancers tend to be detected during surveillance at an earlier stage with a correspondingly better prognosis and that there is indirect evidence that surveillance is likely to be effective at reducing the risk of death from inflammatory bowel disease-associated colorectal cancer [53].

Most of the reported data regarding endoscopic surveillance have focused on its efficacy, yet important questions remain concerning its cost-effectiveness and how the process can be further improved to eliminate the development of advanced cancer without invoking a correspondingly high rate of unnecessary operations. The cost-effectiveness of endoscopic dye spraying or magnification techniques in clinical practice will also need to be formally assessed in future.

Summary

Endoscopic surveillance appears to offer a reasonably effective means to avoid malignancy in most at-risk patients with ulcerative colitis who wish to retain their colon. Biennial surveillance for patients with extensive disease and annual examinations for primary sclerosing cholangitis remain a reasonable trade-off to balance the opposing demands of cancer prophylaxis and cost. Heightened surveillance may be justified in other selected cases, such as those with a positive family history of bowel cancer or chronic active inflammation. Further studies are required to define which patient subgroups can safely undergo less rigorous investigation. Strategies regarding surveillance in patients with left-sided colitis need to be further defined.

The use of dye chromoendoscopy or endoscopic magnification enhances the diagnostic yield of dysplasia and

where possible this option should be used in patients as an adjunct to random mucosal sampling in patients undergoing cancer surveillance for ulcerative colitis.

Crohn's disease

Epidemiological and clinicopathological studies indicate that patients with chronic complicated anorectal or intestinal disease and those with extensive Crohn's colitis are at increased risk of developing carcinoma of the colon or anal canal. A population survey conducted among 11,655 patients from Uppsala, Sweden, showed a 2.5-fold increased risk of colorectal cancer among those with Crohn's disease and a 5.6-old increase for Crohn's colitis [54]. Studies from tertiary referral centers describe even higher rates of colorectal cancer in Crohn's disease. According to a report from Birmingham among patients with extensive Crohn's colitis, the risk of colorectal carcinoma was 18.2 (95% CI 7.8–35.8) compared with expected rates in the general population [55]. These results are similar to that found in patients with ulcerative colitis of an equivalent extent. In a recent meta-analysis of 12 studies, the overall relative risk for colorectal cancer in Crohn's disease was 2.5 (95% CI 1.3–4.7) and for patients with Crohn's colitis it increased to 4.5 (95% CI 1.3–14.9) [56].

Studies have shown that tumors in Crohn's disease occur at sites affected by chronic complicated inflammation. Among 132 patients at Mount Sinai Hospital with colonic stricture, 6.8% developed colorectal cancer compared with 0.7% of patients without a stricture [57]. The development of cancer-complicating internal or external intestinal fistulae is also well described. Carcinoma of the anus or rectum may also arise in patients with chronic complicated anorectal disease including longstanding perianal fistulae, stricture, skin tags or persistent mucosal inflammation [58].

Although the data are not as strong as for ulcerative colitis, increasing disease duration, a positive family history of colorectal cancer and a young age of disease onset are probably associated with a higher future risk of developing of cancer in Crohn's disease. Chronic inflammation of the intestinal epithelium appears to lead to dysplasia and eventually carcinoma in Crohn's disease, although the reported frequency at which dysplasia accompanies cancer in Crohn's colitis varies [59]

Based on the experience in ulcerative colitis, guidelines recommend surveillance in patients with extensive Crohn's colitis. However, formal cancer surveillance programs have not been specifically evaluated in Crohn's disease. According to one study from New York in which 259 patients with Crohn's disease affecting at least one-third of the colon for more than 8 years underwent surveillance, 42 had neoplasia on initial screening (10 with indefinite dysplasia, 23 with low-grade dysplasia, 4 with high-grade dysplasia and 5 with cancer). With ongoing surveillance, the probability of developing definite dysplasia or carcinoma after a negative screening colonoscopy was 22% [60]. These data provide support for endoscopic surveillance in patients with Crohn's colitis, but more information is required to clarify when surveillance should start, how often it is required and what action is required if dysplasia is found. The role of limited resection versus proctocolectomy and end ileostomy in patients with dysplasia complicating Crohn's disease is an important clinical decision that has not yet been addressed by clinical studies.

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Chapter 36
Liver Diseases in Patients with Inflammatory Bowel Diseases

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Summary

- Abnormal serum liver tests are common in inflammatory bowel disease. In acute presentations they are most likely to reflect sepsis but a chronic rise must be carefully investigated in order to diagnose significant hepatobiliary diseases such as primary sclerosing cholangitis (PSC), which complicates up to 5% of cases of ulcerative colitis.
- PSC is an immune-mediated disease and appears to be a genetically complex disorder with susceptibility associated with both extended HLA haplotypes and non-HLA genes. The environmental trigger for the disease has not been identified. There are a number of hypotheses linking the causation of PSC and IBD but none has yet been properly established.
- PSC is a progressive disease with a poor prognosis and predisposes the patient to an unpredictable risk of cholangiocarcinoma in addition to increasing the risk of developing colorectal cancer in patients with concurrent ulcerative colitis.
- Ursodeoxycholic acid is the most widely used treatment for PSC. It has a range of potentially beneficial actions and is well tolerated by patients, but has yet to be proven to be useful in preventing progression of disease. It has a possible role, however, in reducing the risks of colorectal neoplasia in the context of "PSC–IBD".
- There are a number of other hepatobiliary complications of inflammatory bowel disease which may be treatable. These include hepatic abscess, gallstones, autoimmune hepatitis and fatty liver disease. Investigation of liver dysfunction in the context of IBD with imaging and biopsy is therefore usually necessary.

Hepatobiliary complications of inflammatory bowel diseases

The hepatobiliary system and the alimentary tract are closely linked embryologically, physiologically, biochemically and anatomically with all mesenteric venous drainage ascending via the portal vein into the liver. It is not surprising, therefore, that the liver is especially vulnerable to the development of complications of many different gastrointestinal diseases particularly inflammatory bowel disease.

The first association between colonic ulceration and liver disease was made in 1874 by Thomas, who described a young man who died of a "much enlarged, fatty liver in the presence of ulceration of the colon" [1]. The association was confirmed by Lister in 1899, who reported a patient with ulcerative colitis and secondary diffuse hepatitis [2]. Over the next 100 years, it became well established that there is a close relationship between inflammatory bowel disease and various hepatobiliary disorders. These disorders are listed in Table 36.1.

In the last 20 years, a different concept of hepatobiliary disorder in inflammatory bowel disease has emerged. It is now apparent that the major hepatobiliary diseases seen in association with both ulcerative colitis and Crohn's disease, namely primary sclerosing cholangitis (PSC), cirrhosis, cholangiocarcinoma and autoimmune hepatitis, represent different aspects of the same spectrum of hepatobiliary disease.

Prevalence of liver disease

The prevalence of liver disease in patients with ulcerative colitis and Crohn's disease has varied widely in different series. The discrepancy between the series may be largely due to differences in the number of patients included with severe, active or extensive inflammatory bowel disease and also in the method used to investigate liver dysfunction.

Abnormal serum liver tests are found in over half of patients with inflammatory bowel disease requiring surgery

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Table 36.1 Hepatobiliary disorders associated with inflammatory bowel disease.

Disorder	Associated with*	
	Ulcerative colitis	Crohn's disease
Primary sclerosing cholangitis (PSC)		
Large duct PSC	√	√
Small duct PSC	√	(√)
Cirrhosis	√	√
Hepatoma	√	√
Cholangiocarcinoma	√	(√)
Miscellaneous disorders		
Fatty liver	√	√
Granulomas		√
Amyloidosis		√
Hepatic abscess		√
Gallstones		√
Autoimmune hepatitis	√	
Primary biliary cirrhosis	(√)	
Budd–Chiari syndrome	(√)	(√)

*√ = definite association; (√) = possible association.

Table 36.2 Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis.

Country of origin	No. of patients with ulcerative colitis	Percentage with primary sclerosing cholangitis
Oxford, UK [3]	681	2.9
Oslo, Norway [5]	336	4
Stockholm, Sweden [6]	1500	3.7 (5.5 in total colitis)

and are due to a number of factors such as malnutrition, sepsis and blood transfusions with the subsequent risk of viral infection. However, significant liver disease is much less common. The true prevalence of hepatobiliary abnormality is difficult to determine as it has traditionally involved obtaining liver histology and cholangiography on an unselected group of patients with inflammatory bowel disease. Most series, therefore, have relied upon detecting persistent abnormalities on serum biochemical testing before proceeding to hepatic biopsy or endoscopic retrograde cholangiography. Less invasive techniques such as magnetic resonance cholangiography and detection of fibrosis using liver stiffness detection (e.g. FibroScan) or panels of serum markers (e.g. FibroTest) are now available and are likely to change the decision-making process for clinicians considering screening for hepatobiliary problems in the context of inflammatory bowel disease [3].

In an early study from Oxford, 5–6% of 300 unselected adult patients with ulcerative colitis had significant histologic abnormalities on hepatic histology compared with 10% of 100 unselected patients with Crohn's disease. None of these patients underwent cholangiography [4,5]. A group of 336 Norwegian patients with ulcerative colitis and persistently abnormal serum liver tests were investigated using cholangiography [6]. More than 14% of patients were found to have some form of hepatobiliary disease and 4% of all patients had PSC, although most were asymptomatic. Similar results were obtained in 1500 Swedish patients with ulcerative colitis [7]. In this thorough study, endoscopic cholangiography was obtained in 65 of 72 patients with elevated values of serum alkaline phosphatase. Primary sclerosing cholangitis was diagnosed in 3.7% of the ulcerative colitis group. The prevalence was 5.5% in patients with extensive colitis and only 0.5% in patients with distal disease. (Table 36.2) This figure may be an underestimate as PSC can occur without any abnormality in serum liver tests [8].

PSC is also associated with Crohn's disease, but has only been reported in patients with extensive colonic involvement. A study from Norway has suggested that PSC is as common in patients with colonic Crohn's disease as it is in ulcerative colitis [9]. Studies to date have suggested a prevalence of 1.3–13% of PSC amongst Crohn's patients. [9–11]

Significant hepatobiliary abnormalities may also occur in the presence of normal serum liver tests in the context of inflammatory bowel disease (IBD). Only 50% of liver biopsies from 74 Swedish patients with ulcerative colitis and normal serum liver tests were found to be completely normal in Broomé *et al.*'s study in 1990 [12]. The biopsies of three patients with total colitis displayed concentric periductular fibrosis and the rest showed minimal portal inflammation or fatty filtration. The patients were then followed for a mean of 18 years. None of the three patients with concentric fibrosis developed abnormal serum liver tests, although cholangiography was not performed. Two other patients developed liver disease; cirrhosis in one and autoimmune chronic hepatitis and cholangiocarcinoma in the other. A recent study from Oxford considered the MRCP appearance of a group of patents with total ulcerative colitis but persistently normal serum liver tests; 18% of patients had MRCP changes diagnostic of sclerosing cholangitis. The natural history of this asymptomatic group remains to be established [13].

In summary, approximately 5% of adult patients with IBD will have significant hepatobiliary disease. Although the number of patients with hepatobiliary abnormality is approximately the same for ulcerative colitis and Crohn's disease, severe significant liver disease is more commonly seen in patients with ulcerative colitis and when it occurs in Crohn's disease it is usually associated with extensive colonic involvement.

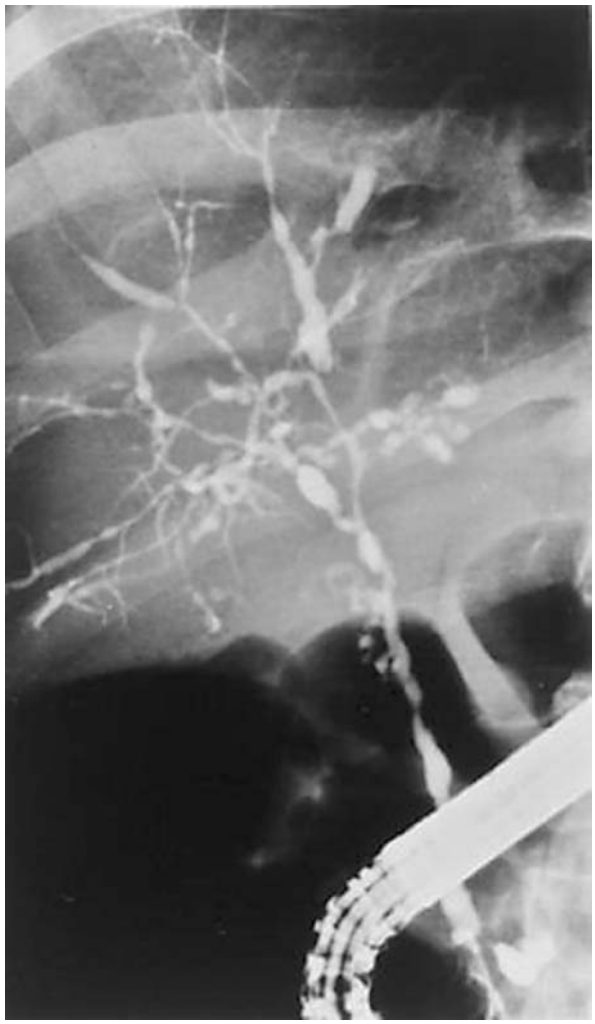

Figure 36.1 Cholangiogram from a patient with ulcerative colitis and abnormal cholestatic serum liver tests showing stricturing and dilatation of extra and intrahepatic bile ducts.

Primary sclerosing cholangitis

PSC is a chronic cholestatic liver disease characterized by an obliterative inflammatory stricturing fibrosis which usually involves the whole biliary tree [14] (Figure 36.1). The changes may sometimes be localized to either the extra- or intra-hepatic bile ducts and the degree of involvement varies considerably from patient to patient.

Epidemiology

PSC is now the major indication for liver transplantation in Scandinavia and the fifth most common indication in the USA [15,16]. The incidence of the disease has been reported as 0.9–1.3 per 100,000 per year in Northern Europe and USA, with a prevalence of 8.5–14.2 per 100,000 [17–19]. PSC appears to be less common in Southern Europe and Asia [20–22]. The most recent UK epidemiology data suggests a lower incidence rate of 0.41 per 100,000 person-years and a prevalence in 2001 of 3.85 per 100,000 [23]. This large study also found that less than 50% of PSC cases were recorded as also having IBD. This is significantly lower than the 75–80% previously reported for northern European PSC patients and may reflect limitations of the use of a GP database to determine the coexistence of these two diseases. Rates of IBD in PSC patients appear to be lower elsewhere in the world with figures of 50% of PSC patients in Spain and Italy and 20% of Japanese patients. With modern methods of diagnosis, the detection of PSC is increasing and its prevalence now appears to be comparable to primary biliary cirrhosis.

Etiology

Genetic susceptibility

The cause of PSC is unknown, but any postulated etiologic mechanism must explain the close association with ulcerative colitis. A number of hypotheses have been proposed (Table 36.3) and it is becoming clear that there is a genetic predisposition to PSC and a number of possible environmental triggers.

Few papers have reported incidents of familial cases of PSC in the English literature [24–28]. Until Bergquist *et al.*'s paper in 2005 [29], a total of only seven affected families had been reported and in all reports the affected family members were siblings. The most striking of these papers is by Jorge *et al.* in 1987, which describes an Argentinean family with 15 siblings, four of whom had well-documented PSC on cholangiography and liver biopsy, with a further brother suffering from chronic cholestasis which might have been caused by undiagnosed PSC [26]. Bergquist *et al.* published the first large study of the familial occurrence of PSC and used a group of 145 PSC patients [29]. A prevalence of 0.7% of PSC in first-degree relatives of PSC patients was demonstrated, which represents an impressive 100-fold increased risk for these relatives compared with the general population in Norway. Even this figure is probably an underestimation as the average age of

Table 36.3 Possible causes of primary sclerosing cholangitis.

Portal bacteremia
Abnormal bile acids
Absorbed colonic toxins
Viral infections
Copper toxicity
Immunological mechanisms
Genetic predisposition
Ischemic arteriolar injury

diagnosis of the disease in this population is 32–42 years, so few of the patients had children old enough to have developed the disease.

A sibling recurrence relative risk (λ_s) can be used as an indicator of importance of a genetic component in the pathogenesis of a disease. It is defined as the ratio of the risk of disease manifestation in siblings of cases compared with the disease risk in the general population [30]. A ratio above unity suggests familial aggregation and λ_s values have been published for a range of autoimmune and immune-related diseases including primary biliary cirrhosis and ulcerative colitis [31,32]. For monogenic disorders λ_s ranges from several hundreds to several thousands, whereas complex traits are usually below 100. The λ_s for PSC has recently been calculated to be approximately 100 compared with 15–35 for Crohn's disease and 6–9 for ulcerative colitis [29,33].

The importance of genetic predisposition in the pathogenesis of PSC has been well established as a result of the work performed over the last 25 years on the genes of the major histocompatibility complex (MHC). This area is the most obvious candidate for investigation of autoimmune disease as it encodes the human leukocyte antigen (HLA) molecules, which are highly polymorphic cell surface heterodimeric glycoproteins, which are essential for cell/cell recognition. Investigation into the HLA in the context of PSC has resulted in the development of a number of extended HLA haplotypes associated with susceptibility and resistance to the disease (see Table 36.4).

Table 36.4 Extended HLA haplotypes associated with susceptibility and resistance to primary sclerosing cholangitis.

Haplotype	Significance in PSC
B8-MICA*008-MICB*24-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
MICA*008-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

Genes within the extended HLA Class I region have been associated with multiple immune-mediated diseases. More recent HLA studies have focused on the MHC class 1 chain-related gene family (MIC genes). These are a group of polymorphic genes localized in the HLA-class 1 region between the HLA-B and *BAT1* (HLA-B-associated *t*ranscripts) genes. *MICA* and *MICB* genes are expressed on gastrointestinal epithelium and can activate natural

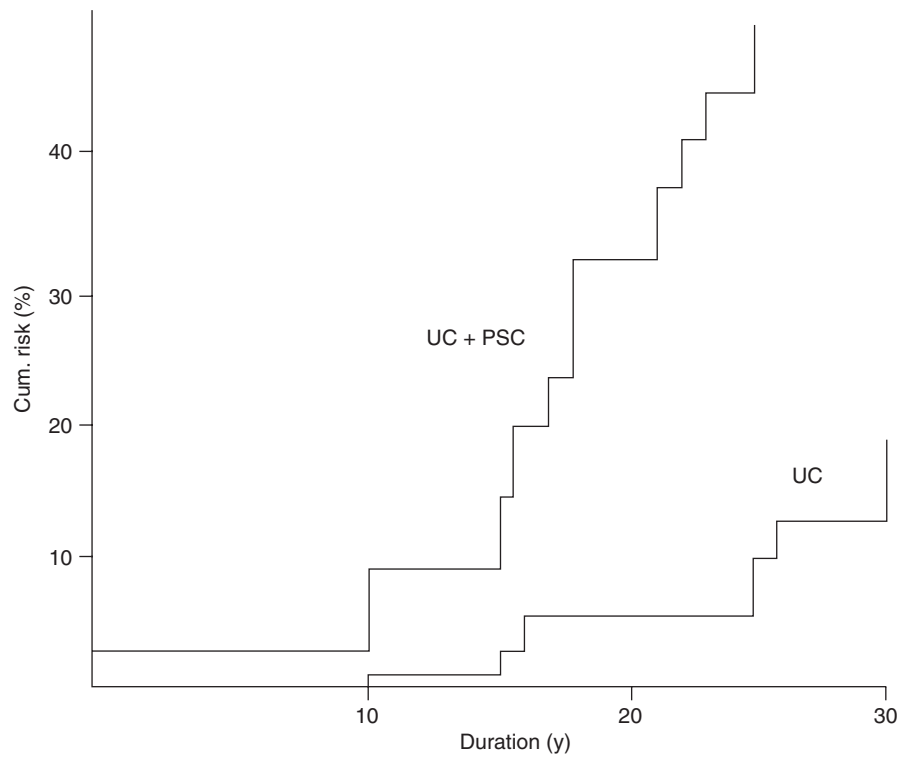

Figure 36.2 The cumulative risk of developing colonic or biliary dysplasia in 40 patients with primary sclerosing cholangitis (PSC) and ulcerative colitis compared with 80 matched controls with ulcerative colitis alone. (Please see page 536.) Reproduced from [98] with permission from John Wiley & Sons, Inc.

killer (NK) cells and γδT lymphocytes. The probability that these genes have a role in immunoregulation and their localization in the HLA class 1 region makes *MIC* genes candidates for PSC. The *MICA*008* allele has been reported as being increased in British PSC patients and an extended haplotype has been proposed with PSC in Norwegian patients being very significantly associated with HLA B8-MICA5.1-MICB24-DR3 [34,35]. The observation in the Norwegian study that B8 and DR3 are only associated with PSC in the presence of both *MICA5.1* and *MICB24* markers is due to the tight linkage disequilibrium between these four genes and the most likely interpretation of this result is that none of the four genes has any real effect on the development of PSC and they are merely acting as markers for the true susceptibility locus in the region. Most recently, it has been recognized that the DRB1*0301-DQB1*0201 and DRB1*1501-DQB1*0602 haplotypes share alleles not only at *MICA* but also at the *HLA-A* and *C* loci, which may result in decreased inhibition of NK cells [36]. The MICA 5.1 allele has also recently been shown to confer protection against development of cholangiocarcinoma [37]. Wiencke *et al.* have gone on to study seven microsatellite markers (MIB, D6S265, D6S2222, D6S464, D6S2223, D6S2225 and D6S2239) and HLA Class II alleles in 219 Norwegian patients with PSC [38]. Results were compared with 282 random controls and 142 HLA-DR3 homozygotes and 187 DR6-positive controls, to control for associations resulting from linkage disequilibrium (LD). This study demonstrated that a gene in LD with the D6S265*122 allele contributes to susceptibility to developing PSC in carriers of DR6 alone [odds ratio (OR) = 3.7, p_c 0.0004]; in addition, a possible protective effect of DR11 was reported.

Although it is clear that HLA genetic variants are important in the development of PSC, there are further layers of genetic complexity to add to the picture. Non-MHC genes are also likely to contribute to the susceptibility to and progression of PSC and numerous candidate gene studies have been performed to attempt to determine which loci might be of importance. Selecting candidate genes and then testing them sufficiently rigorously in large enough populations to show clear statistical significance has proven to be a difficult process. Matrix metalloproteinase 3 (MMP3), intercellular adhesion molecule 1 (ICAM-1) and chemokine receptor 5 (CCR-5) have all been reported as having a positive association with susceptibility to PSC, but none of these associations has been consistently replicated [39–44]. The recent completion of the human haplotype map project (HAPMAP) and advances in high-throughput genotyping technologies should make genome-wide studies feasible for case–control materials [45]. These methods make candidate gene studies obsolete and, if applied to PSC, may identify susceptibility genes which would never have been suspected using current methodology.

Immune mediation

PSC has been described as an "atypical autoimmune disease" due to the presence of autoantibodies, association with "autoimmune" HLA haplotypes and its close association with inflammatory bowel disease. However, PSC lacks a specific autoantigen, affects predominately men rather than women and does not appear to respond well to immunosuppressive medication. The "immune-mediated inflammatory disease" (IMID) model appears to describe the clinical features of the disease better. IMID diseases, which are now thought to include inflammatory bowel disease, rheumatoid arthritis and psoriasis, appear to be mediated by T cells and macrophages [46]. The trigger in these diseases seems more likely to be an environmental antigen than a self-antigen and this trigger produces an inappropriately aggressive immune response resulting in inflammation and tissue damage.

The close association of PSC and IBD led at an early stage to the hypothesis that the environmental trigger for the disease might be bacterial antigen gaining abnormal access to the portal circulation via an abnormally permeable gut wall [47,48]. Portal bacteremia has been described in 24 of 90 patients with ulcerative colitis submitted to colectomy and a number of animal models using T cell receptor and IL-10 knockout mice have indicated that immune responses to bacterial antigens are involved in the generation of colitis and pANNA (anti-neutrophil nuclear antibody) [49–52]. The response of the innate immune system to bacterial antigen is likely to be an initiating step in the pathogenesis of the disease.

Although a range of humoral immune abnormalities have been described in PSC, many are associated primarily with cholestasis rather than PSC specifically. For example, high levels of circulating immune complexes have been demonstrated in PSC but this phenomenon has also been found in other liver diseases [53]. Atypical anti-neutrophil antibodies (ANCA) are present in the serum of up to 88% of patients with PSC [54]. They are not specific for PSC, however, with ANCA being detected in 60–75% of ulcerative colitis patients and 50–96% of patients with autoimmune hepatitis (AIH) [55]. This ANCA is distinct from the pANCA found in microscopic polyangiitis and cANCA in Wegener's granulomatosis. The target antigen is a 50 kDa myeloid-specific nuclear protein and this now appears to be a neutrophil nuclear envelope protein, viz. tubulin-beta isotype 5 [56,57]. Terjung *et al.* suggested that the term pANNA is therefore more appropriate as the recognized antigen originates in the nuclear membrane rather than the cytoplasm [57]. The role of pANNA in the immunopathogenesis of PSC remains unclear, particularly as the myeloid specific tubulin autoantigen is recognized by autoantibodies from patients with both PSC and AIH. However, the titers of pANNA do not change after liver transplantation, which suggests that they are not merely an epiphenomenon.

Animal studies have suggested that pANCAs might be induced by immune responses to bacterial PAMPs (pathogen-associated molecular patterns) or antigens cross-reactive with enteric antigens. Most patients with PSC have antibodies against enterobacterial proteins and 36–46% of PSC patients have antineutrophil cytoplasmic antibodies directed against the bactericidal/permeability-increasing protein (BPI) [58]. This protein is found mainly in the granules of neutrophils and, to a lesser extent, eosinophils and has potent antimicrobial properties with particular effectiveness against gram-negative bacteria. The presence of BPI-ANCA has been associated with inflammation and tissue damage and it has been suggested that BPI-ANCA might promote innate immune reactions by preventing clearance of lipopolysaccharide [59].

Autoantibodies to biliary epithelial cells (BECs) have also been detected in PSC patients. Autoantibodies reacting with antigens on healthy BECs were detected in 63% of PSC patients, compared with 37% of patients with PBC, 16% of AIH patients and 8% of healthy controls [60]. This study went on to show that only the anti-BEC antibodies from the PSC and PBC patients had the capacity to induce cultured BECs to secrete IL-6, a pro-inflammatory cytokine which can stimulate cholangiocyte proliferation and inhibit apoptosis. Furthermore, the IgG and IgM autoantibodies from PSC patients alone could induce the expression of CD44 cell adhesion molecules on BECs. CD44 has a role in the recruitment of lymphocytes to sites of inflammation in AIDs and IMIDs.

Various other autoantibodies may be detected in the sera of patients with PSC [61].These antibodies have been thought to be unlikely to be implicated in disease pathogenesis but may indicate an altered state of immune responsiveness or immune regulation. Preuss *et al.*, however, demonstrated the presence of autoantibodies to recombinant human sulfite oxidase (SO) in PSC patients and a reduction in activity associated with UDCA therapy [62]. Antibody positivity was 56 and 17% in untreated PSC patients and those receiving UDCA, respectively, in comparison with 5% of PBC and 9% of autoimmune hepatitis patients. Furthermore, UCDA treatment resulted in a significant reduction in antibody activity. Additional work is now required to determine how these antibodies may be related to disease etiology or pathogenesis or whether anti-SO antibodies may have a role in the serological diagnosis of PSC.

PSC has been shown to be associated with changes in peripheral lymphocyte subsets and a functional T-lymphocyte portal tract infiltrate [63–67]. There is, however, no consensus about the relative importance of $CD4^+$ and $CD8^+$ cells in the portal infiltrate. Hashimoto *et al.*'s study, probably the most comprehensive published to date, found that $CD4^+$ cells were more common in the portal tracts with $CD8^+$ cells predominating in areas of interface hepatitis [66]. Natural killer cells were reported in this study to constitute around 10% of the portal infiltrate. The variations in the findings of studies in this area probably reflect the focal nature of the disease, with small biopsies being of limited value in predicting the immunohistopathological changes in the whole organ. The stage of the disease is clearly also important as the cellular infiltrate may change as the disease progresses. Mast cells have also been demonstrated in relatively high numbers in the portal tracts of PSC patients and may play a role in fibrogenesis [68].

The initiation and maintenance of the immune cascade is determined not only by MHC recognition but also by the presence of accessory cells and molecules to provide co-stimulatory signals and the production of cytokines to amplify or modify the immune response. The role of Th1 and Th2 cytokines in the pathogenesis of primary sclerosing cholangitis is not yet clearly defined but there is evidence of their involvement in many aspects of the progression of the disease [69].

One of the most interesting clinical features of PSC is that it appears to run a course entirely independent of the associated bowel disease and can even present for the first time after a colectomy. This is in marked contrast to most extra-intestinal manifestations (EIMs) of IBD, which occur at the same time as a flare in the bowel disease. An immunological hypothesis to explain this phenomenon has been developed by Grant and co-workers, who demonstrated the aberrant expression of an adhesion molecule, mucosal addressin cell adhesion molecule (MAdCAM-1), on the endothelial cells of the portal vein and sinusoids [70–73]. This adhesion molecule is usually restricted to the gut. MAdCAM-1 allows adhesion of T-lymphocytes expressing an $\alpha_4\beta_7$ integrin. These T cells also carry chemokine receptor CCR9, which binds to the chemokine ligand CCL25, which is also aberrantly expressed on hepatic endothelium. The source of CCL25 might be activated biliary endothelial cells. Additionally, vascular adhesion protein-1 (VAP-1), which is constitutively expressed on both vascular and sinusoidal endothelium in the liver, has also been found to occur on the vascular endothelium in IBD. It is suggested, therefore, that T cells activated in the gut during attacks of IBD will differentiate into effector cells with the ability to bind to both gut and hepatic endothelium. Some will also persist as memory T cells. Any condition causing hepatic inflammation would then allow recruitment of these T cells to the liver due to the expression of VAP-1, MAdCAM-1 and CCL25 on the hepatic endothelium. Subsequent clonal expansion of memory T cells could lead to the development of inflammatory liver disease such as PSC. The pivotal role of memory T cells could explain why IBD and PSC do not necessarily occur together [72]. A potential flaw in this hypothesis is that the memory T lymphocytes appear to home to the small rather than the large bowel,

which does not appear to fit with the clinical observation that PSC is almost exclusively associated with colonic inflammation.

Clinical features

PSC is mainly a disease of young males, with a male:female ratio of 2:1. The majority of patients present between the ages of 25 and 40 years, although the disease has been diagnosed at any age between 1 and 90 years! The clinical presentation of PSC is variable but commonly includes fatigue, intermittent jaundice, weight loss, right upper quadrant abdominal pain and pruritus [10]. Despite the name of the disease, only a minority of patients suffer attacks of acute cholangitis and these are most common following reconstructive biliary surgery or some form of endoscopic interventional therapy.

Some patients with PSC may present with an established cirrhosis and portal hypertension without any previous symptoms of cholangitis or cholestasis. These patients may be diagnosed and treated as cryptogenic cirrhosis for many years before the diagnosis is established. Physical examination is abnormal in about half of symptomatic patients at presentation. Common abnormalities include hepatosplenomegaly and jaundice, although jaundice often appears only late in the course of the disease. The stigmata of liver disease, including spider naevi, palmar erythema and clubbing, are not usually found. An increasing number of asymptomatic patients with PSC are being diagnosed in whom physical examination is normal. The diagnosis is usually made incidentally when a persistently raised serum alkaline phosphatase is discovered in a patient with IBD [7].

Laboratory investigations

Serum biochemical tests usually indicate cholestasis. However, the levels of alkaline phosphatase and bilirubin may vary widely in an individual patient during the course of the disease, increasing, for example, during periods of acute cholangitis and falling after appropriate therapy. Sometimes the levels may fluctuate for no apparent reason. Modest elevations in serum transaminases are usually found, while hyperalbuminemia and clotting abnormalities are found only at a late stage [10,14].

Low titers of serum antinuclear and smooth-muscle antibodies have been found in patients with PSC but they have no diagnostic significance; serum mitochondrial antibody is invariably absent [10]. Atypical pANCA has the strongest association with PSC, being present in 33–88% of cases [74–76]. This antibody is almost exclusively found in patients with PSC, AIH or IBD. Increased serum IgM concentrations are seen in about half of symptomatic patients and the levels of IgM are similar to those observed in patients with primary biliary cirrhosis. Elevation of IgG is found in about one-third of adult patients tested and 60–80% of children with PSC. The finding of an elevated level of IgG4 in a patient with suspected PSC indicates a likely diagnosis of autoimmune pancreatitis. This is an important differential diagnosis as this condition responds well to corticosteroid treatment; however, as it is not known to be associated with IBD, it will not be discussed further in this chapter.

Radiographic features

The diagnosis of PSC is established by visualization of stricturing and dilatation in the intrahepatic or extrahepatic bile ducts. This has traditionally been performed using endoscopic retrograde cholangiopancreatography (ERCP) or, in some cases, by percutaneous transhepatic cholangiography. The cholangiographic appearances are diagnostic and consist of multiple areas of stricturing and dilatation (beading) of the intrahepatic and extrahepatic bile ducts [14] (Figure 36.1). ERCP, however, carries discomforts and risks, including pancreatitis, cholangitis, intestinal or bile duct perforation and bleeding. More recently, magnetic resonance cholangiopancreatography (MRCP) has proved to be as sensitive as ERCP in diagnosing PSC where the best equipment and operator are available [77,78]. This technique avoids the risks of ERCP and has the advantage of depicting ducts proximal to high-grade strictures. It also allows visualization of bile ducts in patients who have undergone biliary–enteric anastamoses and gastric bypass procedures. Although the limitation of MRCP is that it is purely diagnostic and does not allow for intervention, this technique is usually the best initial approach to the diagnosis of PSC.

Pathological features and staging of PSC

Although the diagnosis of PSC can be made on the basis of typical ERCP or MRCP findings alone, liver biopsy is a useful adjunct to determine the stage of the disease and look for evidence of biliary dysplasia [79]. It is also the only way of diagnosing small duct PSC and therefore should always be arranged for colitic patients with cholestatic LFTs of unknown cause and a normal cholangiogram (see the section on small duct PSC below).

PSC is characterized by damage to bile ducts, leading eventually to their atrophy and loss. Extrahepatic bile ducts appear macroscopically as thickened cords, although the overall diameter is not usually increased. The portal tracts appear sclerotic and inflamed, although the inflammation is often mild in contrast to primary biliary

Table 36.5 Staging criteria for chronic hepatitis associated with PSC.

Stage 1 (portal stage)	Portal hepatitis or bile duct abnormalities or both, with little or no periportal inflammation and fibrosis. The portal tracts are not noticeably enlarged Non-essential features: portal edema and fibrosis may be present; parenchymal changes tend to be mild or absent
Stage 2 (periportal stage or stage of portal enlargement)	Periportal fibrosis with or without periportal hepatitis or prominent enlargement of portal tracts with seemingly intact newly formed limiting plates. Both conditions may coexist. Biliary and fibrosing piecemeal necrosis may not be identifiable Non-essential features: portal edema and fibrosis, proliferation of ducts and ductules and evidence of fibrous, lymphoid or mixed cell cholangitis
Stage 3 (septal disease)	Septal fibrosis or bridging necrosis or both. Non-essential features: the same as in the previous stages. Presence of bridging necrosis is not common. Bile ducts often are severely damaged or absent. In the parenchyma, biliary and fibrosing piecemeal necrosis and associated changes, such as prominent copper deposition, may be found
Stage 4 (cirrhotic stage)	Biliary cirrhosis Non-essential features: they may be the same as in the previous stages, but parenchymal changes usually are more prominent than in stage 3. Bile ducts often have disappeared

cirrhosis (PBC). Typically the bile ducts are surrounded by a cuff of lightly inflamed fibrous tissue, the layers of which are separated by edema, producing an "onion skin" appearance. The ducts eventually atrophy within their fibrous cuffs, leaving a characteristic rounded scar. The histologic picture may be complicated by changes such as canicular cholestasis, which could be attributed to large duct obstruction [80]. The histologic features of PSC can be focal so a single biopsy specimen is not always reliable in diagnosing and staging the disease.

Ludwig *et al.* [81] devised a staging process based on the described histologic observations. The rationale for the staging process is that the large duct disease alone does not account for clinical course and outcome. The progression from isolated portal tract changes to cirrhosis usually determines the clinical course of PSC. Stage 1 disease is marked by bile duct injury and portal inflammation with minimal fibrosis, Stage 2 by expansion of portal tracts, periportal fibrosis and further inflammation, Stage 3 by fibrous septa, bridging fibrosis and progressive ductopenia and Stage 4 by cirrhosis (see Table 36.5).

Natural history

In the majority of patients, PSC is an insidious and progressive disease. The median time of survival from the time of diagnosis of PSC is approximately 12 years in symptomatic patients and spontaneous resolution does not occur. However, 75% of patients with asymptomatic disease are alive after 15 years of follow-up. The majority of patients die in hepatic failure following deepening cholestatic jaundice. However, approximately 10–20% of patients with longstanding PSC develop bile duct carcinoma, which often follows a very aggressive course [11,14]. Up to 21% of patients will have an incidental biliary carcinoma found at the time of transplantation [82]. The mean survival after the diagnosis of cholangiocarcinoma is 9 months.

A number of prognostic models have been developed as both research and clinical tools to attempt to estimate patient survival (see Table 36.6). The most recent, from the Mayo clinic, is the first validated model which does not include histologic stage, thereby avoiding the need for a liver biopsy on all patients [83]. It also avoids the use of variables which are to some extent subjective in nature, i.e. splenomegaly. The risk score is calculated as follows:

$$0.30\,\text{age (years)} + 0.54\ln[\text{bilirubin (mg dl}^{-1})] + 0.54\ln[\text{AST (U l}^{-1})] + 1.24[\text{history of variceal bleeding } (0 = \text{no},\ 1 = \text{yes})] - 0.84[\text{albumin (g dl}^{-1})]$$

(web-based calculator available at http://www.mayoclinic.org/gi-rst/mayomodel3.html). The advantage of this revised Mayo risk score over the Child–Pugh or the Mathematical Model for End Stage Liver Disease (MELD) is that it captures a change in survival probability before the onset of cirrhosis. Once decompensated cirrhosis is present, the MELD score more accurately predicts survival and is more appropriately used in listing for liver transplantation.

The relationship of primary sclerosing cholangitis with inflammatory bowel disease

Association with ulcerative colitis

It has been suggested that the colitis associated with PSC has a specific clinical phenotype and the term "PSC–IBD" has been adopted by some authors [84,85]. The clinical phenotypic characteristics of "PSC-UC" appear to be a total colitis which paradoxically runs a relatively mild clinical course, with a high prevalence of backwash ileitis and rectal sparing compared with patients with ulcerative colitis alone [84,86–89].

Mayo Clinic, 1989 [276]	Kings College, 1991 [277]	Multicenter, 1992 [278]	Stockholm, 1996 [279]	Revised Mayo Clinic, 2000 [83]
Age	Age	Age	Age	Age
Bilirubin	Hepatomegaly	Bilirubin	Bilirubin	Bilirubin
Histologic stage	Histologic stage	Histologic stage	Histologic stage	AST
Hemoglobin	Splenomegaly	Splenomegaly		Albumin
Inflammatory bowel disease	Alkaline phosphatase			Variceal bleeding

Table 36.6 Variables used in prognostic models of PSC.

Smoking

Cigarette smoking has been recognized as a protective factor against the development of ulcerative colitis. Three studies have suggested that cigarette smoking may also additionally protect against the development of PSC. Moreover, this protective effect was more marked in patients with PSC than ulcerative colitis and was seen in patients with and without IBD [90–92]. The mechanism of protection in both disorders remains unknown and small trials of nicotine treatment for PSC have not demonstrated any beneficial effect [93,94].

Onset

Although the symptoms of ulcerative colitis usually develop before those of sclerosing cholangitis, in some patients the onset of PSC may precede the symptoms of colitis by many years. Although large-scale studies have not been performed, there is some evidence that the prevalence of liver abnormality may be higher in children than adults with colitis. Abnormal serum liver tests were detected in 60% of 34 children with ulcerative colitis; abnormalities were most commonly seen in total colitis. Cholangiography was only performed in two patients, one of whom had sclerosing cholangitis [95].

Outcome

The outcome of the hepatobiliary disease is completely unrelated to the activity, severity or clinical course of the colitis. This is borne out by the fact that colectomy makes no difference to the clinical progression or to the mortality of patients with PSC. Indeed, liver disease may develop some years after a total colectomy has been performed [96]. Patients with combined ulcerative colitis and PSC may have a worse prognosis from liver disease than patients with PSC alone [87]. Involvement of the extrahepatic bile ducts alone is more frequently seen in patients who do not have IBD [87].

Biliary and colorectal cancer in ulcerative colitis and primary sclerosing cholangitis

The association between ulcerative colitis and colorectal carcinoma has been recognized since the 1920s [97]. In 1995, Broomé *et al.* suggested that there was an increased risk of colorectal neoplasia in patients with concomitant primary sclerosing cholangitis [98] (see Figure 36.2). This hypothesis has been extensively tested since with conflicting datasets published in recent years (see Table 36.7). Some of this work has been difficult to interpret due to the tendency of the ulcerative colitis associated with IBD to be extensive, which is an independent risk factor for colorectal neoplasia and mild, which might lead to an underestimation of the duration of the disease prior to diagnosis. Nevertheless there is a now general consensus that patients with both colitis and PSC are at increased risk of colonic dyplasia and should undergo annual screening colonoscopy with multiple biopsies.

The possible mechanisms for the increased susceptibility to neoplasia are not clear, but genetic predisposition, alterations in the bile salt pool due to cholestasis and folate deficiency are all possibilities [99]. Recent work has suggested that UDCA may have a role in preventing colonic neoplasia and this would have major significance for the use of this drug in the context of PSC [100,101].

Three studies have all shown that PSC patients consistently develop a more proximal colorectal carcinoma than patients with ulcerative colitis alone [102–104]. This observation may be explained by the higher exposure of the right side of the colon to the carcinogenic properties of the

Table 36.7 Studies considering the effect of PSC on the risk of colorectal carcinoma in patients with ulcerative colitis.

Author (year)	No. of patients	Is PSC a risk factor?
Broomé (1992)	5	Yes
Choi (1992)	5	No
D'Haens (1993)	10	Yes
Broomé (1995)	40	Yes
Gurbuz (1995)	35	No
Brentnall (1996)	20	Yes
Loftus (1996)	178	No
Bansal (1996)	Not stated	Yes
Shetty (1997)	132	Yes
Leidenius (1997)	45	Yes
Kornfeld (1997)	125	Yes
Marchesa (1997)	27	Yes
Nouako (1998)	56	No
Harewood (1999)	110	Yes
Lindberg (2001)	19	Yes

secondary bile acids produced in cholestasis, although this hypothesis remains unproven.

The increased risk of carcinoma of the bile ducts including gall bladder carcinoma in patients with ulcerative colitis is also well established and appears now to occur almost exclusively in the context of pre-existing PSC. Furthermore, one study has suggested that cholangiocarcinoma develops significantly more often in patients with colonic dysplasia or carcinoma, thus suggesting that these patients may constitute a high-risk subgroup requiring increased colonic and biliary surveillance [98]. However, further prospective studies are needed to confirm these findings.

Pouchitis

Patients with ulcerative colitis treated by colectomy with an ileal reservoir (pouch) are sometimes affected by a non-specific inflammation of the pouch (pouchitis). This complication is much more common in patients with PSC and ulcerative colitis (64% of patients affected) than in those with ulcerative colitis alone (32% of patients affected) and is the major complication of this operation [105]. Prior to the development of pouch operations, the Brooke ileostomy was the most commonly performed operation for ulcerative colitis. In patients with coexisting PSC, however, peristomal varices occur in approximately 25% of cases and bleeding from these varices can be difficult to manage [106]. Although the intra- and postoperative complication rates and mortalities are comparable in the two operations, pouchitis tends to present a less difficult management problem than recurrent peristomal variceal bleeds and therefore ileal pouch–anal anastamosis is usually the operation of choice in these patients.

Treatment

There is no curative treatment for PSC, but a plethora of medical, endoscopic and surgical approaches have been advocated. Drug therapy can be divided into those which attempt to influence the course of the disease (the "disease-modifying drugs") and those which can be used to alleviate symptoms. Disease-modifying drugs can be used alongside endoscopic therapies such as biliary duct dilatation and indeed a combination of drug therapy with additional endoscopic treatment as required is the currently recommended management.

Disease-modifying treatment

Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) is a hydrophilic dihydroxy bile acid which has been used for centuries in traditional Chinese medicine for treating a variety of disorders, including liver disease. UDCA is an established treatment for the treatment of primary biliary cirrhosis (PBC) and is also used for intrahepatic cholestasis of pregnancy, cystic fibrosis liver disease, progressive familial intrahepatic cholestasis, chronic graft-versus-host disease and drug and parenteral nutrition-induced cholestasis [107–113].

UDCA has a wide range of potentially beneficial effects in the context of PSC and the relative importance of each in alleviating cholestasis remains unclear [114]. UDCA appears to have a protective effect on the biliary epithelium, possibly by buffering toxic bile acids through modulation of micelle formation and by changing biliary bile acid composition. In addition, UDCA has an anticholestatic effect, stimulating biliary secretion of phospholipids and bile acids by upregulating the synthesis, insertion and activation of transporter molecules in the hepatocyte canalicular membrane mainly via post-translational mechanisms, as shown in experimental models of cholestasis [115]. UDCA also exerts antiapoptotic effects, probably through the mechanism of altering mitochondrial membrane permeability to ions and mitochondrial cytochrome *c* release [116–118]. A number of other less well characterized potential modes of action of UDCA include the reversal of aberrant expression of HLA Class I molecules on hepatocytes in PSC, activation of the glucocorticoid receptor and the suppression of IFN-γ-induced MHC Class II expression.

Unconjugated UDCA is absorbed in the small intestine by passive diffusion and then conjugated with taurine or glycine and excreted into bile [119]. It appears to induce cytochrome P4503A (CYP3A4), which increases the metabolism of substrates of this cytochrome such as cyclosporin, dapsone and nitrendipine [120]. UDCA represents around 3% of the bile acid pool in humans under normal physiological conditions, increasing to around 50% if the standard treatment dose for PBC of 13–15 mg kg^{-1} per day of UDCA is administered [121,122]. Studies on the biliary bile acid composition of PSC patients taking different doses of UDCA have demonstrated that biliary enrichment of UDCA increases with increasing dose and reaches a plateau at 22–25 mg kg^{-1} [123]. If biliary enrichment of UDCA is an important factor for its clinical effect, it may be that these higher doses are more effective. Although it is conventional to prescribe UDCA as a twice daily dose, daily or twice daily dosing has the same effect on biliary enrichment and liver biochemistry [124].

A number of trials have investigated the effects of UDCA treatment in PSC since the first open-label studies by Hayashi *et al.* [125] and Chazouillères *et al.* [126] in 1990. The first double-blind placebo-controlled trial of UDCA by Beuers *et al.* in 1992 demonstrated a significant improvement in serum liver tests and a multiparametric histologic score [127], and similar improvements in serum liver tests were subsequently reported in larger studies by Stiehl *et al.* in 1994 [128] and Lindor in 1997 [129]. Higher doses of UDCA have been trialed to test the hypothesis that these would be needed to provide sufficient

enrichment of the bile acid pool in the context of cholestasis and the Oxford group appeared to demonstrate improvement on both the cholangiographic appearance of PSC and the histologic grade of fibrosis with a dose of 20–25 mg kg^{-1} per day [130,131]. The most recent large UDCA trial on a group of 110 PSC patients using a dose of 17–23 mg kg^{-1} per day for 5 years demonstrated a trend towards increased survival in the UDCA-treated group when compared with placebo, but despite the relatively large number of patients recruited it was still insufficiently powered to produce a statistically significant result [132]. The highest dose trial published to date demonstrated an improvement in Mayo risk score with a 30 mg kg^{-1} target dose with no significant increase in side effects from using a higher dose and, in particular, no worsening of underlying colitis [133]. However, a multicenter study using a dose of 25–30 mg of UDCA in 150 patients over 5 years, based at the Mayo Clinic, has not demonstrated a beneficial effect of UDCA on the progression and outcome of severe PSC and indeed in this study was associated with a higher rate of serious adverse events (Lindor KD, personal communication).[1]

Whilst this work has been ongoing, however, some interesting studies have suggested that UDCA may have another important effect in PSC–IBD patients. Three clinical studies have been undertaken to assess whether UDCA use changes the risk of colorectal cancer in this context. The first, on 59 patients undergoing 3 yearly colonoscopic surveillance, found a significantly reduced risk of colonic dysplasia in the patients taking UDCA [101]. The control population in this study, however, appeared to have an exceptionally high rate of dysplasia compared with other datasets. Two further studies have addressed this issue. The largest, involving 92 PSC patients, demonstrated a reduction in overall mortality of the PSC patients on UDCA but no significant reduction in the risk of colonic dysplasia and neoplasia [134]. Pardi *et al.*, however, have since published promising work on 52 patients with PSC and ulcerative colitis showing a significant reduction in the risk of developing dysplasia [OR 0.14, 95% confidence interval (CI) 0.03–0.64] [100]. The mechanism for this effect may be the inhibitory effects of UDCA on deoxycholic acid (DCA), a secondary bile acid which is present in higher levels in serum and stool in ulcerative colitis patients with colorectal dysplasia or cancer and in non-ulcerative colitis patients with colorectal adenomas and cancers than in control patients [135–139]. UDCA may have an effect on colonic dysplasia by the inhibition of DCA-induced apoptosis and preventing DCA-stimulated growth of colon cancer cell lines [140,141].

[1] Lindor KD, Kowdley KV, Luketic VA *et al.* High dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009;**50**(3):671–3.

Corticosteroid therapy

The role of corticosteroid therapy in PSC remains unclear. Patients with PSC are regularly exposed to courses of corticosteroids when they are prescribed for their coexisting ulcerative colitis and these courses have not been observed to have any impact on their liver disease. Systemic and topical corticosteroid therapy has been evaluated in a number of small and often uncontrolled trials. Two studies of 10 patients with PSC treated with corticosteroids in 1981 and 1984 came to different conclusions regarding efficacy and a controlled but non-randomized trial of 12 patients treated with a combination of low-dose prednisolone and colchicine failed to find any benefit in terms of slowing of disease progression or improved survival [142–144]. There has been a suggestion that there is a small subgroup of patients with overlap syndromes between PSC and AIH who might benefit from steroid treatment, but this has not yet been clearly established [145,146]. Tjandra *et al.* have demonstrated a reduction in glucocorticoid receptors on hepatic T lymphocytes in a rat model of cholangitis and it is possible that a similar mechanism might explain the ineffectiveness of steroids in human PSC [147].

Corticosteroids carry a risk of osteoporosis, particularly when used long term and in the context of cholestasis. Trials have therefore been undertaken on budesonide, a second-generation corticosteroid, which has a high first-pass metabolism and minimal systemic availability. This preparation, however, still appears to cause bone loss [148]. A trial examining the effects of budesonide in combination with ursodeoxycholic acid found no additional beneficial effect [149].

Topical corticosteroids administered through a nasobiliary drain following ERCP have been reported to be beneficial in three small studies, but a controlled trial from the Royal Free Hospital found no benefit with a high incidence of bacterial colonization of the bile, leading to episodes of bacterial cholangitis [150–153].

It is important to be aware of the benign condition of the pancreas; autoimmune pancreatitis (AIP). This disease can cause diagnostic confusion as it causes sclerosing lesions in the bile ducts which look similar to those of PSC. AIP does not, however, appear to be related to IBD and is, in contrast to PSC, very effectively treated with corticosteroids.

Other immunosuppressants

A number of other immunosuppressants have been considered in small trials for the treatment of PSC. Methotrexate, although appearing to improve serum liver tests in PSC patients, does not seem to alter the progress of the disease or confer any additional benefit to UDCA alone [154,155]. An interesting randomized trial of cyclosporin in 34 PSC patients found no deterioration in liver histology over a 2 year follow-up period [156]. The cyclosporin also had useful effects on the course of associated ulcerative

colitis. Tacrolimus (FK506) again appears to produce biochemical improvement but is poorly tolerated in the PSC population [157]. Myophenolate mofetil alone does not appear to have clinically beneficial effects and when combined with low-dose UDCA for 2 years did not appear to offer additional benefits compared with standard doses of UDCA alone [158,159]. Case reports of azathioprine in PSC have been published but no control trials of its use as a single agent have been undertaken to date [160,161]. An interesting trial of a combination treatment consisting of low-dose UDCA (500–750 mg daily), prednisolone 1 mg kg^{-1} per day and azathioprine 1–1.5 mg kg^{-1} per day for a median of 41 months showed that the treatment was well tolerated and all patients had biochemical improvements, including seven patients who had had no biochemical response to UDCA alone until the immunosuppressants were added. In addition, of 10 patients who had a follow-up liver biopsy, six had improved and only one had deteriorated [162].

There have to date been only two small pilot studies of anti-TNF agents in PSC and neither of these has shown a beneficial effect on any measure of disease progression, although it was thought that etanercept might be useful in the treatment of pruritus [163,164].

Antibiotics

In view of the possible role of gut bacteria in the pathogenesis of PSC, antibiotics have been suggested as possible therapeutic agents. Metronidazole in combination with UDCA appears to deliver further improvement in serum liver tests and Mayo risk score than UDCA alone, but has no particular beneficial effect on ERCP appearance or liver histology [165]. A second antibiotic, minocycline, is currently undergoing evaluation, but in this case it is not the antibacterial properties so much as its effects on the production of iNOS (a mediator of inflammation) which are hypothesized as being important.

Antifibrotic therapy

Pirfenidine and colchicine have both been trialed on a small scale in PSC to determine if there is any evidence of a useful antifibrotic effect. Neither has shown clinical efficacy and pirfenidone was associated with significant side effects. Silymarin (milk thistle), a herbal preparation taken by a significant proportion of patients with chronic liver disease, appears to have both antifibrotic and TNF inhibitory properties [166]. A recent trial of silymarin at a dose of 140 mg three times per day appeared to show biochemical improvement in 30 PSC patients, although the Mayo risk score remained essentially unchanged [167]. This agent appears to deserve further evaluation.

Endoscopic treatment for PSC

Endoscopic intervention has been used in PSC both to treat patients with clinical deterioration due to biliary strictures and to attempt to delay the progression of the disease. Tight strictures, especially in the extrahepatic biliary tree, can cause acute deterioration of liver function and more rapid progression to biliary cirrhosis. Such lesions are known as "dominant biliary strictures", occur in 15–20% of PSC patients and can be very difficult to differentiate from cholangiocarcinoma at ERCP. Dominant strictures can be treated endoscopically by balloon dilatation or by placing a stent across the affected area. Alternatively, some non-cirrhotic PSC patients may be best managed by a bilioenteric bypass [168].

The interpretation of results of endoscopic therapy trials for PSC is difficult due to the small numbers of cases tested and the variety of endoscopic techniques used. A retrospective trial of 25 patients presenting with worsening serum liver tests and major bile duct strictures treated with endoscopically inserted biliary stents found that 12 patients (57%) remaining asymptomatic with stable liver function tests during a mean follow-up of 29 months [169]. Short-term stenting has been suggested in one study to have long-term benefits. Thirty-two patients with dominant biliary strictures were treated with endoscopically placed stents for a mean of 11 days. Improvements in symptoms and cholestasis were seen in all patients and these improvements were maintained for several years [170]. Stiehl *et al.* prospectively treated 106 patients with 750 mg UDCA per day for up to 13 years with balloon dilatation of major dominant strictures or placement of biliary stents whenever necessary [171,172]. This combined approach appeared to improve overall survival rates significantly compared with predicted values, but as the study was uncontrolled it was not possible to ascertain whether the UDCA, the endoscopic therapy or the combination of the two was the important factor.

Cholangiocarcinoma

The development of cholangiocarcinoma is a major risk for patients with PSC. It develops in 6–20% of patients with longstanding PSC at a rate of 1–5% per year [173]. Prediction of hepatobiliary malignancy is extremely difficult. In a recent retrospective study of all Nordic PSC patients listed for liver transplantation over a 12 year period, the only independent risk factors for subsequent diagnosis with hepatobiliary malignancy were clinical suspicion of cancer, recent diagnosis of PSC, no previous UDCA treatment and previous colon cancer [174]. High alcohol consumption and smoking have also been implicated as risk factors for cholangiocarcinoma in the context of PSC [175,176].

Early disease is asymptomatic, but patients may present with symptoms of biliary obstruction, including jaundice, pale stools, dark urine and new onset or worsening pruritus. Pain, malaise and weight loss may indicate more advanced disease. Most patients will be investigated initially with ultrasound scanning. The diagnosis should

be suspected when the intrahepatic, but not extrahepatic, ducts are dilated. Color Doppler imaging can detect tumor-induced compression or thrombosis of the portal vein or hepatic artery [177]. Most patients will then go on to cross-sectional imaging. MRI is currently the recommended technique for diagnosis and staging of cholangiocarcinoma as it provides information on the local extent of the tumor, extent of duct involvement, presence of liver metastases and hilar vascular involvement [177].

ERCP usually reveals a particularly narrow bile duct stricture. Brush cytology obtained endoscopically may be useful in confirming malignancy, but suffers from low sensitivity. Neoplasia is particularly difficult to differentiate from the cellular atypia seen in chronic bile duct inflammation in PSC. This technique can, however, be very valuable in diagnosing malignancy and high-grade dysplasia, sometimes at an early enough stage to treat with liver transplantation [79,178,179]. Cytological assessment by endoscopic brushing of dominant strictures to detect early neoplastic transformation is recommended by most centers, but results are dependent on expert cytological examination. Endoscopic ultrasonography can also be used both to visualize biliary strictures and to provide a fine-needle aspirate to diagnose malignancy. Intraductal ultrasonography (IDUS) may allow even better images of the proximal bile ducts and surrounding structures but, despite some interesting preliminary studies, its role in diagnosis and staging of cholangiocarcinoma is not yet entirely clear [177,180–182]. Positron emission tomography (PET) scanning has been used with some success in the early diagnosis of small cholangiocarcinomas and may become a useful tool in differentiating benign and malignant strictures [88]. Open or percutaneous biopsy of potentially resectable cholangiocarcinoma is not generally recommended due to the risk of seeding of the tumor [177].

The level of accuracy achieved in diagnosing malignancy in cytological specimens may be improved by the use of molecular methods. Inactivation or overexpression of a number of genes, e.g. *p53*, *APC*, *Smad*-4, *bcl-2* and *p16* genes, have been observed in PSC [183,184]. Mutated oncogenes have also been demonstrated in a variable proportion of cholangiocarcinomas [185–187]. Telomerase activity is present in 85–90% of all human cancers and *in situ* hybridization has been used to detect telomerase RNA in endoscopic brushings as a marker for malignancy [188]. Although molecular profiling, perhaps using a combination of markers, offers great promise in the future, currently there is no established clinical role for these techniques.

There are no tumor markers which are specific for cholangiocarcinoma and no evidence that measurement of tumor marker levels is useful in monitoring the progression of this cancer. In the context of PSC however, a serum CA19-9 level of greater than 100 U ml^{-1} has been reported to have a sensitivity of 75% and a specificity of 80% for the presence of cholangiocarcinoma [189,190]. The combination of CA19-9 and CEA has been shown to be useful in identifying PSC patients with occult tumors and, more recently, results of brush cytology, DNA analysis, serum CA 19-9 and serum CEA have been combined in an attempt to improve further on this diagnostic sensitivity [189,191].

The tumor usually pursues a progressive course and the prognosis is very poor, with a median survival of 9 months [192]. Endoscopic stenting is usually the best palliative option once the diagnosis of established cholangiocarcinoma has been made. In patients who might be suitable for orthotopic liver transplantation (OLT), biliary manipulation is best avoided as it increases the risk of stricturing and bacterial cholangitis and may jeopardize the chance of a successful OLT.

Orthotopic liver transplantation

OLT is the only option available in young patients with PSC and advanced liver disease. Decisions regarding the optimum timing for transplantation are made difficult, however, by the variable clinical course of PSC and the potential risk of cholangiocarcinoma. Indications for transplantation are summarized in Table 36.8.

Survival rates for patients transplanted for PSC without evidence of cholangiocarcinoma are excellent, with most series publishing 5 year survival rates of more than 75% [82]. Data from the Mayo Clinic have demonstrated that post-transplant survival rates are clearly related to the pre-transplant Child–Pugh stage. The 1, 2 and 5 year survival rates in Child-Pugh A were 98.1, 97.0 and 91.0%, in Child-Pugh B 89.1, 81.0 and 55% and in Child-Pugh C 73.0, 53.0 and 16.0%, respectively [103]. For this reason, and the unpredictable risk of the development of hepatobiliary malignancy, it has been suggested that patients with PSC should be referred for transplantation earlier than other patients with chronic liver disease [193]. Work from the Nordic PSC population found a 5 year survival of 35% in the small group of PSC patients found during or after transplantation to have incidental cholangiocarcinoma ($n = 31$) [174]. However, results of transplantation for patients with established cholangiocarcinoma prior to

Table 36.8 Indications for liver transplantation in PSC.

Accepted indications for transplantation
Cirrhosis complicated by:
• Intractable ascites
• Recurrent cholangitis
• Variceal hemorrhage not controlled by banding or sclerotherapy
• Muscle wasting
• Recurrent bacterial peritonitis
• Encephalopathy
Considerations for transplantation
• Non-cirrhotic patients with intractable itching or fatigue
• Biliary dysplasia

the procedure remain poor, with even the most encouraging results to date still showing survival rates of only 60, 32 and 25% at 1, 3 and 5 years, respectively [194]. Most centers currently consider a definite finding of cholangiocarcinoma to be a contraindication to liver transplantation; however, the discovery of cholangiocarcinoma without clear evidence of extrahepatic spread during transplant surgery should not lead to cancellation of the procedure [174]. Two studies have suggested improved survival in patients transplanted for cholangiocarcinoma who were given adjuvant chemoirradiation [195,196].

PSC recurs in the liver graft in around 20% of cases and the most recent evidence suggests that the presence of active ulcerative colitis in the post-transplant period and the need for maintenance steroids are an independent risk factor for the development of PSC recurrence [197]. Post-transplantation PSC appears have a survival rate similar to that in patients without evidence of recurrence [198]. Surprisingly, there is some evidence that the clinical course of IBD tends to worsen post-transplant despite the immunosuppression given to protect the graft from rejection. A series published by the Royal Free Hospital found an increase in the clinical activity of the associated colitis in 8 of 16 patients transplanted for PSC [199]. Similar data have been published from Birmingham with 9 of 26 patients (35%) noticing a worsening of their symptoms of IBD [200]. In contrast, Gavaler *et al.* [201] and Shaked *et al.* [202] found no worsening of IBD symptoms post-transplant in 23 and 24 patients, respectively . These conflicting data may be explained in part by the differences in immunosuppressive regimens used post-transplantation. The Royal Free and Birmingham centers usually withdraw steroids early and maintain immunosuppression with cyclosporin or tacrolimus with or without azathioprine. The Gavaler and Shaked reports are from units where steroids are used as maintenance immunosuppression.

The risk of colon cancer in PSC increases after transplantation occurring most commonly in the early post-transplant period and is probably associated with high-level immunosuppression accelerating the growth of malignant cells. Colonoscopy with extensive mucosal biopsies is therefore recommended prior to transplantation and annually thereafter in this particularly high-risk group [202].

Symptomatic treatment for PSC

The most common symptoms of PSC are fatigue and pruritus, and neither of these appears to be improved by the use of UDCA. Fatigue is not related to the severity or activity of the liver disease and its pathophysiology remains unknown. Although depression has been thought to have played a role in this symptom, studies have suggested that prevalence of a depressive disorder in PSC patients is not higher than in the general population and a trial of fluvoxamine, a selective serotonin re-uptake inhibitor, appeared to have no beneficial effect on fatigue or quality of life indexes [203,204]. Interestingly, the most recently published data on PSC and fatigue found that fatigue scores were actually significantly lower in a group of 93 PSC patients than the general population, suggesting that this might be an over-emphasized problem for these patients [205].

Pruritus is a frequently occurring and sometimes an extremely distressing symptom of PSC. Although again its precise pathogenesis remains elusive, it may result in part from the accumulation of pruritogenic substances, particularly bile acids, as a consequence of impaired secretion of bile. Although the evidence for this is conflicting, the bile acid-binding resin cholestyramine is certainly very useful for the treatment of pruritus secondary to cholestasis and indeed, due to its favorable side effect profile, it is used as the first-line treatment for most cases [206]. Opioid antagonists such as naltrexone have been shown to be effective in the treatment of cholestasis-associated pruritus [207–210]. They function by preventing binding of endogenous opioid agonists, which are elevated in cholestasis, and this may be effective because of increased opiodergic tone in PSC patients [211]. Ondansetron, a 5-hydroxytryptamine-3 serotonin receptor subtype antagonist has been studied in two small placebo-controlled trials in view of the possible influence of the serotonin neurotransmitter system on pruritus associated with cholestasis [212,213]. Both of these trials showed significant improvements in visual analogue scales recording itch, but the relevance of this improvement in clinical practice remains to be assessed. Rifampicin is also sometimes used an antipruritogenic agent and its action appears to be through the induction of enzymes of the microsomal drug-oxidizing system, promoting the metabolism of endogenous pruritogenic compounds [214,215]. It has also been suggested, however, that it has a direct antimicrobial action on the intestinal lumen, causing a change in the synthesis of secondary bile acids [215]. The use of rifampicin is, to some extent, limited by severe idiosyncratic hypersensitivity reactions and interaction with concomitant medications due to its properties as an enzyme inducer.

Metabolic bone disease (usually osteoporosis) is a frequent complication of advanced PSC [216,217]. General lifestyle measures, such as limiting alcohol intake, regular weight-bearing exercise, cessation of smoking and dietary advice to avoid a low body mass intake, should be discussed with all patients and supplementation with calcium and vitamin D is usually recommended. In addition, if T-scores at hip or spine are <2.5, hormone replacement therapy (HRT) should be considered in postmenopausal women and transdermal testosterone in hypogonadal men. Bisphosphonates, including aledronate and didronel PMO, should also be considered and can be used in addition to HRT or as an alternative if there is no evidence of hypogonadism. There is no true evidence base for any of these treatments in PSC and their recommendation is an extrapolation from experience with PBC.

Small duct primary sclerosing cholangitis (SD-PSC)

Some patients have cholestatic liver function tests and typical histologic features of PSC but a normal cholangiogram. These patients with a clinical diagnosis of PSC but without the typical intra- and/or extra-hepatic cholangiographic changes have been classified as having SD-PSC [218–220]. SD-PSC accounts for less than 10% of all PSC cases and these patients appear to have a better long-term prognosis with less than half the numbers of deaths or liver transplants than the "classic" PSC group [221,222]. There has been a suggestion that a higher proportion of SD-PSC patients have Crohn's disease than in the classic PSC group [222]. Less than 25% of SD-PSC progresses to large duct disease over a 10 year period [223]. The presence of concomitant IBD did not appear to affect the risk of liver death or transplant in SD-PSC patients in a study from the Mayo clinic [224]. Cholangiocarcinoma does not appear to occur in SD-PSC patients unless large duct disease has supervened [223,225].

Autoimmune hepatitis

Patients with IBD are at increased risk of developing other immune-mediated liver disease including AIH [226]. However, interface hepatitis (piecemeal necrosis) on liver histology can accompany the classic bile-duct changes of PSC on cholangiography and so distinguishing between PSC and AIH on liver biopsy can be difficult [14]. AIH and PSC may also occur within the same individual and it remains unclear if this represents the concurrent presence of both diseases, an overlap syndrome or stages in the development of a single disease entity [227–230]. High serum IgG levels, lower ALP, interface hepatitis on biopsy and the presence of autoantibodies (ANA or SMA titer >1:40) should alert the clinician to the possibility of associated AIH and therefore response to steroid treatment [228]. Patients with AIH should be suspected of having associated PSC or AIH-PSC overlap if they have pruritis, ulcerative colitis, bile duct abnormalities on histology, cholestatic liver biochemistry (ALP × 2 ULN) and abnormal cholangiography. These patients tend to be unresponsive to steroids [231,232].

It is unclear from current evidence whether the prevalence of AIH without underlying sclerosing cholangitis is increased in patients with IBD. Ulcerative colitis has been described, however, as being present in 16% of patients with AIH in one study [233].

Cirrhosis

The incidence of cirrhosis associated with IBD has varied in different series between 1 and 5% [234–236]. Most patients in these reports are classified as having biliary cirrhosis and, since patients with sclerosing cholangitis can present with portal hypertension and established cirrhosis with no preceding symptoms, it seems likely that the majority of these patients will have underlying endstage PSC. However, not all patients with cirrhosis and IBD will have PSC and it is possible that some cases may be due, for example, to chronic hepatitis C infection associated with previous blood transfusions. Patients with cirrhosis may present with the typical symptoms of liver failure, including jaundice, ascites and variceal hemorrhage. Although the variceal bleeding usually occurs from veins in the esophagus, patients with concomitant IBD who have undergone total proctocolectomy may bleed from peristomal varices some 6–13 years after formation of the ileostomy [21].

Hepatocellular carcinoma

Two case reports have described the development of fibrolamellar hepatocellular cancer in male patients with ulcerative colitis and PSC. Neither patient was cirrhotic [237,238]. In the single patient who received a transplant, the tumor recurred in the donor liver [238]. In common with most causes of chronic liver disease, patients with PSC and established cirrhosis have an increased risk of developing primary liver cell cancer [237].

Fatty change

Fatty liver or steatosis is the most common type of hepatobiliary lesion found in patients who have IBD (see Figure 36.3). Ultrasound studies have shown the presence of mild-to-moderate to severe liver steatosis in 39.5% of

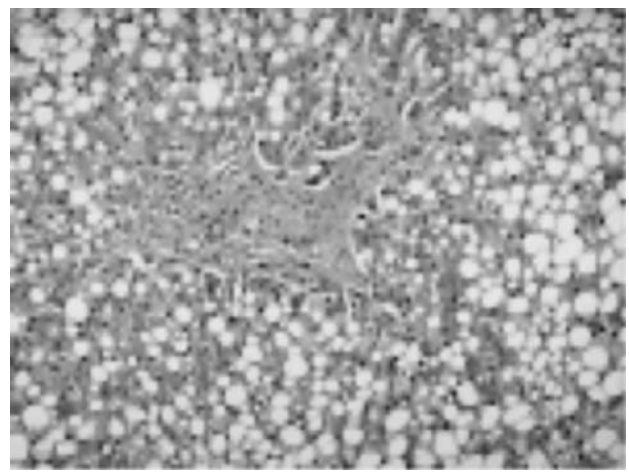

Figure 36.3 Fatty liver. Section shows macrovesicular steatosis

Crohn's disease patients and in 35.5% of ulcerative colitis patients [239]. Fatty change has been recorded as occurring in 45% of patients with ulcerative colitis who undergo colectomy and in 40% of patients with Crohn's disease who undergo similar surgery [240,241]. The presence of fatty liver is related to the general state of health of these patients and the severity of the underlying colitis rather than any other specific factor. The incidence of fatty change in patients with ulcerative colitis at autopsy is similar to that of other debilitated patients. The pathogenesis of fatty liver in IBD is unknown. It is probably multifactorial, secondary to causes such as poor nutrition, drugs, bacterial and chemical toxins and unsuspected alcohol abuse. The steatosis is usually of the macrovesicular type and all types of distribution, including diffuse, periportal and centrilobular, have been described in patients with IBD.

There are no symptoms associated with fatty liver, although on abdominal examination hepatomegaly may be detected. Treatment of the underlying bowel disorder and improvement in the general health of the patient will normally result in a resolution of the fatty change. In view of improvements in the management of IBD, the incidence of fatty change may be falling.

Gallstones

Patients with Crohn's disease of the small bowel have an increased incidence of gallstones. The reported incidence in patients with Crohn's ileitis, ileal resection or intestinal bypass ranges from 11 to 34% and this risk is related to the age of the patient, female sex and previous surgery [239,242–245]. In contrast, the incidence of gallstones in patients with ulcerative colitis and those with Crohn's disease confined to the colon is around 7.5%, which is not statistically different to the general population [239]. Total colectomy with ileoanal anastamosis does not appear to predispose to the formation of cholesterol gallstones [246]. The increased rate of formation of gallstones in patients with inflammation or absence of the terminal ileum is due to a reduction in bile salt absorption, leading to depletion of the bile salt pool. As a result, the concentration of biliary bile salts falls and there is a relative increase in the concentration of biliary cholesterol. Thus, bile may become supersaturated with cholesterol, which in turn increases cholesterol precipitation in the gallbladder and predisposes to the formation of cholesterol gallstones (see Figure 36.4). There may be additional factors predisposing to gallstones in these patients. One study has demonstrated impaired gall bladder contractability, most marked in patients who have undergone bowel resection or have both large and small bowel disease [247].

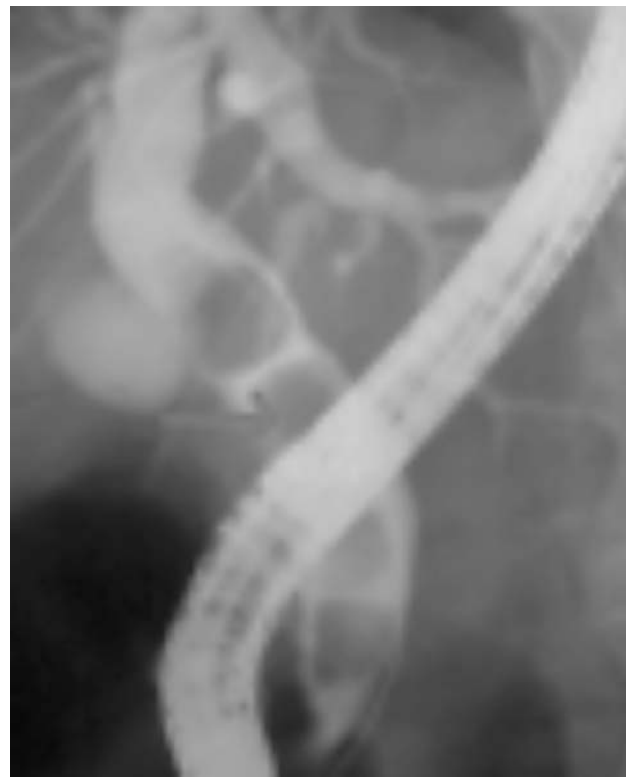

Figure 36.4 ERCP showing gallstones in the common bile duct.

Amyloidosis

Hepatic amyloidosis is a rare complication, occurring in less than 1% of patients with IBD; it is much more commonly associated with Crohn's disease than ulcerative colitis. The development of amyloid can occur in association with Crohn's disease involving either the small or large bowel.

The amyloid deposition in the liver is found in the media of portal blood vessels and in the sinusoidal wall and eventually leads to atrophy and disappearance of hepatocytes. It is most likely to occur in Crohn's patients with chronically active disease. In addition to IBD, most patients who develop amyloidosis have either extraintestinal foci of suppuration or arthropathy. Aggressive anti-inflammatory treatment of the intestinal lesions probably reduces the chance of developing systemic amyloid. More effective recent treatment probably accounts for the reduced prevalence of amyloid. Although regression of amyloidosis has been reported after colectomy in the majority of patients, the overall prognosis is poor [248,249].

Granulomas

Granulomas are occasionally seen in the liver biopsy specimens of patients with Crohn's disease, some of whom may

show a moderate elevation of serum alkaline phosphatase. The granulomas can be present in portal tracts and also in the parenchyma. The presence of hepatic granulomas in patients with Crohn's disease often reflects granulomas in the bowel [250,251]. There has been a single case report of regression of granulomatous lesions in the lungs and liver of a Crohn's disease patient with infliximab therapy [252]. There have been a few isolated reports of hepatic granulomas occurring in association with ulcerative colitis, but the relationship remains unproven. Granulomas are found in 3–4% of liver biopsies from patients with PSC [253].

Liver abscess

Intra-abdominal abscess is a frequent complication of Crohn's disease. However, the development of hepatic abscess in association with IBD is very uncommon, with fewer than 70 cases reported in the literature to date. The abscesses are often multiple and are associated with a high mortality [254]. Most patients who develop liver abscesses have had long-term IBD; however, cases of Crohn's disease presenting with pyogenic liver abscesses are reported increasingly [255]. Streptococci, especially *Streptococcus milleri*, are the most frequent organisms isolated from the abscesses [256].

Primary biliary cirrhosis

While it is clear that cholestatic liver disease in the context of ulcerative colitis is almost invariably caused by PSC or PBC–PSC crossover syndrome, the prevalence of primary biliary cirrhosis in ulcerative colitis does appears to be significantly higher than in the general population and there may be a true immunological link between the two diseases [257–262].

Budd–Chiari syndrome

Hepatic and portal vein thrombosis occurs in an environment of hypercoagulability, thrombocytosis and abdominal sepsis, and these factors can be present in patients with active IBD. Although only a few cases of Budd–Chiari syndrome have been reported in association with ulcerative colitis (and only one with Crohn's disease), the diagnosis should be considered in patients with unexplained sepsis and abnormal liver function tests, as this condition is treatable and can have long-term complications if left undetected [263–265].

Drug-induced hepatitis

Chronic hepatitis, in some cases progressing to liver fibrosis, has been a documented adverse effect of sulfasalazine since the 1970s and this was originally blamed on the sulfonamide as opposed to the 5-aminosalicylate moiety [266]. Similar effects have since been seen with mesalazine. A Committee on Safety of Medicines report in 2002 found a low overall risk of drug-induced hepatitis in patients with IBD caused by mesalazine and sulfasalazine, of the order of 4–5 cases per million prescriptions of these drugs [267]. Azathioprine metabolites, 6-methylmercaptopurine ribonucleotides, are associated with hepatotoxicity due to hypersensitivity, idiosyncratic cholestatic reaction and endothelial cell injury. A review of azathioprine treatment found that only 17 of 517 patients developed abnormal liver function tests after use of the drug and that in all cases this settled after discontinuation of the drug, with no adverse sequelae [268,269].

Another immunosuppressive drug increasingly being used for refractory IBD is methotrexate. Use of this drug in psoriasis has been limited by hepatotoxicity, with fibrosis and cirrhosis detected on liver biopsies in up to 25% of methotrexate-treated patients [270]. A study from the University of Chicago, however, has suggested that cumulative methotrexate doses of up to 5410 mg administered for up to 281 weeks in IBD patients are associated with little hepatotoxicity and that surveillance liver biopsies in these patients are not warranted [271,272].

An association between 6-thioguanine (6-TG) therapy and hepatic nodular regenerative hyperplasia (NRH) in patients with IBD has provoked some interest. One study of 24 patients treated with 6-TG found that six patients (25%) had NRH on liver biopsy and that in two patients (8%) there was clinically significant portal hypertension which resolved on discontinuation of 6-TG therapy. Reversible cholestasis has been reported in a patient with Crohn's disease after an infusion of infliximab [273]. There have also been a handful of case reports of apparent infliximab-induced autoimmune hepatitis, although to date these have been reported in the rheumatology rather than IBD patients. This may, however, simply reflect the wider and earlier use of TNFα inhibitors in this speciality and certainly gastroenterologists should be alert to this potential adverse effect [274, 275].

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Chapter 37
Conditions of the Eyes and Joints Associated with Inflammatory Bowel Disease

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Summary
- Peripheral arthritis occurs in 5–10% of ulcerative colitis patients and in 15–20% of those with Crohn's disease.
- The commonest form is an asymmetric pauciarticular arthropathy associated with active intestinal, predominantly colonic, disease.
- Axial arthropathy in the form of ankylosing spondylitis occurs in about 3% and sacro-iliitis in about 30% depending on the imaging modality used.
- Susceptibility to developing arthropathy is under genetic control.
- Management involves controlling intestinal inflammation, analgesia and physiotherapy.

Introduction

Complications of the eyes and joints are relatively common among patients with inflammatory bowel disease (IBD). Nevertheless, they have not been the subjects of extensive investigation and their pathogeneses remain unclear. Recently, however, a clearer classification has begun to emerge and is leading to new pathogenetic insights.

Arthritis

Peripheral arthritis

In 1895, Hale White described post mortem abnormalities in the joints of patients with ulcerative colitis (UC). Bargen described arthritic complications in association with UC in the 1920s. Initially this was thought to be coincident rheumatoid arthritis, but in 1958 Bywaters and Ansell published a paper demonstrating that the arthritis was inflammatory with a lymphocytic infiltration of the synovium, but that it was not erosive or deforming and was not associated with a positive rheumatoid factor [1].

In the 1960s, an Oxford retrospective study demonstrated a polyarthritis [2]. A prospective study from Leeds, however, showed a large joint arthritis in 11.5% of patients [3]. Subsequent studies in Crohn's disease found a similar distribution of joint disease [4]. In these studies, however, no differentiation was made between arthritis and arthralgia, which potentially is a very important distinction, as was demonstrated by a study from Israel [5]. In this study, investigators compared arthritis and arthralgia in 54 Crohn's disease patients with age- and sex-matched controls; 44% of the Crohn's disease patients complained of arthralgia, but 46% of controls also had joint pains. When objective evidence of joint inflammation was sought, 7.4% of Crohn's disease patients had arthritis, compared with none of the controls. Thus arthralgia is a common problem in both Crohn's disease patients and healthy controls, but inclusion of patients with arthralgia in studies of arthritis may considerably distort the results.

Subsequently, a large study from Oxford studied arthritis in 976 UC patients and 483 Crohn's disease patients with objective evidence of articular inflammation [6]. Two distinct forms of peripheral arthritis associated with IBD were found, both seronegative, with differing patterns of joint involvement and natural histories.

Type 1 (pauciarticular) peripheral arthritis affects less than five joints including a weight-bearing joint and is asymmetric. The swelling is acute and self-limiting and associated with relapse of the IBD in the majority of cases. It lasts for a maximum of 10 weeks, although, like reactive arthritis, 10–20% will develop persistent problems. There is a strong association with other extraintestinal features such as erythema nodosum and uveitis.

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Type 2 (polyarticular) peripheral arthritis affects five or more joints and affects a wide range of joints, but particularly the metacarpophalangeal (MCP) joints. It may cause persistent problems with a median duration of 3 years. It is associated with uveitis, but not erythema nodosum.

Arthritis onset may occur at any time during the course of IBD or before it becomes clinically manifest. It is not related to disease extent in UC but in Crohn's disease there is usually colonic disease. In both types of arthropathy there is little or no joint destruction and patients are seronegative.

In the Oxford series of 1459 patients, the prevalence of Type 1 was 3.6% in UC and 6.0% in Crohn's disease and for Type 2 was 2.5% in UC and 4.0% in Crohn's disease. These figures are slightly lower than in some other studies, which may reflect the strict entry criteria and the retrospective nature of the study. In total, between 5 and 10% of UC patients develop peripheral arthritis and 15–20% of Crohn's disease patients. This study demonstrates that careful clinical characterization of patients may lead to the detection of new clinical entities. This in turn may lead to new pathogenic insights.

Axial arthritis

In some ways, the association of axial arthritis with IBD is clearer than peripheral arthritis. Both ankylosing spondylitis (AS) and isolated sacroiliitis are known to be associated with IBD, but a number of questions remain unanswered.

AS is a seronegative inflammatory arthropathy characterized by sacroiliitis and progressive ankylosis (fusion) of the vertebral facet joints. Patients with AS develop the characteristic "question mark" posture as a result of the progressive fusion, which may also lead to respiratory embarrassment secondary to poor chest expansion and upper lobe pulmonary fibrosis. In about 30% of cases AS is associated with peripheral arthritis. Its prevalence in the general population is between 0.25 and 1%, with a male:female ratio of 3:1 [7,8]. The prevalence of AS in IBD is 1–6% [9,10], varying according to the study population, and the proportion of female patients is higher than in idiopathic AS, accounting for up to 50% of patients in some series. Otherwise the clinical features are identical with those of idiopathic AS and it runs a course independent of the IBD.

In AS sacroiliitis, back pain, restriction of movement and respiratory embarrassment are progressive over a number of years. Isolated sacroiliitis, without these features, has also been described in association with IBD. Its prevalence is largely dependent upon the means of diagnosis and it is often asymptomatic, although in a recent study careful questioning elicited a history of inflammatory back pain in 65% of patients [11].

A prevalence of up to 18% has been documented [10], but more recent studies have suggested a higher prevalence. A large degree of inter- and intra-observer error hampers diagnosis on radiographic grounds alone [12]. Computed tomography (CT) imaging studies have detected sacroiliitis in 32% of patients with IBD [13] and studies using radioisotope scintigraphy have found uptake abnormalities in up to 42% of patients with UC and 52% of patients with Crohn's disease [14]. The significance of these findings is not clear and long-term follow-up studies to demonstrate progression to AS have not been undertaken. It may be that in the majority of patients this is a non-progressive condition.

Pathogenesis

Spondyloarthropathies

IBD-associated arthropathies are often classified in association with spondyloathropathies. AS is the model for this group of rheumatological conditions. It includes reactive arthritis (post-enteric and urogenital), psoriatic arthritis and IBD-associated arthritis [15], which are all seronegative inflammatory arthritides. They share important pathogenic and clinical features, including the presence of inflammatory low back pain, an increased prevalence of AS, an association with erythema nodosum and uveitis and (with the exception of IBD peripheral arthritis) an association with HLA-B27. This association was first recognized in AS, where it is strongest, with 94% of patients possessing HLA-B27 in a recent study, compared with 10% of the general population [16]. Although weaker, the association with peripheral arthritides is still significant: in reactive arthritis approximately 70% of patients possess HLA-B27, although the prevalence varies widely between studies [17]. In patients with peripheral arthritides, those who are HLA-B27 positive develop the long-term complications of disease, namely sacroiliitis, acute uveitis or recurrent arthritis [18,19].

Although controversial, Behçet's disease is sometimes included in the spondyloarthropathy group [20]. The reported prevalence of HLA-B27 is only modestly increased and the association with sacroiliitis is weak. However, there is an increase in mucocutaneous complications such as erythema nodosum and uveitis (although this is characteristically posterior, in contrast to uveitis in spondyloarthropathy, which is anterior).

IBD arthritis

IBD arthritis is likely to be caused by the interaction of genetic and environmental factors.

Axial arthritis

As mentioned above, AS associated with IBD appears clinically to be identical with that of idiopathic AS and it runs a course independent of the IBD. Some authors have suggested that it is generally milder than idiopathic AS, but ascertainment bias is a problem in these studies and currently there is no consensus on this point. The HLA-B27

association of IBD AS is different, being considerably weaker: 50–80% of IBD AS patients possess HLA-B27 [21–23] compared with 94% of idiopathic AS patients [16]. The pathogenic significance of this difference is unclear, but some possibilities are discussed later.

The genetic associations of isolated sacroiliitis are less clear. This is partly because it is not clear what proportion of patients with sacroiliitis have early progressive disease (and will ultimately develop AS) and what proportion have true isolated sacroiliitis. Large follow-up studies are required to address this question. Studies of sacroiliitis in IBD are difficult to undertake, as a substantial proportion of patients may be asymptomatic. The largest study of sacroiliitis performed so far is a study in a population of 134 patients with Crohn's disease, which studied the 70 patients with symptomatic back pain [24,25]. The results demonstrated that 45% of these patients had evidence of sacroiliitis, including 7% who had AS. Those patients with sacroiliitis in the absence of AS were less likely to be HLA-B27 positive. However, a full clinical assessment was not undertaken to differentiate inflammatory from mechanical back pain and the study did not examine patients with asymptomatic sacroiliitis. A smaller study of sacroiliitis in Crohn's disease undertook a full clinical, symptomatic and MRI evaluation of the sacroiliac joints in 56 patients and found that 39% had MRI evidence of sacroiliitis. Interestingly, in this population every patient who possessed HLA-B27 had MRI evidence of sacroiliitis, suggesting that HLA-B27 and Crohn's disease may have an additive effect in determining axial joint inflammation. Hence it is probable that patients with isolated sacroiliitis do not have an increased prevalence of HLA-B27 and that HLA-B27 is a marker of progressive axial disease rather than sacroiliitis *per se*, but clearly long term follow up studies are required. Other genetic associations have also been studied in spondyloarthropathies and there has been the suggestion that possession of polymorphisms in the CARD15 gene on chromosome 16 may predict the development of sacroiliitis in Crohn's disease irrespective of the underlying phenotype of the disease [26]. However, this observation has not been widely replicated.

Peripheral arthritis

Most studies of the peripheral arthritis of IBD have been small and have not made any distinction between different patterns of disease. Possibly as a result of this, they failed to find any association between HLA-B27 and arthritis.

In a recent study of the immunogenetics of IBD arthritis, patients were subdivided according to the clinical classification described above. By doing this, distinct HLA associations were discovered [27]. These are shown in Table 37.1.

Type 1 (the large joint arthritis) is associated with HLA-B27, but Type 2 has a distinct association with HLA-B44. The association of Type 1 with HLA-B27 is, perhaps, unsurprising, as Type 1 peripheral arthritis is clinically very similar to reactive arthritis, in which approximately 70% of patients are HLA-B27 positive. In addition in Type 1 peripheral arthritis, there was a very strong association with the rare HLA Class II allele HLA-DR103 (DRB1*0103). This association is also seen in post-enteric reactive arthritis. Interestingly, in reactive arthritis the HLA-B27 association is twice as strong as in Type 1 IBD peripheral arthritis, whereas the HLA-DR103 association is twice as strong in Type 1 IBD peripheral arthritis.

Table 37.1 HLA associations of peripheral arthropathy in IBD.

HLA type	Type 1 arthropathy ($n = 30$) (%)	Type 2 arthropathy ($n = 30$) (%)	Controls ($n = 603$) (%)
HLA-B27	27*	3	7+
HLA-B35	33**	7	15++
HLA-B44	13	63***	31+++
HLA-DR103	40****	0	3++++

Type 1 vs controls: $+p = 0.001$, RR = 4.0; $++p = 0.01$, RR = 2.2; $++++p < 0.0001$, RR = 12.1.
Type 2 vs controls: $+++p_c = 0.01$, RR = 2.1.
Type 1 vs Type 2: $^{*}p = 0.03$, RR = 8.0; $^{**}p = 0.02$, RR = 5.0; $^{***}p = 0.0001$, RR = 4.8; $^{****}p = 0.0001$, RR = incalculable.

The pathogenic significance of these associations (and any functional significance) is difficult to gauge and the associations described may simply represent linkage disequilibrium with pathogenic genes located close by. This possibility is well illustrated by Type 2 peripheral arthritis. In this condition there is a strong association with HLA-B44, but there is a stronger association with the nearby gene MICA (MHC Class I chain-like gene A). MICA codes for a non-classical HLA molecule which is known to be expressed on the gastrointestinal epithelium and is up-regulated under conditions of cellular stress. It interacts with $\gamma\delta$ T cells, but does not appear to require exogenous antigen for this interaction. Its role appears, then, to be in regulating the immune response and so is a good candidate for involvement in immune mediated inflammation. In a study of patients with Type 2 peripheral arthritis, 99% (44/45) possessed the MICA*008 allele compared with 72% of IBD controls and 73% of healthy controls ($p = 0.001$).

The associations described above provide good evidence that genes in the MHC region of chromosome 6 play an important role in both axial and peripheral arthritis associated with IBD. However, the exact nature of this role remains unclear.

Environmental factors

Little is known about the environmental factors involved in the pathogenesis of arthritis in IBD, but some clues can be gained from studying similar conditions in humans and animal models.

It has been suggested that idiopathic AS is associated with antibodies to *Klebsiella* species [28] and that an abnormal immune response involving HLA-B27 is of pathogenetic importance. In AS associated with IBD, the association with HLA-B27 is less marked and this might suggest that in the presence of an inflamed gut, with increased permeability, more antigen may be presented, allowing other HLA alleles to act pathogenically. However, in IBD no associations between AS and bacteria have been demonstrated, and in idiopathic AS the evidence in favor of *Klebsiella* as opposed to other gut flora is unconvincing.

In contrast, the association between post-dysenteric reactive arthritis and bacterial infection is well established. Gram-negative enterobacteria such as *Salmonella*, *Escherichia coli*, *Yersinia*, *Klebsiella* and *Campylobacter* are all associated with arthritis and bacterial antigens may be isolated from the affected joints. These conditions are clinically very similar to Type 1 peripheral arthritis seen in IBD and so it seems likely that presentation of bacterial antigen may be important in the initiation of Type 1 IBD arthritis. This is plausible given the association between Type 1 arthritis and active disease. In these circumstances, the gut is inflamed and therefore more permeable, a similar situation to acute bacterial enterocolitis. Proliferative T cell responses to the relevant bacteria have been demonstrated from the synovial fluid of patients with reactive arthritis, but interestingly most of these appear to be HLA-DR restricted rather than HLA-B restricted [29–31]. This suggests that the HLA Class II alleles may be more important than HLA-B27 in the peripheral arthritides associated with enteropathy.

Further information about the role of bacteria in initiating arthritis in the presence of gut inflammation has been gained from the study of the HLA-B27 transgenic animal models. Although the rat and mouse models differ in some respects, they provide useful working models. These animals spontaneously develop a colitis and arthritis when reared under normal conditions [32,33]. However, if reared in a germ-free environment the gut and joint inflammation is abrogated [33]. Furthermore, Rath and coworkers have demonstrated that different bacteria induce gut and joint inflammation with differing efficiency, with *Bacteroides vulgatus* and a cocktail of bacteria isolated from Crohn's disease patients being the most efficient, whereas *Escherichia coli* is ineffective [32,34].

Hence it appears likely that bacteria are important in the pathogenesis of the Type 1 peripheral arthritis of IBD and possibly IBD-associated AS, but the mechanisms by which they interact with the immune system are unclear.

The exact site within the gut where this interaction occurs is also a matter for debate and in this area animal models may also provide some useful information:

Rath *et al.* have demonstrated in the HLA-B27 transgenic rat that diversion of the fecal stream away from the cecum abrogates distant inflammation, while leaving the colitis unaffected [35]. They postulated a role for bacterial overgrowth in the cecum in the pathogenesis of extracolonic inflammation. In humans, the cecum is relatively much smaller than in the rat, so these data cannot be extrapolated directly. However, in a study of 434 patients with Crohn's disease, it has been shown that there is a significant decrease in the incidence of new joint complications after resection of the ileocecal region – from one complication for every 89 years of follow-up to one complication every 701 years of follow-up [36]. This is illustrated as Kaplan–Meier survival curves in Figure 37.1. This is highly significant, even when correcting for the time spent in remission from Crohn's disease after surgery. Although this study provides circumstantial evidence that the ileocecal region may be important in the development of arthritis in IBD, it does not help determine whether it is stasis proximal to the ileocecal valve or cecal bacteria that are of importance. Nor is it clear whether it is the interaction of bacteria with upregulated HLA Class II molecules in the ileum or with newly induced Class II molecules in the colon (or another mechanism) that is important.

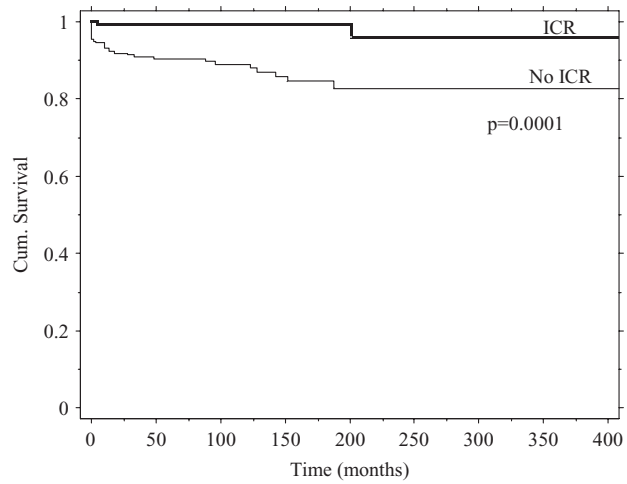

Figure 37.1 Kaplan–Meier curves of survival free of joint complications in Crohn's disease patients who had never undergone surgery and patients after ileocecal resection.

Taking the genetic and environmental data together, it is possible to hypothesize about the mechanisms involved in the arthritides associated with IBD. Classical Type 1 peripheral arthritis of IBD occurs in genetically susceptible individuals and may be caused by the interaction between gut bacteria and HLA Class II molecules in the context of an actively inflamed, and therefore abnormally permeable, gut. Whether this interaction is proximal or distal to the ileocecal valve is unclear and further work is required.

In contrast, the axial complications, such as AS, seem more likely to be mediated by typical or atypical interactions between HLA Class I molecules and the immune system, although intestinal inflammation may make the conditions more favorable for the development of axial

inflammation. This would be consistent with the observation that the HLA-B27 association in IBD is weaker than in idiopathic AS, suggesting that the presence of intestinal inflammation may allow the development of joint disease even in patients without the usual genetic predisposition.

The pathogenetic mechanisms in Type 2 peripheral arthritis are less clear and, although there is a strong HLA Class I association, this may be secondary to linkage disequilibrium with other genes in the region such as the non-classical Class I gene MICA.

All the hypotheses described above remain largely speculative and there are others, including the suggestion that arthritis and other extraintestinal manifestations are an autoimmune reaction to self antigens such as isoforms of tropomyosin found in the gut, eye and joint [37]. However, these do not account for the HLA associations found and the evidence has not yet been widely replicated.

Clinical features

Diagnosis

Axial disease

It has been suggested that the course of AS associated with IBD is less severe than idiopathic AS, but this is not universally accepted. In symptomatic disease, low back pain is a common complaint in both IBD and the general population. In IBD it may represent inflammatory arthritis of the sacroiliac joints (sacroiliitis) or progressive AS. In a patient presenting with back pain, it is therefore important to distinguish between the inflammatory back pain associated with sacroiliitis and AS and mechanical low back pain.

Mechanical back pain may be of sudden onset, is often central and is better after rest, occurring generally in older patients. The clinical features of inflammatory low back pain are an insidious onset over months, morning stiffness and exacerbation of pain by rest and pain radiating into the buttocks (rather than central back pain). It tends to occur in patients under 40 years of age. Plain radiographs of the sacroiliac joints are the conventional means of diagnosis, but X-ray changes occur only after several months and MRI scanning (with or without gadolinium enhancement) is more sensitive and avoids the necessity for a radiation dose.

If radiological studies suggest sacroiliitis, a further assessment is warranted to detect evidence of progressive axial disease, including the modified Schober test of lumbar flexion, lateral lumbar flexion and chest expansion. The most useful blood test in diagnosis is HLA-B27 status, although in IBD a negative HLA-B27 status does not preclude the diagnosis of AS and patients with low back pain or decreased spinal mobility in the presence of sacroiliitis should be treated as having AS.

As many as 20% of IBD patients may have asymptomatic sacroiliitis detectable by plain radiology and it may be diagnosed on the basis of routine abdominal X-rays. In many cases there is no history of inflammatory back pain even on direct questioning. If sacroiliitis is diagnosed, a clinical assessment of spinal mobility should be undertaken along with HLA-B27 status. If there is evidence of decreased spinal mobility, they should be treated as having early AS.

Peripheral arthritis

As mentioned above, there are two distinct forms of peripheral arthritis, and in addition arthralgia may occur. Arthralgias are often seen in conjunction with reducing doses of corticosteroids or the commencement of immunosuppressants such as azathioprine. These normally settle with time and rarely require specific treatment. Both forms of arthritis are rheumatoid factor negative and are not generally erosive or deforming. If there is evidence of erosive disease, then further investigation by a rheumatologist is required. Diagnosis of IBD peripheral arthritis is largely based upon clinical grounds, but other joint disease should be excluded.

The differential diagnosis for Type 1 arthritis includes gout and pseudogout, septic arthritis and arthritis following genitourinary infections such as *Chlamydia* and gonorrhea. When there is no apparent relation to activity of the bowel disease, these causes should be actively sought and serum urate and calcium should be checked routinely. If there is any doubt, joint examination and aspiration should be performed. For Type 2 arthritis, a rheumatoid factor and autoantibody screen should be performed to exclude seropositive rheumatic disease. Arthritis associated with IBD is very rarely erosive or deforming and is not associated with other manifestations of rheumatoid arthritis such as subcutaneous nodules. X-rays of the most severely affected joints should therefore be performed if symptoms are persistent in order to exclude erosive disease.

Management of IBD arthritis

General points

The aims of treatment are to alleviate symptoms and preserve joint function for the future, particularly in axial disease. The management of arthritis in IBD depends on the nature and duration of the arthritis, as discussed below. Because of the relatively small number of patients involved, no controlled trials of treatment have been conducted and most treatment modalities rely on general principles.

Physical treatments (rest, range of movement exercises and physiotherapy) are important components of management and should not be ignored. These may be enhanced

by simple measures such as splinting affected joints and the use of assistive devices such as a walking stick.

Analgesia

Analgesia is a key element in management and is a potentially difficult area as many analgesics have undesirable effects on the gut. A step-up approach to the prescription of analgesics should be used, starting with a simple analgesic such as paracetamol, progressing to stronger analgesics as required. Analgesics containing opioids may cause problematic constipation, particularly in patients with resistant distal colitis.

If at all possible, non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided as they may cause enterocolitis in their own right and may trigger or exacerbate relapse of pre-existing IBD that could be associated with significant lower gastrointestinal bleeding. They should not be used at all in patients with active disease and in these situations other therapies should be used. The recent advent of cyclooxygenase-2 (COX-2)-specific NSAIDs has raised the prospect of fewer unwanted gastrointestinal problems, particularly in the stomach. However, experiments in animals have demonstrated that the enterocolitis of NSAIDs is not mediated by the cyclooxygenase pathway and that the role of the COX isoforms in inflammation may be complex. It is therefore likely that COX-2-specific NSAIDs may not improve the tolerability of these drugs in IBD and this, along with other safety concerns, means that they should still be avoided [38].

Axial disease

Patients with AS should be managed in conjunction with a rheumatologist. Physical therapies, including swimming and spinal therapies, with regular physiotherapy, are of particular importance and all patients should take regular exercise to maintain the mobility of the spine. These forms of exercise should also be recommended to patients with sacroiliitis associated with any form of low back pain or decreased spinal mobility. For patients with severe low back pain or severely reduced spinal mobility, injection of steroid into the sacroiliac joints may help, particularly in association with a period of intensive inpatient physiotherapy. The period of relief from sacroiliac injection alone may be brief.

Drug treatments

Simple analgesics should be used if possible. NSAIDs are the drugs of choice in idiopathic AS and if there is active spinal disease in the absence of active IBD then it is reasonable to use NSAIDs. However they should be stopped if the IBD flares up. Sulfasalazine can be used for both the IBD and joint symptoms but it is most effective in patients with associated peripheral joint problems. Other 5-aminosalicylate (5-ASA) drugs are not as effective as they are thought to be; it is the sulfapyridine component that confers the articular effects rather than the 5-ASA.

If oral steroids are required for active disease, then they will also have a beneficial effect on the spinal disease. However, long-term steroid therapy for spinal disease alone should be avoided. This may be achieved by using methotrexate, which may be effective in both gut and spinal disease.

In difficult inflammatory disease, the advent of monoclonal antibody therapies directed against the action of tumor necrosis factor α (TNFα) has had a dramatic impact upon the treatment of many inflammatory rheumatic conditions, of which AS is one. AS responds to a number of these compounds, including etanercept and infliximab [39,40]. As etanercept has not been effective in Crohn's disease, infliximab is the treatment of choice for patients with active axial inflammation and Crohn's disease, although it is likely that adalimumab will also be useful in this context [41,42].

In severe progressive disease unresponsive to the measures outlined above, radiotherapy remains a last resort; however, the increased risk of hematological malignancy associated with this form of treatment has rendered it rarely used.

Good communication between the patient, gastroenterologist and rheumatologist to maximize the effectiveness of the available therapeutic options is the most important part in the successful management of patients with AS and IBD.

Isolated sacroiliitis should be treated symptomatically with simple analgesia and regular exercise should be encouraged. However, further treatment should not be required in the absence of decreased spinal mobility or severe pain.

Peripheral joint disease

Type 1 arthritis

This is usually self-limiting and so treatment is largely symptomatic. Resting the joint is important and use of a walking stick or splint to take pressure off the joint may lead to a significant improvement. Range of movement exercises should be performed to minimize any periarticular muscle atrophy and to prevent contractures. In severe cases, formal physiotherapy may be required to improve function.

Type 1 arthritis is usually associated with active bowel disease and NSAIDs should not be used. A good, but relatively seldom used, therapy is intra-articular injection of steroid, which may provide very effective symptom relief and may remove the requirement for other treatments. If oral steroids are used to treat the active bowel disease, then these will normally treat the arthritis effectively. If not, an empirical change of 5-ASA drug to sulfasalazine may give good symptom relief. If sulfasalazine fails, then a low dose

of oral steroid specifically for the joint disease may be effective, but this should not be prolonged for more than a few weeks. Long-term treatment is not usually required, although maintenance with sulfasalazine as the 5-ASA of choice may be appropriate, particularly in patients at risk of recurrent disease such as those who are HLA-DR103 positive. In the minority of patients with persistent problems, the treatment options are those of Type 2 arthritis (see below).

Type 2 arthritis

These patients generally have persistent problems and may require long-term treatment. Again, as the disease is usually non-erosive and non-deforming, symptomatic relief is the major aim. Again, splinting of affected joints and rest are important components of management, but the persistent and polyarthritic nature of this condition makes this harder to achieve than in Type 1 arthritis.

For patients with persisting problems, sulfasalazine or low-dose prednisolone may be used. However, prolonged courses of oral steroids should be avoided. Simple analgesia should be tried initially. NSAIDs should only be considered in patients with quiescent disease unresponsive to simple or combination analgesia, but if there is any evidence of an increase in the activity of the bowel disease they should be stopped. In patients with active bowel disease, it may be appropriate to use methotrexate as the first-line immunosuppressant rather than azathioprine in order to treat both gut and joints.

Patients who have evidence of erosive joint disease or a positive rheumatoid factor or who do not respond to the measures outlined above should be managed jointly with a rheumatologist.

Anti-TNFα antibodies have also been used in peripheral arthritis in IBD which has failed to respond to standard therapy. The published experience is limited to case reports but, unsurprisingly, a reduction in inflamed joints and inflammatory markers has been reported [43]. Thus infliximab may well be an alternative treatment in patients with severe resistant joint disease associated with IBD. As in other rheumatic diseases, this may be combined with other immunosuppressants such as methotrexate [44]. However, further studies are required to confirm its efficacy and the best treatment regimens.

Ocular manifestations of IBD

Epidemiology and clinical features

Ocular inflammation in IBD was first documented by Crohn in 1925. If left untreated it is potentially a cause of blindness. However, prompt treatment with topical steroids can minimize this risk.

The prevalence of ocular inflammation varies widely between studies – from 2 to 13% depending on the population and methodology. Various forms have been described, including iritis, episcleritis, scleritis and anterior uveitis. In our retrospective study of 1459 patients (976 UC and 483 Crohn's disease patients), 3% of UC and 5% of Crohn's disease patients had eye complications. The commonest were iritis (60%), episcleritis (30%) and uveitis (10%). However, in most cases the inflammation caused no lasting ocular damage [45]. The female:male ratio was 2.9:1, the eye complications were present at or before diagnosis in 19% of cases and in 72% of cases the eye complications were associated with active IBD. About 30% of patients went on to have recurrent episodes of ocular inflammation. In common with other studies, patients who suffered eye complications were more likely to suffer other muco-cutaneous complications, notably arthritis and erythema nodosum.

Pathogenesis

The clinical observation that ocular inflammation occurs more commonly in those patients with other extra-intestinal manifestations (EIMs) has led to several hypotheses. The first suggested that the eye, the joints, the skin and the liver expressed a particular isoform of tropomyosin, to which an autoimmune reaction was generated [46]. However, although this antigen was found in the target organs, it was not found in the components that became inflamed. Thus it was expressed in chondrocytes but not synovium, and in ciliary muscles but not the iris. In addition, this hypothesis fails to explain why in some patients ocular inflammation may occur as a single manifestation and in others as part of a complex of EIMs.

This phenomenon of the EIMs being distinct but overlapping clinical entities may be explained by a genetic-based hypothesis. This suggests that the presence of the different EIMs is determined by different genes located in the same region. Linkage disequilibrium between these genes would mean that the genes for different manifestations are inherited together more frequently than would be expected by chance, leading to the clinical clustering of the EIMs. Given the HLA associations described above, we studied the HLA-B and DR regions in 52 patients with ocular complications of IBD. This study found that there are associations between ocular complications and HLA-B27 (40% vs 9% in IBD controls, $p < 0.0001$), HLA-B58 (12% vs 1%, $p = 0.002$) and HLA-DR103 (20% vs 8%, $p = 0.001$). These associations appear independent of the HLA-B27 and DR103 associations seen in arthritis [47]. This suggests strongly that there are genes in the HLA region of chromosome 6 which determine the different EIMs and that linkage disequilibrium between these genes may account for the clinical observation of distinct but overlapping clinical syndromes.

Management of ocular manifestations

For a proper diagnosis to be made, the patient should undergo a full ophthalmological assessment including slit

lamp examination. Treatment normally consists of topical steroid treatment initially given frequently (hourly) but reducing subsequently, although a course of treatment lasting 6–8 weeks in total is usual. A cycloplegic agent such as atropine is often added to prevent the formation of posterior synechiae. Occasionally oral steroids may be required for acute anterior uveitis and often in scleritis. Oral or topical NSAIDs may be useful in anterior scleritis, but have no role in anterior uveitis. Their use carries the risks to the IBD described above.

Thus patients with acute ocular inflammation should have a rapid assessment by an ophthalmologist, who should guide their treatment, which normally consists of topical or oral corticosteroid therapy. Like many other of the EIMs of IBD, in resistant cases there may be a role for TNF inhibitors such as infliximab [48]. It is vital to treat ocular inflammation appropriately and aggressively, as failure to treat the inflammation effectively may lead to long-term complications or even blindness, although these situations are rare.

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Chapter 38
Dermatologic Conditions Associated with Inflammatory Bowel Diseases

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Summary
- The orocutaneous manifestations of inflammatory bowel disease are common and often under-recognized.
- Erythema nodosum is the most common cutaneous manifestation of inflammatory bowel disease and is more common in women.
- Many of the orocutaneous and cutaneous manifestations of inflammatory bowel disease follow a clinical course that is independent of the patient's luminal disease.
- Other than a single randomized controlled trial of infliximab for pyoderma gangrenosum, the medical management strategies for the cutaneous manifestations of inflammatory bowel disease are based on case reports and case series.
- It is important to recognize that many orocutaneous and cutaneous manifestations of inflammatory bowel disease are due to nutritional deficiency or are direct side effects of therapy directed at the disease.

Introduction

Inflammatory bowel disease (IBD) is associated with a host of manifestations beyond the gastrointestinal tract that can affect any organ system. Among them, the cutaneous manifestations are not only highly prevalent but, at times, equally debilitating to or more debilitating than an individual's gastrointestinal symptoms (as can be the case in the setting of pyoderma gangrenosum).

The underlying immunopathogenetic mechanisms driving the cutaneous manifestations of IBD are not fully elucidated, but the fact that some parallel intestinal disease activity whereas others seem to run an independent course belie some key differences (Table 38.1). Moreover, cutaneous manifestations can occur as a direct result of medical therapy for, or nutritional consequences of, IBD and are not due to underlying systemic immunologic activity.

Appropriate therapy of cutaneous manifestations first involves recognition of the appropriate diagnosis. The medical options outlined are based largely on the experience of centers specializing in the management of patients with IBD, as there is little controlled trial evidence in this arena.

The importance and clinical significance of cutaneous manifestations of IBD are somewhat dependent on their location and, broadly, these manifestations can be orocutaneous or occur in a more generalized or localized cutaneous distribution. In addition, it is instructive to think of any manifestation of IBD as being related either to the underlying immunologic activity of the disease or, alternatively, to nutritional consequences of disease or as a side effect of therapy directed at the disease.

Orocutaneous manifestations of inflammatory bowel disease

Orocutaneous manifestations of IBD represent an important, common, yet often overlooked consequence of disease. In one study, only 45% of the oral manifestations of Crohn's disease, as assessed by serial dental examinations, were recognized by the treating gastroenterologist [1].

Related to immunologic activity

Aphthous stomatitis

Aphthous stomatitis is one of the most common extraintestinal manifestations of IBD and can occur in patients with both ulcerative colitis (UC) and Crohn's disease (CD). However, aphthous stomatitis seems to be more common in CD with up to 20–30% of patients being affected at some time [2].

Aphthous ulcers tend to be shallow and cluster in the buccal mucosa and usually parallel disease activity (Table 38.1). In some patients in clinical remission, the development of aphthous stomatitis can reliably predict an

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Table 38.1 Relationship of cutaneous manifestations of inflammatory bowel disease to luminal disease activity.

Cutaneous manifestations of IBD	Relation to disease activity
Common	
Erythema nodosum	Parallels disease activity
Pyoderma gangrenosum	Independent of disease activity
Aphthous stomatitis	Parallels disease activity
Uncommon	
Cutaneous Crohn's disease	Independent of disease activity
Sweet's syndrome	Independent of disease activity
Cutaneous vasculitis	Parallels disease activity
Pyostomatitis vegetans	Independent of disease activity
Oral Crohn's disease	Parallels disease activity

impending relapse of their intestinal disease, highlighting the fact that these lesions often closely parallel disease activity.

Therapy of aphthous stomatitis that is related to intestinal disease activity generally requires management of the underlying luminal inflammation. However, symptomatic therapy can include the administration of gels containing lidocaine as a local analgesic.

Pyostomatitis vegetans

Pyostomatitis vegetans (PV) is a vesiculopustular eruption of the oral cavity. It is associated with ulcerated, mucosal plaques and aseptic abscess formation. The prevalence of this condition in patients with IBD is largely undescribed but is generally considered rare, as suggested by an approximately 2% prevalence in a pediatric CD cohort [1]. The course of PV can be independent of intestinal disease activity and has been described in case reports as the only manifestation of clinically asymptomatic UC [3].

Therapy typically involves the use of systemic corticosteroids, but the use of topical tacrolimus or systemic therapy with infliximab and methotrexate has been described [4,5]. In addition, zinc deficiency can be associated with a similar orocutaneous lesion, so assessment of zinc levels or empirical zinc replacement should be considered in the appropriate circumstances.

Oral Crohn's disease (cobblestoning)

Oral CD, otherwise known as mucosal nodularity or mucosal cobblestoning, represents a specific lesion where the buccal mucosa takes on a cobblestone appearance in the setting of CD. It is uncommon, typically parallels luminal disease activity and responds to systemic corticosteroids [6–8].

Related to nutritional deficiency

A number of orocutaneous manifestations of IBD are associated with nutritional deficiencies that are often complications of the luminal disease. Angular cheilitis can be associated with iron and vitamin B_{12} deficiency, both common nutritional states in patients with CD. Zinc deficiency can be associated with a number of orocutaneous manifestations including a PV-like lesion and loss of taste.

Therapy of these manifestations first includes recognition of the nutritional state followed by appropriate replacement. Iron can be given orally but may be poorly tolerated in patients with significant gastrointestinal symptoms, often necessitating the use of iron infusions (iron dextran or iron sucrose). Vitamin B_{12} can be administered as a 1000 μg intra-muscular injection that may be required on a monthly basis permanently if the deficiency state is due to extensive ileal resection in CD.

Related to therapy for IBD

Methotrexate is well known to be associated with the development of aphthous stomatitis. These tend to be deeper than those aphthous ulcers that are associated with the immunologic activity of IBD. Therapy generally involves oral folic acid. Typically, 1 mg of folic acid should be administered orally daily, except on the day a patient takes their methotrexate. In the setting of the development of aphthous stomatitis, a dose increase to 2–3 mg per day or oral folic acid will often alleviate the problem. Occasionally, cessation of methotrexate is required.

Cutaneous manifestations of inflammatory bowel disease

The pathophysiology underlying the cutaneous manifestations of IBD that are not related to nutritional deficiency or therapy is poorly understood. Some insight has been gained in the setting of UC where auto-antibodies that react to a colonic epitope may be cross-reactive with tropomyosin epitopes in extra-intestinal organs, including the skin [9,10].

Similarly to the orocutaneous manifestations of IBD, the cutaneous manifestations should be thought of being related in some way to the immunologic activity of the disease or to nutritional deficiency states or as a side effect of therapy.

Related to immunologic activity

Erythema nodosum

Erythema nodosum (EN) is the most common and most easily recognizable cutaneous manifestation of IBD. The hallmark tender, erythematous raised nodules on the extensor surfaces, particularly over the pre-tibial region, are not easily overlooked (Plate 38.1). Pathologically, there is an acute or chronic venulitis, septal inflammation, hemorrhage and acute panniculitis [11]. Owing to its classical appearance, biopsy is rarely required.

Erythema nodosum tends to parallel luminal disease activity and may take several weeks to resolve. It can be

the presenting feature of IBD and, therefore, IBD should be considered in the differential diagnosis of a patient presenting with EN in the absence of an obvious cause. Erythema nodosum is not unique to IBD and has been described in a number of other conditions, including sarcoidosis, post-streptococcal infections, Behçet's disease, other connective tissue diseases and lymphoproliferative disorders and as a side effect from many medications, most notably oral contraceptive pills [12].

Erythema nodosum occurs in 11% of patients with IBD overall, perhaps more in UC than CD [13]. However, EN occurring in patients with CD tends to be more commonly associated with colonic CD [14]. There is a strong preponderance for EN to occur in female patients relative to their male counterparts, with a female to male ratio of approximately 5.5:1 [14].

The pathophysiology of EN is unknown, but it may be related to immune complex deposition in and around venules in the connective tissue of subcutaneous fat [15]. Genetic associations with EN have been described, including a weak association with HLA-B and a stronger association with the –1031C polymorphism of the TNFα gene [14].

Management of EN consists mainly of managing the underlying luminal inflammation. However, supportive measures such as elevation of the legs may be useful. Nonsteroidal anti-inflammatory drugs (NSAIDs) will usually provide prompt relief but should be used with caution owing to the potential for these agents to lead to an exacerbation of the patient's underlying IBD [16]. Occasionally, systemic corticosteroids are required.

Pyoderma gangrenosum

Pyoderma gangrenosum (PG) is a condition characterized by severe cutaneous ulcers with a violaceous border and a necrotic center (Plate 38.2). The lesions often start as painful skin lesions which then develop into pustules, followed by ulceration [12]. Commonly, PG lesions begin at the site of minor trauma. For this reason, surgical debridement is often avoided as the lesions exhibit pathergy. PG lesions tend to occur in the lower extremity, but can occur anywhere.

Similar to EN, PG can be associated with other disorders, such as rheumatologic disease and myeloproliferative disorders [12]. However, it occurs in the setting of IBD at a greater frequency than with any other associated condition. Prevalence rates of 1–10% for UC and 0.5–20% for CD have been reported, although a population-based study demonstrated a prevalence rate of 0.8 and 1.3% in UC and CD, respectively with no gender predilection [17].

Unlike EN, PG does not parallel luminal disease activity and can, by itself, be more debilitating than a patient's IBD in severe cases and can be recurrent in one-third of patients [18]. The skin lesions of PG can precede bowel symptoms in patients with newly diagnosed IBD.

The diagnosis of PG is typically clinical; however, unlike EN, biopsy is recommended in order to exclude other causes of similar lesions such as fungal, bacterial or mycobacterial skin infections and cutaneous vasculitides or malignancies [12]. The typical pathological appearance is that of a central neutrophilic infiltrate, non-specific inflammation and abscess formation and the absence of vasculitis. The etiology of PG is unknown.

In addition to classic PG, there are other less common variants, which can easily be misdiagnosed. These other variants include pustular PG, bullous PG, vegetative PG and peristomal PG (see below) [19]. Pustular PG presents as coalescing groups of superficial pustules, which are typically painful and tends to be associated only with patients with IBD. It tends to occur on the trunk and extensor surfaces of the limbs [19,20]. Bullous PG is generally seen in hematologic malignancies rather than IBD whereas vegetative PG tends to present as more superficial, unifocal lesions with a better overall prognosis [19].

Management

It is important to recognize and initiate therapy early in the course of PG as these lesions have a propensity to progress and become secondarily infected.

A number of management strategies, both topical and systemic, have been reported for treating PG in the setting of IBD (Table 38.2). Due to the relative infrequency of PG, the evidence for most therapies is in the form of case reports or case series, with the exception of a single randomized, double-blind, placebo-controlled trial of the chimeric IgG_1 monoclonal antibody to tumor necrosis factor α (TNFα) infliximab (see below).

Defining the optimal management strategy for PG depends on the nature and extent of the lesions. Smaller, isolated lesions can often be treated topically, whereas larger lesions will usually require systemic therapy.

Table 38.2 Summary of described therapies for pyoderma gangrenosum in the setting of inflammatory bowel disease.

Topical therapy
Topical or intra-lesional corticosteroids
Topical tacrolimus
Systemic therapy
Infliximab*
Adalimumab
Corticosteroids (oral)
Corticosteroids (intravenous)
Intravenous cyclosporine
Azathioprine/6-mercaptopurine
Tacrolimus
Mycophenolate mofetil
Dapsone
Thalidomide

*Infliximab represents the only therapy for which there is randomized controlled trial evidence.

Topical therapy should include supportive measures aimed at preventing secondary infection such as moist, sterile wound dressings. Intralesional corticosteroids, usually in the form of triamcinolone acetonide 10–40 mg ml^{-1}, is the most commonly used topical therapy [21,22]. The use of topical tacrolimus has been described in the setting of peristomal pyoderma, the unique occurrence of PG lesions at the site of an ileostomy or colostomy after surgery for CD [23]. Other topical therapy that has been described, but is rarely used, is topical sodium cromoglycate, a mast cell inhibitor [24].

Until recently, the most commonly used systemic therapy has been oral or intravenous corticosteroids. Prednisone at a dose of 1–2 mg kg^{-1} per day is generally effective, as are intravenous corticosteroids [25]. Historically, cases of PG that were refractory to corticosteroids were often managed with intravenous or oral cyclosporin at doses ranging from 3 to 5 mg kg^{-1} per day [25–28]. Other systemic therapies have been described including azathioprine, systemic tacrolimus and dapsone [25,29].

However, more recently the chimeric monoclonal antibody to TNFα infliximab has been used increasingly [30–33]. A recently published double-blind, randomized, placebo-controlled trial of infliximab for the treatment of PG represents the only study of its kind for any therapy for cutaneous manifestations of IBD [33]. In this trial, patients with PG were randomized to a 5 mg k^{-1}g infusion of infliximab at week zero, with the primary endpoint being clinical improvement at week 2. Patients who did not improve by week 2 were given open-label infliximab. At week 2, 46% of patients treated with infliximab had a clinical response as opposed to only 6% in the placebo-treated group. Overall, 29 patients received infliximab with clinical improvement occurring in 69%. However, 31% of patients had no clinical response to infliximab, underscoring the challenging nature of this difficult condition. Case reports have now been published describing the beneficial effect of a subcutaneously administered, fully human IgG monoclonal antibody to TNFα, adalimumab, so this remains a reasonable alternative agent in patients who have lost response or developed intolerance to infliximab [31,34].

As PG lesions can exhibit pathergy, surgical debridement can be difficult. However, due to the severity of some lesions, plastic reconstruction is sometimes necessary after a successful course of medical management. Although PG tends to run a disease course independent of that of the luminal disease, occasionally severe PG may be an indication for surgery, particularly colectomy, in patients with IBD.

Sweet's syndrome

Sweet's syndrome is a rare, neutrophilic dermatosis that has been associated with IBD (Plate 38.3). It typically presents as urticarial lesions on the arms, head and neck and is often painful. It is often independent of luminal disease activity but tends to be more associated with colonic disease [35]. Corticosteroids will often lead to resolution of lesions, but other therapies such as dapsone and thalidomide have been described [36].

Cutaneous Crohn's disease

Often referred to as metastatic CD, cutaneous CD represents a spectrum of granulomatous skin lesions distinct from the perineum. It typically presents as ulcerated plaques or nodules, often in the lower extremity [37,38]. These lesions tend to run an independent course of the luminal disease and generally respond to similar immunosuppressive regimens to those used to treat the luminal component of CD.

Cutaneous vasculitis

A cutaneous vasculitis, similar to the leukocytoclastic palpable purpuric lesion of Henoch–Schonlein purpura, can be seen in patients with IBD. The vasculitis typically parallels luminal disease activity and responds to gut-directed immunosuppressive therapy [39].

Related to nutritional deficiency

There are a host of cutaneous manifestations related to specific vitamin and micronutrient deficiency states. Extensive small bowel resection in CD can result in bile salt malabsorption and subsequent deficiency in fat-soluble vitamins such as vitamins A, D, E and K. This can result in follicular hyperkeratosis in the setting of vitamin A deficiency and easy bruising in the setting of vitamin K deficiency. Deficiency in the B vitamins can result in various dermatologic manifestations, including sebhorrheic dermatitis. Zinc deficiency results in a specific syndrome called acrodermatitis enteropathica, which results in, among other things, acro-oral skin lesions. In general terms, these types of nutrient deficiencies are more common in patients with CD of the small bowel than in patients with colonic CD or UC. A comprehensive review of the nutritional status of any patient with IBD is always indicated.

Related to therapy for inflammatory bowel disease

The most common cutaneous manifestation of IBD that is related to therapy is corticosteroid-induced acne (Plate 38.4). This cosmetic side effect of corticosteroids is a frequent cause of non-compliance with respect to medication usage. It typically responds to cessation of the corticosteroids.

Any medication can be associated with other manifestations such as fixed drug eruptions or a hypersensitivity rash, as has been described with azathioprine. Infliximab can be associated with a psoriaform eruption and drug-induced lupus with the associated skin manifestations can

also occur with this agent. In general, any skin lesion that occurs in a patient in IBD could potentially be due to therapy and this should be considered in the differential diagnosis.

Conclusion

The cutaneous manifestations of IBD represent an interesting group of disparate syndromes that offer us a unique glimpse into the immunology of IBD. They serve to underscore the heterogeneity of CD and UC, which applies not only to varied luminal presentations of these disorders, but also to the equally varied cutaneous manifestations. Both CD and UC are associated with an overlapping spectrum of presentations, with erythema nodosum being the most common. However, both nutritional consequences of disease and therapy for disease can be associated with cutaneous manifestations and need to be considered in the differential diagnosis of the IBD patient with oral or skin lesions.

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Chapter 39
Fertility and Pregnancy in Inflammatory Bowel Diseases

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Summary
- The peak incidence of IBD overlaps the prime reproductive years, making the management of IBD patients desiring conception challenging.
- Fertility: (1) women with IBD have similar fertility rates to the general population; surgery, particularly an ileal pouch anal anastomosis, reduces fertility; (2) men with IBD may have lower fertility rates than healthy controls; infertility may be voluntary or related to disease activity, malnutrition, surgery or medication use.
- Pregnancy outcomes: (1) women with IBD have higher rates of low birth weight, small for gestational age infants and preterm birth and should therefore be followed as high-risk pregnancies; (2) the offspring of men with IBD do not seem to have higher rates of perinatal adverse outcomes.
- Pregnancy and IBD: women with IBD have a similar risk of flare during pregnancy to the non-pregnant patient.
- Medications during conception and pregnancy: (1) in women, methotrexate and thalidomide are contraindicated during pregnancy and conception; the majority of other medications used to induce or maintain remission are considered low risk and may be continued after a full discussion with the patient; (2) in men, sulfasalazine causes reversible sperm abnormalities and methotrexate is associated with oligospermia; both agents should be stopped 4–6 months prior to considering conception; other IBD medications are considered low risk.

Introduction

Patients with inflammatory bowel disease (IBD) are affected during their peak reproductive years [1]. Patients are concerned about their childbearing potential – whether they can achieve conception, whether they can have a healthy child and whether the medications they take affect their child's health. As medical therapy for IBD advances, putting more men and women into remission and into a position to consider pregnancy, striking the balance between optimal medical therapy and fetal health becomes more complex.

This chapter summarizes the existing literature on the effects of ulcerative colitis (UC) and Crohn's disease (CD) and of the medications used to treat them on fertility and pregnancy outcomes.

Genetics and inheritance

Patients are often concerned about passing their disease on to their offspring. Unfortunately, family history is the strongest predictor for developing IBD. If one parent is affected, the risks of the offspring developing IBD are 2–13 times higher than in the general population [2,3]. One study estimated that the risk of IBD in first-degree relatives of probands with UC and CD was 1.6 and 5.2%, respectively, and even higher in the Jewish population [4]. If both parents have IBD, the risk of their offspring developing IBD over their lifetime was estimated to be as high as 36% [5].

Several studies suggest that breastfeeding may be protective against the development of IBD in the infant. In a meta-analysis of 17 studies, the eight highest quality studies showed a pooled odds ratio (OR) of 0.45 [95% confidence interval (CI) 0.26–0.79] for CD and 0.56 (95% CI 0.38–0.81) for UC [6]. However, these were not mothers who had IBD themselves.

Fertility and sexual function

Women

Infertility is defined as the diminished ability or the inability to conceive and have offspring. It is also defined in specific terms as the failure to conceive after a year of regular intercourse without contraception. In general, women

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with CD appear to have similar fertility rates to the general population. Older, referral center studies estimated infertility rates between 32 and 42% in women with CD [7,8]; however, community-based and population-based studies suggest infertility rates (5–14%) similar to those in the general population [9,10]. Surgery for CD may decrease fertility compared with medical therapy alone [10].

Women with UC have fertility rates similar to the general population prior to surgery [9–11]. A study by Ording Olsen *et al.* [12] of 290 women with UC versus 661 non-IBD controls found that women with UC had a fecundability ratio (FR) (the ability to conceive per menstrual cycle with unprotected intercourse) equal to the general population (FR = 1.01). However, after surgery for an ileal pouch anal anastomosis (IPAA), the FR dropped to 0.20 ($p < 0.001$). The reduction in fertility may be due to surgery in the pelvis and the consequent adhesions and damage to the reproductive organs. Patients who undergo a proctocolectomy with ileostomy also experience a reduction in fertility [13], as do patients with familial adenomatous polyposis who undergo IPAA [14].

This finding has been confirmed by a meta-analysis and also a systemic review. In a meta-analysis [15] of seven studies, IPAA increased the risk of infertility in women with UC by approximately three-fold. Infertility, defined as failure to achieve pregnancy in 12 months of attempting conception, increased from 15 to 48% in women post-IPAA. The relative risk of infertility after IPAA was 3.17 (95% CI 2.41–4.18), with non-significant heterogeneity. The weighted average infertility rate in medically treated UC was 15% for all seven studies and the weighted average infertility rate was 48% after IPAA. In the systematic review [16], 22 studies, with 1852 females, were included. The infertility rate was 12% before restorative proctocolectomy and 26% after IPAA, among 945 patients in seven studies.

With respect to sexual dysfunction, a German survey of 1000 patients [17] found that women with IBD showed impaired function irrespective of disease activity as compared with healthy controls. High socioeconomic status was a protective factor for several subscores in women and depression was the most important predictor of dysfunction [17]. In the systematic review stated above, an incidence of sexual dysfunction of 8% preoperatively and 25% postoperatively (seven studies, $n = 419$) was reported in women undergoing IPAA.

The risk of infertility and sexual dysfunction should be discussed with the patient prior to surgery as part of the potential risks of the operation. It is unclear if techniques such as laparoscopic IPAA or a subtotal colectomy with rectal stump and ileostomy during the childbearing years are helpful in reducing infertility and sexual dysfunction rates. The drawbacks of the latter procedure include rare ileostomy complications during pregnancy such as obstruction and stoma related problems [18], technical difficulties in creating a functioning pouch several years after the initial surgery and the patient's reluctance to have a long-term stoma.

Women with IBD have also been found to have higher rates of cervical dysplasia compared with the general population, with an increased risk based on the use of immunosuppressants [19] and infliximab [20]. It is recommended that women with IBD, regardless of medication status, have annual papanicolau smears and young women should receive the human papilloma virus vaccine.

Men

In men with IBD, the true rate of fertility is not known and is a difficult endpoint to measure. A case–control study of 42 married men with CD versus 42 married healthy controls noted that prior to diagnosis, both groups had similar numbers of children (1.2 vs 1.5); however, after disease diagnosis, there was a statistically lower number of children born to men with CD than to their age-matched controls (0.4 vs 0.8, $p < 0.05$) [21]. This finding was independent of site of disease and medical therapy with sulfasalzine or steroids. In a survey of 106 men with CD, 62 men with UC and 140 controls, the mean number of pregnancies for the CD patients was significantly lower than the number for controls ($p < 0.02$) [22]. However, in UC patients, the number was not statistically different. Fecundability (the probability of pregnancy per menstrual cycle with unprotected intercourse) was similar between IBD patients and controls, suggesting a high rate of voluntary infertility in men with CD. Surgery, particularly in the pelvis, can also lead to issues with fertility, although this is not well studied in men. Men who undergo IPAA for UC may experience retrograde ejaculation and erectile dysfunction [23], but overall male sexual function is reported to improve after IPAA [24]. In general, men with IBD in remission or with mild disease activity had similar self-reported sexual function to men without IBD [17].

Semen quality is a surrogate marker for fertility and is measured by volume, concentration, motility, progression, total motile count and normal oval forms [25]. These factors are all important in determining fertility, although no single measure is diagnostic of infertility. A small case series noted a 46% rate of oligospermia and reduced sperm motility in men with CD [26]. Abnormal semen quality in CD has been associated with disease activity [27], poor nutritional status [26] and zinc deficiency [28]. These data suggest that being in remission – with inactive disease and good nutritional status – is important for sperm health and, by extrapolation, conception. Therefore, continuing medications to maintain remission during the conception period is of benefit, provided that the medications themselves do not affect fertility or pregnancy outcome.

Overall, patients with IBD have similar fertility rates to the general population. However, surgery, disease activity and certain medications may impact fertility and sexual

function. If a couple is having difficulty conceiving despite a 6 month trial, a referral should be made to a fertility specialist to determine if a correctable cause can be found and whether alternative methods of conception can be offered.

Pregnancy outcomes

The perinatal outcomes of the children of fathers with UC and CD have not been shown to differ from those of the general population [29]. However, in women, population-based studies have shown an increased risk of preterm birth, low birth weight and small for gestational age infants [30–32]. Cesarean sections are also more common in women with IBD [31]. Whether there is an increase in congenital anomalies is unclear and may be related to medication use (see the section on medication below).

A population-based cohort study by Dominitz *et al.* [33] used the computerized birth records of Washington State to compare pregnancy outcomes in 107 UC and 155 CD patients with 1308 controls. Women with CD had significantly higher rates of preterm delivery, low birth weight and small for gestational age infants than the controls. Women with UC, on the other hand, had similar rates to controls, but a significantly higher rate of congenital malformations (7.9 vs 1.7%). The study did not account for medication use and the results have not been replicated in other studies. The Hungarian Case Control Surveillance of congenital anomalies was queried from 1980–1996 [34]. The OR of congenital anomalies in UC patients versus controls was 1.3 (0.9, 1.8), adjusted for parity, age and medication use. However, the risk of limb deficiencies, obstructive urinary congenital abnormalities and multiple congenital abnormalities were increased with OR = 6.2 (95% CI = 2.9–13.1), OR = 3.3 (95% CI = 1.1–9.5) and OR = 2.6 (95% CI = 1.3–5.4), respectively. A case–control study in Italy [35] studied 502 pregnancies in 199 women prior to a diagnosis of IBD and 121 pregnancies in 90 patients after diagnosis of IBD and compared them with 996 and 204 pregnancies, respectively, in the non-IBD control population. Prior to diagnosis, women with CD had higher rates of preterm delivery and low birth weight. After diagnosis, low birth weight was more common among CD patients than in UC patients or controls. In post-diagnosis pregnancies, a higher incidence of congenital anomalies was found in IBD patients (5.5 vs 0.0%) versus controls, with no difference in rates between UC and CD. However, this is likely spurious, as a rate of 0% is not the population norm for congenital anomalies. Also, there was no difference in the rate of congenital anomalies between pre- and post-diagnosis IBD pregnancies, suggesting that the apparent increase in congenital anomalies in the post-diagnosis IBD patients is due to an unusual lack of anomalies in the control population.

A population-representative cohort study of women with IBD in the Northern California Kaiser population [36] compared women with IBD ($n = 461$) matched to controls ($n = 495$) by age and hospital of delivery. Women with IBD were more likely to have a spontaneous abortion, OR = 1.65 (95% CI 1.09–2.48), an adverse pregnancy outcome (stillbirth, preterm birth or small for gestational age infant), OR = 1.54 (95% CI 1.00–2.38) or a complication of labor, OR = 1.78 (95% CI 1.13–2.81). However, there was no difference in the rate of congenital malformations in IBD patients versus controls or individually among CD and UC patients. Independent predictors of an adverse outcome included a diagnosis of IBD, a history of surgery for IBD and non-Caucasian ethnicity. Severity of disease and medical treatments were not associated with an adverse outcome.

A meta-analysis by Cornish *et al.* [37] combined 12 studies totaling 3907 patients with IBD. A clear increase in preterm birth, OR = 1.87 (95% CI 1.52–2.31), low birth weight, OR = 2.1 (95% CI 1.38–3.19), and Cesarean section, OR = 1.5 (95% CI 1.26–1.79), was seen. The risk of congenital anomalies was also increased, with an OR of 2.37 (95% CI 1.47–3.82). The difference was seen in patients with UC, not CD, and was primarily based on the Dominitz study reported above [33] and an older study by Larzilliere and Beau [38]. Overall, the studies regarding the risk of congenital anomalies among the progeny of women with UC are mixed, with some suggesting an increased risk overall [33,38] or for particular anomalies [34], whereas other studies do not suggest an increased risk at all [36]. If there is a risk, the roles of medications, disease activity and other possible contributory factors need to be more clearly defined.

Disease activity

Effect of pregnancy on IBD

In general, women with IBD are as likely to flare during pregnancy as they are to flare when not pregnant. Nielsen *et al.* reported an exacerbation rate of 34% per year during pregnancy and 32% per year when not pregnant in women with UC [39]. Pregnant women with CD also had similar rates of disease exacerbation [40]. In the Kaiser population [36], the majority of patients had inactive disease throughout their pregnancy with no sudden increase in the post-partum (Figure 39.1). This is consistent with other published studies that found the rate of disease flare during pregnancy (26–34%) to be similar to the rate of flare in the non-pregnant IBD population [39,41,42]. Although breastfeeding has anecdotally been associated with an increase in disease activity in the post-partum, this has not been shown to be a contributing factor independent of medication cessation done to facilitate breastfeeding [43].

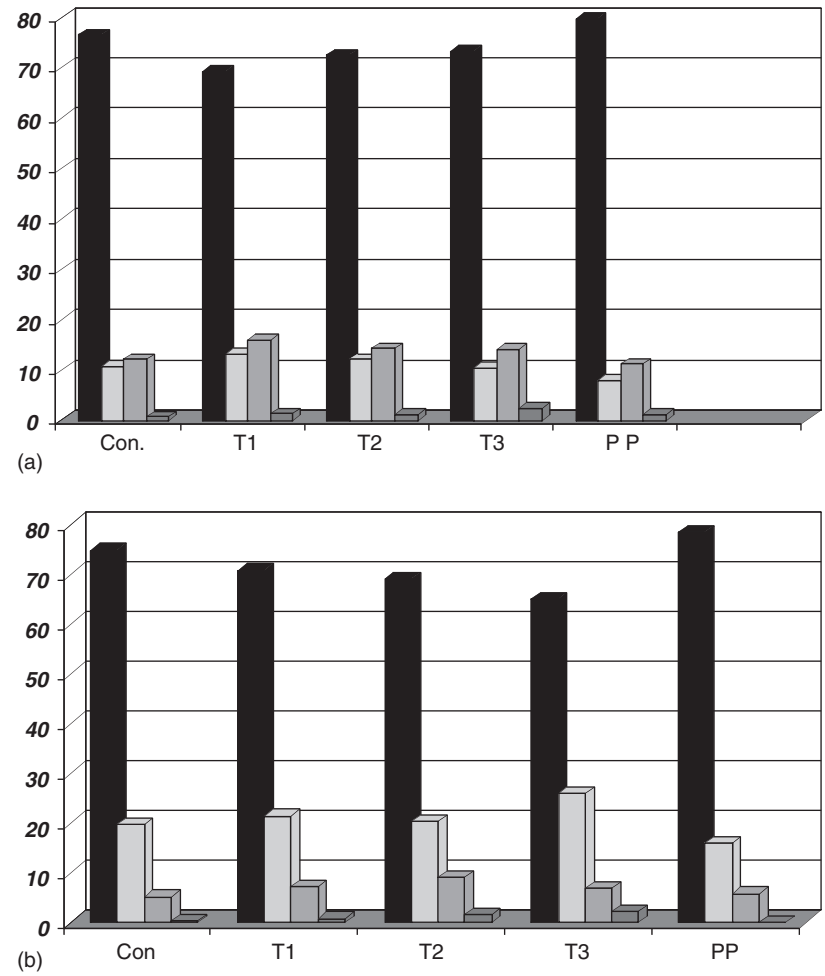

Figure 39.1 Level of disease activity at conception (Con), trimester (T) 1, 2 and 3 and in the post-partum (PP) among patients with Crohn's disease (a) and ulcerative colitis (b). From left to right, the bars represent inactive, mild, moderate and severe disease activity [36].

Disease activity may even be slightly lower during pregnancy [44]. One study found that the rate of relapse may decrease in the 3 years following pregnancy [45]. This was further supported by a study from a 10 year follow-up of a European cohort of patients with 580 pregnancies [46]. Patients with CD who were pregnant during the course of their disease did not have higher rates of stenosis (37 vs 52%, $p = 0.13$) or resection (0.52 vs 0.66%, $p = 0.37$). The rates of relapse decreased in the years following pregnancy in both UC (0.34 vs 0.18 flares per year, $p = 0.008$) and CD patients (0.76 vs 0.12 flares per year, $p = 0.004$). Although the etiology for this is not understood, a possible factor inducing quiescent disease may be disparity in HLA class II antigens between mother and fetus, suggesting that the maternal immune response to paternal HLA antigens may result in immunosuppression that affects maternal immune-mediated disease. This has been demonstrated in rheumatoid arthritis [47] in addition to IBD [48].

Effect of disease activity on pregnancy

Earlier studies suggested that disease activity was a predictor of adverse outcome in pregnancy. Disease activity at conception has been associated with a higher rate of fetal loss [42] and preterm birth [39]; disease activity during pregnancy was associated with low birth weight and preterm birth [49,50]. Other potential predictors of an adverse outcome include ileal CD [51] and previous bowel resection [36,51].

However, in the Kaiser population [36], disease activity was not predictive of an adverse outcome in any category. Even when limited to the presence of moderate to severe disease activity, there was still no association with an adverse outcome. The majority of patients with both UC and CD, however, did have inactive or mild disease throughout pregnancy. Similarly, a population-based study from Denmark [52] reported that women with active disease had adjusted risks of low birthweight, low birthweight at term, preterm birth and congenital anomalies of 0.2 (95% CI 0.0–2.6), 0.4 (95% CI 0.0–3.7), 2.4 (95% CI 0.6–9.5) and 0.8 (95% CI 0.2–3.8), respectively. However, the crude risk of preterm birth was 3.4 (95% CI 1.1–10.6) in those with moderate–high disease activity. Overall, these two population-based studies do not show a significant role of disease activity in predicting adverse outcomes above that expected with the diagnosis of IBD alone.

Labor and delivery

There is an increased rate of Cesarean sections in women with IBD [31]. In general, the decision to have a Cesarean section should be made on purely obstetric grounds. The two exceptions are active perianal disease and the presence of an ileoanal pouch. If a patient has inactive perianal disease or no history of perianal disease, they are not at increased risk for perianal disease after a vaginal delivery [53]. However, if they have active perianal disease, they can risk aggravating their injury with a vaginal delivery. One report noted an increased incidence of perianal disease following episiotomy [54], but this has not been replicated in other studies.

Patients who have an IPAA can have a normal vaginal delivery without fear of damaging the pouch [55]. However, the concern with vaginal delivery is damage to the anal sphincter. While pouch function may deteriorate during pregnancy, after pregnancy it reverts to the pre-pregnancy state [55], but over time damage to the anal sphincter may be compounded by aging and the effects on the pouch will not be seen for several years. The patient, their obstetrician and their surgeon should discuss the theoretical risk to long-term pouch function prior to making a decision on mode of delivery.

Medications

The use of medications during the conception period and pregnancy is a cause of great concern for patients and the physicians caring for them. Overall, the majority of medications used for the treatment of IBD are not associated with significant adverse effects and maintaining the health of the mother remains a priority in the management of these patients. The United States Food and Drug Administration (FDA) classification of drugs offers a guide to the use of medications during pregnancy. The FDA categories are listed in Table 39.1 and are noted for each drug discussed. Table 39.2 summarizes the safety of IBD medications for pregnancy and breastfeeding.

Table 39.1 Food and Drug Administration categories for the use of medications in pregnancy [165].

FDA category	Definition
A	Controlled studies in animals and women have shown no risk in the first trimester and possible fetal harm is remote
B	Either animal studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women or animal studies have shown an adverse effect that was not confirmed in controlled studies in women in the first trimester
C	No controlled studies in humans have been performed and animal studies have shown adverse events or studies in humans and animals not available; give if potential benefit outweighs the risk.
D	Positive evidence of fetal risk is available, but the benefits may outweigh the risk if life-threatening or serious disease.
X	Studies in animals or humans show fetal abnormalities; drug contraindicated

Women

Aminosalicylates

All aminosalicylates (sulfasalazine, mesalamine, balsalazide) are pregnancy category B except olsalazine, which is pregnancy category C. Sulfasalazine is composed of 5-aminosalicylic acid azo-bonded to sulfapyridine. Initial case reports suggested sulfasalazine teratogenicity with evidence of cardiovascular, genitourinary and neurologic defects [56–58]. However, a larger series of 181 pregnant women did not note an increase in congenital anomalies [59]. A population-based study using the Hungarian Case Control Surveillance of Congenital Abnormalities database [60] also did not find a significant increase in the prevalence of congenital abnormalities in the children of women treated with sulfasalazine. Given the concern over potential anti-folate effects of the drug, it is recommended that women take folic acid 1 mg twice daily in the prenatal period and throughout pregnancy. Breastfeeding is also considered low risk with sulfasalazine. Unlike other sulfonamides, bilirubin displacement, and therefore kernicterus, doe not occur in the infant [61]. This may be due to negligible transfer via breast milk.

Case series of mesalamine use in pregnancy do not suggest an increased risk to the fetus [62–64]. This has been supported by a prospective controlled trial of 165 women exposed to mesalamine compared with matched controls with no exposure [65] and a population-based cohort study from Denmark [66]. Neither trial demonstrated teratogenic risk, but there was an increased risk of premature birth, low birth weight and stillbirth. The latter complications may reflect disease effect because the mesalamine group had IBD and the non-exposed group was from the general population.

Breastfeeding while on aminosalicylates has been associated with diarrhea in the infant [67]. Women can breastfeed while being treated with 5-aminosalicylates, but infants should be observed for a persistent change in stool frequency.

Antibiotics

Metronidazole is a pregnancy category B drug. Multiple studies have suggested that prenatal use of metronidazole is not associated with birth defects. These studies include two meta-analyses [68,69], two retrospective cohort studies [70,71] and a prospective controlled study of 228

Table 39.2 Medications used in the treatment of inflammatory bowel disease.

Drug	FDA category	Recommendations for pregnancy*	Breastfeeding [78]
Adalimumab	B	Limited human data: low risk Likely crosses placenta	No human data; probably compatible
Alendronate	C	Limited human data; animal data suggest risk	No human data; probably compatible
Azathioprine/6-mercaptopurine	D	Data in IBD, transplant literature suggests some risk, but low	No human data; potential toxicity
Balsalazide	B	Low risk	No human data; potential diarrhea
Budesonide	C	Data with inhaled drug low risk. No human data for oral drug	No human data
Ciprofloxacin	C	Avoid: potential toxicity to cartilage	Limited human data; probably compatible
Corticosteroids	C	Low risk: possible small risk of cleft palate, adrenal insufficiency, premature rupture of membranes	Compatible
Cyclosporin	C	Low risk	Limited human data; potential toxicity
Fish oil supplements	–	Safe. Possibly beneficial	No human data
Infliximab	B	Low risk: limited human data; crosses placenta and detectable in infant after birth	Limited human data; probably compatible
Mesalamine	B	Low risk	Limited human data; potential diarrhea
Methotrexate	X	Contraindicated: teratogenic	Contraindicated
Metronidazole	B	Given limited efficacy in IBD, would avoid in first trimester	Limited human data; potential toxicity
Olsalazine	C	Low risk	Limited human data; potential diarrhea
Risedronate	C	Limited human data.	Safety unknown
Rifaximin	C	No human data. Animal data report some risk	Safety unknown
Sulfasalazine	B	Low risk. Give folate 2 mg daily	Limited human data; potential diarrhea
Tacrolimus	C	Low risk	Limited human data; potential toxicity
Thalidomide	X	Contraindicated: teratogenic	No human data; potential toxicity

*Low risk is defined as "the human pregnancy data do not suggest a significant risk of embryo or fetal harm."

women exposed to metronidazole during pregnancy [72]. A population-based case–control study found that overall teratogenic risk was low, but infants of women exposed to metronidazole in the second to third months of pregnancy had higher rates of cleft lip with or without cleft palate [73]. This increase was slight and not believed to be clinically significant.

Metronidazole is excreted in breast milk. If a single dose of metronidazole is given, the American Academy of Pediatrics (AAP) recommends that breastfeeding should be suspended for 12–24 h [74]. Potential toxicity exists for longer term use of metronidazole and it is not compatible with breastfeeding.

Quinolones (e.g. ciprofloxacin, levofloxacin, norfloxacin) are pregnancy category C drugs. Quinolones have a high affinity for bone tissue and cartilage and may cause arthropathies in children [75]. The manufacturer reports damage to cartilage in weight-bearing joints after quinolone exposure in immature rats and dogs. However, a prospective controlled study of 200 women exposed to quinolones [76] and a population-based cohort study of 57 women exposed to quinolones [77] did not find an increased risk of congenital malformations. Overall, the risk is believed to be minimal, but given safer alternatives, the drug should be avoided in pregnancy. The data on breastfeeding are limited, but quinolones are probably compatible with use [78].

Rifaximin is a pregnancy category C drug. This is a new agent and little information exists on safety in pregnancy. Rifaximin has not been found to affect fertility or pregnancy outcome in rats [79] or to cause teratogenic complications in rats and rabbits in one study [80], although other studies have noted teratogenicity in rats and rabbits, including cleft palate and incomplete ossification [81]. Safety in breastfeeding is unknown.

In general, given the limited evidence of benefit of these agents in IBD and the extended duration of use in the treatment of CD and UC, they should be avoided during pregnancy. Short courses for the treatment of pouchitis can be considered based on the safety data presented previously. An alternative antibiotic for pouchitis is *amoxicillin/clavulanic acid*, a pregnancy category B drug. A population-based case–control study [82] and a prospective controlled study [83] did not show evidence of increased teratogenic risk and it is compatible with breastfeeding.

Corticosteroids

Corticosteroids are pregnancy category C drugs. A case–control study of corticosteroid use during the first

trimester of pregnancy noted an increased risk of oral clefts in the newborn [84]. This was confirmed by a large case–control study [85] and a meta-analysis that reported a summary OR for case–control studies examining the risk of oral clefts [3.35 (95% CI 1.97–5.69)] [86]. However, the overall risk of major malformations was low [1.45 (95% CI 0.80–2.60)]. A prospective controlled study of 311 women who received glucocorticosteroids during the first trimester did not note an increased rate of major anomalies and no cases of oral cleft were reported [87]. The study was powered to find a 2.5-fold increase in the overall rate of major anomalies. An increased risk of premature rupture of membranes and adrenal insufficiency in the newborn has been reported in the transplant setting [88]. Overall, the use of corticosteroids poses a small risk to the developing infant and the mother needs to be informed of both the benefits and the risks of therapy. Prednisone and prednisolone are compatible with breastfeeding.

There are no published data on the safety of oral *budesonide* in pregnancy. Inhaled or intranasal budesonide is not associated with adverse fetal outcomes based on large clinical series [89,90]. Safety in lactation is not known.

Bisphosphonates

The bisphosphonates alendronate and risedronate are pregnancy category C drugs and the safety in breastfeeding is unknown. Many patients with IBD are started on these medications in conjunction with corticosteroids for prevention of bone loss. Both agents should be avoided in pregnancy because animal studies have shown that alendronate crosses the placenta and is stored in fetal bone, causing anatomic changes [91]. The effects on human fetal bone development are unknown. The half-life of alendronate is more than 10 years and it accumulates in bone. The concern in giving this agent to a woman of child-bearing potential is that the drug is slowly released from bone and may result in a low level of continuous exposure to the fetus throughout gestation. Risedronate has a reported half-life of 20 days. However, an ongoing study by the manufacturer suggests that the half-life may be significantly longer. Although the one study of 24 pregnancies exposed to alendronate did not report an increased risk of adverse events [92], the long-term application of bisphosphonates in women of child-bearing potential should be done with caution and under the guidance of an endocrinologist.

Immunomodulators

The immunomodulators are the most controversial agents used in the treatment of the pregnant woman with IBD.

Methotrexate

Methotrexate, a pregnancy category X drug, is clearly teratogenic and should not be used in women considering conception. Methotrexate is a folic acid antagonist and use during the critical period of organogenesis (6–8 weeks post-conception) is associated with multiple congenital anomalies collectively called methotrexate embryopathy or the fetal aminopterin–methotrexate syndrome [78]. The syndrome is characterized by intrauterine growth retardation, decreased ossification of the calvarium, hypoplastic supraorbital ridges, small, low-set ears, micrognathia, limb abnormalities and sometimes mental retardation [93]. Exposure in the second and third trimesters may be associated with fetal toxicity and mortality [78]. Methotrexate may persist in tissues for long periods and it is suggested that patients wait at least 6 months from the discontinuation of the drug before attempting conception.

Methotrexate is excreted in breast milk and may accumulate in neonatal tissues. The AAP classifies methotrexate as a cytotoxic drug with the potential to interfere with cellular metabolism [94]. It is contraindicated in breastfeeding.

Azathioprine/6-mercaptopurine

6-Mercaptopurine (6MP) and its prodrug azathioprine (AZA) are pregnancy category D drugs. Animal studies have demonstrated teratogenicity with increased frequencies of cleft palate, open-eye and skeletal anomalies seen in mice exposed to AZA and cleft palate, skeletal anomalies and urogenital anomalies seen in rats [95]. Transplacental and transamniotic transmission of AZA and its metabolites from the mother to the fetus can occur [96]. The oral bioavailability of AZA (47%) and 6MP (16%) is low, [95] and the early fetal liver lacks the enzyme inosinate pyrophosphorylase needed to convert azathioprine to 6MP. Both features may protect the fetus from toxic drug exposure during the crucial period of organogenesis.

The largest evidence on safety comes from transplantation studies, where rates of anomalies ranged from 0 to 11.8% and no evidence of recurrent patterns of congenital anomalies emerged [95]. A population-based cohort study from Denmark compared 11 women exposed to AZA or 6MP with the general population [97]. The adjusted OR for congenital malformations was 6.7 (95% CI 1.4–32.4). However, when a single severely ill patient with autoimmune hepatitis and multiple other medications was removed from the cohort, the OR was 3.4 (95% CI 0.4–27.3).

In IBD, multiple case series have not noted an increase in congenital anomalies [98–101], although one study did report a higher incidence of fetal loss in women with IBD with *prior* treatment on 6MP compared with those who never had 6MP exposure [102]. However, recently, a Danish nationwide cohort study [103] found that women with CD exposed to corticosteroids and AZA/6MP were more likely to have preterm birth (12.3 and 25%, respectively) compared with non-IBD controls (6.5%). Congenital anomalies were also more prevalent among AZA/6MP-exposed cases than the reference group (15.4 vs 5.7%) with an OR of 2.9 (95% CI 0.9–8.9).

However, only 26 women were exposed to AZA/6MP during conception versus 628 patients in the reference group and the authors controlled for "disease activity," which they defined as >2 or <2 admissions for disease exacerbation, accounting for only the most severe patients. Regardless, these data are cause for concern and certainly large prospective trials are needed.

Given the potential for severe toxicity in the breastfeeding infant, breastfeeding is not recommended [78]. However, recent small studies in IBD suggest that the overall exposure to the infant is low. Moretti *et al.* [104] reported four women breastfeeding on AZA. In two women, multiple breast milk samples did not have detectable levels of drug by high-performance liquid chromatography and none of the four infants had any complications. Two other studies measured metabolite levels in the breastfeeding infant. Gardiner *et al.* [105] reported four infants with undetectable metabolite levels despite mothers whose levels were in the therapeutic range. Sau *et al.* [106] collected 31 samples from 10 breastfeeding women on AZA/6MP. Only two samples had low levels of 6MP in breast milk (1.2 and 7.6 ng ml^{-1} in one patient versus a serum level of 50 ng ml^{-1}). There were no detectable 6-thioguanine nucleotide (6TGN) or 6-methylmercaptopurine (6MMP) levels in the 10 infants, nor were there signs of hematologic or clinical immunosuppression. Overall, these three studies suggest that transfer of drug to the breastfeeding infant is minimal. The risks and benefits of breastfeeding must be considered carefully; however, at present there does not appear to be an absolute contraindication to breastfeeding.

Cyclosporin and tacrolimus

Cyclosporin is a pregnancy category C drug. A meta-analysis of 15 studies of pregnancy outcomes after cyclosporin therapy reported a total of 410 patients with data on major malformations [107]. The calculated OR of 3.83 for malformations did not achieve statistical significance (95% CI 0.75–19.6). The rate of malformations was 4.1%, which is not different from the general population. The conclusion of the study was that cyclosporin did not appear to be a major human teratogen. In a study published in the obstetric literature [108], a retrospective review of 38 pregnancies in 29 women between 1992 and 2002 was conducted. There were 4 spontaneous abortions and 10 first-trimester terminations for worsening liver function. The mean gestational age was 36.4 weeks and there were no intrauterine or neonatal deaths. Five minor congenital anomalies were noted. The investigators concluded that planned pregnancy at least 2 years after liver transplantation with stable allograft function and continued immunosuppression had an excellent maternal and neonatal outcome.

There are several case reports of successful cyclosporin use during pregnancy to control UC and complete the pregnancy [109–111]. In the setting of severe, corticosteroid-refractory UC, cyclosporin may be a better option than colectomy given the substantial risk to the mother and fetus of surgery during this time.

Cyclosporin is excreted into breast milk in high concentrations. Therefore, the AAP considers cyclosporin to be contraindicated during breastfeeding due to the potential for immune suppression and neutropenia.

Tacrolimus is also a pregnancy category C drug. The earliest experience with this medication was in 1997, with a report of 27 pregnancies with exposure to tacrolimus [112]. Two infants died at weeks 23 and 24, but the mean gestational period was 36.6 weeks. There was a 36% incidence of transient perinatal hyperkalemia. One newborn had unilateral polycystic renal disease. Another study from Germany reported on 100 pregnancies in transplant recipients followed up from 1992 to 1998 [113]. There was a 68% live birth rate, a 12% spontaneous abortion rate and a 3% stillbirth rate; 59% of the infants were premature. Malformations occurred in four neonates with no consistent defects. In a later single-center experience, 49 pregnancies in 37 women over 13 years were followed up prospectively [114]. Thirty-six women survived the pregnancy and two premature babies were seen. One infant died of Alagille syndrome; the rest survived and 78% were of normal birth weight. No other congenital abnormalities were noted. A single case report of a patient with UC who had a successful pregnancy on maintenance tacrolimus was recently published [115]. No other data on IBD have been published so far.

Tacrolimus is contraindicated in breastfeeding because of the high concentrations found in breast milk.

Thalidomide

Thalidomide, a pregnancy category X drug, has some anti-tumor necrosis factor (TNF) effects and has been used successfully for the treatment of CD [116]. However, its teratogenicity has been extensively documented and includes limb defects, central nervous system effects and abnormalities of the respiratory, cardiovascular, gastrointestinal and genitourinary systems [78]. Thalidomide is contraindicated during pregnancy and in women of childbearing age who are not using two reliable methods of contraception for 1 month before starting therapy, during therapy and for 1 month after stopping therapy [117]. There are no human data on breastfeeding, but it is not advised given the potential toxicity.

Biologic therapy

Infliximab

Infliximab, a pregnancy category B drug, is used for the management of CD [118] and UC [119]. Infliximab is an IgG1 antibody, which does not cross the placenta in the first trimester, but very efficiently crosses the placenta in the third trimester [120]. While this protects the infant from exposure during the crucial period of organogenesis,

infliximab levels can cross easily in the third trimester and therefore be present in the infant for several months from birth. There is a growing body of evidence that suggests infliximab is low risk in pregnancy. There were four early case reports on patients with CD. In one case [121], the mother received infliximab during the conception period and first trimester, had active disease throughout and was also on azathioprine, metronidazole and mesalamine. The pregnancy ended in premature birth at 24 weeks and death of the infant 3 days later of intracerebral and intrapulmonary bleeding. In the other three cases, the pregnancy ended in a live birth; two infants were full-term and one was preterm at 36 weeks and the infants were healthy at last follow-up [122–124].

The two largest studies are from the TREAT Registry [125] and the Infliximab Safety Database [126] maintained by Centocor (Malvern, PA, USA). The TREAT Registry is a prospective registry of patients with CD. Patients may or may not be treated with infliximab. Of the 5807 patients enrolled, 66 pregnancies were reported, 36 with prior infliximab exposure. Fetal malformations did not occur in any of the pregnancies. The rates of miscarriage (11.1 vs 7.1%, $p = 0.53$) and neonatal complications (8.3 vs 7.1%, $p = 0.78$) were not significantly different between infliximab-treated and infliximab-naïve patients, respectively.

The Infliximab Safety Database is a retrospective data collection instrument. Pregnancy outcome data are available for 96 women with direct exposure to infliximab [126]. This was primarily exposure in during conception and the first trimester. When patients found that they were pregnant, the treatment was often stopped. The 96 pregnancies resulted in 100 births. The expected versus observed outcomes among women exposed to infliximab were not different from those of the general population. A series of 10 women with maintenance infliximab use throughout pregnancy was also reported [127]. All 10 pregnancies ended in live births, with no reported congenital malformations. Another series [128] reported 22 patients with exposure to infliximab within 3 months of conception, continued until 20 weeks of gestation, at which time the drug was stopped to minimize placental transfer. Several of the patients did have a flare of disease in the third trimester. There were three spontaneous abortions, one missed abortion, one stillbirth at 36 weeks (umbilical strangulation), two preterm births, three low birthweight infants and no congenital anomalies.

Infliximab crosses the placenta and is detectable in the infant for several months after birth. A case report [129] noted higher than detectable infliximab levels in an infant born to a mother on infliximab therapy every 4 weeks. The mother breast fed and continued to receive infliximab, but the infant's infliximab level dropped over 6 months, suggesting placental rather than breast milk transfer. The effect of the high infliximab levels on the infant's developing immune system is not known, although at 7 months the infant had appropriate responses to vaccination. In a case series [130] of six patients (four with CD, two with UC) receiving infliximab during pregnancy, all six patients delivered a healthy infant. The mothers were receiving infliximab 5 mg kg^{-1} every 8 weeks and the mean time between the last infusion and delivery was 64 days (range 2–120 days). The mean infliximab level at birth for the mother, the cord blood and the infant was 9.8, 10.2 and 15.5 μg ml^{-1}, respectively. It took anything from 2 to 7 months for the infant to have undetectable infliximab levels. In every instance, the levels in the infant were higher than in the mother at birth, supporting the fact that IgG1 antibodies are very efficiently transported across the placenta in the third trimester, but the infant reticuloendothelial system is too immature to clear the antibody effectively rapidly. Table 39.3 lists the individual levels for each mother and child.

So far, there has been no reported adverse event associated with elevated infliximab levels in the newborns. In our experience, infants exposed to infliximab *in utero* have appropriate response to standard early vaccinations. In adults receiving a similar agent, adalimumab, pneumococcal and influenza vaccinations were given safely and effectively [131]. However, live vaccinations such as varicella and smallpox are contraindicated in immunosuppressed patients, such as those on anti-TNF therapy [132]. Traditionally, the first live virus encountered by an infant was at 1 year of age (varicella, measles–mumps–rubella) when infliximab levels would be undetectable. However, now, rotavirus live vaccine is given at 2 months of age. Although it is given orally and is significantly attenuated, its safety in this setting is not known and the mother and pediatrician should be cautioned about its use.

Table 39.3 Infliximab (IFX) levels in mothers and infants exposed to therapy during pregnancy.

	Patient No.					
Property	1	2	3*	4*	5*	6
Mean maternal IFX level (μg ml^{-1})	21.3	2	8.3	5.7	6.6	15.3
Days from last infusion to birth	30	2	90	90	120	49
Maternal IFX levels at birth (μg ml^{-1})	15.1	1.4	19.2	3.8	4.8	14.5
Cord blood IFX (μg ml^{-1})	–	2.0	26.5	3.3	8.8	20.5
Newborn IFX at birth (μg ml^{-1})	25.3	2.9	23.6	4.2	8.7	28.2
Month from birth IFX undetectable	5	2	7	2	3	–

*Breastfed.

It is not known whether infliximab is excreted in human milk or absorbed systemically after ingestion. The only available study on infliximab in breast milk found that levels were either not present or were too low to be detected in the single patient studied [133]. Case reports of women who breast fed while on infliximab do not suggest toxicity or elevated infliximab levels in the infant [129,130] and it is considered compatible with breastfeeding.

Adalimumab

Adalimumab, a pregnancy category B drug, has recently demonstrated safety and efficacy for induction of remission in CD [134] and is FDA approved for this indication. Three case reports [135–137] document the successful use of adalimumab to treat CD during pregnancy, including one in which the patient received weekly dosing throughout pregnancy for a total of 38 doses [137]. OTIS (Organization for Teratology Information Specialists) reported 27 women enrolled in a prospective study of adalimumab in pregnancy and an additional 47 adalimumab-exposed pregnant women in a registry [138]. The rate of spontaneous abortion and stillbirth was similar to the diseased comparison and the general population. The rates of congenital malformation and preterm delivery are also within the expected range.

Adalimumab, an IgG1 antibody, would be expected to cross the placenta in the third trimester as infliximab does. However, as adalimumab levels cannot be checked commercially, this has not been confirmed. Adalimumab is considered compatible with breastfeeding, although there are no human data.

Certolizumab

Certolizumab pegol is a PEGylated Fab′ fragment of a humanized anti-TNFα monoclonal antibody. Studies have shown its efficacy for induction and maintenance of remission in CD [139,140], and it is currently under FDA review. A study of pregnant rats [141] receiving a murinized IgG1 antibody of TNFα and a PEGylated FAB′ fragment of this antibody demonstrated much lower levels of drug in the infant and in breast milk with the Fab′ fragment compared with the full antibody. However, one concern may be that the Fab′ fragment may also cross the placenta in low levels in the first trimester, which the IgG1 antibody should not do. Further data in humans are needed once the drug is commercially available.

Fish oil supplements

Many patients with IBD use fish oil supplements as an adjunct to standard medical therapy. Because this is a supplement and not a drug, it is not rated by the FDA. A randomized controlled trial of fish oil supplementation demonstrated a prolongation of pregnancy without detrimental effects on the growth of the fetus or on the course of labor [142]. Fish oil supplementation may also play a role in preventing miscarriage associated with the antiphospholipid antibody syndrome [143]. In women with IBD who may be at increased risk for preterm birth and miscarriage, fish oil supplementation is not harmful and may be of some benefit.

Medications during conception in men

Sulfasalazine has been clearly associated with infertility and abnormalities in sperm number, motility and morphology [144,145]. In a study of 21 patients on sulfasalazine, 86% had abnormal semen analysis and 72% had oligospermia [146]. The effect appears to be reversible: when men were switched from sulfasalazine to mesalamine, semen quality returned to normal [147,148]. An association between sulfasalazine use in the parent and congenital malformations in the progeny has been described [149]. As the lifespan of sperm is 120 days, men desiring conception should either discontinue sulfasalazine or switch to mesalamine at least 4 months prior to conception.

The effect of corticosteroid therapy alone on male fertility and congenital anomalies is not known. Burnell *et al.* [21] did not find an association between steroid therapy and infertility. Corticosteroids are currently used in the treatment of immunologic infertility with no evidence of an increase in congenital anomalies [150]. Based on the limited available data, corticosteroids can be continued during the conception period, keeping in mind that disease activity itself may have a negative impact on fertility and semen quality.

Methotrexate may cause reversible oligospermia in men [151]. There are no case reports to date of congenital anomalies resulting in the offspring of men on methotrexate. It is recommended that men stop methotrexate for at least 4 months before attempting conception.

AZA and 6MP do not appear to reduce semen quality in men with IBD versus men with IBD not on AZA [152]. However, both groups did have abnormal semen quality compared with normal controls. One case report describes a man with CD who had two successful conceptions prior to initiating 6MP, but had secondary infertility after 6MP [153]. Semen analysis revealed oligospermia with a concentration of $8000\,ml^{-1}$ and 90% total motility with 20% forward progressive motility. The couple was able to conceive successfully with intracytoplasmic sperm injection.

Animal data clearly demonstrate dominant lethal mutations in mice receiving AZA/6MP with resulting infertility [154]. A recent study in mice gave intraperitoneal injections of 6MP to male mice for 51 days [155], mated them with females after 45 days of treatment and then examined the products of conception at 13 days of pregnancy. It was found that treatment with 6MP did not affect sperm morphology or sperm production compared with controls. However, pregnancy rates were inversely related to escalating doses of 6MP. The abortion rate was

significantly higher in the 6MP group than the control group, but the incidence of major congenital malformation was the same. It was concluded that the high abortion rate coupled with the normal sperm morphology suggested more occult sperm damage at the genetic level.

This study suggests that semen analysis alone is inadequate to determine sperm damage in men on AZA. In a prospective study of 25 men with IBD [156], in remission, genetic damage among those exposed and unexposed to AZA/6MP was determined using sperm chromatin structure analysis (SCSA), an established technique to study male infertility [157]. SCSA uses flow cytometry to define abnormal chromatin structure as an increased susceptibility of sperm DNA to acid-induced denaturation, which reflects DNA breaks and alterations in the quantity of protamines and in composition and level of disulfide groups. There were 9 unexposed (controls) and 16 exposed patients. With respect to basic semen analysis, semen volume, sperm progression and total motile count were similar between the two groups. The percentage of normal oval forms (7% unexposed, 6.4% exposed) was also similar between groups, but is below the definition of normal for the general population (>14%), perhaps suggesting a role for IBD itself in reducing semen quality. Sperm motility, although above the range of normal in both groups (>50%), was significantly lower among those exposed to AZA/6MP (52 vs 65%, $p = 0.007$). The results of SCSA are presented as the DNA fragmentation index, which is the percentage of cells containing damaged DNA: <15% is excellent, 15–30% is good and >30% is poor. Although the mean for both groups was in the excellent range, there was a trend towards higher fragmentation in the exposed group (13.9 vs 9.1%, $p = 0.071$). Larger numbers of patients are currently being studied.

Reports in humans are mixed with respect to the occurrence of congenital malformations in the offspring of men on AZA/6MP. Two reports support an increased rate of congenital malformations [158,159] whereas a larger case series did not find a difference between fathers on 6MP at conception and those who conceived prior to starting the drug [99]. Recently, a population-based study from Denmark showed a trend towards increased congenital malformations in fathers on 6MP with an adjusted OR of 1.8 (95% CI 0.7–5.0) [160]. In exposed pregnancies, there was a 7.4% rate of congenital abnormalities versus 4.1% in controls. All congenital anomalies in the exposed group were in male infants. The use of AZA/6MP during the conception period remains controversial. At this time, there are no conclusive data to recommend stopping the medication during the conception period.

Infliximab appears to be low risk. Data in mice treated with an analogous antibody to mouse TNFα (infliximab cross-reactivity is limited to only humans and chimpanzees) reported no detrimental effects on male reproduction [161]. In a study by Katz *et al.* of 10 men who received infliximab near the time of conception, there was no increase in congenital anomalies in the progeny [126]. A study of 10 men on infliximab therapy noted a significant increase in semen volume after infliximab infusion and a trend towards a reduction in sperm motility or the percentage of sperm that show flagellar motion [162]. Sperm concentration remained normal; however, in patients receiving infliximab maintenance therapy, there was a significant decrease in the percentage of normal oval forms after infusion, a phenomenon not observed in infliximab-naïve patients. This small study suggests that infliximab may affect sperm morphology in addition to sperm motility and that the effect on morphology is more profound with increased exposure to infliximab. It has also been demonstrated *in vitro* that TNFα effectively and dose dependently inhibits germ cell apoptosis in human seminiferous tubules [163]. Furthermore, in rat seminiferous epithelium, this prosurvival effect can be blocked by infliximab [164]. This suggests that effective anti-TNF therapy in large enough doses may affect sperm count and thereby reduce fertility. The study of human semen quality on infliximab [162] did not show a change in sperm concentration with infliximab infusion. The lack of infliximab-associated changes in sperm concentration in this study could reflect a dose-dependent effect of infliximab on spermatogenesis, the lack of an *in vivo* apoptotic effect of infliximab on spermatogenesis or an insufficient sample size to define this phenomenon. In summary, infliximab treatment in men may decrease sperm motility and morphology. Whether or not these semen analysis findings translate into impaired fertility has not been formally examined. Therefore, at this time, it is not recommended that men receiving infliximab stop therapy if they are considering conception. The risks of stopping treatment include a flare of the underlying IBD and development of antibodies to infliximab that may preclude future use. If infertility is clinically evident and evaluation of the infertile couple suggests that semen quality is abnormal in the absence of other infertility risk factors, then consideration should be given to stopping infliximab treatment at that time.

Conclusion

The use of IBD medication during conception and pregnancy is generally low risk. For a drug to be clearly associated with congenital anomalies, the same defect must be seen repeatedly, a phenomenon not demonstrated with any IBD mediation except methotrexate and thalidomide, both of which are contraindicated. Sulfasalazine and methotrexate are associated with reversible sperm abnormalities in men and should be discontinued 4–6 months prior to conception. The risk of an adverse event must be weighed against the benefit to the health of the parent

from continuing their medication and controlling their underlying disease.

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Chapter 40
Inflammatory Bowel Disease in the Pediatric Population

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Summary

- IBD presents a major, lifelong health threat, challenging the psychological resources of both the affected child and the family. IBD frequently interferes with physical activities, limits social interactions, disrupts education, impairs growth, and delays puberty.
- Differential diagnosis of disorders resembling IBD is very important in treating pediatric patients.
- Highly specific serological tests can be useful adjunctive aids in discriminating IBD in its mild forms from functional bowel disorders.
- In the pediatric age group, it is essential to also ensure normal nutritional status, growth and development.
- Biologic therapies may be employed safely in the pediatric patient; however, specific attention must be paid to potential adverse effects.

Introduction

There are special challenges associated with the diagnosis and management of inflammatory bowel disease (IBD) in children and adolescents. When IBD occurs at the particularly vulnerable period of childhood and adolescence, potentially adverse effects on growth, quality of life and psychosocial functioning are likely. There are also many similarities in terms of the clinical features and therapeutic options, irrespective of the patient's age. In this chapter, we focus on the issues unique to the pediatric population. We will discuss issues pertaining to the transition between pediatric and adult centers of care. This chapter also emphasizes certain clinical dilemmas that are particularly important in this age group, including an approach to dealing with diagnostic uncertainty in the child with recurrent abdominal pain, therapeutics and adherence issues, in addition to psychosocial problems.

Epidemiology of pediatric inflammatory bowel disease

Aside from celiac disease, Crohn's disease (CD) and ulcerative colitis (UC) are the most common chronic immune-mediated bowel disorders of children and adolescents in North America and most of Europe. Between 1940 and 1993, an almost seven-fold increase in the incidence of CD was reported among a primarily Caucasian population in Olmsted County, MN, USA [1]. The peak incidence occurs between the ages of 15 and 25 years. Notably, the proportion of new cases diagnosed in individuals below age 20 years increased to 17% by 1990 [1]. Hence the median age at diagnosis has decreased. Rather than a true increase in disease incidence, data on the incidence of CD among children in Europe, the United States and Canada support the evidence for an earlier age at diagnosis [2]. Another study revealed that the incidence of CD (7–12 per 100,000) and UC (5–6.9 per 100,000) among African-American children in the state of Georgia was similar to that observed in the age-matched population overall [3], revealing that IBD is more prevalent among African-American children than previously thought. In a population-based study in Wisconsin, similar results were observed, with an incidence of IBD of about 7 per 100,000, irrespective of ethnic origin [4]. In our experience in Montreal, the incidence of new cases of pediatric CD has increased four-fold over the past two decades, whereas that for UC has not changed.

Clinical presentations specific to the pediatric population

The signs and symptoms of IBD are generally dependent upon the sites involved, their extent and severity, rather than the age of the patient. However, certain clinical presentations are either unique or more common to the

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Table 40.1 Clinical presentations of IBD particular to the pediatric age group.

Unique	Common
Growth failure	Recurrent abdominal pain
Delayed sexual maturation/puberty	Short stature
	Recurrent unexplained fever
	Arthralgias/arthritis
	Perianal disease
	Anorexia
	Recurrent aphthous mouth ulcers

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pediatric age group (Table 40.1). Notorious among these is growth failure, seen in up to half of patients at the time of diagnosis [5]. The exact prevalence depends upon the criteria used, varying from 36 to 88% for a decrease in height centile exceeding 1 SD (standard deviation) to a decrease in height velocity, respectively [6]. It is important to recognize that this mode of presentation, far more common in CD than UC, may occur in the absence of gastrointestinal complaints. Unfortunately, the paucity of associated gastrointestinal symptoms may lead to a delay in diagnosis, often entailing one or more years until IBD is suspected. Usually, patients have delayed puberty accompanying their poor growth. The management of such patients is a major challenge, in terms of both improving growth and the psychosocial implications of short stature and pubertal delay [6].

Other "atypical" clinical presentations which should raise suspicion of IBD in the pediatric age group are recurrent unexplained fever, arthralgias or arthritis, perianal disease (tags, fissures, abscesses and/or fistulae), mouth lesions such as aphthous ulcers [7] and unexplained anorexia.

Diagnostic approaches

The differential diagnosis of CD and UC in the pediatric age group is summarized in Table 40.2. When the clinical presentation is unambiguous, the recognition and diagnosis of IBD is straightforward in children and adolescents as it is in adults. Thus, for example, a diagnosis of colitis (UC or CD) is strongly suspected in patients who present with typical symptoms, such as bloody diarrhea, urgency and abdominal discomfort. These clinical findings are then promptly confirmed by standard radiological, endoscopic and histologic criteria and an accurate diagnosis is promptly arrived at [8]. On the other hand,

Table 40.2 Differential diagnosis of disorders resembling IBD in the pediatric age group.

Disorder	CD	UC
Infectious etiologies		
Acute appendicitis	++	−
Mesenteric adenitis	++	−
Enteritis (*Yersinia enterocolitica*, enteropathogeneic *E. coli*, *Campylobacter jejuni*, *Salmonella*, *Shigella*, *Entamoeba histolytica*, *Giardia lamblia*, *Dientamoeba fragilis*, *Mycobacterium tuberculosis*, etc.)	++	+
Pseudomembraneous or antibiotic-associated colitis	++	+++
Vascular disorders		
Hemolytic uremic syndrome, Henoch Schoenlein purpura, Behçet's disease, polyarteritis nodosum, systemic lupus erythematosis, ischemic bowel disease, dermatomyositis	+	+++
Immunodeficiency disorders (congenital, acquired)	++	++
Iatrogenic		
Radiation, chemotherapy (typhlitis), graft-vs-host disease	+	+
Obstetric and gynecological causes		
Ectopic pregnancy, ovarian cysts, tumors, endometriosis	+	+
Allergic		
Eosinophilic and allergic gastroenteropathies	+	+
Neuromuscular		
Hirschsprung's disease, pseudo-obstruction syndromes	+	++
Others		
Intussusception, Meckel diverticulum, tumors	+	++

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in children who present with non-specific and indolent intestinal and extra-intestinal symptoms that can be characteristic of both IBD and functional bowel disorders, a diagnostic challenge arises. In such cases, some clinicians may rely on invasive diagnostic testing, including at minimum a barium upper gastrointestinal (UGI) series and small bowel follow-through (SBFT), and also a complete colonoscopy with biopsies in order to confirm or exclude IBD. Performing a colonoscopy on adults over 50 years of age in the setting of a functional bowel disorder is perhaps justifiable on the basis of the merits of screening for colonic tumors. However, in children it is inappropriate to pursue these investigations in the setting where IBD is very unlikely. Generally, children suspected of IBD have not experienced significant health problems prior to onset of their symptoms. Therefore, one should consider the emotional impact of intrusive testing in the patient who very likely

Table 40.3 Contrasting diagnostic approaches to the child suspected of IBD.

A. Clinical index of suspicion high
Ileo-colonoscopy with multiple biopsies
Upper GI and small bowel follow through barium X-rays
Upper endoscopy with multiple biopsies (if clinically indicated)
Capsule endoscopy (if clinically indicated and a stenosis is excluded by a patency capsule or not suspected)
Rule out microbial causes
Exclude immunodeficiency disorder
Exclude autoimmune enteropathy
Exclude allergic disorder
Clinical index of suspicion low
Verify normal physical examination
Verify normal growth parameters
Carry out limited investigations:
CBC: hemoglobin, platelet count, CRP
Serum albumin, iron, ferritin
Other potential investigations:
Fecal markers of inflammation
Serological assays for IBD and for celiac disease
Abdominal ultrasound + Doppler assessment of mucosal vessel density

Modified from Seidman EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

has a functional bowel disorder. Given these clinical challenges, clinical investigators have searched for a marker or a combination of non-invasive tests that may enable clinicians to screen for IBD. The diagnostic approach to the child suspected of IBD should depend upon the level of suspicion (Table 40.3).

In this setting, infectious causes of chronic diarrhea are ruled out (Table 40.2), via stool cultures and search for ova and parasites. Assay for *Clostridium difficile* toxin is frequently indicated, in view of the fact that children are often exposed to antibiotic therapy. A complete blood count is usually obtained, with particular attention paid to the presence of a microcytic anemia and thrombocytosis, commonly seen in IBD [9]. In children suspected of IBD, the combination of anemia and thrombocytosis has been shown recently to have a positive predictive value of 90% for IBD [10]. Hypoalbuminemia, often observed in the presence of a protein-losing enteropathy, is also frequent, but not specific for IBD. Elevated levels of circulating markers of acute phase reactants, such as the erythrocyte sedimentation rate (ESR), C-reactive protein and orosomucoid, are more common in active CD than in UC [9]. In addition to their lack of specificity for IBD, results of these tests may be negative in up to one-third of patients [9–11].

Infectious enterocolitis should always be considered in the differential diagnosis of childhood IBD, even in established cases presenting with a clinical relapse. Most acute, infectious causes of diarrhea resolve after a few weeks. Exceptions include pseudomembranous or antibiotic-associated colitis, particularly prevalent in the pediatric age group. Our recent data show that the yield of *C. difficile* toxin increases when stool samples are obtained via colonoscopy and sent promptly to the laboratory on ice [8]. Infestation with *Giardia lamblia* or *Dientamoeba fragilis*, and also amoebic colitis, can also mimic IBD. The latter classically results in discrete, punched-out ulcers with a rolled, edematous margin. Rectal cultures for gonorrhea and serological testing for *Lymphogranuloma venereum* should be considered in the setting of sexually active patients. Rare causes of infection, such as *Cytomegalovirus*, *Cryptosporidium*, *Microsporidia* or *Stongyloides*, should be sought in the setting of congenital or acquired immunodeficiency disorders.

Highly specific serological tests can be useful adjunctive aids in discriminating IBD in its mild forms from functional bowel disorders [12,13]. Perinuclear anti-neutrophil cytoplasmic autoantibodies (pANCA) have been established as an autoimmune marker most characteristic of UC in the pediatric age group. On the other hand, antibodies to oligomannosidic epitopes of the yeast *Saccharomyces cerevisiae* (ASCA) have been shown to be a reliable marker of CD. Double positivity (both IgA and IgG) for ASCA was found to be 100% specific for pediatric CD [13]. The potential sensitivity of these markers as screening tests for IBD is maximized when the two assays are combined. Table 40.3 summarizes our approach to the child suspected of IBD, depending on the severity of symptoms and the level of clinical suspicion.

Serological testing, along with other biomarkers, comprises adjunctive tests that are insufficient in of themselves for a diagnosis of IBD. As in adults, the standard investigation of the child for whom the index of suspicion for IBD is high includes an ileocolonoscopy with multiple biopsies [8]. Barium UGI and SBFT is then generally used to define the extent of the disease in the small bowel or if strictures of the colon exist. We have previously shown that an upper endoscopy with routine esophago-gastro-duodenal biopsies can demonstrate proximal gastrointestinal CD missed by barium studies [14]. Indeed, the Porto criteria established by the IBD Working Group of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition recommends that an upper endoscopy be done systematically for diagnosis of pediatric CD or indeterminate colitis [15] (Table 40.3). Radiological assessments of the colon and terminal ileum are not adequate diagnostic substitutes for colonoscopy. Images on barium studies and computed tomography (CT) scans have been mistakenly diagnosed as IBD in infectious enteritis or colitis. A recent pediatric study using a new generation magnetic resonance imaging (MRI) with gadolinium contrast showed a high correlation with histology and could be considered

as a complementary tool to endoscopy and biopsies in classifying indeterminate colitis [16]. MRI was found to be inaccurate in terms of assessing inflammation severity [17]. The high false negative rate of technetium-labeled autologous white blood cell scintigraphy precludes its routine use to establish a diagnosis of IBD in children [18]. Scintigraphy can be useful in pediatric patients when total colonoscopy or ileoscopy could not be performed [19]. A recent pediatric study [20] showed that positron emission tomography (PET) offers a non-invasive tool for identifying and localizing inflammation in the bowel. Although active inflammation was identified in 80% of children with IBD and none of those with functional abdominal pain, no inflammatory controls were studies. Moreover, PET scans are not yet widely accessible.

Abdominal ultrasound can be very useful and an easily accessible non-invasive imaging test to screen for thickened loops of bowel. Doppler assessment of intestinal mucosal vessel density has been shown by our group to correlate with activity in CD [21]. We recently reported [22] that wireless capsule endoscopy is a safe and non-invasive method to investigate the entire small bowel. It revealed a diagnosis of jejuno-ileal CD or eosinophilic gastroenteritis in 60% of the cases where the traditional tests (ileocolonoscopy and UGI with SBFT) revealed lesions in only 25% of cases [22]. As in adult patients, a complete colonoscopy is imperative to obtain tissue confirmation of the diagnosis [8] and constitutes the most cost-effective strategy to determine the extent and severity of IBD in children [23]. Biopsies should be routinely taken, as histological features of UC and CD may be uncovered even in zones of macroscopically normal mucosa. In about 10% of cases of so-called indeterminate colitis, endoscopic and histological findings may be insufficiently distinctive to discern unequivocally ulcerative from Crohn's colitis. In such instances, determination of pANCA and ASCA serological tests can be helpful [12,13,24].

Rarely, IBD may present during infancy [25]. The clinical presentation resembles that seen in infants with autoimmune enteropathy [26] or immunodeficiency disorders [27], requiring careful exclusion of these possibilities (Table 40.3).

Disease activity markers

It is not uncommon to be faced with pediatric CD patients who manifest few symptoms, yet present with chronic anorexia and growth failure. It is generally not acceptable to such young patients to re-evaluate disease activity by repeating colonoscopic examination [8]. Follow-up endoscopic studies are reasonable in the case of indeterminate colitis, when surgery is contemplated. It is also justifiable in cases refractory to medical management, in order to exclude other disorders such as concomitant infections of the upper (*Helicobacter pylori*) or lower [*C. difficile*, cytomegalovirus (CMV)] gastrointestinal tract.

Biological markers such as the C-reactive protein, erythrocyte sedimentation rate or serum orosomucoid are often elevated. In addition, iron deficiency anemia, hypoalbuminemia and thrombocytosis may be found. However, these markers are inadequately specific to be utilized to reliably monitor disease activity. An increasing body of evidence suggests that serological markers can be used to predict the phenotype (fibrostenosing, penetrating/fistulizing) and the progression of the disease [28–30]. However, they are not useful as markers to follow disease activity [28]. Several other serological or fecal markers appear to be promising. Assays for fecal calprotectin or lactoferrin are sensitive markers of inflammation and its level correlates well with clinical disease activity indices [31,32].

Our approach employs intestinal wall vessel density as a function of disease activity, using pulsed color Doppler abdominal sonography [21]. Affected bowel loops are thicker in the group of pediatric patients with active CD ($p < 0.001$]. Vessel density is much more frequently moderate or high (2–4 and >5 vessels cm^{-2}, respectively) in active than in quiescent CD. This method is simple to perform in young patients, is non-invasive and accurately monitors the course of the disease [33].

Therapeutic approaches for the pediatric IBD patient

The major goals in managing IBD at any stage of life are to induce and maintain remission of disease activity and to assure an optimal quality of life. In the pediatric age group, it is essential also to ensure normal nutritional status, growth and development. However, the yet obscure etiology and pathogenesis of IBD, along with its highly variable severity, extent and clinical course, render it difficult to achieve an optimal outcome in all cases. Medical therapy for IBD has advanced remarkably in recent years [34,35]. The general approach to treating the child with IBD, as in adults, is based on both the severity of symptoms and the localization and extent of the disease. Relatively few controlled clinical trials have been carried out in children. The doses of the drugs commonly employed in the pediatric IBD population are summarized in Table 40.4. The special issues related to surgery in pediatric IBD are discussed below.

5-Aminosalicylates

As in adults, children with mild to moderate UC are typically treated with oral sulfasalazine or 5-aminosalicylate (5-ASA), either alone or in combination with topical 5-ASA and/or corticosteroid enemas. Young children often retain and tolerate suppositories or rectal foam better than

Table 40.4 Commonly employed medications for IBD in the pediatric age group.

Drug and dose	Potential side effects
• *Aminosalicylates*	
Sulfasalazine (40–60 mg kg^{-1} per day; tid)	Headaches, nausea, hypersensitivity reactions (skin), pancreatitis, pericarditis, granulocytopenia, thrombocytopenia
5-ASA/mesalamine/olsalazine (40–70 mg kg^{-1} per day; bid or tid)	
Topical 5-ASA (enema 2–4 g or suppository 0.5–1 g, both q 12–24 h)	Side effects of topical applications are seldom encountered
• *Corticosteroids*	
Prednisone (1–2 mg kg^{-1} max. 50 mg per day; qam or divided bid)	Acne, moon facies, striae, growth impairment, hypertension, aseptic necrosis or bone fractures, depression or other mood alterations, sleep disorder, osteopenia, myopathy
Topical enemas (hydrocortisone 50–100 mg q 12–24 h or methylprednisolone 10–40 mg in 30–60 ml 0.9% NaCl, q 12–24 h)	
Budesonide (9 mg per day; qam)	Budesonide: less systemic side effects
• *Immunomodulator therapy*	Idiosyncratic: pancreatitis, rash, fever
6-Mercaptopurine (1–1.5 mg kg^{-1} per day)*	Leukopenia, hepatitis (associated with increased metabolite levels)
Azathioprine (2–3 mg kg^{-1} per day)*	Nausea
Methotrexate (25 mg per 1.73 m^2 sc weekly)	Nausea; teratogenicity (methotrexate)
• *Immunosuppressive therapy*	Nephrotoxicity, headaches, paresthesias, hirsutism, oral thrush. Diabetes
Cyclosporin A or tacrolimus (dose adjusted according to drug levels)	
• *Biological therapy*	
Anti-TNFα (infliximab 5 mg kg iv, weeks 0, 2, 6)	Serum sickness-like reactions
(Adalimumab, 160 mg per 1.73 m^2 sc, followed by 80 mg per 1.73 m^2 sc, 2 weeks later)	Lupus-like syndrome
	Lymphoma
• *Antibiotics*	Opportunistic infections
Metronidazole (10–20 mg kg^{-1} per day)	Long-term use: peripheral neuropathy
Ciprofloxacin (restricted to >16 years; 250–750 mg bid, according to weight)	Bone pain; potential for altered bone health

*Dose adjusted according to TPMT genotype and metabolite levels.

Modified from Seidman EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

enemas. However, patients with refractory distal colitis may require high-dose prednisolone enemas in order to obtain remission off oral corticosteroids (Table 40.4). Although often employed, 5-ASA has not been proven to be effective in CD.

Corticosteroids

Patients with moderate to severe disease are generally managed with corticosteroids, orally or intravenously, depending upon symptom severity. Steroid use is associated with numerous side effects, which are often intolerable for many pediatric patients. These include exacerbation of acne, facial puffiness (moon face), hirsutism, striae, cataracts, aseptic necrosis of the hip or knee, growth impairment, hypertension, depression or other behavioral changes, sleep disturbance, myopathy, as well as osteoporosis with compression or pathological fractures (Table 40.4). Therefore, systemic corticosteroids are generally reserved for more severe disease and for a limited period if possible. Side effects can be reduced to a certain extent by adopting alternate-day dosing of prednisone and avoiding a nocturnal dose.

Budesonide is a well-absorbed and rapidly catabolized corticosteroid that has the advantage of causing fewer glucocorticoid-related systemic side effects. A randomized and controlled pediatric study [36] in active CD (CDAI >200) using the ileal release preparation observed a statistically comparable remission rate (55%) compared with standard prednisolone. Moreover, the budesonide-treated group had significantly fewer side effects and adrenal suppression (higher morning cortisol) [36]. The addition of antibiotics (metronidazole and ciprofloxacin) has not been shown to improve the remission rate compared with budesonide alone [37]. Thus, ileal release budesonide is effective and safe for distal ileal or ileocecal CD in children and adolescents, as in adults. Corticosteroids in the form of prednisone have not been shown to be of benefit as maintenance therapy. Alternate-day, low-dose prednisone has been proposed in order to reduce relapses, without inhibiting linear growth [38]. However, confirmation from randomized controlled trials is lacking. Ileal release budesonide at a dose of 6 mg per day has been shown to be effective as maintenance therapy in adult studies [39]. However, its use as maintenance therapy in the pediatric age group has not been studied.

Immunomodulatory drugs

6-Mercaptopurine (6-MP) and its parent drug azathioprine (AZA) are arguably the most effective immunosuppressive drugs for the long-term management of both CD

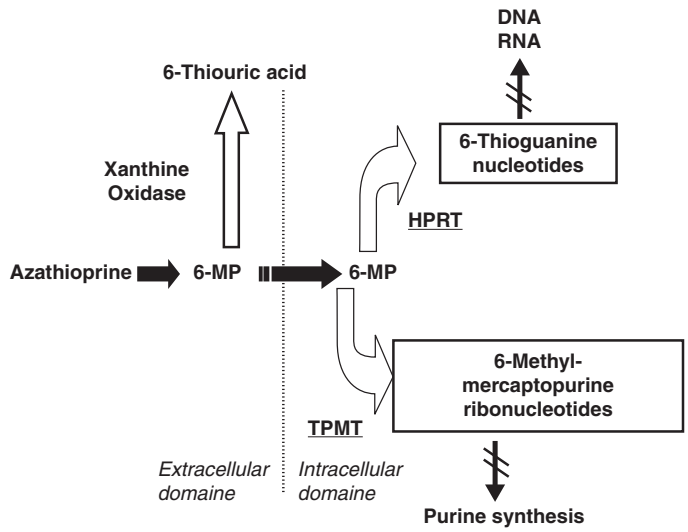

Figure 40.1 Pathways in the metabolism of azathioprine (AZA) and 6-mercaptopurine (6-MP). Oral AZA is rapidly converted to 6-MP by a non-enzymatic process. Initial 6-MP transformations occur along competing catabolic (XO, xanthine oxidase; TPMT, thiopurine methyltransferase) and anabolic (HPRT, hypoxanthine phosphoribosyltransferase) enzymatic pathways. The latter intracellular enzyme (dashed line) transforms the drug into 6-thioguanine nucleotides (6-TG), which have been shown to be the most important parameter associated with treatment efficacy. TPMT converts the drug into 6-methylmercaptopurine ribonucleotides (6-MMP). Patients heterozygous for a mutant allele of TPMT will convert a higher proportion of the drug into 6-TG. This translates into a higher success rate, but with an increased risk of myelosuppression. Reproduced from Seidman EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

and UC [40]. They are proven to be effective for steroid-dependent and also chronically active or steroid-resistant disease. An important pediatric trial showed that 6-MP can dramatically reduce the risks of relapse after steroid-induced remission in CD [41]. This study has revised the approach to the long-term management of CD, supporting the pre-emptive use of AZA or 6-MP in order to improve the natural history of the disease, effectively maintaining long-term remission while preventing steroid dependence or resistance for most cases [42]. Pharmacogenetic advances have led to the development of new strategies in order to optimize and individualize therapy with AZA and 6-MP, maximizing efficacy, while minimizing toxicity [40,43]. AZA is an inactive pro-drug that undergoes a series of enzymatic reactions via competing pathways, leading to two major metabolites (Figure 40.1). One route leads to the production of 6-thioguanine nucleotides (6-TG), shown to be the active metabolite [44]. However, excessive levels of 6-TG are potentially myelotoxic. In the competing pathway, thiopurine methyltransferase (TPMT) yields 6-methylmercaptopurine (6-MMP) ribonucleotides. The latter metabolites appear to be therapeutically inactive but potentially hepatotoxic at high levels [45]. Co-dominantly inherited polymorphic alleles confer variable TPMT enzyme activity levels, with about 11% of individuals heterozygous and 0.3% homozygous for common TPMT mutations. Such individuals have intermediate and low/absent TPMT activity, respectively [43]. Heterozygous patients with intermediate activity generate higher therapeutic 6-TG levels, but are at a greater risk for myelosuppression. The identification of a patient's TPMT genotype or phenotype is recommended by the US Food and Drug Administration (FDA) and the American Gastroenterological Association (AGA) to allow the physician to adjust the dose of the drug accordingly, avoiding early, potentially fatal, leukopenic events in patients with homozygous mutations in TPMT [40]. Patients with sub-therapeutic 6-TG levels, due to either under-dosing, poor compliance or excessive TPMT activity, with excessive 6-MMP and thus high 6-MMP/6-TG ratios (>30), are more likely to be refractory to therapy with these drugs [44–46]. Measuring 6-MP metabolite levels and TPMT activity provides clinicians with useful tools for optimizing therapeutic response to 6-MP/AZA and also for identifying individuals at increased risk for drug-induced toxicity [40,46].

Methotrexate is considered to be in the class of antimetabolite therapies, due to its antagonistic effect on folic acid metabolism. Among adults, almost 40% of steroid-refractory chronically active CD patients have been found to respond to weekly injections of methotrexate (25 mg). No similar, controlled trials have been done in the pediatric age group. In pediatrics, this therapy has generally been employed in moderate to severe CD, refractory to corticosteroids. In two open studies, the majority of pediatric patients achieved a sustained remission with methotrexate [47,48]. Hepatotoxicity is a major concern in long-term therapy. A study in children with juvenile rheumatoid arthritis showed that liver enzyme elevation more than 40% of the time was associated with an increased risk of fibrosis [49]. Obesity may add to the risk.

T lymphocyte immunosuppressive drugs

Cyclosporin A (CsA) is a fungal product whose immunomodulatory effects are achieved by blocking lymphocyte cytokine production, interleukin-2 (IL-2) in particular. This inhibits the activation and proliferation of T helper cells. The efficacy of intravenous CsA among adult patients with severe UC is well established. Although surgery can be often be avoided in the short term, the benefits of CsA must be weighed against the risk of severe

complications, including potentially life-threatening opportunistic infections and the theoretical risk of lymphoproliferative disease. In addition, most patients relapse upon CsA withdrawal. Thus, CsA is generally employed as a bridge to the longer term use of AZA or 6-MP. The latter drugs must be initiated several weeks prior to tapering the dose of CsA. CsA has also been used in CD, especially in cases with severe perianal or fistulizing disease. As noted for severe UC, it is primarily used as a bridge to therapy with a longer acting immunomodulatory therapy, usually with 6-MP/AZA or methotrexate. Intravenous CsA ($4\,mg\,kg^{-1}$ per day) was used in an open-label study in 10 children with severe Crohn's colitis refractory to steroids, with a 70% response in the short term [50]. However, 30% relapsed within 6 months and 6-MP-resistant patients did not respond. CsA may also be effective when given orally, if trough CsA levels between 250 and $400\,ng\,ml^{-1}$ are achieved. Side effects of CsA treatment include nephrotoxicity, headaches, paresthesias and infections (Table 40.4). Until further studies with longer follow-up have been conducted, the use of CsA should be restricted to those IBD patients with severe, steroid-resistant disease activity who require a drug with a rapid therapeutic onset. Other biological therapies, such as anti-tumor necrosis factor (TNF) antibodies, have largely replaced the use of CsA. Another T-lymphocyte inhibitory drug, tacrolimus or FK 506, is considered to be even more potent than CsA. Scant data are available with this drug in IBD, although case reports suggest a beneficial effect in fistulizing CD. An open-label study showed some benefit for oral tacrolimus over the short term for 9 of 14 pediatric cases with severe, steroid-refractory colitis [51]. Blood levels were maintained in the $10–15\,ng\,ml^{-1}$ range and azathioprine or 6-MP was added after about 6 weeks. However, 4 of 9 responders eventually required a colectomy. Thus, fewer than 50% of cases achieved long-term remissions.

Biological therapies

Monoclonal antibodies have been engineered specifically to target integral steps in the cascade that contributes to mucosal inflammation, either by blocking pro-inflammatory cytokines or by administering anti-inflammatory cytokines. Infliximab is a human/mouse chimeric anti-TNFα neutralizing antibody that received approval in both the United States and Canada for pediatric IBD. Studies in adult patients have demonstrated that this antibody is relatively highly effective for rapidly inducing remission in active CD and UC, and also for closing fistulas, particularly in the perineum. A recent controlled pediatric study using infliximab showed satisfactory results in induction and maintenance of remission in moderate to severe pediatric CD [52]. Patients receiving regular infusions every 8 weeks were more likely to have sustained remission than those on an every 12 week infusions.

Although relatively safe, infusion reactions to anti-TNF therapy may be encountered. We observed that fully humanized anti-TNF antibodies (adalimumab) are a safe and effective alternative in cases with infusion reactions or loss of response to therapy due to the formation of anti-infliximab antibodies [53]. An open-label study suggested that CDP571, a humanized anti-TNFα monoclonal antibody is generally well tolerated in pediatric CD [54]. However, proof of efficacy has not yet been established by randomized controlled study.

Recently, several cases of a very aggressive lymphoma (hepatosplenic T cell lymphoma) were reported, all in young patients treated with infliximab concomitant to thiopurines drugs [55]. Our recommendation in pediatric IBD is to avoid exposing pediatric patients to thiopurine drugs if infliximab is definitely to be employed or to stop the thiopurine when the biological treatment is initiated. In terms of concomitant immunomodulation to prevent the development of anti-infliximab antibodies, two options are used in our experience. The infliximab infusions can be used as monotherapy, scheduled on a regular basis (e.g. q 8 weeks) with a bolus of steroid pre-infusion or else by replacing the thiopurine drug with low dose oral methotrexate.

Natalizumab, a humanized monoclonal immunoglobulin-G4 antibody to alpha 4 integrin was tested recently in a single-arm, preliminary pediatric study [56]. A clinical response was observed in 55% of 31 adolescents with moderate to severe CD, while 29% achieved a remission. Although well tolerated, further randomized and controlled trials are needed to establish efficacy and long-term safety.

Antibiotics

As in adults, antibiotic therapy has been most often employed for perianal CD. In one report on 325 pediatric patients, 15% had perianal fistulae, while perirectal abscesses were encountered in 13% [57]. Metronidazole is most often used for this indication and also for treating colonic CD. However, the long-term use of metronidazole has been associated with peripheral neuropathy. Ciprofloxacin has also been advocated for the therapy of perianal CD, in addition to primary therapy. However, concerns about potential toxicity to cartilage in experimental animals has precluded the widespread use of this antibiotic in children under the age of 16 years or those less than Tanner stage IV [58]. In our experience, perianal disease often recurs after cessation of therapy with antibiotics. The use of azathioprine or 6-MP thus represents a better long-term strategy in most cases. Severe fistulizing disease is best managed with infliximab or with surgical drainage and seton placement in cases with perineal sepsis.

Assessment and management of malnutrition

Assessing the problem

The potential complications of growth impairment and pubertal delay are unique to pediatric IBD patients (Table 40.1). Weight loss is an extremely common finding at presentation, occurring in almost 90% of cases. In fact, inadequate energy intake often precedes other symptoms, such that by the time of diagnosis, a majority of pediatric patients are malnourished, with weight falling below the third percentile for age along with arrested growth or a deceleration in its velocity [6,59] An excellent estimation of the degree of acute malnutrition is obtained by calculating the percent ideal weight for height [60]. Very often, growth failure ensues after weight for height fall below 90% of ideal predicted. The causes of malnutrition in children with IBD are summarized in Table 40.5. The most important cause is inadequate intake of calories to meet the energy needs in the growing child. Anorexia is due to a combination of factors, including the abdominal pain and diarrhea brought on by food ingestion, nausea and early satiety, in addition to the pro-inflammatory cytokines that have a suppressive effect on appetite.

Growth and other nutritional parameters to be followed in all pediatric patients with IBD are summarized in Table 40.6. Updating percentile curves for weight and height, and also height velocity, should be part of every assessment at least biannually. Growth failure is characterized by a cessation of linear growth over a period of at least 6 months or by a decrease exceeding one or more standard deviations in height percentile. An easy bedside rule of thumb is that growth velocity generally exceeds 4 cm per year in prepubertal boys and 3.5 cm in girls. Peak height velocity occurs before menarche in female adolescents. Therefore, it is important to recognize and treat malnutrition before it is too late to achieve any catch-up. For many adolescent patients with IBD, impaired growth leading to short stature and the accompanying delayed maturation of secondary sexual characteristics may be more troubling and debilitating than their underlying disease. Therapy for correcting growth failure is detailed below.

Table 40.5 Known causes of undernutrition and growth failure in pediatric patients with IBD.

Potential factor	Details
Inadequate energy/ nutrient intake	Disease induced (cytokines, symptoms)
	Iatrogenic (unacceptable dietary restrictions)
Malabsorption	Diminished absorptive surface area
	Bacterial overgrowth
	Bile salt deficiency
Increased gut losses	Secretory losses: electrolytes, minerals, trace metals
	Protein-losing gastroenteropathy
	Bleeding
Drug–nutrient interactions	Corticosteroids (calcium, protein)
	Sulfasalazine (folate)
	Cholestyramine (fat, vitamins)
	Cyclosporin A (magnesium)
Increased requirements	Sepsis, fever
	Increased cell turnover
	Replace losses: catch-up growth

Reproduced from Seidman EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

Table 40.6 Nutritional parameters in pediatric patients with IBD.

Evaluation of growth/chronic malnutrition:
Height % for age
Growth velocity
Bone age
Bone density (DEXA adjusted to bone or height age)
Evaluation of acute malnutrition:
% Ideal weight for height
Serum albumin (secondary to protein-losing enteropathy)
Tricipital skinfold
Micronutrient parameters:
Iron, total iron binding capacity (TIBC), ferritin
Vitamins: folate, $B_{12,}$ A, D, E
Other minerals: Ca, P, Mg, Zn
Electrolytes (if profuse diarrhea)
Rare deficiencies: vitamin C, K, selenium, copper

Reproduced from Seidman EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

The proper management of short stature in the IBD child requires evaluation of its cause [6,61,62], as summarized in Figure 40.2. The clinician must ascertain whether the growth failure is due to inadequate intake of calories as a result of the IBD itself or to an unlikely hormonal deficiency (growth hormone, thyroxin or cortisol). Chronic inflammation and excessive production of pro-inflammatory cytokines may be contributory. Other causes include constitutionally delayed growth or genetic short stature. In cases due to acute malnutrition and inadequate caloric intake, the weight for height percentile will almost invariably be abnormally low.

Therapy of active CD

The potential role of nutritional therapy in children with IBD can be subdivided into two categories: either as primary therapy in order to induce remission in active CD or

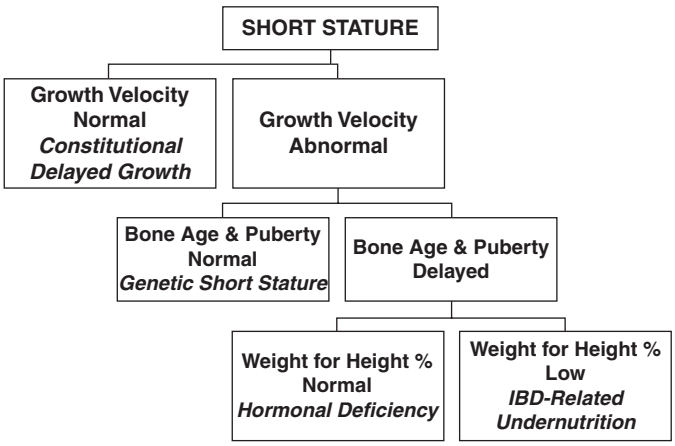

Figure 40.2 Assessment of the cause of short stature in pediatric IBD patients. Reproduced from Seidman, EG & Caplan A, Special considerations in the diagnosis and management of inflammatory bowel disease in the pediatric age group. In: *Inflammatory Bowel Disease: From Bench to Bedside, 2nd edn*, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp. 773–90. With kind permission of Springer Science and Business Media.

UC or as adjunctive therapy to help maintain remission and to enhance growth [6,61,62]. Although several randomized controlled trials have suggested that elemental and semi-elemental diets are as effective as steroids, meta-analyses have shown an overall statistical advantage for corticosteroids [63] Nevertheless, nutrition is still a logical choice as primary therapy for active CD in selected cases, especially those children and adolescents with marked undernutrition and growth failure [6,61,62]. Patients who tend to respond best (75% remission rate) are those with newly diagnosed CD involving the terminal ileum with or without the cecum or proximal colon [64]. Individuals with longstanding disease or extensive colitis generally respond less favorably (50 and 35%, respectively). Although steroids more often induce remission, their use is associated with a net loss of mineral bone density, negative nitrogen balance and impaired linear growth. In contrast, nutritional therapy enhances growth, induces net anabolism with positive nitrogen balance and improves bone health. Another clinical scenario favoring diet as primary therapy is for adolescents with CD who refuse a course of corticosteroids due to concerns for growth, cosmetic or other adverse effects [61,62].

Drawbacks to the use of nutrition as primary therapy for CD include the relatively high cost, the unpleasant taste and monotony of defined formula diets when employed as sole source of nutrition. The availability of flavor packets has improved the acceptance and oral tolerance of elemental and semi-elemental formulas. Another option shown to be effective is the use of polymeric formulas rich in transforming growth factor beta (TGFβ). They have been shown to induce clinical remission associated with mucosal healing [65]. In addition to mucosal macroscopic and histological healing, a fall in mucosal pro-inflammatory cytokines (IL-1, IL-8 and interferon gamma mRNA), but a rise in the regulatory cytokine TGFβ mRNA were observed [66]. These results indicate that these polymeric formulas are influencing the disease process itself and thus suggest that the clinical remission achieved is a result of a reduction in inflammation, in addition to other nutrition effects. Whichever formula is selected, a major objective is to avoid parenteral nutrition, unless the enteral route has failed or is contraindicated. Defined formulas are as effective as, and certainly safer and less costly than, parenteral nutrition. In order to induce remission, we generally administer a semi-elemental diet as sole source of nutrition (40–70 kcal kg^{-1} ideal body weight per day), for four consecutive weeks. The patients drink the formula during the day, along with clear fluids, as tolerated. The balance is administered nocturnally, by nasogastric tube or via a gastrostomy [5,62].

In addition to inducing remissions for active disease, nutritional therapy has been successfully employed on an intermittent, cyclical manner (4 out of every 16 weeks) in order to sustain growth as well as to maintain remission off steroids [67]. Patients who received an intermittent semi-elemental diet had significantly fewer relapses and markedly improved growth velocity than those treated with low-dose, alternate-day prednisone. In addition to improving symptoms, elemental diets reduce excessive intestinal permeability, reverse the protein-losing enteropathy and decrease intestinal and systemic markers of inflammation [68]. An alternative approach, often favored in Canada and Europe, is to employ AZA or 6-MP, as detailed above, after induction of remission using diet therapy.

The mechanisms underlying the beneficial effects of nutritional therapy remain incompletely understood. Several theories have been postulated: removal of dietary antigens, elimination of proinflammatory nutrients such as omega-6 fatty acids and nucleotides, altered eicosanoid and pro-inflammatory cytokine production, bowel gut hormones and flora, and diminished pancreatic, hepatobiliary and intestinal secretions [61,68]. Patients with active IBD have increased production of arachidonic acid metabolites derived from dietary sources of omega-6 fatty acids such as vegetable oils, leading to high levels of pro-inflammatory eicosanoids such as leukotriene B_4, a potent neutrophil chemoattractant [61]. Fish oils contain

eicosapentenoic acid, an unsaturated fatty acid that is metabolized through the cyclooxygenase pathway, leading to leukotriene B_5, 30 times less potent than its leukotriene B_4 counterpart. Studies using fish oil supplements have lent support to the potential role of dietary fatty acids in the pathogenesis and therapy of IBD [61]. One study using enteric-coated fish oil was highly effective in reducing relapses in CD, supporting this hypothesis [69]. Another novel approach to the use of nutriceuticals to alter the inflammatory process in IBD was to employ *N*-acetylglucosamine (N-AG) to assist in tissue repair mechanisms. In a pilot study, treatment resistant pediatric IBD patients were treated with N-AG orally or rectally [70]. There appeared to be some short-term benefit in several cases, including patients with strictures.

Approach to growth failure

The nutritional impact of IBD is particularly severe in the prepubertal patient. Many patients ingest an insufficient quantity of calories in order to meet their energy needs and also the metabolic costs of growth. Hence growth failure is a common, serious complication that is unique to the pediatric age group, encountered in up to half of CD and about 10% of UC patients [6,59–61]. Normal growth is an important indicator of remission and outcome parameter of therapeutic efficacy in pediatric IBD. However, despite "appropriate medical therapy," CD results in permanent short stature in 20–35% of adults who had the disease prior to their puberty [71]. There is a time limit for achieving potential "catch-up" growth because of progressive bone maturation and eventual epiphyseal fusion. Weight gain can be achieved in weeks, whereas growth acceleration requires many months of sustained treatment. In order to be effective, therefore, therapy must be initiated well before bone maturation is complete. Chronic undernutrition resulting primarily from inadequate caloric intake is by far the most important factor. Caloric intake in pediatric IBD is only 54–85% of estimated requirements and anorexia often persists despite clinical remission. Intervention must be initiated early, consistently and aggressively, assuring adequate nutritional support over a sufficient period in order to achieve enhanced growth. When growth is significantly impaired and the disease remains localized and non-progressive, surgical management may be considered. In general, however, surgery is considered for growth failure only if optimal medical and nutritional therapies have failed. Although gains in weight and height are seen postoperatively [72–74], final adult height is not invariably different for the group of patients with CD who had surgery during childhood compared with those who did not [74]. Total parenteral nutrition (TPN) can achieve weight gain and reverse growth arrest in CD. However, metabolic and infectious complications and cost considerations favor the use of enteral nutritional support [5,6]. The sustained administration supplementation of a polymeric formula (40–80 kcal kg^{-1} ideal body weight per day) via nocturnal nasogastric or gastrostomy routes effectively reverses growth failure. Compliance with high-calorie oral supplements generally is poor over the long term. Our high rate of success in having children and adolescents accept and comply with this form of therapy resides largely in the positive attitude of the nutrition support team, and also the patients' high motivation [62].

Hormonal deficiency is rarely a cause of growth failure in IBD, but should be looked for if adequate nutritional therapy fails to improve height velocity. In a pilot study [75], low-dose recombinant growth hormone therapy failed to improve height velocity in children with CD and growth failure. Whether or not growth hormone in conjunction with nutritional therapy would be effective in promoting sustained catch-up growth remains to be determined.

Therapeutic aspects: ulcerative colitis

Nutritional support is considered an adjunctive therapy, as there is no evidence that bowel rest or TPN influences the outcome of UC [5,6,61,62]. Patients requiring hospitalization for a relapse should receive parenteral nutrition if their baseline nutritional evaluation reveals that they are malnourished or if their intake is likely to be curtailed for at least 1 week. Growth failure has been reported in approximately 10% of pediatric patients with UC. Although much less common than in CD, this problem can significantly affect the child's self-esteem, behavior and school performance. Nutritional support for growth failure in IBD is discussed above. In our experience, a modest improvement of disease activity can be achieved by supplementing the diet with fish oils containing omega-3 fatty acids (9 g per 1.73 m^2 per day). The magnitude of clinical benefit is likely contingent upon a concomitant decrease in the ingestion of dietary omega-6 fatty acids (vegetable oils).

Assessment and therapy of micronutrient deficiencies

Patients with IBD often develop micronutrient deficiencies, in addition to energy deficits and growth failure, as reviewed above. Among the many potential mineral deficiencies (Table 40.6), iron is most common, most likely due to chronic gastrointestinal blood loss, iron malabsorption and/or inadequate dietary intake. The evaluation of iron stores is complicated by the confounding interpretation of low serum iron, transferrin saturation and ferritin levels due to chronic inflammation. Mineral deficiencies are not uncommonly encountered and merit monitoring and repletion (Table 40.6). Zinc deficiency may be associated with growth failure [76]. Multiple electrolyte disturbances (K, Ca, P, Mg) due to severe diarrhea may lead to arrhythmias or tetany.

Detection and management of bone disease

Prevention of osteoporosis should begin during childhood, a time of rapid growth and accrual of bone density. The presence of osteoporosis carries a significantly increased risk (30%) of fracture. In order to prevent osteoporosis, one must first identify patients at high risk. Children and adolescents with IBD may have multiple risk factors, including the presence of a chronic inflammatory disorder with the over-production of inflammatory cytokines that can lead to increased bone resorption. This is highlighted by the report [77] of a 12-year-old boy without a history of steroid use, in whom severe osteoporosis and multiple collapsed vertebrae were the presenting manifestations of CD. Other risk factors may include undernutrition, calcium malabsorption, a sedentary life style and glucocorticoid therapy. Therefore, bone mineral density (BMD) should be measured as part of the routine work-up of IBD patients in the pediatric age group. Several studies have confirmed that children with IBD are at risk for osteopenia, when compared with healthy age- and sex-matched controls [78,79]. A recent study [80] showed that decreased bone turnover occurs in children newly diagnosed with IBD. Although indicators of osteoblast activity increased with clinical improvement, bone mineral accrual did not accelerate.

BMD is best assessed by dual-energy X-ray absorptiometry (DEXA) (Table 40.6). Trabecular, rather than cortical, bone is predominantly affected in IBD, as seen in the lumbar spine and femoral neck. Low BMD is much more prevalent in children with CD than in those with UC, especially among females. Children with low BMI should undergo BMD screening, since they are at risk for low bone mass [80]. In order not to overestimate osteoporosis due to growth failure with delayed bone maturation in IBD, BMD values in pediatric patients with CD should take into consideration delayed bone maturation that is often present. Interpretation of BMD on the basis of bone or height age, rather than chronological age, resulted in a diminution of the overall frequency of abnormally low BMD from 44 to 26–30% [78]. The latter prevalence correlates with the results of studies in adult patients. Annual BMD evaluation, corrected for bone or height age, should be part of the management of IBD in children, particularly in CD or in patients who have received corticosteroids.

Strategies to prevent and treat osteoporosis include modifying risk factors, such as correcting malnutrition, optimizing intake of calcium and vitamin D, encouraging physical exercise and limiting glucocorticoid therapy, whenever possible. Several of these goals can be accomplished if nutritional therapy is employed for the management of active disease, rather than conventional corticosteroids [61,62]. If there is no improvement in BMD, bisphosphonate therapy may be considered. These drugs can help maintain BMD even if corticosteroids are used because of their long skeletal half-life. Repeat DEXA should be carried out to confirm adequate response to therapy, since the absorption of these drugs is often problematic. Bisphosphonates are not always well tolerated owing to gastrointestinal symptoms or well absorbed. If necessary, bisphosphonates can be administered intravenously [81]. An open uncontrolled study in adults [82] recently showed that intravenous pamidronate in combination with calcium and vitamin D is a well-tolerated strategy for treating CD-associated osteopenia and osteoporosis. A significant increase in BMD in the lumbar spine was observed. Pediatric trials in IBD are under way.

Psychosocial functioning in pediatric IBD

Although the mortality associated with pediatric IBD is low, it still presents a major, lifelong health threat, challenging the psychological resources of both the affected child and the family. IBD frequently interferes with physical activities, limits social interactions, disrupts education, impairs growth and delays puberty [83]. In its acute phases, IBD can present a serious impediment to daily functioning. Relapses may necessitate hospitalizations, which cause major disruptions in the child's academic, social and family life. The majority of children with CD experience considerable worry, distress and concern about their disease and its effects on school absences, academic achievement and participation in family and social activities away from home [83]. The chronicity of IBD poses persistent demands on children and their families to cope with fluctuating degrees of illness, prolonged use of medications and dietary limitations. The complications of growth failure and delayed puberty in CD add to the psychological stress associated with the disease, particularly as patients approach adolescence.

A meta-analysis indicated that children with IBD have more psychological disturbances than age-matched groups with other chronic illnesses [84]. Problems of low self-esteem, anxiety and depression are frequent [85,86]. Children with IBD were found to be more often either clinically depressed or to show significant depressive symptoms soon after diagnosis, compared with healthy controls and with children having other chronic illnesses [87,88]. Although depression is the most common emotional problem reported, other difficulties associated with IBD include separation anxiety, fearfulness, social withdrawal, relationship problems and problems with body image [89,90]. While a higher incidence of internalizing (self-directed) problems is observed, externalizing (acting-out) problems have also been noted [91].

It is unclear whether depression is associated with diagnosis of IBD, with the disease process itself or with

its treatment. Burke *et al.* [87] demonstrated that in all instances of major depression in pediatric IBD, onset followed the diagnosis. The fact that siblings of patients also show an increased incidence of psychiatric disorders raises the possibility that psychopathology in children with CD is due to factors other than the disease itself. These may include a genetic predisposition, dysfunctional family dynamics or both. There is no evidence for a unique psychological "profile" for children with IBD. However, psychological parameters can be useful in distinguishing CD from anorexia nervosa.

IBD in children may also have considerable consequences on the mental health of family members. Parents frequently have depression, anxiety and somatization [92]. Parents commonly worry about the effects of the disease on their child's school performance and on their future. Siblings also express concern about their ill sibling's well-being [92]. They may resent the time their parents devote to their ill sibling.

Therapeutic interventions may also affect psychosocial functioning in the pediatric patient with IBD. Psychosis induced by corticosteroid therapy is rarely encountered in pediatric CD patients [93]. However, a wide range of intense emotional reactions among children and adolescents is frequently observed. Parents view their children's behavior as "out of character," especially when it comes to challenging parental authority and expressing anger or exhibiting aggression. However, it is unclear to what extent these changes are due to the illness as opposed to the effects of systemic corticotherapy.

The children may report feeling "different inside" or having "changed forever" as a result of their IBD. They frequently feel less in control of their emotions and behavior. Some experience bouts of crying, often without apparent reason. Many state that they have become more withdrawn and unsociable; others report that they no longer feel like enjoying themselves. They may consciously withdraw from recreational activities or social contact because they fear other children will find out that they have a disease and will treat them differently. Others withdraw simply because they feel unable to participate or enjoy themselves. The aversion to being questioned, feelings of embarrassment or reluctance to be confronted with their disease often make it difficult for children and adolescents with IBD to return to school after long absences due to their illness. These reactions of shame and embarrassment often contribute to depressive feelings of psychological isolation, fragility and instability.

Problems related to compliance with therapy are notorious in pediatric populations. For many children, "forgetting" to take their medications is a way of psychologically denying their disease. For others, it may be more conscious and related to the lack of efficacy or side effects of the drug. In addition to the adverse effects of steroids on mood, cognition and behavior, psychological factors in children, parents or the family at large may also contribute to poor compliance and adherence to therapy. Nutritional therapy can also have important effects on psychosocial functioning. During periods of treatment, patients endure prolonged periods of food deprivation and experience frustration due to disruption of social and family activities. Special formula diets are particularly difficult for children who eat their meals at school. They are often already embarrassed about their disease. Thus, nutrition as primary therapy potentially exacerbates the child's feeling of being different due to the disease itself and may contribute to their sense of alienation. There is the additional consideration that the feeding tube and pump apparatus make the disease more visible, both to patients and to those around them. This can accentuate feelings of self-consciousness and heighten embarrassment in social situations. Some patients experience the insertion of a nasal gastric tube as intrusive or aggressive on the part of the medical team. The psychological meaning that patients attribute to treatment procedures, and also their emotional reactions (e.g., anxiety, fear and depression), may be more influential than their physical response in determining treatment success. More data on the psychological effects of medical and nutritional therapies are needed to provide a better understanding of the factors affecting compliance with treatment and the relationship to the medical team [94].

Children with IBD have been reported to have a significantly impaired quality of life [83]. They fear everyday activities and are concerned about future employment. They need sympathetic management and efforts should be concentrated on improving their daily psychosocial functioning, enabling them to lead as normal a life as possible. This can best be achieved by medically controlling their disease activity, achieving normal growth and development through nutritional interventions and providing psychosocial support to them and their family members.

Surgical considerations

As in adults, the surgical approach to the child with IBD must always bear in mind that unlike UC, CD is not a surgically curable disorder. Surgical interventions are therefore reserved either for complications of CD or for symptoms that cannot be managed medically (Table 40.7). CD patients who need a surgical intervention for any cause will experience a symptomatic recurrence of disease in 20–30% of cases within the first year after surgery, with increasing likelihood in each subsequent year [95]. Intractability of symptoms despite medical therapies generally infers a poor prognosis postoperatively, with earlier clinical relapse [96].

Other surgical indications (Table 40.7) include recurrent episodes of partial bowel obstruction or an abscess that fails to respond to conservative measures. Enteroenteric

Table 40.7 Specific surgical indications in pediatric inflammatory bowel disease.

Crohn's disease	Ulcerative colitis
• Intractable symptoms; failure of medical management	• Prolonged steroid dependence, intolerance to immunosuppressive agents
• Hemorrhage	• Hemorrhage
• Fulminant colitis with or without toxic megacolon	• Fulminant colitis, with or without toxic megacolon
• Known or suspected perforation or abscess	• Suspected perforation
• Obstruction of upper and/or lower GI tract	• Chronically active, unremitting disease
• Fistulae: enteroenteral, enterocutaneous, enterovesical, enterovaginal	
• Intractable perirectal disease	
• Growth failure despite nutrition support	• Growth failure despite nutrition support
• High risk of dysplasia, carcinoma	• High risk of dysplasia, carcinoma

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fistulae are not always an indication for surgery. Although an enterovesical fistula is not an absolute indication for surgery, most patients will require an operation eventually. Perianal CD also calls for a conservative approach. Infliximab has been shown to be effective therapy for severe cases. Perianal "sepsis" usually needs to be treated surgically. Abscesses should be drained and fistulae laid flat or treated with setons when possible. Although surgical resection has not been shown to influence the natural history of CD, it can lead to dramatic growth acceleration and attainment of normal adult height in some, but not all, cases [72–74,96].

The principal indications for surgery in pediatric UC [97] include intractable disease (64%), refractory growth failure (14%), toxic megacolon (6%), hemorrhage (4%), perforation (3%) and cancer prophylaxis (2%). Despite the relative success of anti-TNF antibodies, CsA, tacrolimus and other immunosuppressive agents, it must be borne in mind that colectomy can be a life-saving and curative procedure in a child with fulminant colitis or toxic megacolon.

Patients with UC and CD with extensive and/or severe involvement of the colon are at increased risk of developing colon cancer [98,99] and should be enrolled into a colonoscopy surveillance program 8–10 years after disease onset, irrespective of age. Restorative proctocolectomy has been the most commonly practiced and accepted operation. If rectal involvement is mild, some surgeons prefer to carry out a colectomy and a straight ileoanal anastomosis (SIAA) in one stage. Meticulous supervision of the conserved rectum is mandatory, as it might be a site for later cancer. Successful ileoanal-endo-rectal anastomosis after total colectomy and a mucosal proctectomy is often achieved. The rectal mucosa is stripped from its muscular wall and the ileal mucosa is sutured on. The creation of a neo-rectum with an ileal pouch–anal anastomosis (IPAA) affords decreased stool frequency. IPAA has long been the treatment of choice in the pediatric population. However, evidence concerning the optimal method of reconstruction after proctocolectomy is sparse. A meta-analysis recently compared outcomes from IPAA and SIAA [100]. Five studies satisfied the inclusion criteria, comprising a total of 306 patients, 86 of whom (28.1%) underwent SIAA and the remainder IPAA. Pouch failure was more common in the SIAA group [odds ratio (ORR 3.21; 95% confidence interval (CI) 1.24–8.34], as were abdominal salvage procedures (OR 9.5; 95% CI 3.14–28.77). Short-term adverse events were similar between the two groups, with the exception of perianal sepsis, the higher frequency of which, in SIAA, just reached statistical significance. Bowel frequency was lower in the IPAA patients, although few studies presented functional data in a comparable form. Finally, although IPAA does not jeopardize pregnancy and childbirth, it does impair female fecundity [101] and has a low risk of impairment of erection and ejaculation in young males. Traditional colectomy with IRAA has been shown to preserve female fertility in UC [102]. These issues must be discussed with young patients and their parents before undergoing proctocolectomy.

Transition to adult care

The transition of care from pediatric to adult gastroenterology becomes a major concern towards the end of adolescence. Potential difficulties include missed or delayed consultation with interruption in continuity of care, and also issues related to the separation from parents who were typically very involved in medical follow-up previously [103]. As they enter into adult care, parents may not easily accept that their "child" should be seen alone and is invited to make decisions independently, as young adults. The parents lose their leadership role as soon as the patient establishes a different relationship with the doctor,

switching from a triad to a two-way relationship.. This period of transition can be stressful for both the chronically ill adolescent and the parents, who may feel excluded.

In the course of follow-up, special interest is focused on issues particular to the pediatric age group, including growth, development and puberty. Moreover, specialists in caring for adolescents with IBD need be very familiar with psychological and social issues related to that period of life. The adult gastroenterologist who is charged with the responsibility for care of the adolescent and young adult with IBD needs to obtain training and experience with these issues.

The concept of "transition" has the goal of assuring a progressive and respectful move between the pediatric and adult center of care. Successful transition requires collaboration between the two teams in order to assure continuity of care and compliance with medications [104]. It is very important to provide a complete summary of the patient's files to the adult gastroenterologist, as details about previous history, investigations, complications and treatments are not always accurately recalled. The transition has to be planned in advance with the child and parents, in order to prepare them. A key issue is to find a gastroenterologist specialist in IBD who is aware of the particular adolescent's concerns and comfortable in dealing with them. It is then important for the adolescent or young adult to be able to establish an appropriate relationship with the new doctor and team. This can be initiated by the pediatric gastroenterologist seeing the adolescent without the parents when the transition time approaches.

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Chapter 41
Lymphocytic and Collagenous Colitis

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Summary

- The incidence of microscopic colitis appears to be increasing and in some centers approaches that of Crohn's disease. This might relate to an apparent association between microscopic colitis and a wide variety of medications.
- There is a strong association with celiac disease but the microscopic colitis does not respond to gluten withdrawal.
- Microscopic colitis is, by definition, a histopathologic diagnosis and depends on clinical awareness of the condition and obtaining adequate colonic biopsies.
- The pathogenesis of the disease is poorly understood. In the collagenous variety an aberrant activation of intestinal subepithelial myofibroblasts appears to be involved.
- Budesonide has emerged as the treatment of choice for those patients not responding to simple anti-diarrheal medications.

Background

Microscopic colitis is now well recognized as a common cause of watery diarrhea and, in some units, this diagnosis rivals Crohn's disease in terms of numbers of new cases per annum. Microscopic colitis was first reported by Lindstrom in his classical description of collagenous colitis in 1976 [1]. The term lymphocytic colitis was used to distinguish patients who, like those with collagenous colitis, presented with watery diarrhea and a normal colonoscopy, had an expansion of intraepithelial lymphocytes but no increase in the subepithelial collagen layer [2]. Microscopic colitis is now the accepted terminology encompassing both the collagenous and lymphocytic forms [3]. Whether these are two separate conditions or represent ends of a spectrum of a single disease is a moot point.

Initially, it was reasonable to believe that the rising incidence of microscopic colitis related to better awareness of the disease. However, it would appear that the number of cases year on year continues to rise even in units that have had a long interest in this type of inflammatory bowel disease (IBD).

Despite being generally a more benign disease than ulcerative colitis and Crohn's disease, microscopic colitis can prove to be remarkably difficult to treat.

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Incidence

As many cases of microscopic colitis are probably undiagnosed, it is difficult to assess incidence or prevalence figures. Greater awareness of the condition no doubt explains much of the increase in incidence reported in recent years. Despite these reservations, there is good evidence emerging that there might be a true rise in the number of new cases reported per annum over the past 20 years. Two regions in particular have focused on this apparent increase – Örebro in Sweden and Olmstead County, MN, in the United States. Olesen *et al.* in Sweden have shown a fairly dramatic rise in the incidence rates for microscopic colitis from 6.8 to 11.8 per 10^5 over just a 5 year period in the 1990s [4]. A similar increase has recently been reported from Olmstead County by Pardi *et al.* [5]. A study from Iceland has shown similar incidence rates [6]. A Spanish study reports finding microscopic colitis in 9.5% of patients with watery diarrhea and a normal-looking colonic mucosa [7].

Females dominate in most large series, more so in collagenous than lymphocytic colitis [7–10]. Likewise, all studies show a peak in diagnosis in the late 50s and early 60s. Thus, microscopic colitis is now the first disease to consider in the older woman presenting with chronic watery diarrhea.

Etiologic theories and associations

The etiology of microscopic colitis is unknown; indeed, lymphocytic infiltration and collagen formation may be

the histologic end-points of a variety of insults. Theories as to the causation of microscopic colitis should take account of the female preponderance, the peak onset in middle age and the apparent continuing increase in incidence.

The female predominance, especially of collagenous colitis, lends credence to an autoimmune theory and many diseases with a putative immune basis are associated with these conditions. Celiac disease, thyroid disorders, rheumatoid arthritis and diabetes are some of the more common conditions greatly over-represented in patients with microscopic colitis [11–15]. Chande *et al.*, in a retrospective review of 104 patients, noted that 29% had a pre-existing diagnosis of an autoimmune diagnosis [16]. Koskela *et al.* reported a 53% incidence of concomitant autoimmune diseases [17]. However, apart from a non-significant increase in anti-nuclear factor, autoantibodies are not increased in patients with collagenous colitis [18]. The relationship between celiac disease and microscopic colitis is, perhaps, the most intriguing. All major reports on microscopic colitis have highlighted an unexpectedly high concurrence of the two disorders. Bohr *et al.*'s study from Sweden records celiac disease in 8% of 163 patients with collagenous colitis [8]. Although initially associated with collagenous colitis, it is now recognized to occur with both histologic types [19–21]. Gluten does not appear to be the offending substance as many cases of microscopic colitis come to light when the diarrhea of celiac disease fails to resolve on a gluten -free diet despite good histologic recovery of the small bowel pathology [22]. The close relationship between these conditions has led to the investigation of the small bowel in a number of studies which, although based on small numbers, are nevertheless of considerable interest. Fine *et al.* have shown that the class II HLA genes DQ2 and DQ1, 3 found in most patients with celiac disease were also present in the majority of individuals with microscopic colitis and may explain the overlap between the two conditions [23]. Moayyedi *et al.* found small bowel abnormalities in four of nine patients with microscopic colitis; two had significantly elevated levels of intestinal antigliadin antibodies and two had abnormal small bowel permeability [24]. Wolber *et al.* recorded gastric lymphocytosis in two of four celiac patients who had colonic lymphocytosis [19], and flattening of the ileal mucosa has also been reported by others [25,26]. Bile salt malabsorption has been well documented despite normal ileal mucosa [27] and small bowel fluid and electrolyte absorption may be impaired [28]. These studies demonstrate that the small bowel (and perhaps stomach) of patients with microscopic colitis may be involved at a structural, immunologic or functional level, with overt celiac disease being the most recognizable manifestation.

The occurrence of microscopic colitis within families suggests a genetic predisposition for this disease but there is no evidence to support this at present [29].

The peak incidence of microscopic colitis in the sixth and seventh decades raises the question of a drug-induced syndrome. Non-steroidal anti-inflammatory drugs (NSAIDs) have been postulated as a cause of this colitis. In a study by Bohr *et al.*, 34% of patients were taking NSAIDs [14]. In contrast, Veress *et al.*'s study found no association [25]. The most convincing evidence in favor of a cause and effect comes from Riddell *et al.*'s case–control study showing long-term use of NSAIDs in 19 of 31 patients compared with just 4 of 31 controls [30]. The diarrhea improved in three patients on drug withdrawal and recurred in the single patient who was re-challenged. It may be argued that the colitis is related to the arthritis and not the medication, but it is our experience that this association is independent of the type of arthritis. A recent case–control study compared drug consumption of patients with microscopic colitis with controls and, most importantly, with a group of patients with chronic watery diarrhea thought to be of functional etiology [31]. A significant association was found between the use of the selective serotonin reuptake inhibitor (SSRI) sertraline and lymphocytic colitis while a trend was noted with NSAIDs and collagenous colitis.

The difficulty of assigning causality between a particular medication and microscopic colitis was dealt with by Beaugerie and Pardi [32]. Employing a standard tool used in pharmovigilence studies, the authors proposed a scoring system based on the time elapsed between exposure to a drug and the adverse event, the time to resolution of symptoms when the medication is stopped and the return of symptoms if re-challenged with the drug. Combined with these data, the authors added scores for histologic or immunohistologic findings in the case of re-challenge and gave further weight to the strength of literature review. Using this system, they found strong evidence in favor of a causal relationship for acarbose, aspirin, NSAIDs, lansoprazole, ranitidine and sertraline.

The diarrhea of microscopic colitis may respond to bile salt resins [14,33]and this led Ung *et al.* to a careful evaluation of the possible role of bile salt malabsorption in these patients [27]. This study reported a 44% rate of bile salt malabsorption, as measured by the [^{75}Se]homocholic acid taurine test (^{75}Se-HCAT), in collagenous colitis. Eleven of 12 individuals with an abnormal ^{75}Se-HCAT responded rapidly to cholestyramine, as did 10 of 15 patients who had normal measurements. These findings raise the possibility that luminal factors other than bile salts may be involved in the etiopathogenesis of this inflammatory condition.

Andersen *et al.* demonstrated fecal fluid cytotoxicity on cell lines in a patient with collagenous colitis and postulated bacterial toxins as causative agents [33]. There is some indirect evidence to support this hypothesis: fecal stream diversion can lead to clinical and histologic resolution of the colitis [34]; the diarrhea may respond, as mentioned above, to cholestyramine [27]; and bismuth subsalicylate, a drug with antibacterial properties, has been shown to be beneficial for some patients with microscopic colitis [35].

Table 41.1 Differential diagnosis of patients with chronic watery diarrhea and a normal colonoscopy.

Microscopic colitis
Celiac disease
Giardiasis
Infective colitis (e.g. cryptosporidiosis)
Ileal Crohn's disease
Contaminated small bowel syndrome
Idiopathic bile salt malabsorption
Post-cholecystectomy diarrhea
Gastro-colic fistula
Carbohydrate malabsorption, e.g. lactose, sorbitol
Neuro-endocrine tumors
Brainerd diarrhea
Laxative abuse
Irritable bowel syndrome

Clinical presentation and differential diagnosis

The clinical presentation of microscopic colitis is that of chronic watery diarrhea which may be associated with abdominal cramps. Systemic symptoms such as anorexia and weight loss are rare and hematologic and biochemical blood tests are often unhelpful [14]. Microscopic colitis is, by definition, a histopathologic diagnosis and thus depends on clinical awareness of the condition and obtaining adequate colonic biopsies. Rectal histology may be normal in a considerable number of patients with microscopic colitis whereas biopsies taken from the left colon will identify over 80% cases [7,13,36,37]. Other diagnoses to be considered in individuals with chronic watery diarrhea and a normal colonoscopy are outlined in Table 41.1. This is not an exhaustive list and does not include the many infectious causes of watery diarrhea in the immunocompromised patient. The presentation of idiopathic bile salt malabsorption [38] or post- cholecystectomy diarrhea [39] may be indistinguishable from microscopic colitis. The diarrhea associated with neuroendocrine tumors such as gastrinomas or vipomas is usually severe and accompanied by weight loss. The irritable bowel syndrome is placed last on this list and should only be considered after a careful search for other pathology. This is particularly important for those patients presenting for the first time in their fifth or sixth decades.

Pathology

Clinicians need to be encouraged to biopsy the colon even in the presence of normal endoscopic findings. A recent study of 167 patients with chronic diarrhea with normal colonoscopy showed significant diagnostic findings in 32% of patients, the majority of which were microscopic colitis [40]. There is little doubt that pathologists underdiagnose microscopic colitis. Much of the reason for this is due to confusion in the literature with regard to terminology and to a lack of consensus with regard to specific diagnostic criteria. Like others, we use the umbrella term microscopic colitis and subcategorize patients into lymphocytic and collagenous types. There are only rare reports of progression from lymphocytic to collagenous colitis or vice versa [41–43]. Interpretation of serial or post-treatment biopsies may be difficult due to variability in sampling and lack of knowledge of the natural history of the disorders.

Biopsy technique

Colorectal biopsies should be taken to include biopsies from the proximal half of the colon since distally there maybe no collagen band thickening. Rectal or sigmoid biopsy alone may miss a large proportion of cases [13,36]. The transverse colon yielded the largest percentage (83%) of diagnostic biopsies in one study, whereas rectosigmoid biopsies were diagnostic in only 66% [44]. Thus, full colonoscopy with right- and left-sided biopsies may be necessary to maximize the diagnostic yield.

Histopathologic features

Lymphocytic colitis

The most sensitive and specific features are a diffuse intraepithelial lymphocytosis and cuboidal change or flattening of the normal columnar surface colonocytes (Plate 41.1). A detached or denuded surface epithelial layer is often a clue to the diagnosis and may on occasions hinder the evaluation of intraepithelial lymphocytosis. The normal number of lymphocytes within surface colonocytes is 5 per 100 [2,45]. Care should be taken to ignore intraepithelial lymphocytes (IELs) over lymphoid aggregates, as this is a normal phenomenon [46]. In our experience, the mean number of surface IELs in lymphocytic colitis is 40 (range 20–60) [47]. Others who have performed quantitative studies showed a mean of 24.6 [2] and 37 lymphocytes per 100 surface epithelial cells [45]. Our study has used quantitative immunohistochemistry and therefore may be the more accurate. We also find that a confirmatory immunohistochemical stain for common leukocyte antigen (CLA) is useful on occasions (Plate 41.2).

Focal intraepithelial lymphocytosis only (involvement of some but not all biopsies) is insufficient for diagnosis and has been addressed in a few studies. Focal lymphocytic and collagenous colitis have been described as preceding Crohn's disease in one report [48]. Other features, however, including focal involvement of biopsies, abnormal colonoscopic findings, presence of numerous neutrophils and a foreign body-type granuloma between the collagen fibers, helped to distinguish these cases in retrospect. In our experience, differentiation from Crohn's disease is rarely a problem if strict histologic diagnostic

criteria are applied and correlated with clinical and endoscopic findings. In addition, it is always difficult to exclude entirely so-called "Brainerd" or epidemic diarrhea, which is most likely infectious in etiology [45,49].

Using a cut-off of 15 lymphocytes per 100 surface colonocytes, Wang *et al.* have suggested abandoning the name lymphocytic colitis and replacing it with the term colonic epithelial lymphocytosis [20]. In this study, 28 of 40 patients fitted the classical description of lymphocytic colitis but the remaining 12 were atypical, fulfilling the histologic but not the clinical or endoscopic criteria. These patients represented a heterogeneous group of disorders including idiopathic constipation, Crohn's disease and infectious colitis. A recent commentary suggests that the term colonic epithelial lymphocytosis be reserved for these atypical cases which do not satisfy all the criteria for classic lymphocytic colitis [50].

Collagenous colitis

All of the features of lymphocytic colitis outlined above are seen in collagenous colitis, but the number of IELs tends to be lower [47,50]. The *sine qua non* for the diagnosis of collagenous colitis is a thickened subepithelial collagen layer (Plate 41.3). There has been considerable controversy with regard to the exact thickness necessary to make the diagnosis. The upper limit of normal is stated to be 7 μm. A rule of thumb states that >10 μm raises the possibility, while >15 μm establishes a diagnosis in the proper setting. More importantly, in our experience, is the pattern of injury; in particular, the lacy infiltration of the superficial capillaries of the lamina propria is characteristic and in doubtful cases can clinch the diagnosis (Plate 41.4). This has previously been highlighted by Lazenby *et al.*, who stated that no absolute or minimum quantitative measure of subepithelial collagen should be used for the diagnosis [51]. The collagen accumulation is usually diffuse but can be patchy in up to 20% of cases. Prominent eosinophils are an occasional feature. Pericryptal myofibroblasts are also prominent. We have seen one case of collagenous colitis with pseudomembranes [52] and other groups have also reported this finding [53,54].

Diagnostic difficulties

Diagnostic pitfalls in pathologic interpretation are summarized in Table 41.2.

In addition, IBD-like changes, both acute and chronic, have been described in microscopic colitis and may lead to diagnostic difficulty. Cryptitis, crypt abscesses, Paneth cell metaplasia and architectural distortion have been described in lymphocytic and collagenous colitis [55]. The presence of Paneth cell metaplasia was associated with abdominal pain and a higher frequency of bowel motions. A small minority of patients with acute changes had concomitant infections and/or a history of antibiotic use. A rare variant of microscopic colitis, mimics Crohn's disease, by showing extensive granulomatous inflammation [56,57]. A unique case of atypical collagenous colitis was associated with common variably immunodeficiency [58]. One other important differential diagnosis is amyloidosis which can be missed if a Congo red stain is not performed [59].

Table 41.2 Diagnostic pitfalls in histopathology.

Collagenous colitis	Lymphocytic colitis
Tangential sectioning – at least three crypts in the vertical plane need to be viewed	Over-interpretation of patchy IELs (CLA stain useful in confirming lymphocytic colitis)
Misinterpretation of subnuclear cytoplasm of colonocytes as collagen	Over-interpretation of increased cellularity of the lamina propria
Over-reliance on H&E: Trichrome stain is always necessary to confirm diagnosis of collagenous colitis	
If full thickness fibrosis or fibromuscular hyperplasia, consider ischemia and solitary rectal ulcer	
Uncertain significance of thickened basement membrane (e.g. seen near diverticulae)	

Pathogenesis

It now seems clear that collagenous colitis is due to an aberrant activation of intestinal subepithelial myofibroblasts (ISEMF) [60]. It appears that the normal type IV collagen of the basement membrane is unaltered in the disease. A study by Aigner *et al.* showed that pericryptal myofibroblasts normally secrete small amounts of types I, III and VI collagen around the deep parts of the crypts. In the upper part of the crypts and in the subepithelial location, increased amounts of type VI collagen and the matrix glycoprotein tenascin are produced. It is tenascin and type VI collagen that accumulate and comprise the majority of the subepithelial band. It has been suggested by this group that decreased synthesis of matrix metalloproteinases (MMPs) may be responsible for the abnormal collagen accumulation, that is, the delicate balance between collagen production and resorption is disturbed [61]. This finding has been confirmed by Gunther *et al.*, who demonstrated reduced MMP1 but also increased levels of the tissue inhibitor of matrix metalloproteinase 1 (TIMP 1) in 12 cases [62]. They also confirmed the accumulation of tenascin while also showing an absence of undulin. This tenascin–undulin dichotomy is a feature of a rapidly remodeling extracellular matrix as compared with

the high undulin/low tenascin content in long-standing scar tissue. This may explain why these deposits can be removed within periods of a few months upon fecal stream diversion [34]. Controversy persists, however, with a report stating that type III collagen is predominant in the condition [63]. It is interesting that other forms of colonic fibrosis (e.g. Crohn's strictures) are composed of predominantly collagen types I, III and V [64]. Finally, elegant electron microscopy work has shown deficient fibroblast cell processes, focally deficient basal lamina and surface epithelial cells resting directly on a thickened collagen layer in this condition [65].

Immune mechanisms have been proposed as being important in microscopic colitis. The abnormalities detected have been similar in both conditions. These include an accumulation of CD4 positive cells in the lamina propria and abnormal expression of class II MHC molecules on colonic epithelial cells. The immunoregulatory molecule CD1d shows decreased expression in epithelial cells [66] The increased surface IELs are predominantly CD8 positive, T cell receptor (TCR) $\alpha\beta$ phenotype [67]. No increase in TCR $\gamma\delta$ cells was identified. Microscopic colitis demonstrates a TH1 mucosal cytokine profile. Interferon-gamma, interleukin-15 and tumor necrosis factor α are upregulated with induction of nitric oxide synthase and downregulation of interferon-related cell junction proteins [68].

Pathophysiology

The mechanism of the diarrhea seen in microscopic colitis has been evaluated in a small number of patients with conflicting results. Giardiello *et al.* reported net fluid secretion, as distinct from the expected absorption, in the small bowel and colon of one subject and net jejunal secretion and reduced colonic absorption in a second patient. The induction of water and electrolyte secretion by the dihydroxy bile acids is a likely source of the diarrhea in those patients with bile salt malabsorption [15]. In contrast, another study has demonstrated reduced colonic water absorption in all six patients evaluated. These abnormalities were shown to be due to reduced active and passive sodium and chloride absorption and to reduced Cl^-–HCO_3^- exchange [28]. The pathophysiology of the diarrhea appears to correlate with lamina propria cellularity and not the thickness of the collagen layer, suggesting that the inflammatory reaction is the important component in the causation of the diarrhea [69]. Elevated levels of prostaglandin E2 have been postulated as a circulating secretagogue in a single case study [70]. An elegant study by Burgel *et al.* strongly supports the view that, in collagenous colitis, reduced sodium and chloride absorption along with active chloride secretion are the main components of the diarrhea. The subepithelial collagenous band is felt to act as a diffusion barrier while down-regulation of tight junction molecules may contribute to a "leak flux mechanism" [71].

Treatment

Microscopic colitis usually follows a benign course [9,72,73] and treatment options must keep this in mind. Despite the absence of systemic symptoms often associated with Crohn's disease and ulcerative colitis, it is often surprisingly difficult to achieve a remission in these disorders. As outlined above, a careful drug history should be taken and any potentially harmful medication stopped if possible. Until recently, the treatment was usually empirical and involved the patient in a strategy of therapeutic trials. Anecdotally, corticosteroids were effective but an unacceptably high dosage was often required [14] and relapse was common on reduction or withdrawal. This was far from ideal in a population that is predominantly female and post-menopausal. Happily, it is now apparent that the topically acting steroid budesonide is the agent most likely to lead to symptomatic and histologic recovery [74–76]. Indeed, there is little scientific evidence to support any other options [77]. The evidence for the effectiveness of budesonide is strongest for collagenous colitis. The work of Miehlke *et al.* not only confirms the effectiveness of budesonide but also shows that clinical relapse can be treated by its re-introduction [76]. It appears that long-term treatment with budesonine is effective in collagenous colitis but that relapse is common when the medication is withdrawn [78]. As with other steroid-dependent conditions, it may be possible to use immunomodulatory drugs such as azathioprine or mercaptopurine in these circumstances [79]. The mechanism of action of budesonide is not clear. Bajor *et al.*, working on the basis of the earlier studies from the same department in Gothenburg, have conclusively shown that budesonide normalizes the absorption of bile acids in patients with collagenous colitis [80]. This finding fits well with Ung *et al.*'s work showing symptom resolution in 21 of 27 patients given a bile salt-binding agent [27]. These drugs are not always well tolerated and may limit their effectiveness.

Bismuth subsalicylate initially looked promising. Fine and Lee reported that 11 of 13 patients responding to the drug given orally for 8 weeks [35]. This was mirrored by histopathologic improvement, including resolution of the thickened subepithelial collagen in the six patients who displayed this feature prior to therapy. The treatment was well tolerated and nine of the group remained in remission with a follow-up of 7–28 months. The mechanism of action of bismuth is unknown but may relate to its antibacterial properties. Despite this early promise, there have been few subsequent reports and no controlled trials. Thereafter, the choice of treatment is based on little more than case reports and personal experience (Figure 41.1)

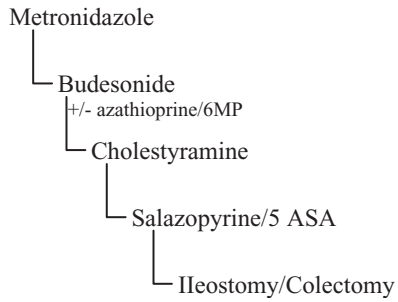

Figure 41.1 Therapeutic options in microscopic colitis.

Antibiotics have been reported to be beneficial in some of these studies – metronidazole being the most quoted [14,73]. The hope of avoiding long-term medication makes this use of antibiotics a reasonable first approach. Thirty-seven of 108 patients treated in a Swedish study benefited from salazopyrine while 45 individuals could not tolerate the medication [14]. Surgery is occasionally required for severe disease [41], but a diversion ileostomy might be a better option and would have the benefit of allowing time for other therapies to act. In our limited experience, diversion does work but relapse occurred on re-anastomosis.

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Chapter 42
Inflammatory Bowel Disease Microcirculation and Diversion, Diverticular and Other Non-infectious Colitides

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Summary
- The microvascular endothelium in the gut microcirculation plays a critical role in mucosal immune homeostasis, regulating the trafficking of circulating leukocytes during health and chronic inflammation.
- Microvascular endothelial cells from human IBD have an increased capacity for leukocyte binding and also an enhanced capacity for binding naïve leukocytes. Targeting leukocyte–endothelial interaction has emerged for refractory Crohn's disease patients with the compound natalizumab.
- In human IBD, microvascular dysfunction with impaired arterioloar vasorelaxation has been identified in both chronically inflamed Crohn's disease and ulcerative colitis tissues
- The differential diagnosis of IBD includes distinct forms of colitis, which may have non-immune mechanisms contributing to inflammation. Diversion colitis is a common complication of ostomy surgery, where a downstream segment of bowel is excluded from short-chain fatty acids derived from non-digested dietary carbohydrates in the fecal stream which are an important source of epithelial nutrition.
- Diverticular colitis is a complication of diverticulosis in older patients which may either respond to antibiotic treatment or require segmental resection for refractory cases. Ischemic colitis is a heterogeneous collection of disorders characterized by impaired colonic perfusion which will frequently improve with supportive therapy, while severe patients will face resection.

Introduction

The intestinal inflammatory response involves not only immune cells, but also non-immune cell populations in the gut, including the microvascular endothelium which lines the arterioles and venules. Endothelial cells are now appreciated to play a critical role in immunity through their ability to undergo activation, express cell adhesion molecules and selectively recruit circulating leukocytes, mediating transmigration into tissues undergoing inflammation [1]. Gut microvascular endothelial cells are known to play an important role in immune trafficking to the intestine during health, in addition to contributing to altered patterns of leukocyte recruitment which are central to the pathogenesis of chronic inflammation in human inflammatory bowel disease [IBD; Crohn's disease (CD), ulcerative colitis (UC)] [2]. In addition to this important "gatekeeper" role in initiating the inflammatory response, the microvasculature regulates tissue perfusion and new evidence suggests that microvascular dysfunction with impaired mucosal perfusion occurs in the chronically inflamed CD and UC mucosa [3]. A third contribution of the microvasculature to the pathogenesis of chronic intestinal inflammation involves angiogenesis, where neovascularization of the bowel contributes to tissue remodeling in both CD and UC [4]. At present, the microvasculature is being explored as a therapeutic target in IBD [5]. The development and implementation of the anti-α_4-integrin blocker natalizumab represents an initial strategy targeting leukocyte-endothelial interaction for the treatment of chronic inflammation in IBD [6].

Additional forms of colitis which mimic IBD, but derive from distinct mechanisms, include diversion colitis, diverticular colitis and colonic ischemia [7]. These entities represent key considerations in the differential diagnosis of IBD,

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but will have distinct outcomes and treatment strategies. Diversion colitis is by definition a complication of surgery, where the creation of an ostomy may leave a downstream colonic segment diverted from the fecal stream [8]. Diversion colitis is felt to result from a "nutritional" loss of short-chain fatty acids which are derived from bacterial fermentation of non-digestible carbohydrates in the colonic lumen during normal passage of the fecal flow [9]. The beneficial effect of short-chain fatty acid enema therapy for patients suffering from diversion colitis has substantiated this pathophysiologic understanding. Definitive therapy will involve either restoration of gut continuity and the fecal flow or resection of the diverted segment of downstream bowel. Diverticular colitis is a complication of diverticular disease, where most typically a segment of the left colon will be affected by chronic inflammation [10]. Treatment initially includes antibiotics and is similar to the clinical approach for diverticulitis. A subset of patients may develop a chronic colitis, which can mimic IBD. These patients can benefit from 5-aminosalicylate (5-ASA) or immunosuppressive treatment and definitive therapy may necessitate surgery with resection of the involved colonic segment. Ischemic colitis is a common entity which will be highly heterogeneous in its causes, presentation, clinical course and severity. Ischemic colitis results from hypoperfusion of the colon, most commonly in a segmental distribution involving watershed areas between the inferior mesenteric artery where it comes into continuity with the vascular perfusion of the superior mesenteric or rectal arteries. Ischemic colitis accounts for over 50% of gastrointestinal ischemic injuries. Its clinical spectrum will vary from mild colopathy, transient colitis, chronic ischemic colitis to severe manifestations with gangrene, stricture formation and fulminant colitis leading to perforation and sepsis. Most cases are idiopathic, but secondary forms of ischemic colitis are linked to estrogen compounds, nonsteroidal agents, cocaine, serotonin agonists used in the treatment of irritable bowel syndrome, vasculitis and surgical repair of the abdominal aorta, among others.

This chapter provides a review of the contribution of the microcirculation to gut health and physiologic mucosal immunity, alterations in vascular function associated with IBD, potential vascular targets for IBD therapy and an overview of distinct forms of colitis mimicking IBD which are linked to non-immune etiologies.

The gut vasculature

The small and large bowel derive their blood supply from the superior mesenteric artery (SMA) (supplying blood to the small bowel, proximal and transverse colon), the inferior mesenteric artery (IMA) (supplying the distal colon) and the middle and inferior rectal arteries (supplying blood to the distal rectum). The exact vascular anatomy will vary from individual to individual, but the SMA will typically give off the middle colic artery which will then branch into the ileocolic artery supplying blood to the terminal ileum, cecum and proximal ascending colon, and the right colic artery providing blood supply to the distal ascending colon, hepatic flexure and proximal transverse colon. The IMA typically makes three major branches which include the left colic artery supplying blood to the distal transverse colon, descending, sigmoid arteries supplying the sigmoid and the superior rectal artery which supplies the rectum. Rectal perfusion will also be derived from the middle and inferior rectal arteries which branch off of the inferior iliac artery. These medium-sized arteries will have watershed areas at the splenic flexure and the distal sigmoid colon, where collateral flow will normally develop. When IMA flow is compromised, collateral vessels will assume an important role in maintaining perfusion of the left colon and these vessels include the marginal artery of Drummond and also the central anastomotic artery and the arc of Riolan which is present at the root of the mesentery.

The gastrointestinal tract is highly vascularized, with a rich microcirculation that branches from resistance arterioles located beneath the muscularis mucosa into arcades of capillaries and venules that reach into the villous tips in the small bowel and crypts in the colon. The individual villi will each possess a fountain-like subepithelial capillary network which will play a critical physiologic role in gut function. Gut blood flow is highly variable, which will fluctuate from high vascular demand to periods of quiescence with low basal flow depending on physiologic need [11,12]. The SMA at rest will demonstrate perfusion flow ranging from 29 to 70 ml min^{-1} per 100 g of tissue, whereas in the fed state this perfusion will increase by 28–132%. Work by Granger and Barrowman demonstrated that mucosal perfusion increases dramatically during the fed state, increasing by up to four-fold. Vascular perfusion will increase in response to partially digested food, bile, bile salts and dietary fats in addition to neurochemical and endocrine regulation [11]. Arterioles regulating the perfusion of the gut have a diameter of 15–20 μm and are extremely dynamic in their ability to regulate perfusion. Small arterioles can change their diameter by 2–3-fold depending on physiologic need, whereas larger arterioles are believed to change their internal diameter by 20–40%. When one considers that vascular flow follows Poiseuille's law of fluid dynamics and flow is dependent on the luminal radius multiplied to the fourth exponential power, then these changes with diameter correspond to dramatic increases in perfusion.

The smallest vessels in the gut microcirculation are capillaries, which are derived from the terminal arterioles. Capillaries have internal diameters of 4–10 μm and are lined by a single tube of endothelial cells. Capillary perfusion is also dynamic, with 20–30% of gut capillaries not

receiving blood flow during resting conditions and these vascular beds opening up during periods of increased demand.

Capillaries will drain into venules, which are larger and not lined by smooth muscle cells. These postcapillary venules are the most heavily involved vascular segments in the inflammatory process, as their intercellular junctions will facilitate leukocyte transmigration into tissues in addition to accommodating flux of plasma proteins [13]. Larger venules will have smooth muscle lining and are located in a paired countercurrent exchange orientation in the submucosa throughout the intestinal tract, which is readily visible during routine endoscopic assessment of the colon. The large of amount of vascular fluid which is secreted into the gut lumen via epithelial cells and glandular structures on a daily basis (in excess of 10,000 ml per 24 h) will be reabsorbed due to this countercurrent exchange process, with only 200 ml of stool output typically occurring on a daily basis.

Endothelial cells play a major role in the regulation of multiple areas of microvascular function. Endothelial cells regulate microvascular perfusion through the generation of nitric oxide from the conversion of L-arginine to citrulline via nitric oxide synthase (NOS) isoforms. Originally described as endothelial derived relaxing factor (EDRF), endothelial derived nitric oxide (NO) will function through potentiation of cGMP in surrounding smooth muscle cells to vasorelax arterioles and increase tissue perfusion. Additional endothelial derived vasorelaxing substances will include prostanoid species, including prostacyclin (PGI_2) and cyclooxygenase-derived PGE_2. Neurogenic mechanisms of vasorelaxation with acetylcholine are dependent on an intact endothelium being present in the resistance arterioles. If the endothelium is disrupted, then vasorelaxing substances, including both substance P and acetylcholine, may function as vasoconstrictors. Additional vasoconstrictor substances specifically produced by the endothelium include endothelins (ET-1, ET-2, ET-3), which can act on vascular smooth muscle cells through ET_A receptors or directly on endothelial cells (through ET_B receptors) to decrease flow. Endothelial cells in the microcirculation will produce angiotensin-converting enzyme (ACE) to stimulate the renin–angiotensin system, leading to vasoconstriction. Additional substances generated by the microvascular endothelium which will induce vasoconstriction include thromboxane A_2 and superoxide anion, which will counteract the effect of NO-mediated vasorelaxation.

Regulation of perfusion is dependent on metabolic demand, as the oxygen tension in tissues decreases with increased function and arterioles will vasorelax in response to either the decreased oxygen or the increased presence of metabolic products, such as adenosine. A final mechanism which plays a role in the regulation of vascular perfusion is flow-mediated vasodilation. In addition to the contribution of endothelial cells in the regulation of regional perfusion, pericytes, a specialized layer of vascular smooth muscle cells which surround endothelial cells in arterioles, will also play a central role in vascular regulation [14]. These cells will contract and restrict flow, and also secrete vasoactive substances which will both increase and decrease vascular diameter and tissue perfusion.

In addition to their role in the regulation of tissue perfusion, endothelial cells play a central role in immune homeostasis in addition to regulating the recruitment of circulating leukocytes during inflammation. The majority of leukocyte recruitment occurs in the post-capillary venule. During the exit from capillaries, hemodynamic forces will cause leukocytes to migrate to the post-capillary venular wall. The interaction of leukocytes with the endothelium is a highly orchestrated process which begins with rolling adhesion. Rolling is a low-affinity interaction mediated by endothelial expression of E-selectin and P-selectin, which will bind to the L-selectin ligand expressed on leukocytes. E-selectin is under transcriptional regulation and is not constitutively expressed in human gut microvascular endothelium. The second stage of leukocyte–endothelial interaction will involve integrins (LFA-1, VLA-4 and $\alpha_4\beta_7$) and their respective endothelial ligands ICAM-1, VCAM-1 and MAdCAM-1. ICAM-1 and MAdCAM-1 are constitutively expressed in human gut endothelial cells and will increase expression following inflammatory activation with cytokines [tumor necrosis factor alpha (TNFα), interleukin 1β (IL-1β)] and bacterial LPS. VCAM-1 is not expressed at baseline in gut endothelial cells and will increase following exposure to inflammatory cytokines and LPS. Studies from animal models have demonstrated a central role for VCAM-1 in mediating chronic gut inflammation, but studies attempting to demonstrate increased expression of this molecule in the inflamed human gut have provided conflicting evidence. Histologic studies have failed to show clear evidence of a microvascular increase, while studies in isolated cultures of human intestinal endothelial cells have readily demonstrated both VCAM-1 gene product and surface expression of protein. Further evidence which suggests an important functional role for VCAM-1 in gut inflammation has come from animal models of IBD, where mice engineered to develop spontaneous colitis have demonstrated a therapeutic effect of anti-VCAM-1 antibodies administered intravenously. Leukocyte interaction with integrin ligands will produce firm adhesion where leukocytes will flatten along the endothelial surface, leading to the third step of extravasation, which is in part mediated by endothelial PECAM-1 (CD31) [15]. It is important to note that selective expression of the molecule MAdCAM-1 by the gut specific endothelium plays a key role in the homing of specific leukocyte populations to the gut through interaction with the $\alpha_4\beta$7-integrin, which functions as a molecular "zip

code" to guide recirculation of leukocyte populations to the mucosal immune compartment in the gastrointestinal tract [16,17].

The microvasculature in IBD

There is extensive evidence for vascular involvement in the pathophysiology of human IBD [18,19]. At a morphologic level, the earliest visible lesion in CD, the aphthous ulcer, will form overlying microvessels and this local accumulation of neutrophils contributes to ulcer formation into the epithelium overlying the vessel. Colonic CD "rake" ulcers will typically form over the mesenteric attachment to the bowel in both the small and large bowel. Ultrastructural studies using transmission electron microscopy revealed abnormalities in endothelial cells lining the microcirculation of areas affected by CD, which included loss of monolayer integrity, tissue edema, extravasation of red blood cells and focal venular necrosis adjacent to areas of undamaged endothelial cells.

Early studies investigating the contribution of endothelial cells to chronic gut inflammation characterized cell adhesion molecule expression in the microvasculature. Histologic studies demonstrated marked increases in endothelial E-selectin and ICAM-1 expression, and also the gut specific homing molecule MAdCAM-1 in both CD and UC specimens [20,21]. Studies by Salmi *et al.* demonstrated that leukocyte homing patterns in the IBD gut are altered, showing that there is preferential recruitment of naïve leukocytes expressing L-selectin and CD45RA by endothelium in chronically inflamed segments of IBD intestine [22]. These findings were confirmed by Burgio *et al.*, who found that both naïve T cells and monocytes were preferentially recruited to the IBD intestine, which overexpressed the peripheral lymph node addressin CD34 sialomucin complex, which normally functions as a ligand for naïve leukocytes in the peripheral immune compartment [23]. The implications for this finding suggest that chronic inflammation will result in inappropriate recruitment of naïve leukocytes to the antigenically rich mucosal immune compartment, which may further perpetuate immune activation and drive inflammation.

To characterize further the role of endothelial cells in IBD, techniques were developed to isolate these local cell populations from resected surgical tissue. These cultures of human intestinal microvascular endothelial cells (HIMECs) demonstrated that IBD-derived endothelial cells have an increased capacity for leukocyte adhesion, which is an acquired alteration that is not present in areas of uninvolved IBD intestine [24–27]. The mechanism underlying enhanced activation and increased leukocyte binding capacity appears to involve a loss of NO production and sustained generation of superoxide anion. NO, derived from the inducible nitric oxide synthase isoform, appears to play a key role in downregulating activation of intestinal endothelial cells and HIMECs derived from chronically inflamed segments of bowel demonstrate a transcriptional loss of this molecule [28,29]. Further studies have demonstrated increased microvascular endothelial expression of arginase II in IBD histologic sections and induced expression in endothelial cells following activation with inflammatory cytokines (i.e. TNFα, IL-1β) and bacterial LPS [30]. Arginase II is an enzyme which degrades arginine, the amino acid substrate necessary for the generation of NO via NOS enzymes. These data suggest that complementary mechanisms will play a role in the loss of NO generation in endothelium exposed to chronic inflammatory stress. Furthermore, these data also suggest that the intestinal endothelium will adapt to the stress of chronic inflammation through an acquired loss of its ability to generate NO, and quench superoxide which accompanies the activation of these microvascular endothelial cells, ultimately leading to sustained inflammatory activation.

Both CD and UC are characterized by refractory, poorly healing mucosal ulceration and damage. Because refractory, poorly healing wounds are often ischemic, studies were specifically performed to determine whether the mucosal perfusion in IBD is impaired, leading to relative ischemia. Studies by Hultén *et al.*, using an intraoperative technetium perfusion assay, reported impaired mucosal perfusion in CD strictures, demonstrating the presence of ischemia in these areas of tissue remodeling [31]. Further evidence supporting the concept of impaired perfusion of the mucosal surfaces was generated by Angerson *et al.*, who assessed IBD mucosal perfusion using endoscopic ultrasound [32]. Similar findings were also demonstrated by Tateishi *et al.*, who used intraoperative Doppler ultrasound, which again demonstrated impaired mucosal perfusion in chronically inflamed IBD [33]. Work performed in freshly isolated resistance arterioles from surgical specimens by Hatoum *et al.* demonstrated that loss of NO-mediated vasorelaxation and excess reactive oxygen species, specifically superoxide anion, were present in vessels isolated from chronically inflamed segments of IBD bowel, but not areas of intestine which were not exposed to chronic inflammatory stress *in vivo* [3]. These studies further demonstrated an increased dependence of IBD intestinal arterioles on vasoactive prostanoid species produced from cyclooxygenase-1 and -2. These studies provided a potential mechanistic understanding for the clinical observation that use of non-steroidal compounds can trigger severe exacerbation of IBD. The inhibition of vasoactive prostanoid production in IBD arterioles by non-steroidal agents would lead to further exacerbation of mucosal ischemia, impaired ability to heal and deterioration of the chronic inflammation in the affected bowel. Novel studies in rodent models of IBD by Harris and colleagues have demonstrated a beneficial effect of vasodilator

compounds and inhibitors or thromboxane, which provide impetus for human clinical studies targeting microvascular dysfunction [34,35].

An additional mechanism which has been a focus of new vascular investigation in IBD is angiogenesis. IBD surgeons have recognized for decades that involved segments of bowel will demonstrate neovascularization on the serosal surfaces overlying areas of chronic mucosal inflammation. Likewise, transabdominal ultrasound studies have demonstrated overall increased vascular perfusion in the areas of active IBD inflammation, which correspond to areas of neovascularization in the chronically inflamed intestine, readily demonstrable as a Comb's sign on computed tomography (CT) enterography [36,37]. When we consider that ischemia is one of the most potent angiogenic signals, then an angiogenic response on the outer layers of the bowel wall may represent a pathophysiologic adaptation to the relative mucosal ischemia which accompanies chronic inflammation in IBD [38]. Thus, serosal hyperemia and mucosal ischemia may coexist and further worsen the angiogenic process which accompanies bowel remodeling during chronic intestinal inflammation.

Danese *et al.* demonstrated increased microvessel density in both CD and UC bowel and a pro-angiogenic milieu in the local IBD tissue environment [4]. Animal models of IBD have confirmed angiogenesis and also clinical and histologic improvement with anti-angiogenic treatment, suggesting a potential role for long-term management targeting this vascular pathway [39].

Targeting the microvasculature for therapy in IBD

Most medications used in the treatment of human IBD do not exert a direct function on the microvascular endothelium. When we consider the importance of the vasculature in immune homeostasis, inflammation, wound repair and overall tissue physiology, this may represent an untapped opportunity to develop novel therapeutic strategies for patients who have failed to respond to current treatment approaches. This point is further supported by that fact that only 30% of longstanding CD patients will achieve complete endoscopic mucosal healing with the most potent biologic agents currently available. Hence the majority of IBD patients with moderate to severe disease may be appropriate candidates for additional therapeutic modalities which target additional pathophysiologic mechanisms including the vascular alterations associated with both CD and UC.

The first therapeutic agent which specifically targeted an endothelial mechanism for the treatment of chronic inflammation in CD was the selective leukocyte adhesion antagonist natalizumab [6,40]. This IgG4 humanized antibody functions as an inhibitor of leukocyte alpha4 integrins, effectively interrupting their ability to interact with and bind MAdCAM-1 on the endothelium in the intestinal microvasculature [41]. Natalizumab demonstrated a significant ability to induce and maintain remission in refractory CD patients in pivotal registry trials, which ultimately resulted in FDA approval for the treatment of refractory CD. However, enthusiasm for natalizumab was dampened when maintenance use was associated with JC virus reactivation, leading to progressive multifocal leukoencephalopathy (PML), an extremely rare but dangerous infectious complication which may occur in approximately 1 in 3000 individuals. Despite these concerns, the compound did gain FDA approval for multiple sclerosis patients and also CD patients who have failed prior treatment with an anti-TNFα agent. Additional, more selective, anti-$\alpha_4\beta_7$ inhibitors which specifically target interaction with MAdCAM-1 but will not interfere with VCAM-1 leukocyte interaction are currently undergoing investigation in both UC and CD.

Additional strategies targeting the microvasculature in the IBD intestine include anti-angiogenic agents and potentially agents which will ameliorate the microvascular dysfunction associated with chronic inflammation [42]. An agent with anti-angiogenic potential which has shown efficacy in open-label case series for the treatment of refractory CD is thalidomide [43,44]. This agent, which was banned due to its terrible legacy of teratogenicity, is a potent anti-angiogenic and has become available on a restricted basis for selected patients who are compliant with a strict prescribing program designed to prevent inadvertent fetal exposure. An additional anti-angiogenic agent which has been shown to help maintain remission in patients with UC is the natural product curcumin [45,46]. This derivative of the household spice turmeric exerts anti-angiogenic activity on human intestinal endothelial cells, and this may represent a therapeutic mechanism which underlies its demonstrated ability to benefit IBD patients.

Animal models of IBD have demonstrated a beneficial effect of agents which target the microvascular dysfunction which is associated with chronic intestinal inflammation. Agents targeting impaired vasorelaxation, specifically excess thromboxane and endothelin, have shown beneficial effect, suggesting future trials targeting these mechanisms in human IBD.

Distinct forms of colitis which may mimic IBD

Diversion colitis

Diversion colitis is a form of IBD which is a direct result of surgical manipulation of the gastrointestinal tract, where a downstream segment of large bowel is removed from the fecal stream due to the creation of a proximal ostomy [47]. Patients who develop diversion colitis need

not have a pre-existing diagnosis of CD or UC and the majority of patients will in fact have an otherwise normal downstream colonic segment which has been bypassed due to diverticulitis, colorectal adenocarcinoma or protection of an anastomosis. The spectrum of diversion colitis can range from minimal, asymptomatic friability of the mucosal lining to gross ulceration contributing to fistula formation, significant anemia and systemic illness [8,48].

The most compelling etiologic mechanisms which are felt to underlie the development of inflammation in the bypassed colonic segment are the loss of luminal short-chain fatty acids (SCFAs), specifically butyrate, which are derived from the fermentation of non-digestible plant carbohydrates consumed in the diet by enteric bacteria. *n*-Butyrate is the preferred metabolic substrate of the colonic epithelium, specifically provided the major source of energy for the left colon and rectum. SCFAs are abundant in the colonic lumen and are readily absorbed into epithelial cells, where they are now known to exert powerful effects on the regulation of cellular homeostasis and the cell cycle. Colonic SCFAs have been demonstrated to function as the preferred metabolic fuel for colonic enterocytes, enhance Na, Cl and water absorption, regulate HCO_3 secretion and acid–base balance, regulate motility, increase cell differentiation, exert a bacteriostatic effect, increase colonic blood flow and increase colonic oxygen consumption, among multiple physiologic functions. The best evidence supporting the hypothesis that the loss of luminal SCFAs is the major etiologic component of diversion colitis comes from clinical studies which administered exogenous enemas of butyrate, effectively treating this form of colonic inflammation [9,49].

Clinical features

Although the true incidence of diversion colitis is difficult to assess precisely, the condition will affect a majority of patients who undergo creation of a Hartman's pouch (i.e. diverted distal colonic segment), with one prospective study demonstrating 70% of patients demonstrating diversion proctitis/colitis within 1 year of surgery [8]. Most patients will demonstrate an increase in frequency and the amount of rectal discharge, which may range from mucoid material to frank blood. Pelvic and rectal pain will occur less frequently. Additional complaints can include anal fissure, tenesmus and low-grade fever, although many patients will demonstrate endoscopic and histologic evidence of inflammation in the absence of symptoms. Patients may also present with end-stage complications of inflammation, including stricture formation.

The classic endoscopic findings in diversion colitis are mucosal friability in the setting of mucosal exudates, loss of normal mucosal vascular pattern and superficial ulcerations due to diffuse inflammation. Distal colonic changes will typically demonstrate the most pronounced changes. Histologic changes on endoscopic pinch biopsy are generally non-specific and will typically demonstrate mucosal edema, surface exudates, mucin depletion, mixed inflammatory infiltrate and crypt abscesses and lymphoid hyperplasia, while true granulomas are not seen [50]. When diversion colitis occurs in the setting of prior IBD colitis, the histologic and endoscopic picture may frequently demonstrate a mixed pattern with features of both diversion and the underlying CD or UC pathology [51].

Treatment

Treatment of diversion colitis has been attempted with administration of SCFA enemas (i.e. butyrate) and also fiber enemas, in an attempt to provide substrate for the bacterial generation of SCFAs. Although steroids and mesalamine products have also been assessed, their efficacy has not been shown superior to placebo. The treatment of choice is to restore continuity to the colonic lumen with surgical reversal of the proximal ostomy and, if that is not feasible, then distal completion proctocolectomy. Patients who have suffered from diversion colitis have been reported to suffer from residual symptoms of abdominal pain and bloating, despite surgical restoration of colonic continuity [52].

Diverticular colitis

Clinical features

Diverticulosis, or the acquired form of diverticular disease, is an extremely common clinical occurrence in the United States and other Western countries, estimated to effect between 5 and 10% of the population over 45 years of age and up to 80% of individuals over the age of 85 years [53]. Most patients with diverticuli will remain asymptomatic, but episodes of acute diverticulitis will effect up to 20% of individuals with diverticuli at some point in their lifetime. In addition to this commonly encountered complication, a segmental colitis associated with diverticular disease was also described in the 1980s and 1990s [54]. This condition bears a striking clinical, endoscopic and histologic resemblance to IBD. Whereas acute diverticulitis is a pericolonic acute inflammatory complication of a microperforation of the bowel wall in the vicinity of the vascular penetration of the vasa recta through the bowel wall due to increased luminal pressure, diverticular colitis, often abbreviated segmental, chronic colitis associated with diverticular disease (SCAD), will occur in individuals with a normal rectum and proximal colon [55]. Patients with SCAD will have a segment of chronic mucosal inflammation in the setting of diverticuli, are typically over age 60 years, will more frequently be male and will present with a constellation of symptoms ranging from painless hematochezia to lower abdominal cramps or altered bowel habits. Rare clinical manifestations will include fever, leukocytosis, nausea and/or weight loss.

Endoscopy is essential to establish a diagnosis of diverticular colitis or SCAD. Colonoscopy will typically reveal mucosal erythema, granularity and/or friability of the sigmoid colon with sparing of the rectum and more proximal colon. Histologic assessment of endoscopic pinch biopsies will demonstrate findings of chronic colitis similar to IBD, while other reports have described histologic changes as being either non-specific or consistent with mucosal prolapse.

Treatment

Therapy of SCAD is sequential and will initially mimic the treatment approach for acute diverticulitis [10]. Following confirmation of negative stool studies for infectious pathogens, including *Clostridium difficile*, the majority of SCAD patients receive a regimen of oral broad spectrum antibiotics for 7–10 days, which will include coverage against Gram-negative organisms. Once the antibiotic course has been completed and symptomatic colitis has resolved, treatment emphasizes a regimen of oral fiber supplementation [56]. The majority of patients will respond to this conservative treatment strategy. Patients who do not initially respond to this treatment course can receive mesalamine compounds and also topical steroid enemas. There is a subgroup of patients with SCAD who develop a more aggressive form of chronic inflammation, which bears a stronger resemblance to CD. These individuals may ultimately demonstrate non-caseating granulomas and fissuring ulcers or fistulae. Many of these patients will require surgical management; however, the majority of these individuals will not go on to develop frank CD over a 6–12 month follow-up period.

There is interest in the etiopathogenesis of diverticular associated colitis, as it may represent an overlap with classic IBD and also pouchitis, the frequently encountered complication associated with ileoanal reconstruction following colectomy. This hypothesis is supported by the fact that both pouchitis and SCAD share histopathologic findings and initial treatment emphasizes antibiotics targeting bowel flora. The fact that bacterial stasis in diverticuli may represent a critical component for the development of diverticular colitis suggests that a defined antigenic drive may be underlying the development of this form of chronic bowel inflammation.

Ischemic colitis

Ischemic colitis is the most common form of vascular injury to the gastrointestinal tract, estimated to occur at rates varying from 5 to 44 cases per 100,000 person-years [57]. Ischemic colitis is the direct result of compromised vascular flow to the large bowel, which may manifest in a wide spectrum of pathologic changes ranging from transient injury to life-threatening fulminant disease with transmural infarction [58]. The triad of acute abdominal pain, bloody stool and low blood pressure are hallmark features of ischemic colitis. Although the majority of patients are females older than age 65 years [59,60], there are subsets of young, otherwise healthy individuals who can also manifest ischemic colitis, with endurance athletes [61], specifically long-distance runners, being one example and patients with irritable bowel syndrome treated with serotonin-modulating agents representing a second [62,63].

Etiologies of ischemic colitis are variable, but share the common mechanism of decreased delivery of oxygenated blood to the large bowel. Among these possibilities, decreased cardiac output, chronic renal failure [64], cardiac arrhythmia, shock [65,66], arterial thrombosis, embolism, complications of surgery (i.e. failed re-implantation of the inferior mesenteric artery following abdominal aortic aneurysm repair) [67], colonic obstruction with increased luminal pressure leading to impaired mucosal perfusion [63], hypercoagulability [68,69], vasculitis [70], intra-abdominal inflammation or infection or complications of drugs [68,71,72] may all predispose to ischemic large bowel injury.

Clinical features

Clinical presentation of ischemic colitis is highly dependent on the severity of the vascular injury, and also the location of the ischemic insult [73]. Ischemic injury in the territory of the IMA involving the left colon is most common. Left colonic injury will typically result in increased bowel movements and rectal bleeding. Ischemic injury to the right colon is more problematic, however, as the thinner walled ascending colon may lead more commonly to complications of transmural bowel injury. Right colonic ischemia may be more subtle, presenting with abdominal pain in the absence of bloody diarrhea or altered bowel function. In general, an acute onset of crampy abdominal pain, with urgency, diarrhea and subsequent bleeding, is the classic presentation. Physical findings are highly variable, but will frequently present with mild tenderness and/or abdominal distention. More severe injury will present with peritonitis and this has been estimated to occur in up to 20% of patients experiencing ischemic colitis.

Diagnostic testing is frequently non-specific in the evaluation of ischemic colitis. In the majority of cases, routine laboratory tests, including blood counts, creatinine phosphokinase, amylase, serum lactate and lactate dehydrogenase, are all normal. In severe cases of ischemic colitis, the white blood count can be elevated and acidosis may be present on electrolyte measurement. Stool analysis is always recommended as part of the diagnostic evaluation of ischemic colitis, as infectious colitis from organisms such as *C. difficile* should be diagnosed promptly and treated.

Radiologic findings are again highly variable and will often parallel the severity of the ischemic injury to the colon. Non-specific radiographic findings will include

bowel dilation, ileus and mural thickening. Thumbprinting, the pathognomonic radiologic finding of ischemic colitis, is identified in only a minority of patients, which may approach 20% of cases. CT scanning is helpful in demarcating the extent of the colonic injury, and also evaluating other bowel segments. Endoscopy and histologic evaluation of pinch biopsies will confirm the diagnosis of ischemic colitis and this has emerged as the diagnostic modality of choice. However, the extent of colonoscopic evaluation must be weighed carefully, as ischemic injury in the bowel will make it more susceptible to perforation due to insertion of a sigmoid loop during intubation and the decision to terminate a procedure must be weighed carefully by endoscopists who are experienced in the assessment of colonic injury. Mucosal appearance in ischemic colitis is dynamic and will frequently manifest a number of changes. Friability, petechial hemorrhages and pale mucosa will predominate. Later in the course of ischemic injury and a frequent presentation in more severe cases, the mucosa may be hemorrhagic and sloughing can occur. The most severe ischemic injury will demonstrate a bluish black, dusky mucosal appearance which is consistent with gangrene. Histopathology will also demonstrate an evolving tissue injury. The initial, acute phase will show mucosal hemorrhage, edema and tissue necrosis. Later injury will show leukocytic infiltration and sloughing of the surface epithelium, and also ulceration of the mucosa. The later stages of ischemic colitis will demonstrate repair, manifesting with granulation tissue and scarring which may become permanent with stricture formation.

Treatment

Treatment of ischemic colitis is largely supportive, with dietary limitation and administration of antibiotics which will cover bowel flora [74]. Moderate to severe ischemic colitis, which will manifest with radiographic changes, requires hospitalization for bowel rest, intravenous antibiotics and pain control. Restriction of diet is recommended in patients suffering from moderate to severe ischemic colitis as bacterial translocation can occur in the injured bowel, and avoiding the physiologic demand for increased enteric blood flow required for digestion may also limit further ischemic insult. Likewise, medications which may impact blood flow, including non-steroidal anti-inflammatory drugs and aspirin, should be avoided during the acute period of bowel injury.

The majority of patients who have required inpatient management for ischemic colitis will recover within the first 48 h during hospitalization. Depending on the extent and severity of ischemic injury, the period for complete resolution may last several weeks. Surgery should be considered in patients who have failed to resolve clinically over a 2 week period or in the setting of impending sepsis. The 20% of patients who experience this protracted clinical course, with a failure to resolve the clinical colitis, are predisposed to develop colonic strictures even if the initial surgical intervention is avoided. Likewise, operative management of patients with refractory ischemic colonic injury must be conservative and careful consideration should always be given to the creation of an ostomy. The recommendation for the diversion of the bowel in an ostomy in the majority of patients requiring surgery stems from the high rate of anastomotic failure and leak, when this is attempted in the ischemic colonic tissues. Close surgical management is necessary in patients with refractory disease, as overt gangrene can emerge in patients who fail to re-establish circulation, which will lead to perforation and high rates of perioperative mortality.

Conclusion

The microcirculation plays an essential role in normal mucosal immune homeostasis and alterations in gut microvascular function have been identified in human IBD. Increased and altered leukocyte recruitment with preferential transmigration of naïve leukocytes has been identified in IBD. New therapeutic strategies targeting endothelial–leukocyte interaction have shown promise, with the initial approval of natalizumab for the treatment of refractory CD. Enhanced endothelial activation linked to a loss of endothelial NO generation have been demonstrated in both chronically inflamed CD and UC microvessels. This loss of endothelial NO production underlies microvascular dysfunction in chronically inflamed IBD arterioles, which leads to impaired vasorelaxation and mucosal perfusion. The vascular pathology in IBD also includes an angiogenic response and new therapeutic strategies have demonstrated therapeutic benefit with anti-angiogenic compounds in animal models of IBD.

The importance of microvascular function in classic IBD also highlights additional forms of colitis which may be linked to distinct, non-immune etiologic mechanisms as well as therapeutic approaches. Diversion colitis results from surgical manipulation of the gastrointestinal tract and creation of an isolated downstream segment of colon. The colonic mucosa in the left colon and rectum excluded from the fecal stream will develop a nutritional-deficit colitis, due to the lack of exposure to SCFAs derived from fermented non-digestible carbohydrates which are necessary for epithelial health. Segmental colitis associated with diverticuli (SCAD) is an increasingly appreciated form of colonic inflammation found in the aging population, which may mimic diverticulitis. SCAD is most commonly treated with antibiotics, but refractory and severe cases may require segmental resection. Finally, ischemic colitis is a heterogeneous condition which is associated with multiple etiologic mechanisms including cardiac dysfunction, drug side effects, increased luminal pressure and surgical manipulation of the intra-abdominal vasculature, among

other reasons. The majority of patients may improve with supportive care, but a sizable minority of individuals will require surgical management due to severe vascular injury.

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Chapter 43
Clostridium Difficile-associated Diarrhea

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Summary

- *Clostridium difficile* carriage rates are increased in IBD patients.
- *C. difficile* diarrhea has increased in frequency throughout the world and recent *C. difficile* epidemics have been linked to a hypervirulent *C. difficile* strain resulting in greater severity of disease.
- Although most mild to moderate cases of *C. difficile* infections continue to respond to metronidazole or vancomycin, refractory and recurrent cases may require alternative therapies.
- Alternative antibiotics, toxin binders, probiotics and immunological therapies can be considered for treatment of acute and recurrent *C. difficile* infections in severe and refractory situations.
- Future approaches to the control of nosocomial *C. difficile* infection may involve active or passive immunization of at-risk individuals

Introduction

Clostridium difficile, first identified in 1935 as a commensual organism in the fecal flora of healthy neonates, was given its name because it grew very slowly in culture and was difficult to isolate [1]. Although it produced cytotoxins and was pathogenic for guinea pigs and rabbits, the organism was considered part of the normal neonatal gut flora that disappeared after weaning. In 1978, Bartlett *et al.* [2] identified *C. difficile* as the source of a cytotoxin found in the stool of patients with antibiotic-associated pseudomembranous colitis. The incidence of *C. difficile* infection has increased dramatically and the organism is now recognized as the most frequent cause of nosocomial infectious diarrhea in developed countries [3–6]. Incidence rates of nosocomial infection range from 0.1 to 43 per 1000 hospital admissions [7–13]. Up to 46% of high-risk patients, such as those admitted to acute care general medical wards and receiving antibiotics, may be colonized with *C. difficile* [14–18]. In community populations, the reported prevalence of *C. difficile*-associated diarrhea ranges from 8 to 25 per 100,000 person-years [9,19,20].

The sequence of events leading to *C. difficile* diarrhea and colitis in susceptible individuals are disturbance of the normal colonic microflora, exposure to and colonization by *C. difficile*, toxin production and toxin-mediated intestinal injury and inflammation (Figure 43.1). Depending on host factors, especially the immune response to *C. difficile* toxins, the outcome of colonization is either asymptomatic carriage or a spectrum of disease ranging from mild diarrhea to life-threatening pseudomembranous colitis, a spectrum termed *C. difficile*-associated disease (CDAD) [21,22]. The normal human colonic microflora of adults and of children over 2 years old is usually capable of preventing colonization by *C. difficile*. Colonization rates with *C. difficile* of 25–80% have been reported in infants and children up to age 2 years; despite the presence of toxin, however, they rarely develop *C. difficile*-associated diarrhea [23,24]. Immaturity of the enterocytes with absence of toxin receptor expression is a possible mechanism for this clinical phenomenon [25].

During the three decades since its identification as a pathogen, our understanding of the epidemiology, pathogenesis and management of disease caused by *C. difficile* has increased dramatically. Yet despite this increased knowledge there has been no substantial decline in the frequency of hospital-acquired *C. difficile* diarrhea and colitis. The introduction of new antibiotics and their more frequent use, especially fluoroquinolones, and the emergence of a resistant and hypervirulent BI/Nap1/027 strain of *C. difficile* have resulted in an increased incidence of CDAD with increased morbidity and mortality in some parts of the United States and Canada, especially Quebec province [5,11,26,27]. The causative strain of the 2002 *C. difficile* epidemic in Quebec has also been found in a number of hospitals of England, The Netherlands, Belgium, Austria and France and greater dissemination of this virulent and fluoroquinolone-resistant strain in North America, Europe and other parts of the world may lead to changes in the epidemiology of CDAD, with resultant increased

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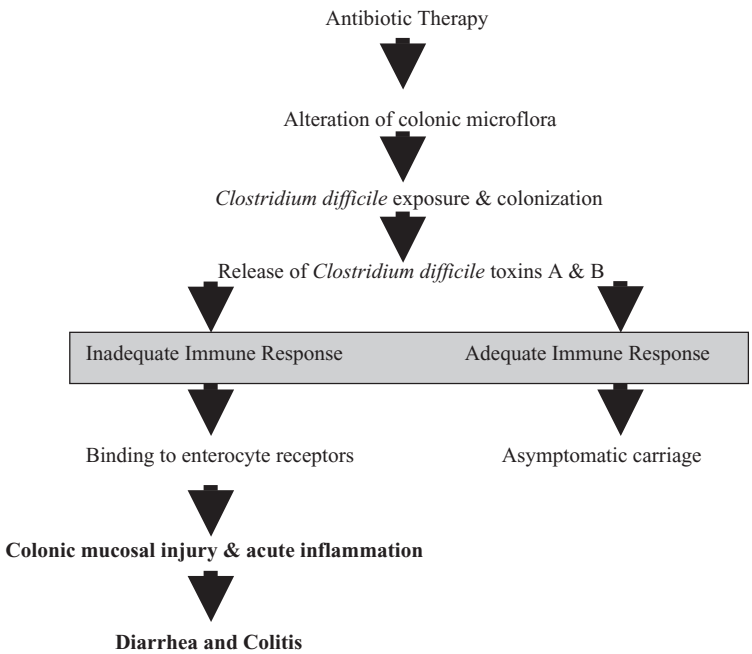

Figure 43.1 Pathogenesis of *C. difficile* diarrhea and colitis. Reproduced from Farrell RJ, Kyne L, Kelly CP, Pseudomembranous colitis and Clostridium difficile infection. In: *Inflammatory Bowel Disease: From Bench to Bedside*, 2nd edn, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp.823–44. With kind permission of Springer Science and Business Media.

prevalence, more severe disease and more fatalities [12,14,15,28,29].

Clostridium difficile

C. difficile is a Gram-positive, spore-forming, obligate anaerobic rod. The organism's ability to form spores allows it to survive in harsh environments and withstand antibiotic therapy. *C. difficile* grows best in a selective medium containing cycloserine and cefoxitin and enriched with fructose and egg yolk. This medium can detect as few as 2000 organisms in a stool sample [30].

Pathogenesis

C. difficile diarrhea is a toxin-mediated disease. Pathogenic strains of *C. difficile* produce two potent protein exotoxins, toxin A and toxin B. Toxins A and B are encoded by two genes, *tcdA* and *tcdB*, that map to a 19.6 kb pathogenicity locus (PaLoc) containing additional regulatory genes [31,32]. The two toxins are structurally similar and show 49% homology at the amino acid level [32]. These high molecular weight proteins are believed to bind receptors on the luminal aspect of the colonic epithelium and are then transported into the cytoplasm. Binary *C. difficile* toxin (CDT) is a potent cytotoxin and its reported prevalence in CDAD cases ranges between 1.6 and 9.3% [33,34]. CDT is encoded by *cdtA* and *cdtB* genes which are located outside pathogenicity locus. Prior to the binding of these toxins to receptors, *C. difficile* interacts with apical microvilli of the intestinal epithelial cells through its own surface proteins, which include adhesions (the flagellar cap protein FliD, the flagellin FliC, the cell wall protein Cwp66 and the Cwp84 protease) which helps it to adhere to host determinants [35]. Specific cell surface receptors for toxin A or toxin B have not been characterized as yet. In rabbit ileum, the brush border ectoenzyme sucrase-isomaltose binds *C. difficile* toxin A and functions as a cell surface receptor [36]. Since this enzyme is not present in human colonic mucosa, other membrane surface glycoproteins presumably serve as toxin receptors. Both toxins potently activate cell signaling molecules including NF-κB and MAP kinases in human monocytes leading to the production and release of pro-inflammatory cytokines including interleukin (IL)-1β, tumor necrosis factor alpha (TNFα) and IL-8. These pro-inflammatory effects appear to precede toxin internalization and may be mediated by cell surface receptor binding [36].

There is some evidence that binary toxin CDT production and the presence of an 18 bp deletion in the pathogenicity locus gene, *tcdC*, particularly in the highly virulent BI/NAP1/027 strain, may be associated with increased incidence and disease severity [4,33,37–40]. However, their definite role in pathogenesis of CDAD remains to be elucidated.

The amino-terminal regions of both toxins carry a series of repeated protein sequences that are believed to mediate toxin binding to the host cell membrane, whereas the carboxy-terminal regions of both toxins possess similar glucosyltransferase activity. Once internalized, both toxins inactivate Rho proteins, a family of small GTP-binding proteins. The critical enzymatic action is the glycosylation of a specific, conserved threonine amino acid on Rho [41,42]. The rho protein targets of toxins A and B are rhoA, rac and cdc42, key cell signaling molecules that direct gene expression and are essential to maintain the actin cytoskeleton. Consequently, toxin-mediated rho inactivation

results in depolymerization of actin filaments, disruption of the cytoskeleton, cell rounding and cell death [36,42,43]. In contrast to cholera toxin or *Escherichia coli* heat-stable toxin, *C. difficile* toxins have no effects on intracellular levels of cyclic AMP or GMP. However, a number of other bacterial toxins target Rho proteins in a similar manner. For example, the cytotoxins from *C. sordellii* and *C. novyi* add a glucose to Rho and toxins from *Bacillus cereus* and *Staphylococcus aureus* also modify Rho family proteins. Hence it appears that *C. difficile* toxins and other structurally unrelated bacterial cytotoxins modify host cell structure and function by attacking Rho family proteins that are vital for maintenance of normal cell architecture and function.

Toxin A is an inflammatory enterotoxin that induces fluid secretion, increased mucosal permeability and marked enteritis and colitis when injected into the intestinal lumen of animals [43]. Toxin A also possesses weak cytotoxic activity against cultured cells [44]. Initially toxin B was considered an extremely potent cytotoxin but without enterotoxic activity [43,45]. This led investigators to believe that toxin B did not participate in the pathogenesis of CDAD in humans. However, studies over the last decade have shown that toxin B is not only pro-inflammatory and cytotoxic but also enterotoxic in human colon [46–48]. First, toxins A and B have been shown to cause injury and electrophysiological changes in human colonic strips *in vitro*. In fact, toxin B is 10 times more potent than toxin A in inducing these changes [49]. Second, there have been several reports isolating toxin A-negative/toxin B-positive (A^-B^+) strains of *C. difficile* from patients with antibiotic-associated diarrhea and colitis [48,50–53]. Outbreaks caused by toxin A^-B^+ strains are rare but have been reported from Europe, Canada and Japan) [51]. The estimated prevalence of toxin A^-B^+ strains of *C. difficile* causing CDAD varies widely and ranges from 0.2 to 56% [48,50–52]. Toxin A^-B^+ strains accounted for 44% of the isolates in a recent study from Ireland [48].

Both toxins of *C. difficile* bind to and damage human colonic epithelial cells [49]. *C. difficile* toxins produce colonic injury as a result of damage to the enterocyte cytoskeleton and disruption of tight junction function [49,54]. The toxins also cause a severe inflammatory reaction in the lamina propria with the formation of microulcerations of the colonic epithelium that are covered by an inflammatory pseudomembrane. A characteristic of *C. difficile* infection is the intense acute neutrophilic inflammation seen in pseudomembranous colitis patients and in animal models of the disease. In contrast to cholera toxin, which stimulates massive intestinal fluid secretion without a significant inflammatory response, *C. difficile* toxin A stimulates fluid secretion accompanied by considerable mucosal edema, inflammatory cell infiltration and necrosis.

Interactions between neuropeptides and inflammatory mediators released from inflammatory cells of the intestinal lamina propria and from epithelial cells are also critical initiators of the toxin A-induced inflammatory process (Figure 43.2). Pothoulakis *et al.* reported the release of the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) from sensory nerves and degranulation of mast cells within 15 min of luminal application of toxin A in animal intestine [55]. This is followed by release of TNFα from macrophages and upregulation of adhesion molecules on endothelial cells, allowing neutrophil attachment and invasion. Pretreatment of rabbits with a monoclonal antibody directed against the neutrophil adhesion molecules CD18 prevented neutrophil infiltration and substantially reduced toxin A-induced secretion and mucosal injury [56]. The importance of sensory neuropeptides in *C. difficile* diarrhea is also demonstrated by a report that prevention of SP and CGRP release from sensory neurons by administration of specific SP or CGRP antagonists substantially inhibit toxin-A mediated diarrhea and inflammation. Moreover, mice genetically deficient in the NK-1 (SP) receptor are largely protected from the secretory and inflammatory changes induced by toxin A and mast cell-deficient mice have markedly diminished responses to the toxin [57]. Neurotensin (NT) also appears to be involved in CDAD [58]. NT receptors are markedly up-regulated within 15–30 min of toxin A exposure and an NT receptor antagonist reduced the intestinal effects of toxin A, including mucosal mast cell activation [58].

Recent work also suggests that the proinflammatory chemokine macrophage inflammatory protein-2 (MIP-2) plays a pivotal role in mediating the early interaction between sensory nerves and mast cells and macrophages of the intestinal lamina propria following luminal exposure to toxin A. Intestinal epithelial cells release MIP-2 within 15 min of exposure to toxin A, well before the onset of fluid secretion or inflammation [59,60]. Moreover, an antibody to MIP-2 substantially inhibited intestinal secretion and inflammation in this model, supporting the view that release of this chemokine is critical for pathogenesis. These results suggest that inflammatory mediators such as MIP-2 and IL-1β released from enterocytes in response to toxin A activate sensory nerves in the subjacent lamina propria. Sensory nerves then release proinflammatory neuropeptides such as substance P and CGRP which in turn stimulate inflammatory cells leading to release of proinflammatory cytokines such as TNFα and leukotrienes that elicit neutrophil recruitment via activation of adhesion molecules on vascular endothelial cells.

Risk factors

As shown in Table 43.1, almost all antibiotics have been associated with *C. difficile* diarrhea and colitis, including metronidazole and vancomycin [61,62]. However, the precise risks associated with individual agents are difficult to establish [3,63]. While the duration of

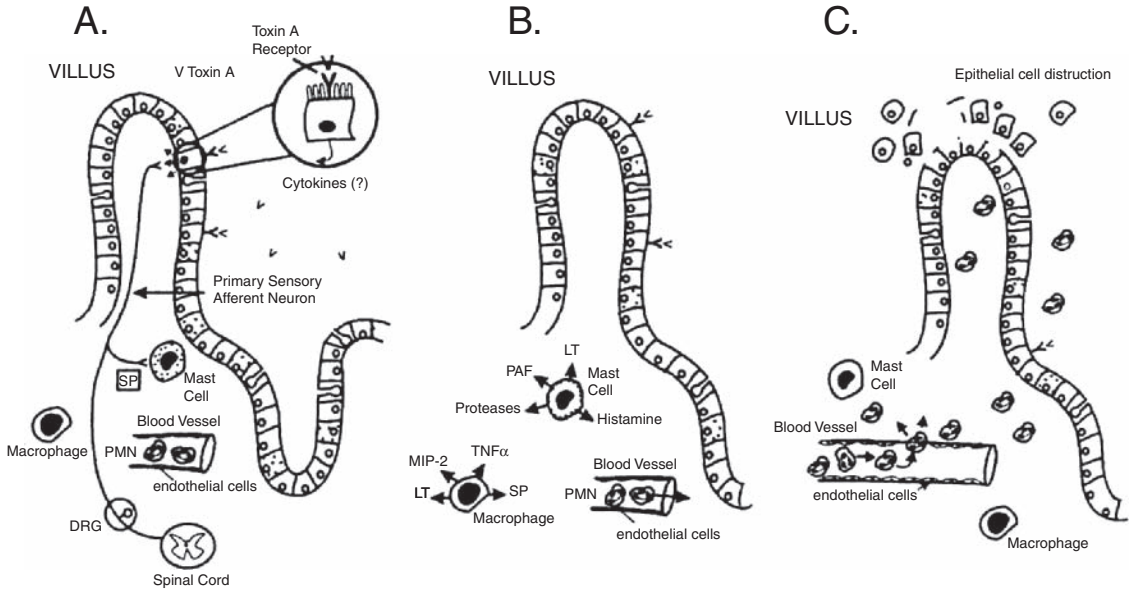

Figure 43.2 Pathogenesis of inflammatory diarrhea caused by *C. difficile* toxin A [189]. (a) Toxin A binds to its brush-border receptor(s) on intestinal epithelial cells, causing release of cytokines from these cells which diffuse into the lamina propria and activate primary sensory afferent neurons whose cell bodies are present in the dorsal root ganglia (DRG). Activation of primary sensory neurons causes early release of substance P (SP) and calcitonin gene-related peptide (CGRP), which stimulate mucosal mast cells and other resident immune cells, such as macrophages. (b) Significant mucosal mast cell degranulation occurs early after toxin A administration, releasing several proinflammatory mediators, such as histamine, platelet-activating factor (PAF), leukotriene C_4 (LT) and proteases (rat mast cell protease II). Activated intestinal lamina propria macrophages also release potent inflammatory mediators, such as macrophage inflammatory protein-2 (MIP-2), LT, tumor necrosis factor alpha (TNFα) and SP. These mediators directly stimulate fluid secretion from epithelial cells and also upregulate expression of adhesion molecules on endothelial cells and polymorphonuclear neutrophils (PMNs). (c) PMNs subsequently enter into the intestinal mucosa and release more proinflammatory mediators, which act on epithelial cells, causing acute destruction and necrosis of villus enterocytes 2–3 h after toxin A exposure. Toxin A can also directly damage enterocytes by inactivating Rho proteins and by damaging the enterocyte cytoskeleton. Adapted with permission from Pothoulakis C, Castagliuolo I, LaMont JT. Neurons and mast cells modulate secretory and inflammatory responses to enterotoxins. *News Physiol Sci* 1998; 13:58–63.

antibiotic therapy, the number of different antibiotics used and the route of administration significantly influence the risk of *C. difficile* diarrhea [27,63,64], pseudomembranous colitis associated with a single pre-operative antibiotic dose has been reported. The "big four" classes of antibiotics predisposing to *C. difficile* diarrhea are fluoroquinolones, clindamycin, cephalosporins and ampicillin/ amoxicillin [27,63]. Whereas early work focused attention on the prominent role of clindamycin and cephalosporins as inducing agents, recent studies, especially from North America, have shown that fluoroquinolones are the most common agents implicated in *C. difficile* diarrhea, especially in nosocomially acquired cases [14,27,65]. In a study by McDonald *et al.*, all current but none of the historic BI/NAP1 isolates were resistant to gatifloxacin and moxifloxacin [14]. Frequent nosocomial use of fluoroquinolones may encourage the spread of the highly virulent, fluoroquinolone-resistant BI/NAP1/027 strain leading to increased incidence and severity of CDAD [14,27,28]. Ampicillin, amoxicillin or amoxicillin–clavulanate (Augmentin) are also common causes, especially in outpatients. Less commonly implicated antibiotics include penicillins other than ampicillin/amoxicillin, macrolides (erythromycin, clarithromycin and azithromycin), tetracyclines, sulfonamides, trimethoprim and chloramphenicol. Antibiotics

Table 43.1 Antimicrobial agents that predispose to *C. difficile* diarrhea and colitis [190].

Frequently	Infrequently	Rarely or never
Quinolones	Tetracyclines	Parenteral aminoglycosides
Cephalosporins	Sulfonamides	Metronidazole
Clindamycin	Macrolides (including erythromycin)	Bacitracin
Ampicillin and amoxicillin	Chloramphenicol	Vancomycin
	Trimethoprim	

Adapted with permission from Kelly CP, LaMont JT. Treatment of *Clostridium difficile* diarrhea and colitis. In: *Gastrointestinal Pharmacotherapy* (ed. MM Wolfe), Philadelphia: WB Saunders, 1993, pp. 199–212.

that are rarely or never associated with *C. difficile* infection include parenteral aminoglycosides, vancomycin, bacitracin, nitrofurantoin or antimicrobial agents whose activity is restricted to fungi, mycobacteria, parasites or viruses. An increased rate of community-acquired severe CDAD in individuals previously considered at low risk has been reported and, in some cases, without prior antibiotic exposure [20,66–68]. Antineoplastic agents that possess antibacterial properties, principally methotrexate and 5-fluorouracil, have occasionally been implicated. Presumably, these agents induce a sufficient disturbance of the intestinal microflora to allow colonization with *C. difficile* [69,70]. Although three fairly recent studies have shown an increased risk of CDAD in patients receiving gastric acid-suppressive agents [66,71,72], controversy still exists and a large, prospective, controlled interventional study may be required to resolve the issue.

Immune factors and host defense

The first line of defense against *C. difficile* infection is the normal bowel microflora that inhibits growth of this pathogen *in vitro* and *in vivo* [73,74]. Normal adults not exposed to antibiotics are rarely infected with *C. difficile* but there have been reports concerning an increase in incidence and severity of CDAD among individuals who were previously considered at low risk [20,27,66]. Although *C. difficile* is frequently cultured from the stools of healthy neonates, it is seldom part of the normal colonic microflora in healthy children above age 2 years and adults. Colonization by *C. difficile* follows alteration of the endogenous microflora by antibiotics or cancer chemotherapy agents. The protective effect of the normal stable intestinal flora is frequently referred to as "colonization resistance." Disruption of this barrier by antibiotics and subsequent infection with *C. difficile* was originally demonstrated in animal models [75]. *C. difficile* can colonize the intestine of "germ-free" mice. Wilson and Freter demonstrated that inoculation of these animals with fecal flora from normal mice led to the disappearance of *C. difficile*, confirming the importance of the normal flora in preventing colonization [75]. The phenomenon of "colonization resistance" has also been demonstrated *in vitro* where the growth of *C. difficile* is inhibited by emulsions of feces from healthy adults but not by sterile extracts. Aas *et al.* showed that administration of donor stools via nasogastric tube resulted in prevention of recurrence of CDAD in 16 of 18 patients due to re-establishment of the normal gut microflora [76]. The specific organism or group of organisms of the normal adult microflora that exclude *C. difficile* is not entirely clear, but anaerobic species including *Bacteroides* may be especially important. For example, treatment with lyophilized *Bacteroides* species can inhibit the growth of *C. difficile* in the stool of patients with chronic recurrent infection. A UK study by Hopkins and MacFarlane showed that whereas *Bacteroides* species diversity was increased in the feces of healthy elderly people, bifidobacterial species diversity decreased with age, with *Bifidobacterium adolescentis* and *Bifidobacterium ngulatum* being the most common isolates [77]. CDAD patients were characterized by a greater diversity of facultative species, lactobacilli and clostridia, but greatly reduced numbers of bacteroides, prevotella and bifidobacteria [77]. Such bacterial population changes in the normal microbiota could result in metabolic conditions favorable for the establishment of pathogenic microorganisms, such as *C. difficile*. Healthy neonates and infants have poor "colonization resistance" because they have not yet developed a stable complex colonic microflora [73]. Colonization rates with *C. difficile* of 25–80% have been reported in infants and children up to the age of 2 years; however, despite the presence of toxin, they rarely develop *C. difficile*-associated diarrhea [3,78]. Although cats, dogs, horses, rabbits, Syrian hamsters and donkeys are colonized by *C. difficile*, there is no evidence that animals serve as reservoirs for colonization of humans [79].

The humoral immune system provides a second line of defense against *C. difficile*. Immunization of laboratory animals against toxin A protects against a subsequent challenge with *C. difficile* [80]. Infant hamsters who drink milk from mothers immunized against toxins A and B are also protected [80]. The fact that only one-third of *C. difficile* carriers develop diarrhea [81] suggests that the host's ability to produce anti-toxin antibodies may play a similar role in humans in modifying disease expression. Serum antibodies against *C. difficile* toxins are present in the majority of the adult population. Secretory IgA anti-toxin is present in colonic secretions and can inhibit binding of toxin A to its specific brush border receptor providing a possible mechanism of immune protection [82]. A selective reduction in mucosal IgA-producing cells and macrophages is associated with colonic disease in *C. difficile*-infected patients and severe reduction in colonic IgA-producing cells may predispose to recurrence of CDAD [82]. High levels of serum and intestinal antitoxin antibodies may be associated with mild colitis or asymptomatic carriage of *C. difficile* [83,84]. Conversely, a deficient antibody response may predispose to severe, prolonged or recurrent *C. difficile* colitis [84,85].

In a prospective study of nosocomial *C. difficile* infection, 41% of 47 patients who acquired *C. difficile* remained asymptomatic [17]. At the time of colonization, serum levels of IgG antibody against toxin A were three times higher in asymptomatic carriers than patients who developed *C. difficile* diarrhea (Figure 43.3). Multivariate analysis indicated that patients with a low serum level of IgG anti-toxin A were 48 times more likely to develop *C. difficile* diarrhea than patients who had high antibody levels ($p < 0.001$). Although no protective association was found for serum IgG anti-toxin B levels, these were significantly

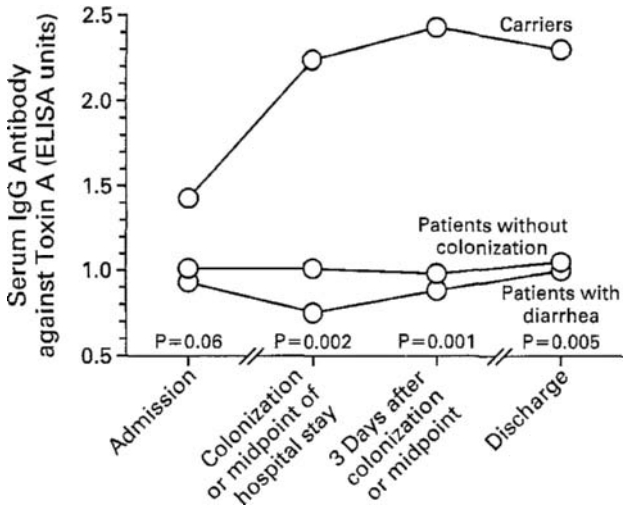

Figure 43.3 Serum IgG antibody levels against toxin A in asymptomatic carriers and patients with *C. difficile* diarrhea during hospitalization [17]. The median levels of IgG antibody against toxin A are shown for 28 patients in whom *Clostridium difficile* diarrhea developed, 19 asymptomatic carriers and 187 patients without colonization at the time of hospitalization, at the time of colonization by *C. difficile*, 3 days after colonization and at discharge. The median interval between admission and colonization was 3 days (range, 3–33 days) and the median interval between the third day after colonization and discharge was 12 days (range, 2–56 days). Serum levels of IgG antibody against toxin A were three times higher in asymptomatic carriers compared with patients who developed *C. difficile* diarrhea. The *p*-values refer to the comparison among the three groups (by the Kruskal–Wallis test). Reproduced with permission from Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000; 342:390–7. Copyright ©2000 Massachusetts Medical Society. All rights reserved.

correlated with IgG levels against toxin A in the asymptomatic carriers. While we await controlled trials, open-label studies have demonstrated the efficacy of passive immunotherapy using pooled human immune globulin (containing anti-toxin A IgG antibody) in patients with recurrent or refractory *C. difficile* diarrhea [86]. In a more recent small study, *C. difficile* toxoid vaccine induced immune responses to toxins A and B in patients with CDAD and was associated with resolution of recurrent diarrhea. The results of this study support the feasibility of active vaccination against *C. difficile* and its toxins in high-risk individuals but must be validated in larger, randomized, controlled trials.

A third protective factor is gastric acid, which kills most of the vegetative cells and probably reduces the number of viable spores of *C. difficile* [71,72,87,88]. Normal intestinal peristalsis is also important as a defense mechanism by eliminating *C. difficile* and its toxins. Conversely, antidiarrheal medications that reduce intestinal peristaltic activity may delay clearance of the organism and its toxins and worsen the duration or intensity of illness.

Epidemiology of *C. difficile* infection

In the 1980s and early 1990s, nosocomial *C. difficile* infection caused approximately 250,000–500,000 cases of diarrhea and colitis each year in the United States [16], compared with only 20,000 cases per year in outpatients [19]. However, based on hospital discharge data, the number of cases doubled in the United States between 1996 and 2003 [5] and it has recently been reported that approximately 3 million cases of CDAD occur annually in the United States [40,89,90]. It is also estimated that a case of CDAD carries an average cost of $4000 and can prolong hospital stay by almost 4 days [91,92]. The new millennium has witnessed a significant increase in the incidence, severity, recurrence and relapse rates and also mortality rates associated with nosocomially acquired CDAD, especially in epidemic areas of North America and Europe [5,12,20,26,93,94]. Similar but less pronounced trends have been observed with community-acquired CDAD [9,20,23,28].

Dissemination of the hypervirulent NAP1/027/BI strain which produces 16 and 23 times as much toxins A and B, respectively, as compared with historical isolates and its resistance to fluoroquinolones, poor sanitary conditions, lack of optimal cleanliness in hospitals, problems with infection control strategies and an aging population are some of the factors contributing to a recent dramatic rise in the incidence and severity of CDAD. The incidence of CDAD among patients aged 65 years or older in Quebec province, Canada, increased from 102 per 100,000 population in 1991 to 867 per 100 000 in 2003 [12]. Furthermore, the proportion of complicated cases increased from 7.1% in 1991–92 to 18.2% in 2003 [12].The Quebec Health Ministry reported a total of 7004 cases of *C. difficile* infection in 2003–2004 with 1270 deaths (a crude mortality rate of 18%) [11]. Loo *et al.* [4] reported an attributable mortality of greater than 10% in those aged over 60 years. McDonald and colleagues (Centers for Disease Control and Prevention, Atlanta, GA, USA) demonstrated that a diagnosis of CDAD, based on US hospital discharges using National Hospital Discharge Survey (NHDS) data, doubled from 82,000 cases or 31 per 100,000 population in 1996 to 178,000 cases or 61 per 100,000 in 2003 [5]. The CDAD rate was calculated at 228 per 100,000 among persons aged 65 years or older compared with 40 per 100,000 among persons 45–64 years old. In the UK, the number of CDAD cases increased from 28,000 in 2002 to 43,000 in 2004 [94].

Carriage of *C. difficile* is rare in healthy adults not taking antibiotics; intestinal carriage rates of 0–3%, have been reported in American and European populations [95,96]. It remains unclear whether carriage is a temporary or permanent state. In contrast, the incidence of *C. difficile* carriage is unusually high following admission to hospital and treatment with antibiotics. In one study, 7% of patients admitted to an acute care hospital had positive stool

cultures for *C. difficile* and another 21% became colonized with *C. difficile* during their hospital stay [16]. At the time of discharge, 82% of hospital carriers were still excreting *C. difficile* in their stools [16], accounting for the high rate of infection in nursing homes and chronic care facilities. Elderly, debilitated patients in hospitals and nursing homes are particularly vulnerable and colonization rates as high as 73% have been reported in some facilities. Subsequent hospital studies reported similar *C. difficile* carriage rates [10,17]. Two recent studies have shown that 44% cases of CDAD are community acquired while 56% are nosocomial [97,98].

C. difficile survives in the hospital environment as antibiotic-resistant spores that are ingested by patients. Infected patients, thermometers, blood pressure cuffs, environmental surfaces, inanimate objects and the hands of healthcare workers are all potential sources of *C. difficile* in the hospital setting [99,100]. In one study, patients sharing a room with a *C. difficile*-carrying room-mate acquired *C. difficile* more rapidly than patients who were in single rooms or with room-mates who were culture negative (mean time to acquisition, 3.2 days compared with 18.9 days, respectively) [16]. The same group of investigators also cultured *C. difficile* from the hands of 59% of hospital personnel caring for patients with positive *C. difficile* cultures and also from bedrails, commodes, toilets, floors, scales, call-buttons, windowsills and dustmops and in the rooms where these patients were nursed [16]. Thus, cross-infection may occur by patient-to-patient spread or through environmental contamination [16,101]. The spread of infection can be interrupted by careful hand washing with soap and water after examining patients and by the use of disposable gloves [102]. Alcohol gel hand rubs do not kill *C. difficile* spores and hands must be cleaned with soap and water [102,103]. In a recent study, 18–60% of the initial inoculation of *C. difficile* spores on a contaminated hand were readily transferred by a handshake after using commercially available alcohol gel and handwashing with soap and water was effective in removing *C. difficile* from contaminated hands [103].

Although asymptomatic carriers rarely go on to develop *C. difficile*-associated diarrhea [17,81], they can contaminate the hospital environment and serve as a reservoir of infection. McFarland *et al* demonstrated that 29% of cultures taken from rooms of asymptomatic carriers were positive for *C. difficile*, whereas only 8% of cultures from rooms of culture-negative patients were positive [16]. Asymptomatic carriers have also been implicated as the source of strains of *C. difficile* that caused *C. difficile*-associated diarrhea in other hospital inpatients. In antibiotic-treated animals, the infective dose of toxigenic *C. difficile* may be as low as two organisms [95]. If human susceptibility is similar, control of *C. difficile* infection in hospitals will continue to be a major challenge as the organism is excreted in high numbers in liquid feces (up to 10^9 organisms per gram) [101]. Furthermore, *C. difficile* can be cultured in a hospital room 40 days after discharge of an infected patient and it is likely that spores of *C. difficile* may persist for many months in hospital wards, as they are particularly resistant to oxygen, desiccation and many disinfectants [101,104].

Although antibiotic exposure is the most important risk factor for *C. difficile* infection, other risk factors include increasing age (after infancy) and severity of underlying disease [37,98]. In England and Wales, 75% of all reports of *C. difficile* to the Public Health Laboratory Service Communicable Disease Surveillance Centre between 1992 and 1996 occurred in patients over 64 years of age [105]. Pepin *et al.* from Canada showed that the increase in incidence was more marked in patients over 65 years of age and it increased from 102.0 per 100,000 population in 1991 to 866.5 per 100,000 population in 2003 [12].A recent similar study by McDonald *et al.* from the United States also demonstrated that increasing age is an independent risk factor for CDAD [5]. Independent of age, sicker patients are also more likely to acquire *C. difficile* [17,98]. In a study of antibiotic recipients, patients with severe underlying disease at the time of hospital admission were eight times more likely to develop *C. difficile* infection than patients who were less severely ill [17]. In a recent study, Vanjak *et al.* reported a significantly higher rate of CDAD among patients with autoimmune hepatitis [106].

An increased incidence of *C. difficile* infection in oncology and HIV patients appears to be related to specific risk factors among these groups of patients. Higher numbers and prolonged courses of different antibiotics during hospitalization, low intensity of chemotherapy, reflecting a lower frequency of neutropenia, lack of parenteral vancomycin use and hospitalization within the previous 2 months were independently predictive of *C. difficile* colitis in hospitalized oncology patients [107,108]. In a recent study, administration of IL-2 either during hospitalization or in the 30 days preceding admission was seven times more likely to have occurred in CDAD cases [108]. A $CD4^+$ cell count less than 50 mm^{-3}, and also clindamycin and penicillin use, were independent factors significantly associated with *C. difficile* colitis among HIV-infected patients. Although most of the HIV patients with CDAD have low white cell counts, a number of cases with intense leukemoid reactions have been described [109]. In a large study by Sanchez *et al.* from the United States, *C. difficile* was the commonest recognized cause of bacterial diarrhea among patients infected with HIV and the risk of *C. difficile* diarrhea increased with increased severity of HIV disease [110].

Given the documented association between IL-8 polymorphism and the development of traveler's diarrhea, Jiang *et al.* assessed the rates of IL-8 promoter polymorphism among patients with CDAD [111]. Of 42 CDAD patients, 39% were positive for the polymorphism

compared with 16% and 17% of control patients with *C. difficile*-negative diarrhea and no diarrhea, respectively. This is the first indication of genetically determined variations in an individual's risk for symptomatic CDAD.

Other reported risk factors for *C. difficile* infection include the presence of a nasogastric tube, gastrointestinal surgery, non-surgical gastrointestinal procedures, intensive care unit stay and duration of hospital stay [63,91]. The strengths of the associations of these risk factors with *C. difficile* vary from study to study. Because these factors are often markers of disease severity and/or older age, the strength of their association with *C. difficile* often loses statistical significance after controlling for confounding variables [17,87].

Pathology

When the human colon is exposed to *C. difficile* toxins, loss of actin filaments leads to cell rounding and shedding of cells from the basement membrane into the lumen, leaving a shallow ulcer on the mucosal surface. Serum proteins, mucus and inflammatory cells flow outwards from the ulcer, creating the characteristic colonic pseudomembrane. The spewing forth of the inflammatory exudate from the mucosal microulceration produces the typical "volcano" or "summit" lesion of *C. difficile* colitis (Figure 43.4). On gross or sigmoidoscopic inspection of the colonic or rectal mucosa, pseudomembranes appear as yellow or off-white raised plaques 0.2–2.0 cm in diameter scattered over a fairly normal appearing intervening mucosa [112]. Up to 30% of patients with pseudomembranous colitis (PMC) have no characteristic findings in the rectum and sigmoid colon and pseudomembranes are restricted to the more proximal colon [113]. Edema and hyperemia of the full thickness of the bowel wall are common and this is reflected by the typical radiographic appearance of "thumbprinting" or massive wall thickening on computed tomography (CT) scanning of patients with pseudomembranous colitis.

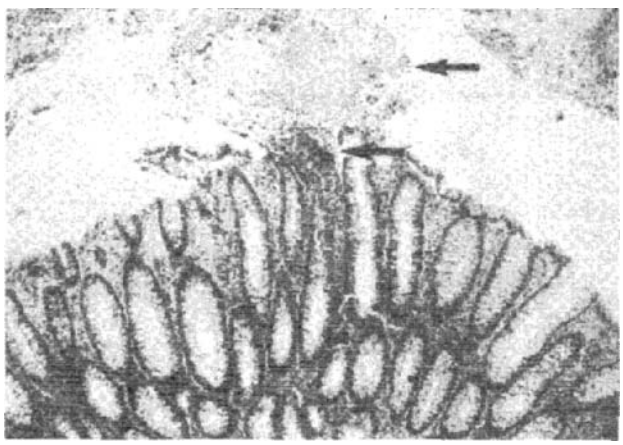

Figure 43.4 Endoscopic-biopsy specimen from a patient with pseudomembranous colitis and a "summit" or "volcano" lesion (hematoxylin and eosin stain, ×55) [3]. Focal ulceration of the colonic mucosa (lower arrow) is evident, with exudation of a pseudomembrane (upper arrow) made up of inflammatory cells, fibrin and necrotic debris. The adjoining mucosa is intact. Reproduced with permission from Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. *N Engl J Med* 1994; 330:257–62. Copyright ©1994 Massachusetts Medical Society. All rights reserved.

The patchy distribution of the pseudomembranes is probably related to a toxin dose–response effect. For example, when human colonic mucosal strips *in vitro* were exposed to different concentrations of toxin B, cellular damage was very patchy at low concentrations but, as the toxin concentration was raised, the area of damage increased until it was nearly confluent [62]. Similarly, some patients with early PMC have only scattered lesions on the colonic mucosa, whereas others exhibit a confluent pseudomembrane covering the entire mucosa.

The pathologic features of PMC have been classified into three distinct types [114]. In type 1 PMC, the mildest form, the major inflammatory changes are confined in the superficial epithelium and immediately subjacent lamina propria. Typical pseudomembranes and summit lesions are present and crypt abscesses are occasionally noted. Type 2 PMC is characterized by more severe disruption of glands and marked mucin secretion and more intensive inflammation of basal lamina. Type 3 PMC is characterized by severe, intense necrosis of the full thickness of the mucosa with a confluent pseudomembrane. In practice, colonic histology is often normal in mild cases and may reveal only type 1 changes in the majority of cases, while the classical type 3 pseudomembranous colonic changes are only seen in a minority of patients. Small bowel enteritis in human beings is exceedingly rare [115] but common in rabbits and foals where pseudomembranes are present in the small bowel and histological findings are similar to PMC [79,115]. There are only a handful of case reports of pseudomembranous ileitis and some of these were complicated by ileal perforation [115]. Most but not all of these cases had prior colectomy, either recently or in some cases several years or decades previously. *C. difficile*-related diversion colitis has also been reported and can be difficult to differentiate endoscopically and microscopically from recurring ulcerative colitis or diversion colitis [116]. There are also rare case reports of refractory pouchitis secondary to *C. difficile* infection [117].

Clinical features of *C. difficile* infection

Infection with *C. difficile* can produce a wide spectrum of clinical manifestations ranging from the asymptomatic

carrier state in infants and adults to fulminant colitis with megacolon or perforation.

Asymptomatic carrier state

Asymptomatic carriage of *C. difficile* is common in hospitalized patients. Approximately two-thirds of infected hospitalized patients remain asymptomatic [118]. Several large epidemiological studies have demonstrated that 10–30% of patients in hospital may be carriers of the organism [15–17,119]. Carrier states in adults who have received antibiotics could be as high as 46% [18]. Despite the fact that over 50% of *C. difficile* isolates from these patients are toxigenic, they do not appear to be at an increased risk of developing symptomatic disease [6,28,41,84]. The basis for this variability in response is not entirely clear, but as mentioned above, the host immune response appears to be more important than bacterial virulence factors. Other important host response factors may include toxin receptor density, the presence or absence of the normal barrier flora.

Antibiotic-associated diarrhea

Mild diarrhea is fairly common during treatment with antibiotics, but it is related to *C. difficile* in only 12–33% of cases [89,120]. *Clostridium perfringens* enterotoxin and *Staphylococcus aureus* are other important infective causes of antibiotic-associated diarrhea (AAD) and prevalence rates of 3.3% and 0.2%, respectively, have been reported [120]. Most antibiotic-associated diarrhea is related to an osmotic effect of unabsorbed carbohydrate [89]. In normal individuals, unabsorbed dietary carbohydrate delivered to the large intestine undergoes fermentation by the microflora to short-chain fatty acids, hydrogen, methane and other metabolites. However, during antibiotic therapy this normal fermentation process is interrupted, allowing accumulation of carbohydrates that bind water and cause diarrhea. Diarrhea is watery, containing mucus but not blood. Sigmoidoscopic examination reveals normal colonic mucosa or mild edema or hyperemia of the rectum. Obvious colitis or pseudomembrane formation does not occur. Systemic symptoms are absent and diarrhea stops when antibiotics are discontinued in the majority of patients.

C. *difficile* diarrhea without pseudomembrane formation

This is the most common clinical manifestation of *C. difficile* infection. The incubation period for diarrhea after colonization is not known but is likely to be less than a week, with a median time of onset of approximately 2 days [10,16,17]. *C. difficile* diarrhea is a more serious illness than simple antibiotic-associated diarrhea. *C. difficile* diarrhea is typically watery and foul smelling. Mucus or occult blood may be present but visible blood is rare [121]. Some patients present with fever, leukocytosis and crampy abdominal pain. Extraintestinal manifestations of *C. difficile* infection such as cellulitis, necrotizing fascitis, prosthetic device infection, septic arthritis, septicemia, osteomyelitis, bacteremia, brain empyema, splenic or pancreatic abscess may occur but are extremely rare [115,122,123], but asymmetric arthropathy affecting large, weight-bearing joints is more common [123]. Fecal leukocytes may be present in the stools but are not a reliable indicator of *C. difficile* colitis, as they were absent in 72% of toxin-positive stools in one study. However, when present, fecal leukocytes indicate that severe infection and a high chance of detecting *C. difficile* toxins [124]. Sigmoidoscopy may reveal a nonspecific diffuse or patchy erythematous colitis without pseudomembranes.

Pseudomembranous colitis

This entity is the classic manifestation of full-blown *C. difficile* colitis and is accompanied by similar, but often more severe, symptoms than observed in *C. difficile* diarrhea. Lee *et al.* recently showed that advanced age and long hospital stay may make patients with presumed antibiotic-associated diarrhea more susceptible to PMC, and when both risk factors are present, the positive predictive value of developing PMC was 0.86 [125]. Sigmoidoscopic examination reveals the classic pseudomembranes, raised yellow plaques ranging from 2 to 20 mm in diameter scattered over the colorectal mucosa (Figure 43.5).

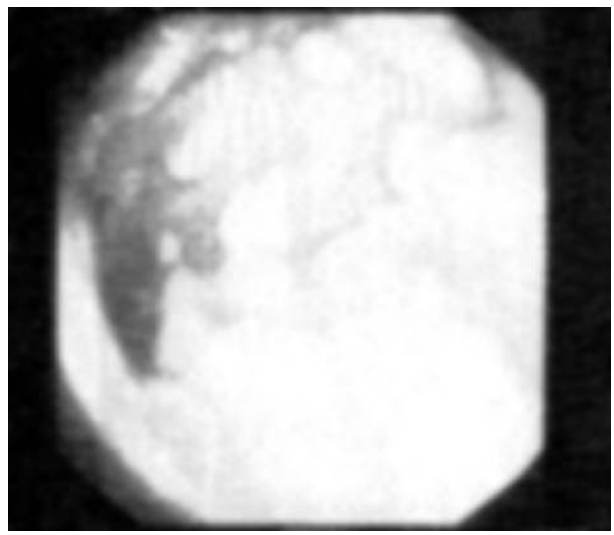

Figure 43.5 Colonoscopy view of pseudomembranous colitis resulting from refractory *C. difficile* infection. In the lower part, coalescing pseudomembranes are visible (raised, adherent yellow plaques on the colonic mucosa that vary in size from 2 to 20 mm). In the upper part, there is nonspecific erythema of the colonic mucosa, with isolated pseudomembranes visible. Reproduced from Farrell RJ, Kyne L, Kelly CP, Pseudomembranous colitis and Clostridium difficile infection. In: *Inflammatory Bowel Disease: From Bench to Bedside*, 2nd edn, (ed. SR Targan, F Shanahan & LC Karp), 2003, pp.823–44. With kind permission of Springer Science and Business Media.

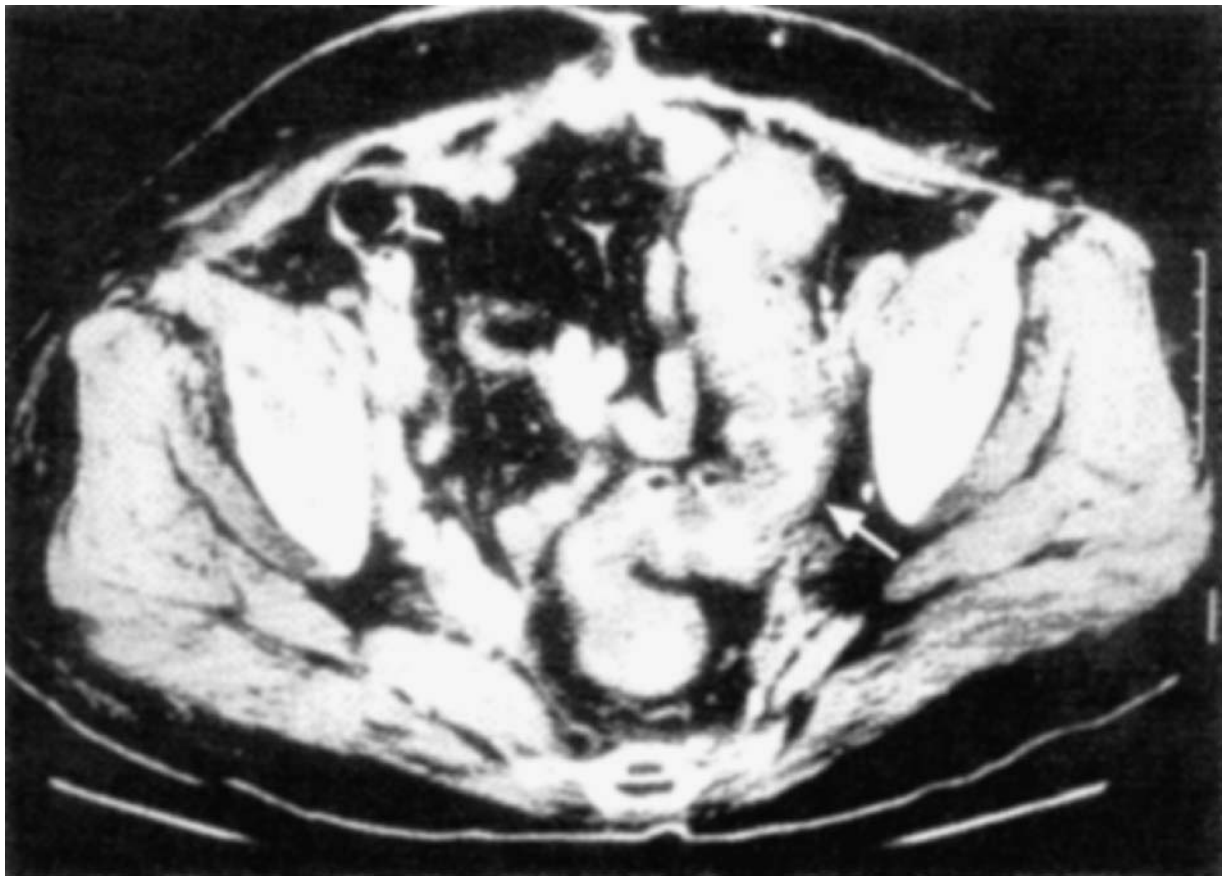

Figure 43.6 Computed tomograph of the abdomen in *C. difficile* colitis [12]. There is marked thickening of the colonic wall in the sigmoid colon (arrow). The accordion pattern is evident, produced by a series of broad edematous colonic haustral folds. Reproduced with permission from Linevsky JK, Kelly CP. *Clostridium difficile* colitis. In: *Gastrointestinal Infections: Diagnosis and Management* (ed. JT LaMont), New York: Marcel Dekker, 1997, pp. 293–325.

In severely ill patients, white blood cell counts of 20,000 or greater and hypoalbuminemia of $3.0\,g\,dl^{-1}$ or lower may be observed. Fecal leukocytes are present in a greater proportion of patients (40–50%) in this clinical setting and peripheral leukocytosis is more common and may serve as a surrogate marker of this infection [126].Three patterns of leukocytosis have been described: a sudden rise in the white blood cell count correlating with symptoms of *C. difficile* colitis, and exacerbation of pre-existing leukocytosis plus nonspecific symptoms of *C. difficile* infection or leukocytosis preceding *C. difficile*-associated symptoms [126]. In the last two scenarios, the diagnosis of *C. difficile* colitis is generally delayed and *C. difficile* infection as a cause of unexplained leukocytosis should be considered [126]. Nursing home residency and pre-hospitalization acid-reducing treatment are both independently associated with increased risk of recurrent PMC, while a serum albumin less than $25\,g\,l^{-1}$, a white blood cell count of above 20,000 and pre-hospitalization nasogastric tube feeding carried higher mortality rates [127]. Most patients with PMC have involvement of the rectosigmoid area but as many as one-third of patients have pseudomembranes limited to the more proximal colon. There have been a few reported cases of pseudomembrane formation involving the small intestine [115]. A number of these were in post-surgical patients and included involvement of a defunctionalized limb of a jejunal–ileal bypass, an ileal conduit or an end-ileostomy. Although an abdominal CT scan in patients with PMC is not highly specific, it may reveal mucosal edema, thumbprinting, pancolitis, pericolonic inflammation and pronounced thickening of the colonic wall that may involve the entire colon, collections of fluid in the lower abdomen or pelvis and also the characteristic "accordion sign" of contrast trapped among the thickened folds [128,129] (Figure 43.6). In a recent study by Ash *et al.*, 50% of patients with *C. difficile* colitis had abnormal CT scans, with segmental colonic disease more common than diffuse disease [129]. Specific CT findings in this study did not correlate with clinical parameters and did not predict surgical treatment [129]. A neutrocytic ascites with a low serum-to-ascites albumin gradient may occur in patients with hypoalbuminemia. Ascites may even be the

presenting manifestation of PMC. A recent radiological review of typical sonographic appearances of common colonic diseases reported ascites in 64% of patients with PMC, compared with 24% of patients with diverticulitis, cancer and inflammatory or ischemic bowel disease [130].

Fulminant colitis

Fulminant colitis in *C. difficile* infection occurs in approximately 3% of patients [131–133]. As the incidence and severity of *C. difficile* infection has significantly increased over last decade, the proportion of fulminant colitis has similarly increased [12,26]. In a study by Pepin *et al.* from Quebec, Canada, a high leukocyte count (>20,000) and an elevated creatinine level (200 μmol l^{-1} or greater) were strongly associated with worse outcomes. In a 2003 study, 41% of 110 patients diagnosed at the Centre Hospitalier Universitaire de Sherbrooke with a high leukocyte count and/or high creatinine level had fulminant colitis and 26% of these patients died within 30 days after diagnosis [12]. Longo *et al.* reported an even higher overall mortality rate of 47% among their 67 patients who developed fulminant *C. difficile* colitis [132]. Transmural inflammation in fulminant *C. difficile* colitis can lead to serious complications, including perforation, prolonged ileus, megacolon and death [133]. Patients with fulminant colitis may complain of severe abdominal pain, diarrhea and abdominal distention. Some patients exhibit high fever, rigors, dehydration and marked leukocytosis. Diarrhea is usually prominent, but may be minimal in patients who develop toxic megacolon or an ileus resulting in the pooling of secretions in the dilated, atonic colon [68]. Diarrhea may actually decrease in the absence of clinical improvement, as a result of paralytic ileus and acute colonic dilatation. Hypoalbuminemia may also occur because of severe protein-losing enteropathy. An abdominal radiograph may reveal a dilated colon (>7 cm in its greatest diameter), consistent with toxic megacolon. Patients with megacolon may also have dilated small intestine on plain abdominal radiographs with air–fluid levels mimicking an intestinal obstruction or ischemia (pseudo-obstruction) [78]. In some patients, fulminant *C. difficile* infection may present with signs and symptoms of bowel perforation. Typically, these patients have abdominal rigidity, involuntary guarding, rebound tenderness and reduced bowel sounds. Abdominal radiographs may reveal the presence of free abdominal air. Those patients who need surgery should undergo a total rather than partial colectomy. A UK study by Koss *et al.* showed a mortality rate of 100% with left hemicolectomy compared with 11% with total colectomy [134]. Patients who present with acute abdomen (without obvious perforation or toxic megacolon) secondary to *C. difficile* infection generally do not need surgery, but in cases of severe leukocytosis (white blood cell count of 30,000–80,000], hypotension or metabolic acidosis, colectomy may be indicated for impending or existing perforation [135].

C. difficile infection in patients with inflammatory bowel disease

Infection with *C. difficile* may complicate the course of ulcerative colitis or Crohn's disease [136,137]. Two recent studies have demonstrated an increased CDAD incidence in patients with inflammatory bowel disease (IBD) compared with non-IBD populations, with *C. difficile* carriage rates of 65–75% among IBD patients attending outpatients [136,137]. In contrast to the high rate of antibiotic-associated CDAD in the non-IBD population, only 61% of IBD patients had recent exposure to the antibiotics [137]. Colonic involvement and immunomodulator therapy appear to be independent risk factors for development of CDAD among IBD patients. *C. difficile* infection negatively impacts clinical outcomes in IBD patients [137] and IBD patients infected with *C. difficile* may develop diarrhea, abdominal pain and low-grade fever mimicking a flare of their IBD. The diagnosis is established by identification of *C. difficile* toxin in a stool sample and several studies have shown that all IBD patients who require hospitalization for diarrhea should be routinely tested for *C. difficile* on admission, even if pre-hospital testing has been negative, in view of the substantial false negative results with enzyme-linked immunosorbent assay (ELISA) [119,136,137]. While pseudomembrane formation appears to be rare in the setting of IBD [137], *C. difficile* diarrhea and colitis generally respond promptly to appropriate therapy with metronidazole or vancomycin with the exception of complicated CDAD which is associated with more complications and poor outcomes. Response to metronidazole, especially in patients with severe CDAD, has recently been reported as suboptimal with some investigators recommending that all IBD cases with complicated CDAD should be treated with vancomycin [15,137]. Some patients have developed *C. difficile* at the onset of their first attack of IBD, a situation that can lead to considerable diagnostic confusion. Infection with *C. difficile* in patients with ulcerative colitis or Crohn's disease requires prompt diagnosis and management since failure to diagnose the infection may lead to inappropriate treatment with corticosteroids or immunosuppressive agents.

Diagnosis

The diagnosis of *C. difficile* diarrhea or colitis is usually based on a history of recent or current antibiotic therapy, development of diarrhea or other evidence of acute colitis and demonstration of infection by toxigenic *C. difficile*, usually by detection of toxin A and/or toxin B in a stool sample with ELISA tests for toxins A and B [3,68,89].

The diagnosis of *C. difficile* diarrhea should be suspected in any patient with diarrhea who has received antibiotics within the previous 2 months and/or whose diarrhea began 72 h or more after hospitalization [5,74]. However,

Table 43.2 Stool tests for diagnosis of *C. difficile* infection [112].

Test	Detects	Advantages	Disadvantages
Cytotoxin *assay*	Toxin B	"Gold standard" Highly sensitive and specific	Requires tissue culture facility Takes 24–48 h
Enzyme immunoassay	Toxin A or B	Fast (2–6 h) Easy to perform High specificity	Not as sensitive as the cytotoxin assay
Latex agglutination assay	Bacterial enzyme–glutamate dehydrogenase	Fast Inexpensive Easy to perform	Poor sensitivity and specificity
Culture	Toxigenic and non-toxigenic *C. difficile*	Sensitive Allows strain typing in epidemics	Requires aerobic culture Not specific for toxin-producing bacteria Takes 2–5 days
Polymerase chain reaction	Toxin A or B genes in isolates or directly in feces	High sensitivity and specificity	Requires expertise in molecular diagnostic techniques

Adapted with permission from Linevsky JK, Kelly CP. *Clostridium difficile* colitis. In: *Gastrointestinal Infections: Diagnosis and Management* (ed. JT LaMont), New York: Marcel Dekker, 1997, pp. 293–325.

CDAD should also be considered in symptomatic IBD patients with colonic involvement who require hospitalization and are on immunomodulators regardless of any recent antibiotic exposure. Recent studies have shown that up to 40% of patients with *C. difficile* diarrhea at tertiary referral centers are symptomatic on admission to hospital [16,17]. Therefore, it is also prudent to consider the diagnosis in patients who present to hospital with antibiotic-associated diarrhea, especially if there is a history of recent discharge or transfer from hospital or nursing home.

Testing of non-diarrheal stools for *C. difficile* by culture or toxin assay is not recommended because many patients in hospital may be asymptomatic carriers of the organism [16,17,74,121]. Treatment of asymptomatic carriers is not recommended, as it may prolong the carrier state. For the same reason, "test-of-cure" of *C. difficile* in asymptomatic patients with recent episodes of *C. difficile* diarrhea is not indicated. The duration of stool carriage of this organism following an episode of *C. difficile* diarrhea is unclear, but may persist for at least 3–6 weeks.

Fecal specimens

If *C. difficile* diarrhea is suspected, a freshly taken fecal sample should be submitted immediately to the laboratory in a clean, water-tight container, to test for the presence of toxigenic *C. difficile* (usually by the detection of fecal toxin A or B). Anaerobic culture and the use of transport media do not enhance recovery of the organism or its toxin and therefore are not recommended [99]. Storage at ambient temperature leads to possible denaturation of fecal toxin. Therefore, samples should be tested immediately for toxin or frozen for future testing. Freeman and Wilcox recommended storing samples at 4 °C to minimize toxin degradation [138]. They also demonstrated that buffering fecal samples with phosphate-buffered saline allowed *C. difficile* to survive for prolonged periods [138]. As *C. difficile* readily forms spores, culture of the organism from stools is largely unaffected by ambient storage.

Laboratory tests for *C. difficile*

A variety of laboratory tests are available for the diagnosis of *C. difficile*-associated diarrhea (Table 43.2). Enzyme immunoassays (EIAs) to detect both toxins A and B in the stool are increasingly used in clinical practice [68]. These tests have the advantages of being relatively inexpensive, quick and highly specific. However, their sensitivity is not ideal, leading to frequent false negative results. The tissue culture cytotoxicity assay is more sensitive, leading to greater diagnostic accuracy, but it is also more costly and time consuming.

Tissue culture cytotoxicity assay

The "gold standard" diagnostic test to identify *C. difficile* toxins in the stool of patients with antibiotic-associated diarrhea is the tissue culture cytotoxicity assay [15,28,139,140]. By inactivating rho proteins (see above), toxins A and B effect a disintegration of the actin cytoskeleton of mammalian cells leading to cell rounding. A suspension of diarrheal stool in phosphate-buffered saline is centrifuged and filtered. The filtrate is then inoculated on to a monolayer of cultured cells [usually fibroblasts or Chinese hamster ovary (CHO) cells]. The monolayer is examined after overnight incubation and again at 48 h for cell rounding. The specificity of the test result is established by preincubating the sample with specific neutralizing antitoxin antibody [141].

The advantages of the cytotoxicity assay include its high sensitivity (67–100%) and specificity (85–100%), if

performed correctly [28,99]. However, the sensitivity of the test may be reduced by inactivation of toxins during transport and storage, by the age and type of cell line used and by the dilution titer of the stool sample [138,142]. Therefore, a negative cytotoxicity test does not completely rule out *C. difficile* as the cause of diarrhea. A positive stool cytotoxin test in a patient with antibiotic-associated diarrhea indicates that it is highly likely that *C. difficile* is the cause of diarrhea. Disadvantages of the cytotoxicity assay are that it is relatively expensive, requires a cell culture facility and is slow, requiring incubation of the fecal filtrate for 24–48 h [15,28]. Furthermore, nonspecific cytopathic effects observed in approximately 2% of cases can make interpretation of test results difficult [143].

Enzyme-linked immunoassay tests for toxin A or toxins A and B

There are several commercially available EIAs for the detection of toxin A or toxins A and B of *C. difficile* in stool specimens [48,139,140,144,145]. Most of the tests initially used in clinical practice were designed to detect toxin A by using a monoclonal antibody that reacts with an epitope located on the amino-terminal region of the toxin A molecule. The advantages of EIA tests are that they are easier to perform than the cytotoxicity test, they are relatively inexpensive, results may be available within 2–6 h and they have high specificity (75–100%) [15,28,99]. Their main disadvantage is that they are less sensitive than the cytotoxicity test (63–99%) [15,28,74,99]. In addition, if stool samples are tested for toxin A only, *C. difficile* diarrhea due to a toxin A-negative, toxin B-positive strain will not be diagnosed [51,53]. For this reason, commercial kits that detect both toxins A and B have an advantage over those that detect toxin A alone [48,51,144] and are currently used in most hospitals in the United States [68]. A recent study demonstrated ELISA sensitivity rates of 54, 75 and 78% for first, second and third stool samples, respectively [137], and therefore three stool samples for ELISA, which tests both toxins A and B, are often considered necessary to exclude CDAD reliably [28].

Latex agglutination assay

The latex agglutination test was designed to detect toxin A in the stool but instead recognizes glutamate dehydrogenase, another bacterial protein present in nontoxigenic strains of *C. difficile* and other nonpathogenic clostridia [146]. Thus the test is nonspecific and is also not sufficiently sensitive (23–59%) for the diagnosis of *C. difficile* diarrhea [3,147].

C. difficile culture

The stool culture test for *C. difficile* is sensitive (89–100%) but is not specific for toxin-producing strains of *C. difficile* [3,99,147]. *In vitro* testing for toxin production by isolates cultured from toxin-negative stools may improve specificity, but this is not a routine laboratory procedure and is costly and time consuming. An advantage of stool culture of *C. difficile* is that it permits strain typing of individual isolates. The latter facilitates recognition of outbreaks of nosocomial infection. After the outbreak of BI/NAP1/027 in Quebec, North America and some parts of Europe, a renewed interest in culturing *C. difficile* emerged in an effort to learn more about toxigenic strains and antimicrobial susceptibility [4,14,15,68]. Recently described methods used for strain typing of clinical isolates of *C. difficile* include pulsed-field gel electrophoresis, polymerase chain reaction (PCR) ribotyping, restriction-endonuclease analysis typing and toxinotyping [14,15,68].

Polymerase chain reaction for detection of toxin A or toxin B

PCR, with the use of specific primers based on the gene sequences of toxins A and B, has been used to detect toxigenic *C. difficile* in clinical isolates [140,148,149]. Although this is a highly sensitive (100%) and specific (94–100%) test [149], it is laborious and requires initial culture of *C. difficile*. PCR methods for the detection of toxin genes directly in feces have been developed recently [48,147,148]. Using a nested PCR assay for the detection of toxin B in fecal specimens, Alonso *et al* reported 99% concordance with the cytotoxicity assay and sensitivity and specificity of 96.3% and 100%, respectively [150]. Application of PCR methods in the clinical laboratory will require expertise in molecular diagnostic techniques and may not prove to be any more rapid or less expensive than stool cytotoxicity assay. PCR is now increasingly being used for strain typing, especially after outbreaks due to new hypervirulent BI/NAP1/027 strain and reported higher prevalence of toxin A-negative, toxin B-positive CDAD [4,14,48,50].

Endoscopic diagnosis of *C. difficile* diarrhea and colitis

Sigmoidoscopy or colonoscopy is not indicated for most patients with *C. difficile* diarrhea [74]. Endosocopy is helpful, however, in special situations, such as when the diagnosis is in doubt or the clinical situation demands rapid diagnosis [74,89]. In these situations, limited flexible sigmoidoscopy or colonoscopy may be performed at the bedside. In severe pseudomembranous colitis, because of the risk of perforation, only minimal amounts of air should be introduced. The presence of pseudomembranes in the rectum or sigmoid colon is sufficient to make a presumptive diagnosis of *C. difficile* colitis [13,151]. It is important to note that the results of endoscopic examination may be normal in patients with mild diarrhea or may demonstrate nonspecific colitis in moderate cases. The finding of rectal pseudomembranes in a patient with antibiotic-associated diarrhea is virtually pathognomonic for *C. difficile* colitis. Pseudomembranes and fibrinopurulent eruptions are rarely seen in IBD patients who develop *C. difficile* colitis

[137,151]. Some patients without any diagnostic features in the rectosigmoid will have pseudomembranes in the more proximal areas of the colon [13,74]. Other endoscopic findings include erythema, edema, friability and nonspecific colitis with small ulcerations or erosions.

Treatment

Management of mild to moderately severe *C. difficile* diarrhea and colitis

The first step in the management of *C. difficile* diarrhea and colitis is to discontinue the precipitating antibiotic(s) if possible [3,13,68]. Diarrhea will resolve in approximately 15–25% of patients without specific anti-*C. difficile* therapy [8,89]. Supportive therapy with hydration and correction of electrolyte abnormalities is important in patients with *C. difficile* diarrhea. It is not clear whether mild cases require antibiotic treatment against *C. difficile* [15,152]. Conservative management alone is not indicated in patients who are severely ill or who have multiple medical problems, as it is difficult to predict who will improve spontaneously and who will have ongoing diarrhea. If it is not possible to discontinue the precipitating antibiotic, because of other active infections, the patient's antibiotic regimen should be altered to make use of agents less likely to exacerbate *C. difficile* diarrhea, for example, parental aminoglycosides, tetracyclines, macrolides, sulfonamides, narrow-spectrum beta-lactams and urinary antiseptics [15,152,153].

Anti-peristaltic agents such as diphenoxylate plus atropine (Lomotil), loperamide (Imodium) or narcotic analgesics should be avoided as they may delay clearance of toxin from the colon and thereby exacerbate toxin-induced colonic injury or precipitate ileus and toxic dilatation [13,15]

Specific therapy to eradicate *C. difficile* should be used in patients with severe symptoms and in patients whose symptoms persist despite discontinuation of antibiotic treatment. Currently, the most widely accepted antimicrobials for the treatment of *C. difficile* diarrhea are metronidazole (250–500 mg four times per day for 10 days) and vancomycin (125–500 mg four times per day for 10 days). However, there have been several recent reports of high failure rates with metronidazole, especially in cases of severe CDAD [15,23,28,154–156]. Bacitracin, teicoplanin, nitazoxanide and fusidic acid have also been used to treat CDAD, but have few if any advantages over conventional antimicrobials. In a systematic review of the efficacy of different treatments of *C. difficile* intestinal disease, none of these agents showed clear therapeutic superiority in terms of response rates. Ramoplanin, PAR-101 and rifalazil are highly active against *C. difficile in vitro* [157]. Rifaximin has been shown to be as effective as vancomycin and may be more effective in recurrent CDAD [157,158]. Rifampicin, tinidazole, doxycycline and linezolid have also been used with some success [141]. The advantages and disadvantages of specific therapeutic agents are outlined below.

Metronidazole

Metronidazole is the drug of first choice in the treatment of *C. difficile* diarrhea and colitis [68,159,160]. It is inexpensive ($0.50 per 250 mg tablet) and is highly effective for the treatment of this condition. A number of clinical studies have demonstrated that metronidazole therapy results in the resolution of diarrhea and colitis in most patients treated [8,152,160,161]. In a prospective randomized clinical trial, metronidazole(250 mg four times per day for 10 days) was found to be as effective as vancomycin (500 mg four times per day for 10 days) in terms of response and relapse rates for the treatment of *C. difficile* diarrhea. However, in the presence of recent increasing evidence of higher treatment failure rates with metronidazole in cases of severe CDAD [12,15,23,154], vancomycin may be used preferentially in this subset of patients [15,28].

Metronidazole, unlike vancomycin, is well absorbed when administered orally. Fecal concentrations are low or absent in healthy individuals or asymptomatic carriers of *C. difficile*, but higher concentrations are observed in patients with *C. difficile* colitis. In patients with diarrhea, metronidazole may be secreted through an inflamed intestinal mucosa or decreased intestinal transit times may result in decreased absorption [91]. Intravenous metronidazole (500 mg four times per day) may be used if patients who cannot tolerate oral medication or have ileus or toxic megacolon because it is excreted into bile and can accumulate in bactericidal levels in the inflamed colon [68,157].

Systemic side effects may occur with the oral use of metronidazole [160]. However, this antibiotic is remarkably well tolerated. In one institution, in a 10 year period, over 600 patients received metronidazole for the treatment of *C. difficile* diarrhea; only 1% of those treated experienced significant side effects [8]. Adverse effects include nausea, vomiting, diarrhea, furred tongue, headache, urticaria, ataxia, angioedema, metallic taste, peripheral neuropathy (with prolonged therapy) and a disulfiram-like reaction with alcohol. Metronidazole may potentiate the anticoagulant effects of warfarin resulting in prolongation of the prothrombin time. Its use in pregnant and nursing women is cautioned because of the unknown effect of metronidazole on fetal organogenesis and reports of tumorigenicity in rodents. Its safety in children has not been documented. Poor response to metronidazole has been reported when offending antibiotics being used for a concurrent infection could not be stopped [23,156,162]. Treatment failure with metronidazole has been associated with intensive care unit stay, albumin levels less than 25 $g\,l^{-1}$ and antimicrobial use during treatment for CDAD [156,162].

Although the majority of *C. difficile* isolates are sensitive to metronidazole, resistant isolates have been reported, as have occasional cases of *C. difficile* diarrhea induced by metronidazole [15,59,159,163].

Vancomycin

Vancomycin has been successfully used for the treatment of *C. difficile* colitis since 1978 [164] and oral vancomycin remains the only FDA-approved treatment for CDAD [15,23]. Its pharmacokinetic properties make vancomycin an ideal agent for the treatment of *C. difficile* diarrhea [3,15]. When given orally, it is neither absorbed nor metabolized and is excreted virtually unchanged, in high concentrations, in the feces. A number of controlled trials have confirmed the efficacy of vancomycin in the treatment of *C. difficile* colitis [154,164]. Symptomatic improvement is usually evident within 72 h of initiating therapy and complete resolution of diarrhea and colitis occurs in the majority of patients (80–97%) by the end of a 10 day treatment course [23,161]. A number of recent studies have shown vancomycin to be more effective than metronidazole in cases of severe CDAD [12,15,154] and vancomycin may be preferentially used in cases of severe and refractory CDAD [28]. In one observational study of 122 patients treated with vancomycin at one institution, the response rate, drug intolerance rate and relapse rate were 99, 1 and 10%, respectively [8].

Fekety *et al* demonstrated that vancomycin at a dose of 125 mg four times per day is as effective as vancomycin 500 mg four times per day [165]. The lower dose is recommended for patients with mild to moderate colitis but the higher dose is recommended if the patient is critically ill or has impending ileus, colonic dilatation or fulminant pseudomembranous colitis. Vancomycin may be administered by mouth, nasogastric tube or enema [8,68,154,159]. In the case of ileus or toxic megacolon, a combination of intravenous metronidazole and vancomycin administered by nasogastric tube or rectally may be more effective [68,157]. It should not be given intravenously as effective luminal concentrations of the agent cannot be obtained via this route [13]. Systemic side effects associated with the use of oral vancomycin are rare.

Despite the many advantages of therapy with vancomycin, it is considered a second-line agent for the treatment of mild to moderately severe CDAD. There are two main factors discouraging the use of oral vancomycin: first, it is expensive (a 10 day course may cost up to $800), and second, its use may encourage the spread of vancomycin resistance amongst nosocomial bacteria. Oral vancomycin therapy should be reserved for patients who are intolerant of or fail to respond to metronidazole, have severe pseudomembranous colitis, are pregnant or are under the age of 10 years [68,89,158,159]. Table 43.3 compares metronidazole and vancomycin therapy of *C. difficile* diarrhea.

Table 43.3 Metronidazole and vancomycin for treatment of *C. difficile* diarrhea [191]

Parameter	Metronidazole	Vancomycin
Dose (mg)	250–500	125–500
Frequency	tid or qid*	tid or qid*
Duration (days)	10–14	10–14
Route	Oral or intravenous	Oral
Response rate (%)	>96	>96
Cost (10 day oral course) ($)	20	800
Disadvantages	Systemic side effects Rare resistant strains of *C. difficile*	Encourages growth of nosocomial vancomycin-resistant bacteria

*tid, three times per day; qid, four times per day.
Reproduced with permission from Kelly CP, LaMont JT. Treatment of *Clostridium difficile* diarrhea and colitis. In: *Therapy of Digestive Disorders* (ed. MM Wolfe), Philadelphia: WB Saunders, 2000, pp. 513–22.

Other antibacterial agents

Bacitracin (25,000 U four times daily for 7–10 days) has been studied in several clinical trials and is less effective than metronidazole or vancomycin for the treatment of *C. difficile* diarrhea [161]. The overall response rate is only about 80% and the relapse rate (>30%) appears to be higher than with conventional therapy. Due to its adverse side effect profile, bacitracin has rarely been used as treatment for CDAD [157]. In two randomized therapeutic trials, teicoplanin 100 mg twice per day for 10 days was shown to be at least as effective as vancomycin for the treatment of *C difficile* diarrhea [166]. It also appears to be associated with a lower relapse rate (approximately 7%) [157,166] and a Cochrane review suggests that it is slightly better than vancomycin for bacteriologic and symptomatic cure [152]. However, teicoplanin is relatively expensive and, like bacitracin, it is not readily available for oral administration in the United States. The efficacy of fusidic acid for the treatment of *C. difficile* diarrhea has been tested in a limited number of patients [13,166]. Once again, it is less effective than metronidazole or vancomycin and is associated with a relapse rate of approximately 28% [157,166]. Recent studies have shown rifaximin not only to be effective for the first episode of CDAD but also very promising for treating refractory/recurrent CDAD [158,167].

Management of severe pseudomembranous colitis

As with mild to moderate cases of *C. difficile* diarrhea, the first step in the management of severe pseudomembranous colitis is to discontinue precipitating antibiotics if possible and start therapy with vancomycin or

metronidazole. As a number of recent studies have shown vancomycin to be more effective than metronidazole in cases of severe CDAD [12,15,154], vancomycin is recommended as a first-line agent if the patient is critically ill [15,28,68,154,159]. Intravenous metronidazole should be given if oral medication is not tolerated. Intravenous vancomycin is not recommended, for the reasons outlined above. For patients with ileus or toxic megacolon, a combination of intravenous metronidazole (500 mg every 6 h) and vancomycin administered rectally or via a nasogastric tube (500 mg every 6 h) with intermittent clamping may be more effective [8,68,157]. In one series, six of eight patients with severe ileus were successfully treated using a combination of vancomycin administered by nasogastric tube, intravenous metronidazole and vancomycin-retention enemas (500 mg of vancomycin in 100 cm^3 of normal saline administered every 6 h via an 18F Foley catheter inserted into the rectum); patients treated with this regimen responded within 5–17 days [8]. Intracecal infusion of vancomycin has been reported but is not recommended because of the risks associated with placement of a narrow-bore tube over a guidewire at colonoscopy in patients with severe active colitis.

Passive immunization with immunoglobulin products have been shown to be effective for patients with severe colitis who do not respond to therapy with metronidazole or vancomycin [83,168]. Patients with severe or prolonged *C. difficile* diarrhea have low serum and fecal concentrations of antibody against *C. difficile* toxins [85,104,168]. Pooled normal intravenous immunoglobulin (IVIG) has been used in a limited number of patients with severe/refractory *C. difficile* colitis with some success. In initial small case series [169,170], symptom resolution with IVIG was observed in a significant number of patients but these were observational uncontrolled studies. A recent, larger, retrospective, comparative study in patients with severe CDAD by Juang *et al.* from Pittsburgh failed to show a reduction in colectomies, mortality or length of hospital stay in patients who were treated with IVIG in combination with intravenous metronidazole and/or oral/rectal vancomycin as opposed to the group who received antibiotic therapy alone [171]. A vaccine, based on formalin-inactivated *C. difficile* toxins, has been developed and tested in human subjects [168]. The *C. difficile* toxoid vaccine may be used to stimulate anti-toxin antibody responses in healthy volunteers and thereby produce a hyperimmune IVIG against *C. difficile* to treat patients with severe or recurrent *C. difficile* diarrhea and colitis. *C. difficile* surface proteins (FliD, FliC, Cwp66 and Cwp84) are being evaluated as vaccine antigens to diminish intestinal colonization [35,118] and some success has been shown in animal models with flagellar cap protein FliD [118].

The presence of extreme leukocytosis (30,000 mm^{-3} or greater), fever, hypotension and metabolic acidosis despite medical therapy are danger signs in fulminant colitis and may indicate the need for emergency laparotomy and colectomy in patients with impending or actual perforation [135,172]. However, surgical intervention in this setting is also associated with a high mortality rate of 32–57% [131,157], making the decision to operate difficult. Ramaswamy *et al.* determined that the following factors predicted increased mortality in severe *C. difficile* colitis: a low serum albumin on admission to hospital (<2.5 g dl^{-1}); a fall in albumin greater than 1.1 g dl^{-1} at the onset of symptoms; exposure to more than three antibiotics; and persistent toxin in the stools 7 days or longer after therapy [173]. If surgery is required, the operation of choice is a total colectomy [134].

Management of recurrent *C. difficile* diarrhea

Approximately 10–25% of patients treated successfully for their first episode of CDAD will have recurrence of diarrhea in association with a positive stool test for *C. difficile* toxin, while 33–60% can be expected to relapse after the second episode [9,23,98,157,159,174]. Recurrence rates as high as 45% have been reported in infectious disease wards [9]. Recurrence is manifested by the reappearance of diarrhea and other symptoms usually within 1–2 weeks or up to 6–8 weeks of stopping treatment with metronidazole or vancomycin and is either due to relapse from the original organism or re-infection by a different organism. Most relapses occur within 2 months [174]. CDAD due to new hypervirulent BI/NAP1/027 strain is more likely to relapse [15,23,27,93]. Symptomatic recurrence is rarely due to treatment failure or antimicrobial resistance to metronidazole or vancomycin. It may result from germination of *C. difficile* spores persisting in the colon despite treatment. Recent evidence suggests that recurrence most likely results from re-infection with the same or a different strain of *C. difficile* from the environment [13,15,174]. Using DNA fingerprinting, Wilcox *et al.* demonstrated that 56% of clinical recurrences of *C. difficile* diarrhea were due to infection with a different strain of *C. difficile* [175]. It is worth emphasizing that therapy with metronidazole or vancomycin perpetuates disruption of the colonic microflora and therefore predisposes to re-infection with *C. difficile*. An impaired host immune response to *C. difficile* toxins may also increase the risk of recurrent *C. difficile* diarrhea [3,13,21,68,83,84].

Regardless of the mechanism of recurrence, treatment of this form of disease can be problematic. Approaches to management include conservative therapy or treatment with specific anti-*C. difficile* antibiotics, the use of toxin-binding polymers or anion-binding resins, therapy with probiotics or prebiotics and immunoglobulin therapy. The basic principles of management involve (a) treatment of *C. difficile* diarrhea and (b) reduction of the susceptibility of the individual to *C. difficile* re-infection and/or *C. difficile* toxin-mediated colonic injury.

Conservative therapy

As with initial episodes of *C. difficile* diarrhea, conservative management of recurrent diarrhea may be preferable to re-treatment with metronidazole or vancomycin. Although diarrhea usually responds to these agents, they do little to eradicate *C. difficile* spores within the colon or in the environment. They also perpetuate the disturbance of the normal intestinal flora and the associated loss of "colonization resistance" [15,159]. In clinical practice, however, it is often impossible to withhold antibiotic therapy, as many patients with recurrent disease are elderly and infirm and are not able to tolerate diarrhea. Even in healthier patients, persisting or worsening diarrhea caused by recurrent *C. difficile* infection are clear indications for active treatment.

Re-treatment with specific anti-*C. difficile* antibiotics

The most common therapy for first recurrence of CDAD is a second course of the same antibiotic used to treat the initial episode [153,157,159]. Vancomycin is preferable for multiple recurrences and especially if the relapse/recurrence is severe [12,23,28,68,154]. As mentioned above, there has been a dramatic increase in the incidence, severity, recurrence and relapse rates over the last decade [5,12,15,26,93] and there are several reports of treatment failure with metronidazole, especially in cases of recurrent and severe CDAD [12,15,23,154]. Of 163 cases of recurrent CDAD, tapering and pulsed doses of vancomycin was significantly more effective than metronidazole [178], with vancomycin therapy clearing *C. difficile* cultures and/or toxins in 89% of recurrent CDAD cases compared with 59% with metronidazole. Fortunately, there is no definite evidence to suggest that sequential episodes become progressively more severe or complicated.

A variety of treatment schedules have been suggested for patients with multiple recurrences of *C. difficile* diarrhea (Table 43.4). One approach is to give a prolonged course of vancomycin (or metronidazole) using a decreasing dosage schedule followed by pulse therapy [177]. The unproven rationale for this treatment course is that pulse therapy with antibiotics allows *C. difficile* spores to vegetate on the off days and then be killed when the antibiotics are taken again [68,157,176,177]. A combination of vancomycin 125 mg four times per day and rifampicin 600 mg twice per day for 7 days was used successfully in a study of seven patients with relapsing disease. Rifampicin 300 mg twice daily in combination with vancomycin is still occasionally used in some centers [68], but there is no evidence that this combination of antibiotics has any unique activity against recurrent *C. difficile* [159]. Recent studies have shown rifaximin to be an effective drug for treating refractory/recurrent CDAD [158,167], but resistance was reported in one patient (3%) [158] and its use for treating CDAD at present cannot be recommended. There is an ongoing study in the United States to elucidate the role of rifaximin in recurrent CDAD. Nitazoxanide has *in vitro* activity against *C. difficile* and in one recent study was found to be non-inferior to metronidazole [157]. A Phase III study comparing outcomes with nitazoxanide versus vancomycin in patients who have failed previous therapy with metronidazole is in progress. Other than antibiotics, donor stool enemas have been shown to be effective for treating recurrent CDAD in a small number of patients [68]. However, for obvious reasons, including the potential spread of other infections, donor stool is rarely used.

Table 43.4 Approach to management of recurrent *C. difficile* colitis [112]

First relapse:
- – Confirm diagnosis
- – Symptomatic treatment if symptoms are mild
- – 10–14 day course of metronidazole or vancomycin

*Second relapse:**
- — Confirm diagnosis
- – Vancomycin† taper
  - 125 mg q6h for 7 days
  - 125 mg q12h for 7 days
  - 125 mg qod for 7 days
  - 125 mg qod for 7 days
  - 125 mg every 3 days for 7 days

*Further relapse:**
- – Vancomycin in tapering dose as above plus cholestyramine 4 g bid or
- – Vancomycin 125 mg qid and rifampicin 600 mg bid for 7 days or
- – Therapy with microorganisms
  - e.g. Saccharomyces boulardii in combination with metronidazole or vancomycin or
- – Intravenous immunoglobulin

*q6h, every 6 h; q12h, every 12 h; qod, every other day; bid, twice daily; qid, four times daily.
†Metronidazole may be substituted for vancomycin, although there are no published data regarding its efficacy in this treatment regimen.

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Anion-binding resins

Cholestyramine (4 g three or four times daily for 1–2 weeks), an anion-exchange resin, binds *C. difficile* toxins and may be used in conjunction with antibiotics to treat frequent relapses [157,178]. Because cholestyramine may bind vancomycin in addition to toxins, it should be taken at least 2–3 h apart from the vancomycin [157]. Treatment with colestipol (10 g four times daily) is associated with a very low response rate (36%) and is not recommended as primary therapy for *C. difficile* diarrhea [157].

Biotherapy

Biotherapy (therapy with microorganisms or "probiotics") is an attractive approach to the management of recurrent *C. difficile* diarrhea because it aims to restore the "colonization resistance" of a normal colonic flora. Several agents and routes of administration have been evaluated, including a mixture of colonic bacteria in saline administered as a rectal infusion, fresh feces administered as a rectal enema, *Lactobacillus rhamnosus* GG given as a concentrate in skim milk, oral administration of non-toxigenic *C. difficile*, brewer's or baker's yeast (*Saccharomyces cerevisiae*) taken by mouth and *Saccharomyces boulardii* (*S. boulardii*) given in capsule form [28,179,180–182]. Unfortunately, many of these studies have been small, open-label and uncontrolled.

S. boulardii is a nonpathogenic yeast that has been reported to reduce the incidence of antibiotic-associated diarrhea [181,182]. Previously, a randomized, double-blinded, placebo-controlled trial involving 124 patients examined the efficacy of *S. boulardii* (500 mg twice per day for 4 weeks) in combination with metronidazole or vancomycin in patients with *C. difficile* diarrhea. *S. boulardii* significantly reduced recurrences compared with placebo in patients with multiple episodes of *C. difficile* diarrhea (recurrence rate 35% versus 65%, $p = 0.04$), but not in those with an initial episode of *C. difficile* diarrhea (recurrence rate 19% versus 24%, $p = 0.9$). The preparation of *S. boulardii* used in this trial is not currently approved for use in the United States but is available in other countries. Recently, there have been conflicting reports on the efficacy of *Lactobacillus* species and *S. boulardii* to reduce the occurrence and recurrence of CDAD [68,180,182] and at present there is insufficient evidence to recommend routine clinical use of probiotics to prevent or treat CDAD [68,180]. Treatment with *S. boulardii* should be avoided in immunocompromised individuals after case reports of *Saccharomyces cerevisiae* fungemia in immunocompromised patients who had been treated with a probiotic mixture of *S. cerevisiae* and *S. boulardii* [28].

Prebiotics

Prebiotics are nondigestible food components (starch or fiber) that stimulate the growth of bifidobacteria, a type of bacterium thought to play a major role in inhibiting the establishment of opportunistic pathogens in the intestine. There are only limited data available on their use in recurrent CDAD. A randomized trial of 142 CDAD cases treated with standard antibiotics with or without an adjunctive prebiotic oligofructose was conducted by Lewis *et al.* [183]. Significantly fewer patients (8.3%) in the prebiotic-treated group had a recurrence within 60 days compared with those on placebo (34.3%, $p < 0.001$) [23,183].

Immunoglobulin therapy

As mentioned earlier, there is now substantial evidence that the immune response to *C. difficile* toxins plays a major role in determining host susceptibility to disease [17,23,83,104,168]. Several investigators have found that serum antibody levels against *C. difficile* toxins are low in patients with recurrent *C. difficile* diarrhea [23,68,85,104,168]. In a study of six children with relapsing *C. difficile* colitis, Leung *et al.* found that these children had low serum levels of IgG antibody against toxin A. Treatment with normal pooled intravenous gamma globulin, which contains IgG anti-toxin A, was associated with a marked increase in serum antitoxin antibody levels and resolution of recurrent *C. difficile* diarrhea. Although this approach to the management of recurrent *C. difficile* diarrhea is promising, further controlled studies are required before gamma-globulin can be recommended as a standard therapy for this condition. A recent study in which 18 patients who had a severe initial bout of CDAD as opposed to recurrent CDAD received IVIG failed to demonstrate any significant benefit [171]. A toxoid vaccination of formalin-inactivated toxins A and B has been shown to be effective in reducing recurrence of CDAD in a small group of patients [184] and is currently in Phase 1 trials [68]. A study by Young *et al.* demonstrated efficacy and safety of an anti-*C. difficile* whey protein concentrate (anti-CD WPC) for use as a medical food for the dietary management of patients with CDAD to prevent a relapse of the infection [185]. Anti-CD WPC is prepared from the milk of cows that have been immunized against *C. difficile* and its toxins. Large, randomized controlled studies will be required to validate the efficacy and safety of anti-CD WPC before it can be recommended as a treatment option for recurrent CDAD.

Toxin-binding polymer

Tolevamer, a novel non-antibiotic polymer, a sodium salt of styrene sulfonate polymer, binds noncovalently to *C. difficile* toxins A and B [68,186]. In a Phase 1 trial of 222 patients with CDAD, high-dose tolevamer (6 g per day) demonstrated noninferiority to 500 mg per day of vancomycin, with a trend towards reduced recurrence [23,186]. CDAD resolved by day 10 of treatment in 83% of patients treated with tolevamer compared with 91% of those treated with vancomycin 500 mg per day in divided doses. The recurrence rate was 10% in the tolevamer group compared with 19% in the vancomycin group ($p = 0.05$) [68,186]. Tolevamer does not affect gut microbiota and is well tolerated except for mild hypokalemia. A higher dose of tolevamer (9 g per day) is currently being examined in a Phase 3 multicenter study. [186]

Prevention

Published management strategies and practice guidelines for the prevention of *C. difficile* diarrhea emphasize restriction of antimicrobial use (Table 43.5) [23,27,68,99,159]. Although limitation or restriction of antibiotics known

Table 43.5 Practice guidelines for prevention of *C. difficile* diarrhea [159].

1. Limit the use of antimicrobial drugs
2. Wash hands between contact with all patients
3. Use enteric (stool) isolation precautions for patients with *C. difficile* diarrhea
4. Wear gloves when contacting patients with *C. difficile* diarrhea or their environment
5. Disinfect objects contaminated with *C. difficile* with sodium hypochlorite, alkaline glutaraldehyde or ethylene oxide
6. Educate the medical, nursing and other appropriate staff members about the disease and its epidemiology

Reproduced by permission from Macmillan Publishers Ltd: Fekety, R. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 1997; **92**:739–50.

to be associated with *C. difficile* diarrhea (such as fluoroquinolones or broad-spectrum cephalosporins) may be difficult to implement, observational studies demonstrate that this approach may be beneficial in preventing *C. difficile* diarrhea [15,23,27,68]. Other important recommendations include hand washing with soap or chlorhexidine between contact with patients, the use of gloves for the handling of body substances of patients with *C. difficile* diarrhea and the use of enteric isolation precautions [99,102,103,159]. Alcohol gel hand rubs are not appropriate cleansing agents as they do not kill *C. difficile* spores and hands must be cleaned with soap or chlorhexidine [23,102,103]. Although there is a paucity of data regarding the efficacy of different cleaning agents and disinfectants for *C. difficile*, the American College of Gastroenterology recommends that instruments and contaminated surfaces in rooms of patients with *C. difficile* diarrhea should be disinfected using sodium hypochlorite or ethylene oxide [159]. The Society of Healthcare Epidemiologists of America (SHEA) and the Centers for Disease Control and Prevention (CDC) continue to recommend that electronic thermometers should be replaced with disposable thermometers if rates of *C. difficile* diarrhea are high and to use unbuffered 1:10 hypochlorite solution rather than detergents to disinfect surfaces [23,99]. The recommendation of using disposable thermometers is supported by the results of two studies, one of which employed a randomized crossover design, in which replacement of electronic rectal with disposal thermometers was associated with a decreased incidence of *C. difficile* diarrhea [187].

A recommendation that is likely to have a substantial impact on the spread of *C. difficile* infection emphasized by Fekety is that medical, nursing and other hospital staff members receive education on *C. difficile* diarrhea and its epidemiology [159]. Aggressive infection control policies which involve multiple disciplines are often necessary to control nosocomial infection with *C. difficile*. Strict implementation of major infection-control measures has resulted in a significant reduction in the incidence of outbreaks of CDAD [4,188]. Patient education regarding the mode of transmission of *C. difficile* is also likely to be important, but may be difficult in the patient population who are commonly affected, namely the old and infirm. A strategy involving treating CDAD patients with non-antibiotic drugs such as the toxin neutralizer tolevamer rather than with antibiotics and vaccinating high-risk individuals could prove beneficial.

Future approaches to the control of nosocomial *C. difficile* infection may involve active or passive immunization of at-risk individuals [83,168,184]. A combination of toxoids and surface proteins in a mucosal vaccine could display a complementary protective effect both on the colonization step and on the toxin's activity.

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Chapter 44
Colitides of Infectious Origins

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Summary

- Infections of the gastrointestinal tract, particularly those due to invasive enteropathogens which cause an ileo-colitis, may mimic ulcerative colitis or Crohn's disease.
- Increased vigilance in patients with diarrhea, particularly bloody diarrhea, and an appropriate search for an infective agent are essential before a final diagnosis of non-specific inflammatory bowel disease is made.
- Delay in administering an appropriate antibiotic for a colonic infection may increase morbidity, including the likelihood of developing complications.
- Precision in diagnosis is important to ensure that antibiotics are made available for those infections in which there is a proven role for antimicrobial chemotherapy in reducing the duration and severity of disease, and also to distinguish infective colitis from non-specific inflammatory bowel disease.

Introduction

Many infections of the gastrointestinal tract, those that are transient, self-limiting illnesses, are distinguishable from chronic non-specific inflammatory bowel disease. Others, particularly those due to invasive enteropathogens which cause an ileo-colitis, may be less easy to distinguish from ulcerative colitis or Crohn's disease [1]. Because of the differences in therapeutic approaches, it is important to differentiate these conditions rapidly because use of corticosteroids or immunosuppressive drugs inappropriately may adversely affect outcome. Similarly, delay in administering an appropriate antibiotic may increase the likelihood of complications and therefore increase morbidity. Despite administration of steroids and mesalazine, some self-limiting colonic infections will resolve, but it is clearly inappropriate to commit an individual to life-long maintenance therapy when further problems would not be expected following a single attack of infective colitis. Thus, an accurate initial diagnosis of the etiology of an attack of colitis is essential.

In developing regions, where the microorganisms responsible for infectious colitis are endemic, clinicians routinely search for infectious causes for colitis before making a diagnosis of non-specific inflammatory bowel disease. In the industrialized world, for several decades the reverse was true, with inflammatory bowel disease always being the most likely diagnosis. In recent years; however, reports of intestinal infection to agencies responsible for the surveillance of infectious diseases have increased in the United Kingdom and the United States. There has been a steady increase in reports of *Salmonella* spp. and *Campylobacter jejuni* infections in the UK (Figure 44.1) and a number of important outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) infection with a reported mortality of 1–2% and a relatively high incidence of serious complications such as the hemolytic–uremic syndrome. Increase in foreign travel has further contributed to the importance of infectious colitis in individuals living in the industrialized world [2], as has the increasing use of broad-spectrum antibiotics and the well-recognized association of antibiotic-related diarrhea and pseudomambranous colitis due to infection with *Clostridium difficile*. In the UK, there has been an alarming increase in nosocomial *C. difficile* infections in hospitalized patients with an associated high mortality. Carriage in the community is now also regarded as important in the epidemiology of this infection.

Intestinal infectious disease remains a significant problem which can be attributed to a number of factors, including transmission of enteropathogens through food, despite the widespread implementation of public health measures to minimize transmission of intestinal enteropathogens in the industrialized world. Thus, in patients with diarrhea, particularly bloody diarrhea, clinicians must exercise increased vigilance and ensure that an appropriate search is made for an infective agent before a final diagnosis of non-specific inflammatory bowel disease is made.

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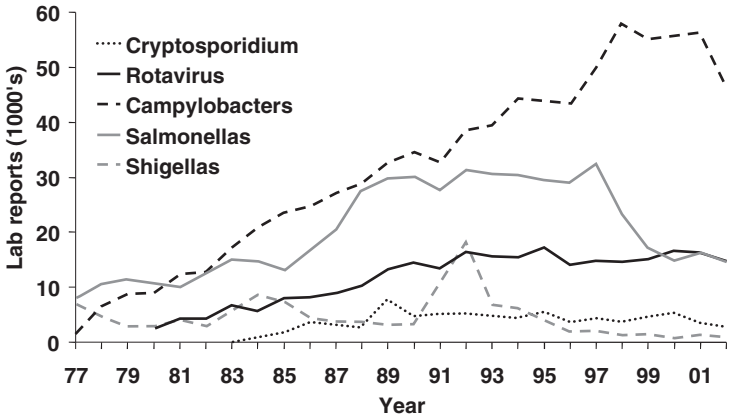

Figure 44.1 Reports of enteropathogens in England and Wales to the Public Health Laboratory Service 1977–2002.

Epidemiology of infectious colitis

The enteric pathogens responsible for infectious colitis are distributed throughout the industrialized and resource-poor countries of the world [3,4]. The relative prevalence, however, varies between different geographic locations; for example, amebiasis is found most commonly in the Indian sub-continent, sub-Saharan Africa and Latin America particularly Mexico. In contrast, it is an endemic infection of low prevalence in many areas in the northern hemisphere, including North America and the United Kingdom. During the last two decades, *Salmonella* spp. and *Campylobacter jejuni*, important food-borne pathogens, have been reported with increasing frequency in the United Kingdom. Up to 70% of chicken carcasses in UK supermarkets may be contaminated with these human pathogens.

A survey of general practice confirmed the high prevalence of intestinal infections in the United Kingdom [5] and concluded that many infections are not reported either to general practitioners or to communicable disease surveillance agencies. Humans continue to be important reservoirs of the organisms responsible for infective colitis; some infections such as shigellosis and amebiasis are restricted to humans. Other infections such as those due to *Salmonella* spp. and *Campylobacter* spp. are zoonoses since animal reservoirs have been shown to be increasingly important for the rising incidence of these infections in humans.

Fecal–oral is nearly always the transmission route. Water may become contaminated either by inadequate separation of domestic water supplies and sewage systems or by human contact. In addition to the risks associated with contaminated drinking water, infectious colitis may also be acquired by swimming in contaminated swimming pools, freshwater lakes and seawater [6,7]. Outbreaks of shigellosis and EHEC infection have been well documented following recreational swimming in a freshwater lake in Oregon [8]. Intensive food production methods have increased the risk of contaminating poultry carcasses (*Salmonella* spp. and *Campylobacter jejuni*), beef (EHEC) and pork (*Yersinia enterocolitica*) [9]. Although contaminating organisms will be destroyed by adequate cooking, bacteria may survive on the hands of a food preparer and then be transferred to uncooked salads, fruit or vegetables by direct contact. EHEC has also been responsible for outbreaks following ingestion of hamburger meat, presumably as a result of inadequate cooking [9] and from contaminated vegetables.

Transmission also occurs person to person between children in schools and day-care centers and during sexual contact. Direct spread of infection between humans is particularly common with infections such as shigellosis, in which only 10–100 organisms are required to initiate clinical infection.

A number epidemiological settings and factors increase the risks of acquiring infectious colitis (Table 44.1). Infants and young children and the elderly are at increased risk of acquiring intestinal infection, partly because of relative impairment of host immune defense mechanisms but also in the case of young children, due to increased exposure

Table 44.1 Risk factors for infectious colitis.

Risk factors	Groups at risk
Age	Infants and young children The elderly
Non-immune host defense – gastric acid	The elderly Hypo- and achlorhydria Recipients of acid inhibitory drugs
Immunodeficiency	Congenital immunodeficiency HIV/AIDS Cancer and cancer chemotherapy Undernutrition
Increased exposure to enteropathogens	Travelers Contaminated food and water
Antibiotics	Especially the elderly and cancer patients

during the weaning period. Breast-fed infants are relatively protected during the first few months of life. Innate host defense, particularly gastric acid, presents an important barrier to invading enteropathogens. There is now compelling evidence that the risks of acquiring *Salmonella*, *Campylobacter* and *C. difficile* infections are increased by acid inhibitory drugs, such as the H_2 receptor antagonists and proton pump inhibitors [10,11]. Acid secretion also decreases in the presence of gastric atrophy (as a result of *Helicobacter pylori* infection or pernicious anemia).

Travel from industrialized countries to resource-poor parts of the world will result in an attack of traveler's diarrhea, almost always due to intestinal infection, in about 30% of individuals. The most common cause of acute watery diarrhea in travelers is enterotoxigenic *E. coli*; however, invasive enteropathogens such as *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni* and *Entamoeba histolytica* all occur in travelers and can produce infectious colitis. Travelers to the developing world have increased exposure to these enteropathogens, not only by ingestion of contaminated food and beverages [12–14] but also through recreational activities such as water sports.

It is now well established that immunocompromised individuals, particularly those with HIV infection, are at increased risk of infectious colitis, particularly that due to cytomegalovirus (CMV) infection. *Entamoeba histolytica* is also often isolated from homosexual men but invasive amebiasis is uncommon since infection is usually with the non-pathogenic *Entamoeba dispar*. However, the classic invasive enteropathogens such as *Salmonella* and *Shigella* spp. are commonly isolated from HIV-infected individuals.

The widespread use of broad-spectrum antibiotics both in the community and in hospitalized patients has been another contributory factor to the increase in intestinal infection, particularly that due to *C. difficile*. A new virulent epidemic strain, characterized as toxinotype III, PCR ribotype 027, has been identified initially in North America but now in many other countries. It is associated with increased morbidity and mortality due at least in part to massively increased toxin A and B production. There is increasing evidence that *C. difficile* infection accounts for only a proportion of antibiotic-associated diarrhea and it is likely that other *Clostridia* are also involved.

Etiology of infectious colitis

Many of the broad spectrum of viruses, protozoa and helminths known to produce infectious colitis (Table 44.2) are found worldwide, whereas others clearly have major geographic restrictions. *Mycobacterium tuberculosis* infection of the gastrointestinal tract has predominated in the developing world, although it is now increasingly common in some parts of Europe and North America following the arrival of immigrants from parts of Asia and particularly the Indian subcontinent [15]. The enteropathogenic protozoa *Entamoeba histolytica* and *Balantidium coli* are again predominantly pathogens of the tropics and subtropics, although cases of intestinal amebiasis are not uncommon in the northern hemisphere and are not all necessarily acquired as a result of foreign travel. Infection with *B. coli* is limited almost exclusively to communities who have a close coexistence with pigs, such as the inhabitants of Papua New Guinea.

Table 44.2 Enteropathogens responsible for infectious colitis.

Type	Pathogen
Viruses	Cytomegalovirus
Bacteria	Enteroinvasive *E. coli*
	Enterohemorrhagic *E. coli*
	Shigella spp.
	Salmonella spp.
	Campylobacter spp.
	Yersinia enterocolitica
	Clostridium difficile
	M. tuberculosis
	Aeromonas hydrophila
	Plesiomonas shigelloides
	Klebsiella oxytoca
Protozoa	*Entamoeba histolytica*
	Balantidium coli
Helminths	*Schistosoma* spp.
	Trichuris trichiura

Intestinal schistosomiasis and trichiuriasis also are diseases of the tropics and subtropics and schistosomiasis occurs only in areas that are inhabited by the freshwater snail which is its specific intermediate host. Acute schistosomiasis is recognized to occur in travelers to these endemic areas when it usually presents as the acute infection syndrome, Katayama fever.

CMV infection is limited almost exclusively to the immunocompromised, particularly those with HIV infection, and *C. difficile*-related pseudomembranous colitis is almost always associated with treatment with broad-spectrum antibiotics. Recently, *Klebsiella oxytoca* has been identified as the causative organism of some cases of antibiotic-associated hemorrhagic colitis [16].

Pathogenesis

A diverse series of mechanisms produce infectious colitis that result from microbial enteropathogens [17–19]. The most well-recognized mechanism for destruction of the colonic epithelium and associated inflammation is that of invasion, a mechanism utilized by many of the classic bacterial enteropathogens such as *Shigella* spp., *Salmonella enteritidis* and *Campylobacter jejuni*. Some organisms such

as enterohemorrhagic *E. coli* attach and then disrupt the apical membrane of the epithelial cell and subsequently enter the mucosa through specialized M cells which overlay lymphoid cell collections in the intestine. Others, such as *Entamoeba histolytica*, are able to destroy host epithelial cells merely by attaching to the apical membrane of the colonocyte. Other organisms rely predominantly on the production of cytolethal substances such as cytotoxins, which is perhaps best exemplified by *C. difficile*. Finally, acute and chronic inflammatory responses in the mucosa and sub-mucosa may be stimulated by the presence of microorganisms such as *M. tuberculosis* or the ova of *Schistosoma* spp. or the direct presence of a helminth such as *Trichuris trichiura*, which is also able to induce local anaphylaxis.

Pathogenetic processes in infectious colitis

Contact-dependent cytolysis

Entamoeba histolytica is often considered to be an invasive enteropathogen; however, this is not strictly true. *E. histolytica* produces its cytolethal effects through cell–cell contact. Initial engagement depends on a surface membrane-associated galactose-binding lectin which mediates adherence to the host epithelial cell [20,21] followed by release of a variety of hydrolytic enzymes and pore-forming proteins. Considerable attention has been directed towards the pore-forming protein amebapore, which is similar to complement, and is released and inserted into the host cell membrane, producing high-conductance ion channels leading to a rapid increase in intracellular calcium and cell death [22]. *E. histolytica* also synthesizes a number of other potentially cytolytic proteins, including hemolysins, proteolytic enzymes including a cathepsin B proteinase, an acidic proteinase and a collagenase. These proteinases appear to be involved in disruption of the extracellular matrix, which enables the trophozoites to penetrate into the deeper layers of the mucosa and sub-mucosa. Amebic cytolytic activity is dependent on a number of parasite factors including calcium and a calcium-dependent phospholipase A and microfilament function.

Adherence and effacement

The locus of enterocyte effacement (LEE) is a series of genes contained by enterohemorrhagic *E. coli* and enteropathogenic *E. coli,* which encode proteins that produce the attaching and effacing lesions [23–25]. This lesion is characterized by localized effacement of microvilli, intimate attachment of the bacillus to the host cell membrane and formation of a pedestal-like structure in the host cell which contains cytoskeletal proteins including polymerized actin, α-actinin, ezrin and myosin. The LEE encodes for a type III secretion system that produces a number of secreted proteins which mediate these process. EspA, which is present on small filamentous projections of the organisms, is now thought to mediate initial attachment [24]. The bundle-forming pillus, previously thought to be involved in mediating non-intimate attachment, is now thought to be primarily involved in allowing EHEC organisms to adhere together. EspA filaments are then thought to stimulate release and entry of the translocated intimin receptor (previously known as host protein 90, which then becomes the receptor for intimin, an EHEC attachment protein also encoded by the LEE. Following intimate attachment, EspB and EspD are then also translocated and are considered to be important for the polymerization of actin and other cytoskeletal rearrangements which lead to the formation of the pedestal lesion.

EHEC are not classic invasive enteropathogens; however, they do penetrate the mucosa, probably through M cells, where they proliferate and release Shiga toxins. These are potent cytolethal toxins that act by inhibiting protein synthesis locally in the gut. They are also released into the systemic circulation and are probably responsible for one of the most devastating complications of this infection, namely the hemolytic–uremic syndrome.

Cytotoxin-induced colitis

A few enteropathogens, particularly *C. difficile*, rely almost exclusively on cytotoxin production to cause disease. *C. difficile*, which is a non-invasive, spore-forming anaerobic bacillus, releases two potent toxins. Toxin A (308 kDa) is a potent "enterotoxin." Toxin B (270 kDa) is an extremely potent cytotoxin but is devoid of enterotoxic activity in rabbit ileum [26,27]. Toxin A also possess cytotoxic and hemagglutinating activity and is a potent neutrophil chemoattractant. Both toxins are lethal when administered parenterally to animals. The genes for these toxins have been cloned and sequenced and are located on the bacterial chromosome. Toxin A is generally referred to as an enterotoxin; however, it does not produce intestinal secretion by the classic pathways of *E. coli* LT and ST and cholera toxin. Toxin A primarily elicits an inflammatory response with an inflammatory infiltrate in the lamina propria and increased concentrations of PGE_2 and LTB_4 accompanied by fluid secretion. In addition, there is increased intestinal permeability, which appears to be related to disaggregation of the actin-containing filaments in the peri-junctional actinomyosin ring. Increasing evidence suggests that it is the presence of neutrophils and neutrophil products that mediate these changes. Toxin B also elicits fluid secretion and increases permeability, and releases PGE_2 into the intestinal lumen, although its precise role in pathogenesis is less well characterized. There is evidence, however, that the effects of toxins A and B are synergistic. Specific receptors for toxin A have been identified in some mammalian microvillus membranes. The receptor is a glycoconjugate with a trisaccharide, galactose-α1→3-galactose-β1→4-*N*-acetylglucosamine, which contains

the binding site [28]. Like the Shiga toxin receptor, toxin A receptor is not present in mammalian neonates and this probably explains the absent of disease in human infants infected with *C. difficile*. Both toxins utilize the same intracellular signal transduction system since they act as enzymes to catalyze the glucosylation of Rho, which is a family of proteins which are small GTPases and are essential for maintenance of the actin cytoskeleton [29–32]. The glucosylation of Rho causes disaggregation of actin filaments, cell rounding and cell death. Recent evidence indicates that autocatalytic cleavage of toxin B is dependent on host eukaryotic signals [33].

Invasion of the epithelium

The ability of *Salmonella*, *Shigella* and enteroinvasive *E. coli* to enter the host epithelial cell depends on their ability to trigger a signaling cascade which leads to cytoskeletal rearrangements that allow the organism into the cell in an endocytotic vesicle. Following contact of the organism with the host surface membrane, there is rearrangement of actin filaments and capping of host surface proteins and localized membrane ruffling [34].

Invasion by *Salmonella* involves internalization of bacteria in vacuoles within minutes and is associated with calcium influx and activation of the inositol phosphate transduction pathways [35]. CD42, one of the *ras*-related superfamilies of small GTPases, appears to regulate the cytoskeletal rearrangement [36]. *Salmonella* has a chromosomal pathogenicity island SP-1 containing several invasion operons including *inv/spa*, which are homologous with invasion genes of other invasive bacteria [37,38].

Shigella invasion also involves actin polymerization, which in this case is dependent on the small GTPase Rho [39]. Three surface effector proteins mediate invasion, IpaB, IpaC and IpaD, which trigger the host endocytotic process followed by release of shigellae from the vacuole, mediated by IpaB [40]. These invasion mechanisms exemplify the stealth of enteropathogenic bacteria in using their own surface or secreted proteins to subvert host cell structures to advance the colonization process [41,42].

Shigella is able to use the contractile protein actin to propel it through the cell and to penetrate and invade other cells in the epithelium. It is also able to release shiga toxin, which causes irreversible inhibition of protein synthesis by a highly specific action on the 60s mammalian ribosomal subunit.

The host epithelial cell is stimulated by most invasive enteropathogens to produce a variety of chemokines, notably IL-8 [43,44]. These are potent chemoattractants that promote a rapid influx of neutrophils into the lamina propria. The presence of large numbers of neutrophils enhances the inflammatory cascade and ultimately increases tissue damage and the secretory response within the epithelium. Administration of antibody to the cell adhesion molecule CD18 to inhibit neutrophil influx reduces the mucosal inflammatory response and structural damage and also diminishes fluid and electrolyte secretion by epithelial cells [45].

Inflammatory responses to mucosal enteropathogens

In addition to the acute and chronic intestinal inflammatory responses that are associated with invasive enteropathogens, chronic inflammation is the major pathway for pathogenesis of *Schistosoma* spp. and *Mycobacterium tuberculosis* infection. The adult worms of *Schistosoma* spp. reside in the tributaries of the portal vein and the female worm releases a continuous supply of ova which are disseminated through the portal venous system to a number of sites, including the liver (*S. mansoni*), the bladder (*S. hematobium*) and the intestine (*S. mansoni, S. hematobium* and *S. japonicum*). The ova release a number of antigens which produce a chronic inflammatory response including granuloma formation. Chronic inflammation can lead to epithelial destruction and hemorrhage, with healing by fibrosis sometimes complicated by stricture formation. Similarly, *M. tuberculosis* bacilli in the intestinal wall induce a chronic inflammatory response, again with granuloma formation, although in this case the granulomata are caseating. Chronic inflammation can produce ulceration and stricture formation.

The colonic helminth *Trichuris trichiura* directly invades the intestinal mucosa with its thin anterior end buried usually in the proximal colon. However, in heavy infections worms may be found from the terminal ileum to the rectum. Mucosal inflammation usually only occurs at the site of attachment of the worm and may consist of breaches in the epithelium, sub-epithelial hemorrhages and infiltration with eosinophils, lymphocytes and plasma cells. A local immediate hypersensitivity reaction in the colonic mucosa may be involved in the pathogenesis of the so-called trichuris dysentery syndrome.

Clinical features

The distinction between infectious colitis and non-specific inflammatory bowel disease (ulcerative colitis and Crohn's colitis) can present major difficulties to the clinician. Although infectious colitis usually has an abrupt onset often associated with fever and cramping abdominal pain, these features are not always present and are not exclusive to intestinal infection. Infectious colitis can have a more indolent course typified by the gradual onset of symptoms in amebic colitis which may occur over many days or weeks. A history of foreign travel may be helpful, although in one series 15% of patients returning from abroad with persistent diarrhea were presenting for the first time with non-specific inflammatory bowel disease [46]. Similarly, a history of diarrhea that followed a meal

in which food or water could be a potential vehicle for infection, particularly if other family or friends had similar symptoms, might suggest an infective origin for the symptoms. Finally, infectious colitis is usually self-limiting and will often resolve without any specific interventions. However, when symptoms persist beyond 7–10 days, clinical evaluation alone is usually inadequate to make a firm diagnosis and thus further investigation is usually required.

Bacterial colitis

Salmonella spp.

Non-typhoidal salmonellosis is a common cause of food poisoning, usually occurring 12–48 h after eating contaminated food. Nausea, vomiting and headache occur early in the illness usually followed by fever, cramping abdominal pain and watery diarrhea. In moderate to severe infections, a dysenteric form of the illness can develop with small, frequent, mucoid, bloody stools, severe cramping abdominal pain and tenesmus. Recovery usually occurs in 2–14 days. Some types of *Salmonella* such as *S. dublin* are highly invasive and produce a typhoid fever-like illness. These invasive organisms can produce focal infections including mycotic aneurysms, septic arthritis (0.2–2.5%) and osteomyelitis. Occasionally *Salmonella* infection can cause cholecystitis and cholangitis, meningitis and splenic abscess.

Shigella spp. and enteroinvasive *E. coli*

Shigellosis gradually evolves through an incubation period of 1–4 days which is then followed by fever, headache and anorexia. These constitutional symptoms are followed by watery diarrhea, which then declines but is generally followed by small-volume, mucoid, bloody stools almost invariably accompanied by moderate to severe lower abdominal cramping pain and tenesmus. *Shigella* infections are often accompanied by extraintestinal manifestations in children including seizures, the hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura. Focal infections are uncommon. Enteroinvasive *E. coli* has a similar presentation to shigellosis as these organisms can be regarded as non-shiga toxin-producing *Shigella*. The complications due to shiga toxin, namely seizures and hemolytic–uremic syndrome, are therefore not associated with EIEC infection.

Enterohemorrhagic *E. coli*

The incubation period is usually 3–9 days although it may be more prolonged in children. A typical presentation is of bloody diarrhea, blood usually appearing in the stool early in the illness. This is usually accompanied by lower abdominal pain and tenderness, abdominal distension and, in children, rectal prolapse. As EHEC produces shiga toxin, seizures and hemolytic–uremic syndrome occur as in shigellosis, the latter being responsible for many of the deaths associated with this infection [9]. The majority of infections resolve in 7–21 days but mortality continues to be 3–5%.

Campylobacter jejuni

The incubation period is 2–3 days, which is followed by a prodrome of headache, myalgia and fever. Diarrhea and severe cramping lower abdominal pain then supervene. Nausea, vomiting, anorexia and overt rectal bleeding occur in about 50% of patients. The illness usually resolves within 7–21 days. The severity of the abdominal pain may suggest the presence of an acute surgical abdomen such as appendicitis. *Campylobacter* infection may be complicated by reactive arthritis or a full Reiter's syndrome (arthritis, urethritis and conjunctivitis), particularly in patients who are HLA-B27 positive. *Campylobacter* infections are also complicated by the Guillain–Barré syndrome, which usually occurs 1–3 weeks after the intestinal infection [47]. The Guillain–Barré syndrome which follows *Campylobacter* enteritis is predominantly a motor syndrome with a relatively poor outcome because of residual motor deficit. *Campylobacter* is now one of the most common causes of the Guillain–Barré syndrome [48]. There is now evidence to suggest that *Campylobacter* infection is associated with immunoproliferative small intestinal disease and indeed may be a causative agent [49].

Mycobacterium tuberculosis infection

The presentation of ileo-cecal and colorectal tuberculosis is usually slowly progressive and may produce a variety of symptoms, including chronic diarrhea with or without blood, abdominal pain and a tender abdominal mass in the right iliac fossa and symptoms suggestive of sub-acute small bowel obstruction [15]. In addition, there are usually non-specific symptoms, including fever, general malaise and weight loss. Anorectal symptoms may be those of ulceration, anal fissure, perianal or ischiorectal abscess and fistula.

Yersinia enterocolitica

Infection usually produces watery diarrhea, but in up to 25% of patients bloody diarrhea, fever and abdominal pain may be major symptoms. There is often a marked terminal ileitis with enlargement of local mesenteric lymph nodes which in some situations results in a presentation similar to acute appendicitis. *Yersinia* is also characterized by extra-enteric manifestations including erythema nodosum and erythema multiforme-like skin rashes. Reactive arthritis and Reiter's syndrome also occur and, like *Campylobacter* infection, are more common in individuals who are HLA-B27 positive.

A serious complication of *Yersinia enterocolitica* infection is septicemia, which is particularly prevalent in individuals with iron overload such as hemochromatosis and those with chronic hemolytic anemias such as thalassemia.

Aeromonas and *Plesiomonas* spp. infections

Although the pathogenicity of these organisms has been controversial, there is compelling evidence that they can produce infectious colitis which is indistinguishable from ulcerative colitis.

Viral colitis

Cytomegalovirus colitis, although well recognized to occur in the immunocompetent, occurs predominantly in immunocompromised individuals with HIV infection. CMV colitis is usually of acute presentation with abdominal pain and diarrhea with or without blood. At least 50% of patients develop fever and pain is a frequent symptom. The symptoms of infection usually persist for several weeks and in the immuncompromised usually do not resolve until anti-viral treatment is administered. The presentation may be accompanied by an abdominal mass and when severe there is a substantial risk of perforation when infection is segmental in the colon.

Protozoal colitis

Entamoeba histolytica

Infection with *E. histolytica* can be asymptomatic or acute, with fulminant colitis complicated by perforation. The onset of amebic colitis is usually insidious, with abdominal discomfort and loose stools often initially without blood or mucus. When fulminant, however, symptoms may be abrupt in onset and progress rapidly. Constitutional symptoms are often mild. Fulminant amebic colitis is usually the result of extensive colonic ulceration and may be indistinguishable from fulminant ulcerative colitis. There may be the clinical picture of toxic megacolon with abdominal distension and tenderness, high fever and tachycardia. Complications include severe hemorrhage and perforation and may progress to amebic liver abscess.

Balantidium coli

The clinical features of this infection resemble those of amebic colitis. An asymptomatic carrier state can exist, although infection may present as acute colitis which can be fulminant. In the acute form of the disease, onset of diarrhea with blood and mucus can be abrupt and is often associated with nausea, abdominal discomfort and marked weight loss. Again, colonic dilatation and perforation with peritonitis can occur with this infection. Occasionally balantidial appendicitis occurs.

Helminthic colitis

Trichuris trichiura

Light infections which only involve the proximal colon are often asymptomatic. However, heavy infections may result in the trichuris–dysentery syndrome which is characterized by frequent low-volume stools with blood and mucus. The predominant symptoms may be blood and mucus alone without significant diarrhea. There may be accompanying abdominal pain, tenesmus and rectal prolapse, with constitutional symptoms including anorexia and weight loss. Anemia and finger clubbing are also associated with severe infection and in children there may be impairment of growth and development.

Table 44.3 Infective proctitis.

Type	*Species*
Bacteria	*Chlamydia trachomatis* non-LGV*
	Chlamydia trachomatis LGV
	Neisseria gonorrhoeae
	Treponema pallidum
	Mycobacterium tuberculosis
Viruses	Herpes simplex virus
	Cytomegalovirus
Helminths	*Schistosoma* spp.

*LGV, lymphogranuloma venereum.

Schistosoma spp.

Acute schistosomiasis may be evident clinically 4–6 weeks after infection and is characterized by fever, cutaneous and respiratory allergic manifestations, enlargement of the liver and spleen, lymphadenopathy and peripheral eosinophilia. This early clinical syndrome is sometimes known as Katayama fever. Intestinal schistosomiasis may present clinically months or years after infection. The major symptoms are of colitis with diarrhea which may contain blood and is often associated with non-specific abdominal pain. There may also be constitutional symptoms including tiredness, anorexia and weight loss. Symptoms may be particularly severe in *S. mansoni* infection, which is known to be associated with multiple inflammatory colonic polyps. These can result in severe blood and protein loss from the colon. *S. japonicum* also produces colitis and with long-standing disease there is an increase risk of developing colorectal cancer. *S. hematobium* is particularly associated with disease in the distal colon and rectum. Inflammatory masses may also occur in the colon; these bilharziomas can masquerade as a colorectal neoplasm.

There are some organisms that typically produce an isolated infectious proctitis (Table 44.3).

Diagnosis

The majority of episodes of acute infective diarrhea resolve without the need for making a specific diagnosis of the etiologic agent responsible; however, for persistent diarrhea, particularly when associated with blood, further investigation is indicated. Precision in diagnosis is important to distinguish infective colitis from non-specific

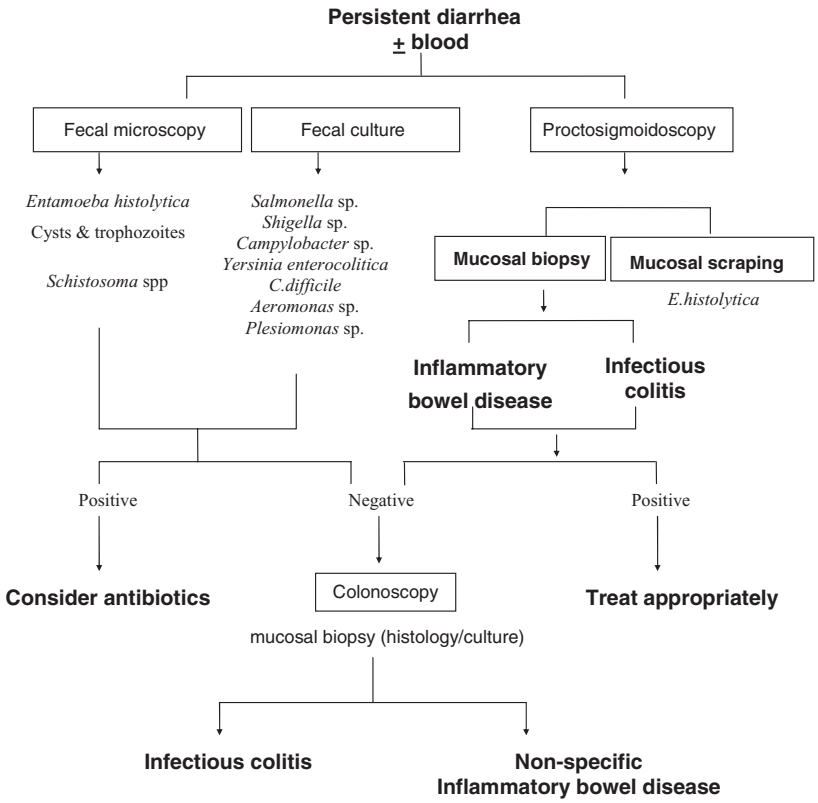

Figure 44.2 An approach to the investigation of infectious colitis.

inflammatory bowel disease and to ensure that antibiotics are made available for those infections in which there is a proven role for antimicrobial chemotherapy in reducing the duration and severity of disease. Delay in starting appropriate treatment in severely ill patients might significantly alter the outcome. Clinical assessment is important for guiding management during the early phase of the illness because confident exclusion of an infective etiology is rarely achieved in less than 24–48 h.

History

Identification of potential risk factors for intestinal infection may be acquired from the clinical history (Table 44.1). An infective etiology should be considered in all patients with inflammatory bowel disease who are apparently in relapse, because even patients with established diagnosis of inflammatory bowel disease are at risk of acquiring intestinal infection.

Examination

Unless there are obvious perianal stigmata of Crohn's disease, other signs suggestive of HIV infection (Kaposi's sarcoma, hairy leukoplakia or oral candidiasis) or typical extraintestinal manifestations of inflammatory bowel disease, such as erythema nodosum, aphthous ulceration, pyoderma gangrenosum and arthralgia, general physical examination rarely distinguishes between infection and non-specific inflammatory bowel disease However, as mentioned above, joint symptoms may accompany infection due to certain invasive enteropathogens and may form part of a Reiter's syndrome.

Acute infectious colitis is rarely distinguished from non-specific inflammatory bowel disease by abdominal examination. Any form of severe colitis can give rise to abdominal tenderness, distension and, in some cases, reduced bowel sounds due to ileus. Rigid sigmoidoscopy should form part of the initial clinical assessment and may confirm the presence of a colitis. Although some forms of infective proctitis do have specific features which might favor a diagnosis of infection (Table 44.4), ulceration in the rectum occurs in both infection and in non-specific inflammatory bowel disease; pseudomembrane may be a feature of ischemic colitis and also *C. difficile* infection. In addition, the endoscopic appearances in the rectum may be normal in many forms of infective colitis that may be limited to the right colon (Table 44.4).

Routine blood tests

Routine laboratory investigations also are rarely informative. In acute infectious colitis there may be anemia, elevated neutrophil count and evidence of an inflammatory process with a raised ESR, C-reactive protein and platelet count. However, these abnormalities also occur in ulcerative colitis and Crohn's colitis.

Clinical assessment is important for assessing the severity of colitis, but is rarely sufficient to differentiate

Table 44.4 Endoscopic appearances of the rectum in infectious colitis and proctitis.

Endoscopic appearance	Clinical diagnosis
"Colitis" but may be normal	Salmonellosis
	Shigellosis
	Campylobacteriosis
	Yersiniosis
	Tuberculosis
	Clostridium difficile infection
	Amebiasis
Deep ulcers	Amebiasis
	Tuberculosis
	Syphilis
Pseudomembrane	*Clostridium difficile* infection
Vesicles	Herpes simplex virus
Beads of pus	Gonorrhea

infection from non-specific inflammation. A variety of other diagnostic procedures are required.

Fecal testing

Microscopy

The standard approach to identify an enteropathogen responsible for an infectious colitis is microbiological examination of feces or rectal swab. Light microscopic examination of three sequential fecal specimens is important for the identification of protozoa such as *Entamoeba histolytica* and *Balantidium coli*. Fragile trophozoites of *E. histolytica* begin to lose their motility at room temperature and rapidly disintegrate, hence it is essential to examine fresh specimens. *E. histolytica* cysts are more robust and survive storage. *Balantidium coli*, a large motile ciliate, can sometimes be seen with a hand lens. Fecal microscopy is also of potential value in identifying the ova of *Schistosoma* spp. and a skilled parasitologist can identify the various subspecies by ova morphology. A "triple test" using several stool specimens and a combination of fixation, concentration and the use of a permanent stain has been shown to increase significantly the sensitivity of detecting stool parasites [50].

Culture

Provided that appropriate culture conditions are employed, stool culture will usually isolate the classic invasive enteropathogens. One of the major disadvantages of fecal culture is the inevitable delay before culture information is available to the clinician. It is unusual for a bacterial enteropathogen to be identified in less than 24–48 h and for slower growing organisms, such as *Yersinia enterocolitica* and *Campylobacter jejuni*, information may not be forthcoming for 3–5 days.

Toxin detection

C. difficile toxins A and B are routinely sought in feces using immunological techniques such as enzyme-linked immunosorbent assay (ELISA). The presence of toxin is generally regarded as evidence of active infection as the presence of the organism alone may only reflect carriage.

DNA-based tests

DNA-based technology, usually directed towards specific virulence factors of common enteropathogens, offers the opportunity to make a rapid, highly specific diagnosis. These techniques are widely used in research laboratories to investigate the role of potential virulence factors in pathogenesis and will ultimately be incorporated into clinical practice [51]. There continue to be difficulties in developing tests that will work on crude fecal extracts, but this technology had been satisfactorily used on tissue and other body fluids, particularly for the identification of *M. tuberculosis*. Immunomagnetic separation techniques have also been developed and have been shown to be more sensitive than PCR-based tests and have the advantage that they also yield the organism for culture and drug susceptibility testing [52].

Specific blood tests

Blood culture

Invasive organisms that produce an enteric fever-like illness including *Salmonella* spp., *Campylobacter jejuni* and *Yersinia entercolitica* can be detected by this approach. Blood culture should be performed in all ill patients with fever and other systemic symptoms.

Serology is generally disappointing as a method of diagnosis for the majority of intestinal infections. In amebic colitis, however, serology will be positive in 80–90% of patients and is an important screening test alongside fecal microscopy if amebiasis is an important diagnostic candidate. Serology is also useful in detection of *Yersinia enterocolitica* infection, although results are not usually positive for at least 10–14 days after the onset of the illness and therefore the results may only become available as the diarrhea resolves. ELISAs are now available for the diagnosis of strongyloidiasis and schistosomiasis and should be regarded as first-line screening tests for these infections. These tests are particularly useful in travelers returning from endemic areas but are of less value in the indigenous population once an infection has been diagnosed and treated, since antibodies may persist for months or even years after the initial infection.

Serotyping continues to be available for a number of bacterial enteropathogens, including *Salmonella* spp., *Campylobacter jejuni* and enterohemorrhagic *E. coli*, and are still of value in identifying the source of outbreaks and monitoring their extent.

Abdominal imaging

Imaging techniques may be useful both in establishing a primary diagnosis and in the assessment of the severity and extent of the disease.

Ileo-colonoscopy

If rigid sigmoidoscopy reveals the presence of colitis in the rectum, then a more extensive examination of the colon and distal ileum is usually unnecessary. A rectal biopsy should be taken for microscopic examination. If the rectum is normal, then it is usually appropriate to examine the proximal colon endoscopically. Appearances of *Shigella*, *Salmonella* and *Campylobacter* infections are macroscopically indistinguishable from non-specific inflammatory bowel diseases, hence differentiating acute infectious from other forms of colitis can be difficult. These infections may produce a predominantly right-sided colitis that macroscopically resembles ulcerative colitis; this finding should raise the index of suspicion for infection. Ileocecal and rectal tuberculosis may produce identical endoscopic appearances to Crohn's disease. However, an experienced endoscopist may be able to identify the typical amebic ulcers, which are shallow lesions with undermined edges often covered with a yellow exudate. The intervening mucosa appears normal, which distinguishes it from ulcerative colitis and other invasive bacterial infections of the colon. However, the diagnosis must be confirmed by microscopic examination of ulcer slough for trophozoites.

Pseudomembranes in the colon appear as pale, white/yellow excrescences on the epithelium which when removed leave an area of spontaneous bleeding separated by areas of normal mucosa. The presence of pseudomembranes is generally indicative of *C. difficile* infection, although pseudomembrane is not specific for this condition and may occur in ischemia.

Strictures are classic features of intestinal tuberculosis and schistosomiasis. Multiple colonic polyps are well recognized to occur in severe colonic schistosomiasis, usually due to infection with *S. mansoni*. Endoscopic polypepectomy has been advocated when polyp formation is extensive and associated with hypoalbuminemia. Larger mass lesions should also be examined endoscopically and biopsies to exclude neoplasia but benign inflammatory masses are well recognized in amebiasis ("amebomata") and schistosomiasis ("bilharziomas").

Endoscopic examination of the terminal ileum might reveal strictures (tuberculosis) or discreet ulceration (yersiniosis). Biopsies should be taken for microbiological culture and histology.

Endoscopic examination of the colon may also reveal the colonic helminth *Trichuris trichiura*. where multiple worms can be seen to be adherent to the colonic mucosa. In some instances it may be possible to detect directly the presence of an enteropathogen in tissue (*M. tuberculosis, E. histolytica*, *Schistosoma* spp. and CMV); therefore, multiple colonic biopsies should always be taken.

Plain abdominal radiograph

A supine plain abdominal radiograph can be invaluable in assessing the severity and extent of infectious colitis. Loss of haustration and colonic dilatation are indicative of severe inflammation. A gas-filled colon devoid of feces is consistent with total colitis. The examination is also useful for detecting free air in the abdominal cavity, a sign of colonic perforation.

Cross-sectional imaging

A trans-abdominal ultrasound scan may reveal bowel wall thickening in invasive ileo-colitis and enlarged lymph nodes in yersiniosis and abdominal tuberculosis. The examination may also be invaluable in detecting complications of intestinal infections such as amebic liver abscess and in confirming the presence of hepatosplenomegaly and portal hypertension in schistosomiasis. An abdominal CT scan also detects bowel thickening (>10 mm) in infective colitis and may be abnormal in up to about 50% of cases [53].

Histology

The colonic mucosa in the later stages of infection with invasive enteropathogens such as *Salmonella* spp., *Shigella* spp. and *Campylobacter jejuni* is often histologically indistinguishable from those of non-specific inflammatory bowel disease. Features which might suggest infectious colitis if biopsies are taken within the first 24–72 h include mucosal edema, straightening of the glands and an acute inflammatory infiltrate including polymorphonuclear leukocytes which can sometimes be seen penetrating the epithelium [54,55]. In *C. difficile* infection, an acute inflammatory infiltrate is also apparent combined with the typical "erupting volcano" lesion which is the histological counterpart of pseudomembrane. Colonic and ileal biopsies in tuberculosis may reveal caseating granulomata and occasionally acid-fast bacilli can be detected by light microscopy, although when tuberculosis is suspected, biopsies should always be sent for microbiological culture. Trophozoites of *E. histolytica* and the "owl's eye" inclusion bodies indicative of CMV infection are other organisms that can be identified in mucosal biopsy. Ova of *Schistosoma* spp. can also be detected in mucosal biopsies and it may be possible to differentiate one species from another on the basis of egg morphology.

Treatment

The treatment of infectious colitis may be considered at two levels, namely (i) general supportive and symptomatic therapy and (ii) specific antimicrobial

chemotherapy aimed to alter the natural history of infection and reduce duration and severity of the illness.

General supportive therapy

Although infectious colitis is usually not associated with major fluid and electrolyte losses, many infections such as *Salmonella* spp. and *Shigella* spp. begin with acute watery diarrhea, which can lead to deficits in fluid and electrolyte balance. These should be replaced by oral rehydration therapy with a glucose–electrolyte solution. This is particularly important in infants and young children and in the elderly who have a low tolerance for dealing with these losses, although otherwise healthy adults can usually replace fluid and electrolytes informally by increasing oral fluid intake by taking salty soups (sodium), fruit juices (potassium) and a source of complex carbohydrate such as rice, bread or pasta [56,57].

Anti-diarrheal agents such as loperamide are frequently used to reduce bowel frequency in acute watery diarrhea [58–61]; however, their use in dysentery is generally not encouraged. These drugs act predominantly by decreasing intestinal motility and there is concern that in dysentery these agents may prolong colonization with the enteropathogen and possibly also increase the risk of colonic dilatation. However, the evidence that these adverse effects are directly related to the use of these agents is poorly established and a more recent study suggests that loperamide is safe in bacilliary dysentery.

Antimicrobial chemotherapy

The microorganisms responsible for infectious colitis can be categorized as to whether antibiotics have been shown to be *definitely effective* in treating the infection, conditions in which these agents are *possibly effective* and finally conditions in which antimicrobial agents are *probably not effective* or possibly even *hazardous* (Table 44.5). There is evidence that clearly shows that antibiotics can reduce the severity and duration of some forms of infectious colitis, particularly in severe invasive bacterial infections and in colitis due to colonic protozoa and CMV.

Antibiotics are indicated for the treatment of dysenteric shigellosis [62–67], *C. difficile*-associated diarrhea [68–71], amebiasis [72] and balantidiasis [73] (Table 44.6). Antibiotics are also of value in *Yersinia* septicemia and when there is associated bone and joint infections [74,75], but its value in milder forms of *Yersinia* enteritis has not been established, probably because the antibiotic has been administered late in the natural history of infection [76]. The role of antibiotic therapy in *Campylobacter* infection remains controversial [77,78]. There is good evidence that the natural history of the illness is not altered if treatment is begun more than 4 days after the onset of symptoms. One randomized controlled trial has shown that treatment with erythromycin early in the infection significantly reduces the duration of the illness in children [79], although another study failed to confirm these findings [80].

Table 44.5 Efficacy of antimicrobial chemotherapy in bacterial and protozoal colitis.

Efficacy	Bacterial colitis*	Protozoal colitis
Proven efficacy	Dysenteric shigellosis Severe salmonellosis (dysentery, fever)	*Entamoeba histolytica* *Balantidium coli*
	C. difficile diarrhea *Yersinia* septicemia *Campylobacter* dysentery/sepsis	
Possible efficacy	EIEH *Campylobacter* enteritis	
Doubtful efficacy	*Salmonella* enterocolitis *Yersinia* enteritis (uncomplicated)	
	EHEC	
Hazardous	?EHEC	

*EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; EHEC, enterohemorrhagic *E. coli*.

Treatment of enteroinvasive *E. coli* infection with antibiotics has not been established, although in severe cases with evidence of systemic involvement it would be reasonable to treat along the same lines as those recommended for dysenteric shigellosis. A very controversial issue is whether antibiotics should be used in EHEC infection, although the balance of evidence at present is that antibiotics, particularly when given after infection is well established, do not significantly improve the outcome [81]. In addition, there is increasing evidence that administration of antibiotics at this stage can promote the development of the hemolytic–uremic syndrome [82,83]. This is presumed to relate to the massive lysis of organisms and release of shiga toxin and endotoxin. A recent systematic review indicates that although there is some evidence that quinolones and fosfomycin may decrease the risk of developing HUS, other studies show that there is an increased risk in those treated with antibiotics. The final conclusion was that further randomized controlled trials are required before definitive advice can be given [84]. Current evidence therefore suggests that antimicrobial chemotherapy should not be used in children with proven EHEC infection. Anti-viral agents such as ganciclovir and foscarnet are effective in CMV colitis but prolonged courses may be required in the immunocompromised [85–87].

Intestinal tuberculosis should be treated according to the current recommendations of the British Thoracic Society; isoniazid (330 mg) and rifampicin (450–600 mg) should be given for 6 months, with pyrazinamide (20–30 mg kg^{-1} daily, maximum 3 g daily) included for the first 2 months [88]. A fourth drug, such as ethambutol

Table 44.6 Antimicrobial chemotherapy for infectious colitis.

Species*	Drug of choice*	Alternative(s)*
Viruses		
Cytomegalovirus	Ganciclovir 5 mg kg^{-1} per 12 h, 14–21 days [85,86]	Foscarnet 60 mg kg^{-1} per 8 h [87], 14–21 days. Maintenance therapy may be required
Bacteria		
Shigella spp.†	TMP-SMX 2 tablets twice daily, 5 days [62]	Short-term quinolone [65–67]
	[3]Ciprofloxacin 500 mg twice daily, 5 days [63]	[1]Cefixime 400 mg daily, 5–7 days
		Nalidixic acid 1 g four times daily, 5–7 days
Salmonella spp.‡	[2,3]Ciprofloxacin 500 mg twice daily, 10–14 days	TMP–SMX, ampicillin, amoxycillin
C. jejuni§	Erythromycin 250–500 mg four times daily, 7 days [77–80]	[3,4]Ciprofloxacin 500 mg twice daily, 5–7 days
		Azithromycin 500 mg daily, 3 days
Y. enterocolitica	[3]Ciprofloxacin 500 mg twice daily, 7–10 days [74,75]	Tetracycline 250 mg four times daily, 7–10 days [74,75]
C. difficile	Metronidazole 400 mg three times daily, 7–10 days [68]	Vancomycin 125 mg four times daily, 7–10 days [68–70]
		Nitazoxanide [89], fusidic acid, teicoplanin [71]
EIEC	?As *Shigella* spp.	
EHEC	?None (see text)	
Aeromonas hydrophila	Ciprofloxacin 500 mg twice daily, 7–10 days	TMP–SMX 2 tablets twice daily, 7–10 days
Plesiomonas shigelloides	Ciprofloxacin 500 mg twice daily, 7–10 days	TMP—SMX 2 tablets twice daily, 7–10 days
Protozoa		
E. histolytica	Metronidazole 750 mg three times daily, 5 days [72]	Paromomycin 25–35 mg kg^{-1} three times daily, 7–10 days [72]
	Diloxanide furoate 500 mg three times daily, 10 days [72]	
Balantidium coli	Metronidazole 400 mg three times daily, 10 days [72,73]	Tetracycline 500 mg four times, daily 10 days [72,73]
Helminths		
Schistosoma spp.	Praziquantel [5]40–[6]60 mg kg^{-1} per day	
Trichuris trichiura	Albendazole 400 mg single dose	Mebendazole 100 mg twice daily, 3 days

*EIEC, enteroinvasive *E. coli*; EHEC, enterohemorrhagic *E. coli*; TMP–SMX, trimethoprim–sulfamethoxazole.
†Multiple resistance to tetracycline, TMP–SMX, ampicillin and chloramphenicol in South America, Greece, Spain and Thailand.
‡Chronic carrier state, norfloxacin 400 mg twice daily, 28 days.
§May only shorten duration of illness when given early.
[1]And other third-generation cephalosporins.
[2]Usually only for bacteremia.
[3]And other fluoroquinolones such as ofloxacin, norfloxacin, fleroxacin and cinoxacin.
[4]Increasing resistance to quinolones being recognized.
[5]*Schistosoma mansoni*, *Schistosoma hematobium*.
[6]*Schistosoma japonicum*.

or streptomycin, should be added initially if drug resistance is suspected, particularly in patients who may have imported the disease from a developing country.

Antimicrobial chemotherapy is also indicated for *C. difficile* [89], protozoal [72,73] and helminth infections in the colon (Table 44.6). Infectious proctitis of bacterial, viral [85,87,90,91] and helminth origin is also responsive to antibiotic therapy (Table 44.7).

Non-specific colitis and intestinal infection

There is a continuing controversy as to whether intestinal infection can act as a trigger for relapse in patients with ulcerative colitis and Crohn's colitis. There are numerous case reports in the literature demonstrating close temporal relationships between the onset of an attack of colitis and the detection of the presence of an enteropathogen in the stool. Several pathogens have attracted particular attention, including *C. difficile*, *Campylobacter* spp., *Aeromona*s spp. and cytomegalovirus infection. There is no question that these and other infections can occur in association with established non-specific inflammatory bowel disease (IBD) and may be isolated during an apparent relapse, but it is not clear as to whether they are of etiologic importance in precipitating IBD in a previously healthy individual.

There are limited prospective studies of sufficient power to answer this question. One study examined 64 patients

Table 44.7 Treatment of infectious proctitis.

	Drug of choice	Alternative(s)
Bacteria		
Chlamydia trachomatis		
Non-LGV*	Doxycycline 100 mg twice daily for 7 days	Erythromycin 500 mg four times daily
LGV	Doxycycline 100 mg twice daily for 3 weeks or more	TMP-SMX 2 tablets twice daily
Neisseria gonorrhoeae	Ceftriaxone 250 mg i.m.†	Oral cefixime, ciprofloxacin or ofloxacin
Treponema pallidum	Benzathine penicillin G 2.4 million units, single oral dose	Procaine penicillin 600,000–900,000 units i.m. for 10 days Doxycycline 100 mg twice daily oral for 15 days
Mycobacterium tuberculosis	See text	
Viruses		
Herpes simplex virus	Acyclovir 5 mg kg^{-1} i.v. 8-hourly for 7–10 days [90]	Forscarnet 40–60 mg kg^{-1} i.v. 8-hourly for 2–3 weeks [91]
Cytomegalovirus	See Table 44.6	
Helminths		
Schistosoma spp.	See Table 44.6	

*LGV, lymphogranuloma venereum.
†Plus doxycyline 100 mg twice daily, oral, 7 days.

during their first attack of ulcerative colitis and 30 others during a relapse of known disease and found no evidence that bacterial enteropathogens were important triggers in either group. Another study searched for enteropathogens in 64 patients with a relapse of known IBD. *C. difficile* was isolated in six patients, *Campylobacter jejuni* in one patient and *Salmonella typhimurium* in one patient. It was considered that these infrequent isolations indicated that intestinal infection played only a minor role in exacerbations of IBD. However, these studies confirm that the two conditions can coexist and that an etiologic agent should be excluded in patients presenting for the first time with possible IBD, and many clinicians would routinely test for infection in patients with known IBD on the basis that intestinal infections are common even in industrialized countries and that it is virtually impossible to distinguish clinically between a simple relapse of IBD and a relapse associated with a co-infection.

Intestinal infection associated with a relapse of IBD should be treated in the same way as recommended for infection in an otherwise healthy individual. In such patients with a relapse of non-specific colitis, it is usually wise to treat both the infection and the inflammatory bowel disease, particularly when the patient is moderately or severely affected. In mild disease, it may be possible just to treat the infection while closely monitoring the activity of the colitis.

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Chapter 45
Recent Advances in the Understanding of HIV and Inflammatory Bowel Diseases

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Summary

- There are approximately 1 million cases of HIV infection in the United States with 40,000–50,000 new cases each year.
- Recent data suggest that acute HIV infection is associated with a profound loss of mucosal CD4-positive lymphocytes; a finding that may influence the pathogenesis and natural history of inflammatory bowel diseases (IBD) in patients with both IBD and HIV infection
- Effective antiretroviral therapy has significantly reduced the incidence of HIV-associated gastrointestinal infections, but these should always be included in the differential diagnosis of IBD flares.
- Use of immunosuppressive therapy in patients with HIV and IBD needs very careful monitoring due to the increased risk of opportunistic infections and also bone marrow toxicity from antiretroviral therapy.
- The incidence of lymphogranuloma venereum caused by rectal infection with *Chlamydia trachomatis* (serovars L1, L2, L3) has increased in men who have sex with men and may present with a proctocolitis that can be confused with Crohn's disease.

Introduction

In order to evaluate potential interactions between HIV infection and inflammatory bowel disease (IBD), it is important to review the contemporary epidemiology, natural history and treatment of HIV infection. Much of the relevant literature on HIV-associated IBD was written in a period of absent or suboptimal HIV therapy when patients had a uniformly gloomy prognosis and their HIV-associated disease was characterized by a high incidence of opportunistic enteric infections and malignancies. In addition, little was known of the mucosal immunopathogenesis of HIV infection. Much has changed and it is likely that the pathogenesis, clinical manifestations and treatment of HIV-associated IBD will be very different to the observations of the first 25 years of the HIV pandemic. This chapter briefly reviews the contemporary epidemiology and natural history of HIV infection. It then summarizes recent data on the mucosal pathogenesis of HIV infection, which has direct relevance to HIV-associated IBD. The question of whether a specific HIV colitis exists is then addressed. The published anecdotal literature on the clinical manifestations of HIV-associated IBD is reviewed to evaluate whether concurrent HIV infection does indeed alter the natural history of IBD. The challenges associated with treating HIV-infected patients with IBD are discussed, paying particular attention to the use of immunosuppressive therapy and biological agents such as inflixamab. Finally, the recent resurgence of lymphogranuloma venereum (LGV) is described. LGV is a sexually transmitted disease seen most commonly in men who have sex with men (MSM) that often presents as an inflammatory proctitis and can be misdiagnosed as Crohn's disease.

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Epidemiology, natural history and treatment of HIV infection

The first cases of acquired immunodeficiency syndrome (AIDS) were identified in groups of homosexual men in North American urban centers in 1981 [1]. At first, the disease was thought to be restricted to certain high-risk groups such as homosexuals, hemophiliacs and individuals of Haitian descent. However, the AIDS pandemic has generalized and affects all communities and populations, albeit with variable incidence and prevalence. In some regions, such as Sub-Saharan Africa, the incidence and prevalence rates of 5 and 35%, respectively, are not uncommon. In the United States, the prevalence of HIV infection in the general population is estimated to be <1%. It is thought that 1 million Americans have HIV infection and

that more than 40,000 new infections occur each year [2]. However, in specific subgroups such as African-American MSM, the prevalence of HIV infection can be as high as 30% [3].

Early natural history studies suggested that HIV infection was followed by a period of clinical latency when patients appeared well. This was followed by the onset of minor infections such as oral candidiasis and subsequently major opportunistic infections including *Pneumocystis jiroveci* pneumonia, malignancies such as Kaposi's sarcoma (KS) and ultimately death. The average period between HIV infection and death was approximately 10 years. The first specific antiretroviral agent, zidovudine, appeared in 1986 and clinicians began to treat patients with single and dual therapy as new agents became available. This resulted in clinical improvement but the effects were transient. The regimens were not sufficiently potent to suppress viral replication, the virus developed resistance to the antiretroviral therapy (ART) and the disease progressed. In 1996, the development of protease inhibitor (PI)-based triple therapy regimens began a new era in HIV therapeutics [4]. The PI-based regimens suppressed viral replication, arrested disease progression and significantly reversed HIV-associated immunosuppression. One major consequence of the availability of PI therapy was a significant fall in the prevalence of HIV-associated opportunistic infections. In gastroenterology clinics, opportunistic enteric infections such as cryptosporidiosis, microsporidiosis and cytomegalovirus (CMV) colitis became become far less prevalent amongst the patients receiving ART [5]. In the last 10 years, there have been further advances in HIV therapeutics. In the United States there are now more than 20 drugs licensed for the treatment of HIV infection (Table 45.1). ART has been simplified and it is now possible to treat most patients with once-daily regimens. In 2006, a new HIV drug, Atripla (Gilead Sciences, Foster City, CA, USA) was licensed. This is a single tablet containing three antiretroviral agents; tenofovir disoproxil fumarate, emtricitabine and efavirenz. This combination provides the opportunity for once-daily dosing with one tablet. Clearly, this development will facilitate patient adherence and maximize the benefits of ART.

Table 45.1 Licensed drugs for the treatment of chronic HIV infection.

Class of drug			
Nucleoside/ nucleotide RT inhibitors	Non-nucleoside RT inhibitors	Protease inhibitors	Fusion and integrase inhibitors
Didanosine	Delavirdine	Amprenavir	Enfuvirtide
Emtricitabine	Efavirenz	Atazanavir	Maraviroc
Lamivudine	Nevirapine	Darunavir	Raltegravir
Stavudine	Etravirine	Fosamprenavir	
Tenofovir		Indinavir	
Zidovudine		Lopinavir	
Abacavir		Nelfinavir	
		Ritonavir	
		Saquinavir	
		Tipranavir	

The changes in the epidemiology and natural history of HIV infection, combined with improved ART, have significant implications for potential interactions between IBD and HIV. As mentioned above, approximately 1 million Americans have HIV infection, a figure that is very similar to the prevalence of IBD. This suggests that it is possible that both conditions might occur in the same patients. With the advent of potent ART, the differential diagnosis of IBD-like symptoms in HIV-positive patients receiving ART has been simplified. Previously, opportunistic enteric infections such as CMV, tuberculosis or intestinal KS had to be excluded before a diagnosis of IBD could be considered likely in an HIV-positive patient. However, all of these diagnoses are extremely rare in patients on ART with fully suppressed viral infection. Such HIV-positive patients presenting with clinical, endoscopic and histologic evidence of IBD are significantly more likely to have IBD rather than HIV-associated opportunistic enteric infection. ART is also known to modulate the mucosal immunopathogenesis of HIV infection. This is likely to have significant implications for the interaction between HIV and IBD and is discussed in more detail below.

Mucosal immunopathogenesis of HIV infection

The most striking immunologic deficit associated with HIV infection is the progressive $CD4^+$ T cell lymphopenia that occurs in the peripheral blood following HIV infection. The CD4 receptor on T cells was initially recognized as the facultative receptor for HIV [6]. The specific mechanism of CD4 lymphopenia remains controversial [7] but the phenomenon is a well-recognized feature of HIV pathogenesis. Several studies have documented CD4 lymphopenia in gut associated lymphoid tissue (GALT) using immunohistochemical and flow cytometric techniques [8–10]. In general, these early studies focused on samples collected from patients with advanced HIV infection and there were limited data on GALT in early HIV infection. More recently, a number of investigators have characterized the mucosal pathogenesis of simian immunodeficiency virus (SIV) or recombinant SIV/HIV (SHIV) in non-human primates [11–13] and of early HIV infection in humans [14,15]. The findings from these studies have been dramatic. It appears that approximately 50% of GALT CD4 lymphocytes are lost within weeks of acute HIV/SIV infection and this observation was seen in both human and primates studies. The pathogenesis of this

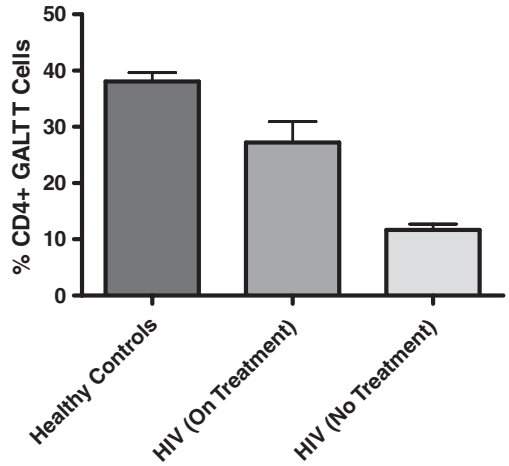

Figure 45.1 Mucosal T cell phenotype in patients with HIV infection. CD4 counts were measured in T cells isolated from intestinal biopsies collected at three time points over a 4 week period from 16 individuals (8 healthy controls, 4 HIV-positive patients on treatment with undetectable plasma viral load and 4 HIV-positive patients not receiving treatment). Although CD4-positive T cell reconstitution is seen in the HIV-positive patients receiving treatment, it is incomplete [20].

process is unclear. Initially, is was felt unlikely that it could not be a direct effect of viral replication leading to cell lysis because the prevalence of infected $CD4^+$ T lymphocytes was less than 1% in the peripheral circulation [16]. However, Mattapallil *et al.* were able to demonstrate that 60% of GALT $CD4^+$ T cells are infected in the primate acute infection model [13]. This observation suggests that the acute loss of $CD4^+$ T cells maybe indeed be directly related to viral infection. Further studies have documented that the use of ART is associated with recovery of the GALT CD4 lymphopenia (Figure 45.1), although the timing and magnitude of recovery remain controversial [14,15,17–20].

GALT CD4 lymphopenia is not the only mucosal abnormality associated with HIV infection. Early studies documented partial villous atrophy and crypt hyperplasia in small intestinal biopsies from patients with HIV infection, a histologic appearance which became known as HIV enteropathy [21]. These changes appeared to be exacerbated in HIV-positive patients with enteric infections such as cryptosporidiosis. However, others have argued that enteropathy associated with HIV infection is relatively modest compared with the mucosal lesions seen in untreated celiac disease [22]. Less contentious is the observation that mucosal cytokine expression is increased in HIV infection and the degree of abnormality appears to be related to the level of mucosal viral replication [23,24]. Using immunohistochemical techniques, Olsson *et al.* were able to show increased expression of the proinflammatory cytokines RANTES, MIP-1α and MIP-1β equivalent to the changes seen in active IBD [25]. Similar findings have been described using reverse transcriptase polymerase chain reaction (RT-PCR) quantification of cytokine mRNA [23,24]. More recently, Brenchley *et al.* suggested that a key step in the mucosal pathogenesis of HIV infection is a disruption of the epithelial barrier that allows passage of luminal bacteria into the lamina propria resulting in low-grade infection that stimulates mucosal inflammation [26].

HIV infection and inflammatory bowel disease

It has been argued that an HIV-associated idiopathic proctitis/colitis can occur in the absence of enteric infection that is distinct from Crohn's disease or ulcerative colitis and responds to treatment with thalidomide [27–32]. It is presumed that the nonspecific histologic changes seen in HIV-associated proctitis/colitis (Plate 45.1) occur secondary to the mucosal inflammation induced by HIV infection.

The mucosal pathogenesis of IBD is complex and described in more detail elsewhere in this book. However, the key features are mucosal inflammation of varying severity that may be continuous (ulcerative colitis) or segmental (Crohn's disease) and superficial (ulcerative colitis) or transmural (Crohn's disease). Perturbation of both humoral and cellular immunity has been described in IBD. Mucosal T cell dysfunction plays a critical role in IBD pathogenesis and it is therefore logical to hypothesize that the T cell changes associated with HIV infection might alter the pathogenesis of IBD in patients who have both conditions. It has been suggested that CD4 lymphopenia might reduce the severity of Crohn's disease whereas the increase in TH_2 cytokine responses seen in HIV [33] might exacerbate ulcerative colitis. The anecdotal HIV/IBD case literature does not provide comprehensive support for these hypotheses and the picture is likely to be further confused by the degree of immune reconstitution associated with ART.

Reported cases of HIV and IBD

The case literature of IBD occurring in patients with HIV, or conversely patients with HIV infection developing IBD, is summarized below in Tables 45.2 and 45.3. As discussed above, one practical problem is that much of the literature was published before patients had access to potent combination ART. As a consequence, the clinical and histopathologic manifestations of their IBD might have been influenced by the virologic and immunologic sequelae of active mucosal HIV viral replication. Another problem is that these patients would have been at risk of concurrent opportunistic enteric infection. Although most case reports tried exhaustively to exclude enteric infection, the diagnosis of infections such as microsporidiosis was, and to some extent still is, an inexact science. A final complication is the phenomenon of immune reconstitution inflammatory

Table 45.2 Reported cases of Crohn's disease in association with HIV infection.

Year	No. of patients	Initial diagnosis	CD4 count	Location*	Ref.
1984	1	AIDS	230	Colon, TI	36
1988	1	Crohn's	410	Colon	37
1994	1	HIV	480	Colon, TI	38
1996	1	Crohn's	270	Colon, TI	39
1996	1	HIV	210	NA	61
1996	2	Crohn's	336, 442	Colon, SI	41
1997	1	HIV	100	Colon, TI	40
1998	4	Crohn's	320, 50, 162, 34	Colon, TI	42
2006	1	HIV	517	Colon	62
2006	1	HIV	555	Colon	53
2006	1	Crohn's	>750	Colon	52

*TI, terminal ileum; SI, small intestine; NA, not available.

syndrome (IRIS). This syndrome occurs most commonly when treatment-naïve individuals with CD4 lymphopenia start ART and experience a clinical deterioration despite favorable immunologic and virologic responses to ART [34]. Most commonly IRIS manifests as an infectious complication such as CMV or tuberculosis, but a case report described four patients who developed appendicitis within 6 months of starting ART [35], raising the possibility that GALT might participate in IRIS-related disease.

Table 45.3 Reported cases of ulcerative colitis in association with HIV infection.

Year	No. of patients	Initial diagnosis	CD4 count	Location	Ref.
1986	1	UC	NA	NA	43
1990	1	UC and HIV	500	Transverse colon	46
1991	1	UC and HIV	546	Pancolonic	45
1992	1	HIV	170	Transverse colon	47
1996	1	HIV	NA	Pancolonic	51
1996	2	HIV	680, 700	Proctitis, NA	61
	2	UC	530,130	NA, right side of colon	
1996	5	HIV	460, 270,4 62, 228, 283	NA	41
	1	UC	256	NA	
1997	1	HIV	450	Pancolonic	63
1999	4	HIV	930	Transverse colon	64

Crohn's disease

The first possible case of Crohn's disease in association with AIDS was reported by Dhar *et al.* [36] and involved a heterosexual male who presented with esophageal candidiasis and within a year developed Crohn's disease requiring surgical intervention. Definitive serology was not available at the time of his death in 1983, but he was found to have CD4 lymphopenia and a reversed helper suppressor ratio. In 1988, James described an individual with an 18 year history of Crohn's disease whose symptoms resolved after the acquisition of HIV infection [37]. The authors postulated that the development of CD4 lymphopenia was a key factor in the resolution of the patient's symptoms associated with Crohn's disease. Although this is an attractive hypothesis, subsequent case reports were variable in its support. Some did not see obvious resolution of Crohn's disease in the face of progressive CD4 lymphopenia [38–40], whereas others did suggest that advanced HIV disease lead to a resolution of Crohn's disease [41,42].

Ulcerative colitis

The first case report was of ulcerative colitis and AIDS was described by Liebowitz and McShane [43]. The patient was a 40-year-old male who presented in 1970 with bloody diarrhea. He had a strong family history of ulcerative colitis and a barium enema demonstrated a pancolitis. Initial treatment with sulfasalazine and subsequently prednisone led to clinical remission but the patient had relapsing disease and in 1984 colonoscopy and histology were more suggestive of Crohn's disease. A month later the patient developed cutaneous KS. As with Crohn's disease, the question arises as to whether HIV-associated immunodeficiency might exacerbate or moderate the natural history of ulcerative colitis. In theory, the TH_1 to TH_2 switch described in HIV pathogenesis [33] might be expected to exacerbate ulcerative colitis, which is often regarded as a TH_2 disease [44]. However, as with Crohn's disease, the jury is somewhat divided, with reports of clinical improvement [43] and deterioration [41,45–47] with advanced HIV disease. An additional diagnostic problem is that intestinal KS may manifest in both HIV-positive [48,49] and -negative patients [50].

Diagnosis of IBD in patients with HIV infection

It is critical that HIV-infected patients who present with symptoms suggestive of IBD receive a thorough diagnostic evaluation to exclude enteric infection. As discussed above, the incidence of opportunistic enteric infection is low in patients with well-controlled HIV infection but it is an important differential diagnosis (Table 45.4) that needs to be excluded [51]. Specific diagnoses that can occur in HIV-positive patients include KS, CMV and mycobacterial disease, all of which can masquerade as IBD.

Table 45.4 Differential diagnosis of IBD in patients with HIV infection.

Infection	Bacterial colitis
	Lymphogranuloma venereum
	Cytomegalovirus
	Herpes simplex
	HIV
	Mycobacterium avium complex
	Mycobacterium tuberculosis
	Amebiasis
	Isospora belli
	Histoplasmosis
Non-infectious	Ischemia
	Drug-induced colitis
	Peridiverticulitis
Neoplasm	Kaposi's sarcoma
	Non-Hodgkins lymphoma

Treatment of IBD in patients with HIV infection

Once enteric infection and malignancy have been excluded, the differential diagnosis lies between idiopathic HIV colitis [32] and IBD. As with non-HIV-associated IBD, the diagnosis can be difficult but is based on a combination of clinical, radiologic and histologic evidence. Once a diagnosis of IBD has been made, the treatment is likely to be similar irrespective of HIV status and most of the clinical case reports document clinical improvement in IBD associated with the use of 5′-ASA products and/or steroids. The concern about steroid-induced immunodeficiency in patients with HIV is probably more theoretical than practical in the era of potent combination ART. More contentious is the use of immunomodulatory and biological therapy in HIV-associated IBD. The evidence base here is extremely limited and treatment will need to be evaluated on a case by case basis. Two recent case reports have described successful treatment of Crohn's disease in HIV-positive patients with the use of infliximab [52,53]. Obviously, it is important to exclude latent or active mycobacterial disease before the initiation of treatment with drugs such as infliximab [54].

Lymphogranuloma venereum (LGV)

LGV is a sexually transmitted infection caused by *Chlamydia trachomatis* (CT), serovars L1, L2 and L3. Although rare in North America, LGV rates are increasing in select populations, specifically HIV-positive MSM. Clinical features of rectal LGV include diarrhea, constipation and hematochezia and individuals may often present to the gastroenterologist for evaluation. The endoscopic appearances of LGV can be confused with those of idiopathic IBD. However, the correct identification and treatment of LGV will obviate more serious sequelae such as rectal stricture and fistulation.

LGV is endemic in Africa, Southeast Asia and the Caribbean and until recently was only infrequently diagnosed in North America and Europe. However, since 2003 outbreaks and cases have been described in The Netherlands [55] with subsequent cases identified in the United Kingdom, [56] North America [57] and Australia [58]. This outbreak has been seen most commonly with an LGV variant (L2b) that is likely identical to a strain previously identified in San Francisco in the 1980s.

The key pathologic feature of LGV is thrombolymphangitis and perilymphangitis. About 3–10 days following infection, a papular/ulcerated lesion develops at the site of inoculation (cervicovaginal/oral/rectal), then resolves without scarring. In 2–4 weeks the draining lymph glands of the primary lesion become inflamed and asymmetrically enlarged to form a bubo with associated constitutional symptoms. Inguinal bubos are common in heterosexual men, although women and MSM with pelvic and rectal infection, respectively, may have silent deep iliac lymph node enlargement.

Early symptoms of rectal infection are those of proctitis and may include pruritis, anal discharge, diarrhea, constipation, pain, tenesmus and hematochezia [59]. Endoscopic inspection will reveal a hyperemic mucosa, with ulceration and contact bleeding with a purulent exudate clinically indistinct from active IBD [60]. Histologic examination will reveal crypt abscesses and non-caseating granulomata. Untreated colorectal infection can lead to whole thickness involvement with fibrosis, stricture, stenosis and fistulation.

Laboratory diagnosis of LGV requires detection of CT using a nucleic acid amplification test with molecular confirmation of L1, L2 or L3 genotypes and may often necessitate the use of reference laboratories. Serology is more widely available but less specific. Absence of definitive microbiological diagnosis should not delay treatment of suspected LGV, especially in high-risk populations such as MSM. The recommended regimen for LGV is doxycycline 100 mg orally twice per day for 21 days.

Conclusion

At a global level, the AIDS pandemic continues unabated and even within the United States the annual number of cases of new infections continues to increase. In this setting, gastroenterologists will be referred HIV-positive patients with symptoms suggestive of IBD. The clinical priorities are to ensure that the patients' ART is optimized, opportunistic infection and malignancy have been excluded and that there is clinical, radiologic and histologic evidence to support the diagnosis of IBD. Treatment should then follow the conventional guidelines for IBD. Use of newer biological agents may be considered for refractory IBD, in the absence of mycobacterial infection,

but should be considered on an individual basis. Unfortunately, despite the increased breadth of ART, there will be patients with long-standing HIV disease that has proved resistant to all available agents. It is these patients who may progress to opportunistic infections that will recapitulate the early case literature of HIV/IBD interactions.

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Chapter 46
Bone Metabolism and Inflammatory Bowel Disease

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Summary

- Crohn's disease and ulcerative colitis are associated with an increased risk of osteoporosis and bone fractures. Initial studies suggested very high rates of osteoporosis in IBD based on bone mineral density (BMD) measurements; however, more recent studies suggest that BMD is often normal in IBD and average short-term changes are small. Doctors managing patients with IBD will have to consider a variety of risk factors and not just BMD measurements in assessing fracture risk.
- The evolution of knowledge regarding receptor for activated factor of nuclear factor kappa B (RANK), its ligand RANKL and osteoprotegerin (OPG) that serves as a decoy receptor, has enhanced our understanding of osteoporosis and also T cell immunobiology.
- Recent clinical studies in subjects with IBD have revealed that serum OPG levels may be elevated and inflamed intestinal tissue secretes OPG. It is suspected that OPG levels are elevated as a counter-regulatory response to low BMD as serum OPG levels in IBD have been found to be inversely associated with BMD. OPG may ultimately prove to be a useful therapeutic target for managing both low bone mass and colitis in patients with IBD.
- Currently, an underpinning of therapy for all IBD patients should include supplemental calcium and vitamin D, particularly for patients using corticosteroids.
- Postmenopausal women and men over age 50 years should be considered for BMD screening (in conjunction with an assessment of other risk factors for fracture) and if this suggests high fracture risk then pharmacological intervention may be appropriate. Those with spontaneous or low-impact fractures that are typical for osteoporotic fractures (e.g., hip fractures, one or more moderate to severe vertebral compression fractures) should be considered to have established disease with high risk for further fractures and also require intervention.

The interplay between factors that affect bone and immune homeostasis

Osteoporosis and fractures are well-recognized extraintestinal complications of IBD. The biology of bone homeostasis has been shown to intersect T cell activation and apoptosis through the receptor for activated nuclear factor kappa B (RANK) and its ligand (RANKL) [1]. The binding of RANK to RANKL induces a signaling and gene expression cascade that results in the differentiation, maturation and activation of osteoclasts, which favors the development of osteoporosis. A circulating decoy receptor, osteoprotegerin (OPG), binds to RANKL and interferes with osteoclastogenesis. RANKL is also a regulator of T cell-dendritic cell interaction in the immune system and is a critical factor in early lymphocyte development and lymph node organogenesis [2]. RANKL gene-deficient mice are unable to support osteoclast differentiation and develop abnormally dense bones (even in the presence of bone resorbing factors such as vitamin D_3, dexamethasone and PGE_2), no evidence of bone remodeling and a lack of all lymph nodes [2]. Activated T cells can directly trigger osteoclastogenesis through RANKL leading to bone loss, an effect that is blocked by OPG [2–4]. Hence this system links systemic and/or mucosal inflammation with altered bone metabolism and, ultimately, osteoporosis. Recently, a phase III clinical trial has documented the efficacy of denosumab, a humanized monoclonal antibody to RANKL that blocks its binding to RANK thereby potently suppressing osteoclast activation, for increasing BMD and reducing fracture risk [5].

In human IBD, it has been shown that circulating OPG is elevated in females with Crohn's disease and in males with Crohn's disease using prednisone compared with healthy controls [6]. It has been speculated that this might

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reflect a counter-regulatory response to low bone mineral density (BMD) or elevated bone resorbing factors such as tumor necrosis factor alpha (TNFα). A second study showed that OPG levels were significantly increased in both Crohn's disease and ulcerative colitis compared with controls [7]. In both studies, serum RANKL levels were no different between IBD patients and controls, although one of these studies found that serum RANKL levels were significantly lower in subjects using corticosteroids [7]. The latter also measured BMD in their IBD subjects and found a significant inverse association between serum OPG and femoral neck BMD ($r = –0.41$, $p = 0.007$) and lumbar spine BMD ($r = –0.38$, $p = 0.017$). OPG was also identified in supernatants from colon explant cultures [7]. OPG levels were significantly increased in specimens derived from inflamed tissue from both Crohn's disease and ulcerative colitis subjects, while uninflamed tissue from disease groups and from healthy controls yielded similar results. By immunohistochemistry, OPG was expressed on few cells in control tissue but was abundantly expressed in cells from Crohn's disease and ulcerative colitis tissue including epithelial cells, dendritic cells, macrophages and in lymphoid aggregates. Hence the actions of OPG may not be limited to bone metabolism alone.

It has been difficult to distinguish the role of systemic corticosteroids in promoting osteoporosis versus the role of the underlying inflammation that the corticosteroids are being used to treat. This has been problematic in IBD where corticosteroids are frequently used to treat the patients with the most active inflammatory disease. While the relative contributions of active inflammation and corticosteroid use on reduced BMD have been difficult to discern, a recent prospective study found that the only measurable parameter that correlated with a fall in BMD was the serum C-reactive protein (CRP), a nonspecific marker of inflammation [8].

Further proof of the association of osteoporosis and systemic inflammation, independent of corticosteroids, comes from animal models of colitis. One study used the interleukin-2 (IL-2)-deficient mouse model of colitis, which is known to develop both osteopenia and colitis [9]. Study animals had elevated levels of bone marrow mononuclear cell expression of RANKL and OPG mRNA and also increased circulating serum RANKL and OPG compared with control littermates. Osteopenia was not evident in IL-2-deficient mice crossbred to be T cell deficient, but osteopenia could be induced by adoptive transfer of T cells from IL-2-deficient mice. These data suggest that activated T cells are critical in mediating osteopenia. Exogenous OPG administration reversed both the osteopenia and the colitis. The colitis was abrogated by a specific reduction in colonic dendritic cells, while circulating inflammatory cytokines were unaffected by exogenous OPG. These data highlight the importance of OPG in the etiopathogenesis of osteopenia and colitis in IL-2-deficient mice and the central role of activated T cells in mediating these conditions and provide direct experimental support for the finding of elevated OPG levels in humans with IBD. Increased OPG in Crohn's disease may result as much from intestinal inflammation as in response to osteopenia. The IL-10 knockout murine colitis model is also associated with diminished bone mass [10]. In two rat models of arthritis (causing both arthritis and osteopenia in the animals), RANKL protein as measured by ELISA and *in situ* hybridization was elevated in the serum and joints, respectively, of these arthritic animals [11]. Exogenous OPG administration prevented osteopenia. Serum RANKL levels increased rapidly during arthritis development in the rat models, implicating RANKL as a pro-osteoporosis factor. Hence it has become increasingly convincing that bone mass is diminished by active colonic inflammation independent of corticosteroid use. Exogenously administered OPG may even warrant clinical investigation as a novel therapeutic approach in Crohn's disease.

OPG is constitutively produced in human intestinal epithelial cells and is upregulated by TNFα [12]). Furthermore, OPG is also expressed by endothelial cells [13,14] and the media of arteries in wild-type mice [15], suggesting a possible role in vascular biology. OPG can prolong endothelial cell survival by inhibiting apoptosis [16]. If Crohn's disease is a vasculitis [17], elevations seen in serum OPG may reflect vascular release of OPG in response to inflammation.

Interleukin-1β (IL-1β) and TNFα, the same proinflammatory cytokines known to be important in IBD, are also implicated in impaired bone modeling [18]. IL-1β and TNFα act synergistically to inhibit longitudinal growth of fetal rat metatarsal bones, whereas IL-6 has no effect on bone growth. This effect is linked to inhibition of chondrocyte proliferation and increased chondrocyte apoptosis, but can be abrogated by administration of antibodies to TNFα or IL-β and also by co-culture with insulin-like growth factor-I (IGF-I). It has been shown that stimulating human articular chondrocytes with IL-1β or TNFα turns on a specific gene program with the production of proteins involved in remodeling cartilage matrix [19]. It has been suggested that TNFα exerts a negative effect on osteoblasts by interfering with the *Phex* gene, which encodes for a protein that is important in bone mineralization [20]. Hence, research into the shared mechanisms between bone and T cell homeostasis suggests novel mechanisms that may be exploited for immunotherapy of intestinal inflammation and osteoporosis simultaneously.

Multiple studies have confirmed the relationship between aging and osteoporosis in IBD [21–24]. Sex hormone deficiency could therefore contribute to the osteoporosis seen in IBD as the highest fracture rate is seen among those over age 60 years [21]. Elderly patients are more likely to be sex hormone deficient and osteoporosis amongst the population of males over age 50 years and

postmenopausal females is well established [25,26]. Much attention has been paid to understanding osteoporosis in hypoestrogenemic females, with relatively little investigation directed toward older males. One study reported reduced testosterone levels in 8% of males with IBD [27]. In a recent study testosterone levels were found to be decreased in 44% of males with IBD [28]. In this same study, low dehydroepiandrostenedione sulfate (DHEAS) levels were found in 51%. After controlling for age, body weight, corticosteroid use and disease activity, DHEAS levels correlated significantly with BMD (lumbar spine, $r = 0.49$, $p < 0.01$; femoral neck, $r = 0.51$, $p < 0.02$). Only 35% of the subjects were current corticosteroid users and 22% were never corticosteroid users. The authors argued that low endogenous DHEAS may be secondary to active disease, which in turn is associated with low BMD [28]. A greater understanding of the interplay between corticosteroids, sex hormones, inflammation and bone homeostasis in IBD is needed.

Vitamin D, well known for its role in bone homeostasis, also has anti-proliferative effects and promotes cell differentiation or apoptosis in different cell lines. Macrophages can synthesize 1,25-$(OH)_2$-vitamin D and the vitamin D receptor (VDR) is expressed in macrophages, peripheral blood monocytes and activated T cells. Ligand-bound VDR can inhibit IL-2 and interferon-γ production from Th1 cells and also inhibits IL-12 production from antigen-presenting cells [29,30]. Vitamin D can also modulate T cell differentiation towards a Th2 phenotype [31]. Hence, beyond its role in bone homeostasis, vitamin D likely has a role in T cell and antigen-presenting cell functions.

Vitamin D intake has been shown to be suboptimal in IBD [32–34]. The administration of vitamin D to the IL-10 knockout mouse model of enterocolitis has a beneficial effect on intestinal inflammation and survival [35]. It has been shown that colitis can be generated by transferring T cells from $CD45RB^{high}$ into immunodeficient mice. Applying this principle, transfer of $CD45RB^{high}$ T cells into immunodeficient mice from VDR knockout mice led to more severe colitis than T cells from wild-type mice. VDR/IL-10 double knockout mice had more severe colitis than IL-10 knockout mice [36]. Therefore, vitamin D may be crucial for subjects with IBD to maintain bone health and vitamin D deficiency may also contribute to an aberrant inflammatory response.

Finally, gene mutations at loci important in regulating bone metabolism might be important in osteoporosis development in IBD. Polymorphisms of the *VDR* gene have been weakly linked with BMD in healthy women [37]. VDR is an important regulator of calcium metabolism and bone cell function. *VDR* polymorphisms affect calcium absorption from the intestine [38]. Since randomized controlled trials in IBD have found that calcium and vitamin D are effective at enhancing bone mass [39,40], it follows that *VDR* might be important in determining bone mass in IBD. However, in a study of 245 subjects with Crohn's disease, there was no association between *VDR* polymorphisms and BMD [41]. IL-6 is involved in bone remodeling and a significant difference in BMD between IL-6 genotypes has been reported [42]. An IL-6 polymorphism at position –174 involves the substitution for a G with a C. Those carrying the IL-6 CC genotype have significantly higher BMD at both the lumbar spine and the hip than the GG genotype. IL-6 polymorphisms have also been associated with a lower BMD in postmenopausal females and in healthy males [43,44]. The CC IL-6 genotype is associated with lower levels of plasma IL-6. Serum IL-6 levels have been shown to be increased in both adults and children with Crohn's disease [45,46] and, in pediatric ulcerative colitis, IL-6 levels correlate with BMD ($r = –0.65$) [47]. Another study assessing the role of cytokine polymorphisms in IBD found that subjects with ileocolonic Crohn's disease were more likely to possess the IL-6 –174 GG genotype than those with non-ileocolonic disease ($p = 0.006$) [48]. An increased number of Crohn's disease patients with colonic disease possessed the IL-6 –174 CC genotype compared with those with non-colonic disease ($p = 0.032$). It is interesting that the IL-6 –174 CC genotype is associated with both isolated colonic disease (typically a less aggressive phenotype than ileal disease) and higher BMD.

BMD in IBD

The most widely used skeletal measurement in IBD is BMD testing with dual-energy X-ray absorptiometry (DXA) testing. The prevalence of low BMD in IBD has been reviewed elsewhere [49]. The relatively high prevalence of osteoporosis defined by BMD (approximately 15%) does not translate into comparably elevated fracture rates [49]. It is likely that earlier studies reporting high rates of low BMD in IBD were biased by a predominance of referral center subjects with more severe IBD, greater steroid exposure and co-morbidities. The fracture rate observed in a large population-based study was approximately one fracture per 100 patient-years [21]. High rates of low BMD have also been reported in children including those who are naïve to corticosteroid use [50]. BMD is a powerful fracture predictor in postmenopausal females and older men [51], but its role in premenopausal females and younger males has not been clearly defined. Even the approach to reporting BMD measurements differs between older and younger individuals. The *T*-score (number of standard deviations above or below young adult mean BMD) are preferred for postmenopausal women and men aged 50 years and older, whereas *Z*-scores (number of standard deviations above or below age-matched mean BMD) are

preferred in females prior to menopause and males under age 50 years [52].

Although some IBD subjects do have low BMD, many IBD patients have normal BMD. In two population-based studies from Canada, persons aged over 50 years with self-reported IBD [53] or adult IBD patients who had the onset of their disease in childhood usually had normal BMD [54]. IBD subjects in a durable remission are a group whose BMD is usually within the normal range and those in remission for more than 3 years have a normal mean *Z*-score that is significantly higher than those with active disease [55]. There was a significant trend for higher *Z*-scores (lumbar spine, $r = 0.43$, $p < 0.0001$; and femoral neck, $r = 0.28$, $p < 0.001$) with increasing duration of remission (active disease, remission for less than 1 year, remission for 1–3 years and remission for more than 3 years).

A longitudinal study has evaluated BMD at baseline and after 2 years in 94 patients with IBD [56]. The subjects had a mean age of 36 years (Crohn's disease) and 38 years (ulcerative colitis). Most of the subjects (59%) had used corticosteroids and 27% had previous fractures, both factors suggesting a high-risk population. On average, there were no changes in BMD over the 2 years of follow-up. Although significant bone loss was evident in 24% at the femoral neck, spine or total body, this was more than balanced by significant bone gain at one of these same sites in 44%. Even corticosteroid use during the follow-up period did not lead to a significant decrease in BMD. Furthermore, patients with the lowest initial BMD at the lumbar spine and femoral neck had the greatest subsequent increase in bone mass. Given the limited change (or even an increase) in BMD, these authors cautioned against prophylactic use of potent antiresorptive agents in the absence of fracture. Overall these recent studies provide further evidence that BMD measurement should be used selectively and not done simply as a matter of course in subjects with IBD [57].

Fracture risk in IBD

Population-based studies show only a modest excess fracture risk in IBD subjects – approximately 20–40% greater than matched controls [21–24]. The discordance between high rates of low BMD and fracture rates suggests that there are many subjects with IBD who have low BMD but do not fracture and also subjects with normal BMD who do fracture. This is consistent with large studies showing that the proportion of fractures attributable to osteoporosis (based on a BMD *T*-score $\leq$–2.5] ranges from <10 to 44% [58] and the majority of osteoporotic fractures actually occur in patients with BMD that is not in the osteoporotic range [59]. This paradox arises from the fact that there is considerable overlap in BMD values for fracture and non-fracture subjects and the frequency distribution is such that there is only a tiny number of BMD values that are in the extremely low range.

Some reports suggest a high prevalence of vertebral fractures in IBD patients. A study examining the effect of budesonide on BMD included 38 subjects (14.2%) with asymptomatic vertebral fractures [60]. The same group previously reported that the average BMD *T*-score of patients with asymptomatic vertebral fractures (height reduction 20% or more) was not correlated with BMD ($p = 0.73$) or lifetime steroid dose ($p = 0.83$) and that 55% of fracture patients had normal BMD [61]. In women, but not in men, the fracture rate correlated with older age ($p = 0.009$). These results need to be interpreted very cautiously, as the use of a 20% vertebral height reduction criterion for vertebral fracture has been criticized as generating too many false positives [62,63]. Furthermore, prevalent vertebral compression deformities are more common in men than women prior to menopause and this likely reflects a non-osteoporotic etiology [64].

The frequent discordance between low BMD and fractures has been previously described not only in postmenopausal females but also in IBD patients [65,66]. In 293 patients with Crohn's disease from a specialty clinic, those with BMD *T*-scores below –1 ($n = 156$) underwent thoracolumbar spine X-rays with combined visual and quantitative vertebral morphometry to identify compression fractures [65]. Of these, 34 (22%) had 63 osteoporotic vertebral fractures (based upon a 20% reduction in vertebral height), most of which (88%) were asymptomatic. Only 38% of the fracture cases actually had spine BMD that was in the osteoporotic range. Hence a finding of low BMD has low predictive value for fractures and even those with a relatively normal BMD can still fracture.

It is clear that there are multiple risk factors for fracture that affect risk independent of BMD [67]. Older age, previous fractures (particularly if related to a low impact injury), family history, smoking, sedentary lifestyle, hypogonadism and risk of falls must also be considered. Prior vertebral fracture is the strongest risk factor for future vertebral fractures [relative risk (RR) 4.4, 95% confidence interval (CI) 3.6–5.4] and risk is high even when BMD is normal [68,69]. Vertebral fractures also predict non-vertebral fractures (including hip fractures with RR 2.3, 95% CI 2.0–2.8). Steroid use is a specific concern in IBD patients and it is worth noting that a recent meta-analysis found that current corticosteroid use approximately doubled the risk of fracture independent of risk determined from age and BMD alone [70]. The optimal approach to managing corticosteroids in IBD should still include attempts to minimize corticosteroid use, use of the lowest effective dose and withdrawal of corticosteroids as soon as possible.

There is an emerging consensus that an expression of fracture risk utilizing absolute 10 year fracture risk is preferable to relative risk categorization based upon

BMD alone. In March 2008 the World Health Organization (WHO) fracture risk assessment tool (FRAX™) was released. The FRAX tool (available online at http://www.shef.ac.uk/FRAX/) was developed from a series of meta-analyses to evaluate fracture risk of patients based on models that integrate the risks associated with multiple clinical risk factors with the option of also including femoral neck BMD [71]. The FRAX algorithms give the 10-year probability of fracture, both for hip fracture and for any major osteoporotic fracture (clinical spine, forearm, hip or shoulder). The performance of FRAX was validated in eleven independent population-based cohorts and confirmed the incremental value of combining clinical risk factors with BMD [72]. FRAX allows for the incorporation of country-specific fracture data in order to calibrate the model to the specific population being assessed. Currently there are 17 country models including four ethnicity-specific models for the USA. Based upon cost-effectiveness analyses for the USA, the National Osteoporosis Foundation has proposed a treatment threshold for postmenopausal women and men over age 50 years of $\geq$3% for hip fracture risk and $\geq$20% for major osteoporotic fracture risk (other indications for treatment are prior hip fracture, prior vertebral fracture, and BMD in the osteoporotic range) [73,74]. Other countries have different treatment algorithms based upon FRAX [75,76].

The implication for gastroenterologists is that the algorithm for assessing and managing osteoporosis risks in IBD patients will not be as simple as getting a BMD test and using these results as the sole basis for intervention. In fact, assessing clinical risk factors may direct gastroenterologists away from even ordering BMD measurements on many patients, some of whom require intervention regardless of BMD and some of whom will have such a low probability of osteoporotic fracture that even a finding of reduced BMD will not reach the intervention threshold [77]. Ultimately, more accurate fracture risk estimation should improve a physician's ability to select patients who need treatment while avoiding unnecessary treatment in low-risk individuals. This position is consistent with the AGA Technical Review on Osteoporosis in Gastrointestinal Diseases [49].

The issue of childhood onset of IBD as an important risk factor for fracture also needs to be addressed. In a population-based study of premenopausal adult females who were diagnosed with IBD prior to age 20 years had normal BMD and no apparent increase in fractures [54]. In a recent study of children with IBD and their siblings, there was no difference in the prevalence of fractures amongst the affected probands and their siblings [78].

Corticosteroid use in Crohn's disease has been associated with fractures in a population-based matched cohort study, but the mean age of the fracture cases was 61 years [79]. This is consistent with younger age as a protective factor. Hence there are legitimate concerns about which corticosteroid users need aggressive bone protective therapy. Few dispute that corticosteroids are implicated in bone loss; however, at issue is whether the initiation of corticosteroid warrants bone specific therapy in young persons with IBD who may be discontinuing the corticosteroids within months and in whom the risk of fracture over the next 10 years is still relatively low.

A fracture risk assessment tool for corticosteroid users has been developed based on the General Practice Research Database [80]. Individual risk factors for osteoporotic fractures were combined in a predictive model for 10 year absolute fracture risk based on 191,752 oral corticosteroid users aged 40 years or older of whom 7412 experienced an osteoporotic fracture. Characteristics that independently contributed to the fracture risk score were corticosteroid dose, age, gender, fall history, fracture history, body mass index, smoking, previous diagnoses, use of medication, recent hospitalization and indication for corticosteroid treatment. Table 46.1 summarizes this scoring system for predicting any osteoporotic fracture in an IBD patient during 5 years of continuous corticosteroid exposure. This same group has shown that intermittent high-dose oral corticosteroids (daily prednisone dose $\geq$15 mg) in IBD patients age 40 years and older was associated with a non-significant excess risk of osteoporotic fracture (RR 1.34, 95% CI 0.75–2.40) if cumulative exposure was low (<1000 mg) [81]. Fracture risk was greater for higher cumulative doses (RR for 1000–5000 mg 1.57, 95% CI 0.86–2.85; for >5000 mg RR 3.20, 95% CI 1.93–5.30). Clinical vertebral fractures showed a stronger association with cumulative predisone dose (RR for <1000 mg 1.45, 95% CI 0.33–6.33; for 1000–5000 mg RR 6.06, 95% CI 2.51–14.55; for >5000 mg RR 11.58, 95% CI 5.08–26.41).

Treating osteoporosis in patients with IBD

Corticosteroids

One basic intervention for preventing bone loss is to reduce or eliminate bone-damaging agents. Although it may be contentious as to the specific role corticosteroids play in initiating or perpetuating osteoporosis (*vis-à-vis* active inflammation) or the degree to which osteoporosis occurs, corticosteroid use does serve as a marker of osteoporosis risk. A population-based study from Canada showed that patients with Crohn's disease (but not ulcerative colitis) who fractured were significantly more likely to be corticosteroid users [79].

Patients with rheumatoid arthritis who stop using corticosteroids can have at least partial reversal of previous bone loss [82]; this has also been confirmed in patients with IBD [83]. Fifteen patients (mean age 35 years) with active Crohn's disease who received a 2 month course of corticosteroids (prednisolone starting at 40 mg per day then

Table 46.1 Risk score of any osteoporotic fracture for 5 years of continuous corticosteroid exposure, age, sex and other risk factors.

(a) Points awarded for each variable:

Factor	Age category (years)		
	50	65	80
Daily dose prednisone equivalent 7.5 mg	8	6	5
Daily dose prednisone equivalent 15 mg	11	9	7
Age	4 per decade		
Male sex	−6		
Body mass index $<20\,kg\,m^{-2}$	3		
Body mass index $\geq 26\,kg\,m^{-2}$	−1		
Smoker	1		
History of fall in the prior 6 months	8		
Fracture history prior to corticosteroid use	6		
Disease/drug risk factor (anemia, dementia or cerebrovascular disease; use in the previous 6 months of anticonvulsants, antiarrythmics, hypnotics/anxiolytics, antidepressants or anti-Parkinsonian drugs)	2 for each factor		
IBD as indication for corticosteroid treatment	1		
Hospitalization for IBD in the last 12 months	4		

(b) Fracture risk for each total score:

Score	Osteoporotic fracture risk (%)	
	Next 5 years	Next 10 years
10	1	2
15	2	3
20	2	4
25	4	7
30	6	12
35	10	19
40	16	28
45	25	40
50	36	56
55	52	75

Example:
50-year-old woman, using prednisone 7.5 mg daily for 5 years = 8
– For each 10 years of age = 5 × 4 = +20
– Smoker = +1
– Fracture history prior to steroids = +6
– Anemia and currently taking antidepressants = +4
– IBD as indication for corticosteroid initiation = +1
Total = 40 gives ~16% osteoporotic fracture risk next 5 years.
Adapted from Van Staa *et al.* [80].

reduced each week by 5 mg per day) were matched for age, sex and site of disease with 19 patients with inactive Crohn's disease who did not require corticosteroids [83]. The study groups were fairly young and did not include any estrogen-deficient or postmenopausal women. Average BMD was near normal at baseline and there was no reduction in the lumbar spine for either group even in the absence of bone-preserving therapy. A transient reduction in femoral neck BMD was seen for the group with active disease after 2 months, with recovery at 8 months; the loss was not statistically significant compared with the inactive disease group (the more reproducible total hip site was not reported). The need for pharmacological preventive therapy in younger women is therefore questionable.

Budesonide

For those patients with IBD who require corticosteroids, it was hoped that budesonide, a locally active corticosteroid with high first-pass metabolism that is indicated for ileal and right colonic Crohn's disease, would help minimize bone loss. In a randomized study of budesonide versus prednisolone in active Crohn's disease, Schoon *et al.* [60] found no difference in serially -measured BMD among patients who were current corticosteroid users and past users. In corticosteroid-naïve patients, there was less BMD loss at 24 months among those receiving budesonide than prednisolone (1.04 versus 3.35%; $p = 0.0084$), although this difference was not statistically significant by a per protocol analysis owing to the small number of patients remaining in the study at 24 months. These data fall short of proving that budesonide affects fracture rates, the only bone-related outcome that really matters, and a much larger study powered to assess fracture outcomes is needed. The study by Schoon *et al.* [60] failed to report whether BMD recovered after corticosteroids were stopped. If short-term corticosteroid-induced bone loss is partially reversible and if patients can withdraw from corticosteroids within 3 months, will it matter which agent is used to treat IBD?

Infliximab

Another approach to limiting bone loss secondary to corticosteroid is to use a non-steroidal agent to treat active disease. It has been reported that infliximab use over 1 year was associated with improved BMD [84], but concluding that infliximab was responsible for the change is confounded by concurrent reductions in corticosteroid dose and inflammation. One group suggested that infliximab can improve serum and/or urinary bone markers of resorption and formation in patients with Crohn's disease [85]. Seventy-one patients underwent infliximab therapy with single infusions for luminal disease and triple infusions (baseline, 2 weeks and 6 weeks) for fistulizing disease. Serum and urinary bone marker concentrations were measured 8 weeks after the cessation of infliximab therapy. A complete clinical response was evident in 57% of patients. There was no correlation between changes in the Crohn's Disease Activity Index (CDAI) and bone marker concentrations, although the analysis did not distinguish between clinical responders and non-responders. A significant change in the levels of at least two markers of bone

formation or two markers of bone resorption was evident in 59% of patients. These improvements were unrelated to demographic or clinical characteristics of patients or to clinical response. It was proposed that there was a specific beneficial effect of the drug, but these data do not provide definitive proof. No BMD or fracture outcomes were provided. This study revealed a heterogeneous response in a heterogeneous group of patients. If infliximab has a beneficial effect on bone metabolism in Crohn's disease, it is still unknown whether this relates to the direct effect of TNFα blockade, an indirect action (via reduction in inflammation) or other infliximab-related mechanisms (such as inducing T cell apoptosis).

Similar results were obtained in a study of 24 patients with active Crohn's disease treated with single-dose infliximab [86]. Serum levels of markers of bone formation increased significantly whereas levels of a serum marker of bone resorption did not change. There was no significant difference in the concentration of bone formation markers between the group who achieved a clinical response (n = 17 based on CDAI reduction of 70 points or greater) and the group who did not (whereas = 7). The sample size was too small to establish whether infliximab improves bone formation independent of disease activity and the study did not assess bone density. Another study also reported an increase in markers of bone formation with no change in markers of bone resorption among Crohn's disease patents receiving infliximab. However, the majority of these patients were also withdrawing from corticosteroids, which makes it difficult to discern a steroid withdrawal effect from an effect of the infliximab [87]. Although uncoupling between bone formation and resorption would be predicted to increase BMD, the magnitude of the change in the concentration of the markers is modest compared with the robust changes seen when potent anabolic agents such as human parathyroid hormone (PTH) are used [88]. Much like BMD measurements, bone marker levels are proxy measures for what really is important, namely fracture rates, and it would take a very large study to prove that infliximab could actually impact on this. A recent study suggested that the combination of infliximab and bisphosphonates had a greater impact on bone mass than bisphosphonates alone; however, infliximab alone had no effect on bone mass [89]. Hence although infliximab might limit the bone loss that might otherwise be seen with corticosteroid use, the expense and potential side effects of this drug limit its role as a bone-sparing alternative. In patients who have suffered major complications of corticosteroids, bone sparing might be an incidental benefit of infliximab.

Bisphosphonates

For those at high risk of osteoporotic fracture, bisphosphonates are often considered for their bone-enhancing effects. There are few randomized trials of a bisphosphonate therapy in patients with IBD and not all of the agents that have been approved for use in postmenopausal women have been evaluated in IBD patients. The various bisphosphonates are distinguished by their potency and route and frequency of administration and may differ in terms of onset/offset of action and side effects. Although gastrointestinal side effects from oral bisphosphonates have not been prominent in the pivotal clinical trials, in clinical practice many patients prove to be intolerant [90]. This may contribute to one of the greatest challenges of using these medications – poor persistence on therapy. Many studies show that about half of patients will stop bisphosphonate therapy by the end of the first year [91]. Adherence and persistence are only slightly better with weekly than with daily dosing regimens and are still suboptimal [92]. It remains to be seen whether even less frequent dosing schedules using intravenous bisphosphonates (ibandronate 3-monthly or zoledronic acid annually) will be more successful. Although serious side effects are rare, patients must be informed about erosive esophagitis (oral route only) and osteonecrosis of the jaw (predominantly but not exclusively from intravenous bisphosphonates).

In a small study 61 patients with IBD were randomized to 12 months of risedronate or placebo. All received a 600 mg calcium supplement. Forty-eight patients completed the trial. Compared with the placebo group, risedronate resulted in a 2.0% (95% CI 0.02–3.97] and 1.9% (95% CI 0.21–3.62) improvement in bone density at the spine and hip, respectively [93].

Siffledeen *et al.* enrolled 154 patients with Crohn's disease (both active and inactive) and reduced BMD (*T*-scores below –1.5) [39]. Patients were randomized to receive open-label intermittent cyclic etidronate (400 mg per day orally for 14 days) or nothing, followed by calcium (500 mg per day) and vitamin D (400 IU per day) for 76 days. This cycle was repeated every 3 months for 2 years. BMD was measured at 6, 12 and 24 months. Both groups significantly increased their BMD at 24 months; however, there was no difference between the groups. Etidronate provided no added benefit over calcium and vitamin D supplementation alone. The study design had some limitations. The study was not blinded and the control group, who knew they were not getting the active treatment, might have enhanced their intake of calcium and vitamin D. Food records were not reviewed on a regular basis and it is not known whether the study population was ingesting suboptimal calcium and vitamin D at baseline (although it can be predicted that they were [94–96]) and whether calcium and vitamin D intake remained stable over time. Enrollment was predominately patients with BMD in the low bone mass range (*T*-scores between –1.5 and –2.5) rather than osteoporotic (*T*-scores at or below –2.5). The majority of the patients were using corticosteroids and their BMD not only did not worsen but actually improved significantly. Even those patients with BMD *T*-scores in

the osteoporotic range had a substantial increase in their BMD at 24 months. Four fractures occurred, two in each group (J.S. Siffledeen and R.N. Fedorak, personal communication), but in a 2 year study of 200 patients only four fractures might be expected [21]. Studies of this nature must be much larger and longer to accrue more fractures, otherwise surrogate endpoints must be used.

Another important message of this paper is that young patients with IBD generally do well regarding their BMD. In this study 34% of subjects were <35 years old and postmenopausal women were excluded. Our group has previously shown that the average BMD in young females diagnosed with IBD before age 20 years was normal when assessed before menopause [54].

Bartram *et al.* [40] studied 74 patients with Crohn's disease and BMD *T*-scores ≤–1.5 at the lumbar spine and/or hip, most of whom were current or past long-term corticosteroid users. All patients received calcium (500 mg per day) plus vitamin D (400 IU per day) and half were then randomized to receive four open-label infusions of intravenous pamidronate (30 mg every 3 months). The mean ages were 45 years (pamidronate group) and 44 years (controls) and 17 were postmenopausal females not receiving estrogen replacement therapy. Once again, both groups increased their spine and hip BMD at 1 year and there was no statistically significant difference on comparing the changes in BMD between the two groups.

The studies by Siffledeen *et al.* [39] and Bartram *et al.* [40] provide reassurance that bisphosphonates are rarely needed in patients with IBD, most of whom are relatively young and have BMD that is not severely reduced. These investigators, like most others, have used BMD as the primary endpoint. While enhancing BMD, or at least limiting its diminution over time, is clearly desirable, BMD is not the entire story when it comes to fracture risk. *T*-scores of –1.5 to –2.5 overlap with the BMD of normal healthy people and the need to treat a young patient in this range with anything more than calcium and vitamin D might be unnecessary. The AGA technical review suggested that clinicians might consider bisphosphonate use in patients with *T*-scores of –1.0 to –2.5 who were chronic users of corticosteroids. There has been a recent change in BMD reporting advocated by the International Society for Clinical Densitometry such that for males under age 50 years and premenopausal females the *Z*-score (and not a *T*-score) is preferred [52]. In these individuals, a *Z*-score of –2.0 or lower is considered abnormal. The WHO densitometric classification based upon *T*-scores (normal if –1 or higher, osteopenic if between –1 and –2.5 and osteoporotic if –2.5 or lower) should not be applied to younger individuals since their fracture risk is usually low even when BMD is reduced.

Another study assessed the impact of risedronate 35 mg per week versus placebo in 90 osteoporotic postmenopausal women with IBD in remission and not using corticosteroids [97]. The groups were well matched at baseline (lumbar spine *T*-score –3.4 ± 0.5 in the treatment group and –3.6 ± 0.6 in the control group) and about half had prevalent vertebral fractures. Calcium intake was maintained at a minimum of 1500 mg per day either through dietary intake or when necessary supplementation. Vitamin D supplements were given to those with 25-OH-vitamin D levels less than 40 nmol l^{-1}. Vertebral fractures were assessed from spinal radiographs taken at baseline at the end of the study (12 months). BMD at all sites was significantly improved in the risedronate group versus the placebo group whereas a significant decrease was observed in the placebo group. Over the 12 month study the incidence of new vertebral fractures was significantly lower in the risedronate group than in the placebo group (12.5 vs 34.1%, $p < 0.05$). The relative risk for developing a new vertebral fracture after 1 year of risedronate administration was 0.36 (95% CI 0.14–0.85).

Calcium and vitamin D

There is evidence in the healthy elderly that calcium and vitamin D supplements together reduce the rate of both vertebral and nonvertebral fractures [98–100]. Vitamin D_3 (cholecalciferol) is more than three times as potent as vitamin D_2 (ergocalciferol) at increasing serum 25-OH-vitamin D levels [101]. Furthermore, in the very elderly, a meta-analysis has shown that vitamin D supplements are effective at preventing falls [102], presumably by acting through skeletal muscle receptors to increase muscle strength [103].

The optimal level of serum 25-OH-vitamin D remains a source of debate, in part reflecting poor standardization in the currently available clinical assays [104,105]. In rickets and osteomalacia, the serum 25-OH-vitamin D levels are usually below 25 nmol l^{-1}, whereas vitamin D "insufficiency" has been defined as a milder reduction in serum 25-OH-vitamin D. The definition of the lower limit of the optimal (normal) range is a source of debate. Many investigators define the beginning of the normal range as the serum 25-OH-vitamin D level associated with a serum PTH which is not further suppressed by increasing vitamin D intake. This translates to an optimal lower limit of 25-OH-vitamin D of 75–80 nmol l^{-1} [106,107].

The data from the studies of Siffledeen *et al.* [39] and Bartram *et al.* [40] suggest that calcium and vitamin D are sufficient to preserve or enhance BMD in patients with IBD. In a small pilot study of corticosteroid users (n = 17), calcium (1000 mg per day) and vitamin D (250 IU per day) were no better than placebo, although overall BMD did not decrease over 1 year in either group [94]. Unfortunately, the Siffledeen study did not include a placebo arm, so it is not clear whether calcium and vitamin D alone led to increased BMD or if there was a spontaneous improvement (perhaps related to reduced inflammatory mediators). Nonetheless, dietary supplements are simple,

inexpensive and seem to be a reasonable treatment choice for patients with reduced BMD (*T*-score –1.5 or lower), those using corticosteroids and after age 50 years.

Recent studies support vitamin D supplementation in patients with IBD. In a group of 42 patients with inactive Crohn's disease, extensive small bowel resection and evidence of steatorrhea (mean fat malabsorption of 47 g per day), 38% had levels of 25-OH-vitamin D less than 8 ng ml^{-1} (20 nmol l^{-1}), indicating severe deficiency [108]. Low levels of 25-OH-vitamin D levels correlated with elevated serum PTH, elevated urinary markers of bone resorption and lower BMD. Bone biopsies were not performed and it is unclear whether the low BMD was secondary to PTH-induced bone resorption or osteomalacia. Whether vitamin D deficiency has an adverse effect on bone because of osteomalacia or because of secondary hyperparathyroidism, it is appropriate to supplement patients with adequate vitamin D. Supplementation can be based on vitamin D intake but is more objectively determined by measuring serum 25-OH-vitamin D levels. It was recently shown that 25-OH-vitamin D levels are abnormal in up to 52% of Canadian patients with Crohn's disease and ulcerative colitis and that in nearly half of these patients their dietary intake of vitamin D was adequate [34]. A group from southern California failed to confirm a high prevalence of low 25-OH-vitamin D levels in patients with these disorders, perhaps reflecting the importance of latitude and sunlight exposure in the production of vitamin D [109]. Given the high prevalence of suboptimal vitamin D status combined with the safety and low cost of supplements, empiric vitamin D supplementation could be considered for IBD patients, particularly for those living in northern climates, older than age 50 years or on oral corticosteroids. Although not required in all cases, measuring 25-OH-vitamin D levels would be appropriate in all subjects, but particularly following small-bowel resection or when there is a documented history of fractures or low BMD. Another approach is to supplement all subjects with IBD, with selective measurement of 25-OH-vitamin D.

In addition to the traditional functions of vitamin D – calcium economy and musculoskeletal health – there are emerging data on non-traditional actions of vitamin D. The latter have been linked to malignancies, autoimmune diseases, diabetes and cardiovascular disease. Receptors for calcitriol and the enzyme involved in its synthesis (1-hydroxylase) are expressed by many tissues other than those concerned with calcium regulation. The vitamin D receptor has been identified in brain, prostate, breast, gonads, colon, pancreas, heart, monocytes and lymphocytes. A recent systematic review of the PubMed database yielded 63 observational studies of vitamin D status in relation to cancer risk [110]. The majority of these studies found a protective relationship between sufficient vitamin D status and lower risk of cancer supporting a possible role for vitamin D in their pathogenesis. Of particular relevance to IBD patients, a systematic review and meta-analysis was conducted for colorectal cancer by Gorham *et al.* [111]. Eighteen studies were included in the analysis, four based upon serum levels of 25-OH-vitamin D and 14 based upon oral intake. A majority (10 of 18) studies found that inadequate vitamin D status was significantly associated with higher risk of cancer of the colon and/or rectum. Individuals with 1000 IU per day or more of oral vitamin D ($p < 0.0001$) or serum 25-OH-vitamin D concentrations $\geq$82 nmol l^{-1} ($p < 0.01$) had a 50% lower incidence of colorectal cancer compared with reference values. A 4 year randomized controlled trial conducted in 1179 community-dwelling postmenopausal women showed that daily supplementation with calcium 1400–1500 mg and vitamin D_3 1100 IU substantially reduced all-cancer risk [112].

Is it possible to have too much of a good thing? Abreu *et al.* [109] retrospectively assessed calcium metabolism in patients with Crohn's disease ($n = 138$) and ulcerative colitis ($n = 29$). Mean serum 25-OH-vitamin D and PTH levels were normal, but there was a significant increase in levels of serum 1,25-$(OH)_2$-vitamin D, the active form of vitamin D, in 42% of the patients with Crohn's disease. There was greater expression of 1α-hydroxylase in the lamina propria of colonic biopsies from patients with active Crohn's disease than in healthy controls or patients with inactive Crohn's disease. Activated mononuclear cells express 1α-hydroxylase activity and can carry out extra-renal conversion of 25-OH-vitamin D to 1,25-$(OH)_2$-vitamin D. This study found a positive correlation between disease activity (modified Harvey–Bradshaw index) and serum 1,25-$(OH)_2$-vitamin D ($r = 0.266$, $p = 0.024$). Higher 1,25-$(OH)_2$-vitamin D levels were correlated with lower BMD ($r = -0.301$, $p = 0.005$). Although 1,25-$(OH)_2$-vitamin D can enhance *in vitro* bone resorption, it is more likely that the association is confounded by the role of local and systemic inflammation in elevating 1,25-$(OH)_2$-vitamin D and reducing BMD, respectively. It is premature to discount the large volume of clinical and scientific data on the salutary benefits of vitamin D sufficiency, but this provocative finding merits further study of the role of this vitamin D metabolite in Crohn's disease and prospective assessment of its response to inflammation and possible role in bone loss.

Conclusion

It is encouraging that more studies are being performed to define the unique features of bone disease in IBD patients. These are urgently needed to help determine the natural history of bone disease in these patients, which treatments are effective and perhaps if treatment is needed at all. Just as children are not little adults, patients with

IBD and low BMD cannot be approached in the same way as postmenopausal women. Factors related to the inflammatory state, medications and nutrition are obvious contributors to a unique pathophysiologic milieu that will probably require its own specific clinical approach. Until such time as adequate data are available on how to assess reliably fracture risk and the need for intervention in IBD patients, guidelines will necessarily be based upon expert opinion. BMD testing can be recommended for those with significant risk for osteoporosis such as those with long-standing active disease, corticosteroid use or a collection of risk factors including older age, hypogonadism, smoking and a sedentary lifestyle. Calcium and vitamin D supplementation can be recommended for all and in particular those using corticosteroids. Advances in understanding the biology of the RANK–RANKL–OPG receptor–ligand pathway have highlighted a role for OPG as a counter-regulatory protein in osteopenia and the enterocolitis of IBD. Agents that target this system need to be explored as a therapeutic option in IBD.

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Chapter 47
Comprehensive Approach to Patient Risk: Risks Versus Benefits of Immunomodulators and Biologic Therapy for Inflammatory Bowel Disease

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Summary
- Immunomodulator and biologic therapy for IBD carry rare, but real, risks of serious and life-threatening adverse events.
- Due to the nature of reporting of adverse events, there is uncertainty as to the exact amount of risk.
- The opposing pulls of early aggressive therapy and long-term safety issues is complicating the treatment approach to IBD.
- It is important to educate patients properly regarding risks and benefits of IBD therapy, so that they can make informed choices in their care.
- We need to be able to select properly which patients most deserve aggressive treatment and which patients are at most risk for serious side effects.

Introduction

The treatment of inflammatory bowel disease (IBD) is evolving. New medications are rapidly being developed and tested and more aggressive treatment algorithms are being proposed. The recognition that we have failed to alter disease natural history despite our best efforts [1] with standard treatments has led to a paradigm shift towards more potent medications earlier in the disease course (e.g., "early-aggressive" or "top-down" therapy). Further studies will be needed to determine how these new treatments and more aggressive strategies impact disease, but critical questions regarding safety need to be answered. Even if we can effectively alter natural history, we need to be certain that the risks of this approach will be acceptable to both patients and physicians.

Although some safety issues have been raised with 5-aminosalicylates, long-term antibiotic use and certainly corticosteroids, most concerns (patient and physician) revolve around immunomodulators and biologic therapy. Commonly used immunomodulators for IBD include azathioprine (AZA), 6-mercaptopurine (6MP) and methotrexate. Currently available biologics include infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia) and natalizumab (Tysabri).

Where do safety data come from?

Historically, safety data have predominantly come from clinical trials. Although we have controlled studies of AZA/6MP in IBD since the early 1970s [2] and biologics from the late 1990s [3], any clinical trial safety data are of limited value due to the rarity of serious side effects and the denominator of included patients. Therefore, most published data on the safety of pharmaceuticals comes from post-marketing surveillance. There are a number of methods to gather post-marketing information. The US Food and Drug Administration (FDA) receives adverse drug reaction reports from manufacturers, healthcare professionals and consumers and is an important source of safety information. The Adverse Event Reporting System (AERS) is a computerized database designed to support the FDA's post-marketing safety surveillance program for all approved drugs. Healthcare professionals and consumers submit reports voluntarily through the FDA's MedWatch program and these data are then incorporated into the AERS. The MedWatch program has been in place since 1996 and is publicly accessible. By the nature of data reporting to MedWatch and open accessibility to both patients and healthcare providers, the quality of the

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data is unconfirmed. Furthermore, the comprehensiveness of MedWatch is suspect as spontaneous post-marketing reporting is notoriously poor. It is estimated that only 1% of serious adverse reactions are reported at all [4]. Single medical center experiences have some value [5,6], but due to limited patient numbers, short follow-up time and problems with referral bias, results are unreliable. The statistical tools of meta-analysis, systematic analysis and decision analysis try to compensate for small patient numbers and estimates risks and benefits. These have been used in IBD to study risks and benefits of immunomodulators [7,8] and biologics [9], but results are meant to apply across large populations as opposed to individual patients. National databases and population-based analyses [10,11] have the advantage of large numbers of patients from diverse care settings, but review has been retrospective in nature. Prospective registries are promising tools to document adverse events as they occur. The TREAT registry has been in place for infliximab and now includes over 15,000 patient-years of follow-up [12]. The major limitation of this and other registries is the uncertainty of how patients are selected for inclusion, causing concern that significant events could be missed. Clearly, there is uncertainty about the existing data, but they are the best we have and we need to be able to communicate the information (and its limitations) to both physicians and patients.

Moving forward

Even if we are able to calculate accurate assessments of risk, a critical step will be to understand whether patients and physicians will accept the tradeoffs of risk for expected therapeutic benefit. Some work has been completed in this field and results show that patients are willing to accept risks higher than we have been seeing in published reports [13]. However, it is dependent on individual patient characteristics, most notably how sick they are and how much benefit they can expect to receive from treatment [13–15]. At the same time as we are beginning to believe that early aggressive therapy may be the right answer for many patients, concerns of hepatosplenic T cell lymphoma (HTSCL) and other severe adverse events have tempered enthusiasm and led some to discontinue combination immunomodulator and biologic therapy. The opposing pulls of altering natural history of disease and long-term safety issues are complicating our treatment algorithms. This chapter defines what we know about risk of IBD medications, puts this risk in perspective and introduces the topic of how to best communicate this complicated matter to our colleagues and patients.

Risk assessment

Risks of the disease

When discussing risks of therapy with patients, we need to make clear that the disease itself carries risk (Table 47.1).

Table 47.1 Risks associated with Crohn's disease*.

Event	Frequency
Require surgery	18% (18/100) [16]
Death from surgery	0.08% (8/10,000) [7,9]
Death from flare	0.15% (15/10,000) [7,9]
Lymphoma	0.02% (2/10,000)†
Death from lymphoma	0.006% (6/100,000) [7,9]

*Without immune suppressive therapy.
†Same annual risk as the annual rate in the general population [11,52].

Approximately 18% of patients with Crohn's disease require surgery within the first year of diagnosis and up to 80% after a 20 year period [16]. Nearly 30% of patients with ulcerative colitis undergo colectomy over the course of their lives [17]. There is probably an increased risk of colon cancer associated with long-standing ulcerative colitis (and Crohn's colitis [18]) that had been estimated as high as 35–40% after having disease for 35 years [19]; however, more recent analyses suggest a much lower risk (either no increased risk [20] or an 10.8% risk after 40 years [21]). Operative mortality is low, but measurable (approximately 8/10,000), as is the rate of dying from a flare of Crohn's disease (15/10,000) [7,9]. The financial burden of IBD on the healthcare system is huge [22,23] and patients with moderate to severe Crohn's disease have nearly a 40% unemployment rate, with 25% of these patients receiving disability benefits [24].

The idea of early aggressive therapy is to prevent disease complications before they occur. Waiting to initiate immunomodulator or biologic therapy until after a complication of disease has developed is often too late. There appears to be a window of opportunity to treat patients and although it may be more acceptable to patients and physicians to wait to use "strong" treatments until they are convinced that it is needed, this tactic is likely grossly to under-treat the majority of patients.

IBD has a major impact on the quality of patients' lives. Utility scores are one of many methods to estimate a patient's quality of life. Utility scores range from 0, which is equivalent to death, to 1.0, which represents perfect health. A utility score for severely active Crohn's disease was calculated to be 0.74, which in comparison is a worse quality of life than class III/IV (severe) angina [25]. A utility score for active ulcerative colitis of 0.32 is significantly lower than that of a patient with renal failure who requires routine hemodialysis (0.43) [15,25]. However, a patient with Crohn's disease in remission had a utility score of 0.96. Therefore, if effective treatment is initiated at the appropriate time, although there will be some risk of medication side effects, there is an opportunity to impact patients' lives greatly. The question for individual patients

will be how much benefit will they gain and at what cost?

Benefits and risks of immunomodulators

Immunomodulators are the mainstay of IBD therapy. 6MP and AZA have been studied for the treatment of IBD since the early 1970s [2,26] and long-term safety data were reported as early at 1979 [27]. These medications can be very effective for Crohn's disease with a reported response rate of up to 67% with an ability to decrease or discontinue corticosteroids in 75% of patients [28]. A classic study by Candy *et al.* showed a long-term remission rate of 42% (versus 7% placebo) [29]. A Cochrane analysis of the efficacy of 6MP and AZA showed an odds ratio (OR) of 2.36 [95% confidence interval (CI) 1.67–3.53] for a clinical response [30] and OR 3.86 (95% CI 2.14–6.96) for a steroid-sparing effect. This corresponds to a number needed to treat (NNT) of five for a clinical response and three to observe a steroid-sparing effect in one patient. A second Cochrane review looked at the long-term maintenance of remission using 6MP or AZA for Crohn's disease. This analysis showed an OR of 2.16 (95% CI 1.35–3.47) for maintenance of remission and OR 5.22 (95% CI 1.06–25.68) for long-term steroid sparing [31]. This corresponds to an NNT of seven to maintain remission and three for a steroid-sparing effect. Extrapolations from Crohn's data and more recent clinical trial data [32] have led to widespread use in ulcerative colitis also. Continued long-term use appears to be necessary to maintain clinical remission for both Crohn's disease [33,34] and ulcerative colitis [35].

Methotrexate has been shown to have similar efficacy in Crohn's disease both for treating active disease [36] and for maintaining remission [37]. Although methotrexate has been studied for ulcerative colitis and has not proven to be effective [38,39], the trials were small and dosing may have been suboptimal.

Immunomodulators are potent and effective medications; however, they carry the risk of rare but potentially serious toxicity (Table 47.2). 6MP/AZA have risks of both direct and indirect toxicity. Direct toxicity was reported in a series of 396 patients and refers to events such as pancreatitis (3.3%), bone marrow suppression (2%), allergic reactions (2%) and drug-induced hepatitis (0.3%) [40]. Similar frequencies were seen in a Cochrane analysis and the number needed to harm (NNH) was 14 (for one patient to have an adverse event leading to withdrawal from the study) [31].

Indirect toxicity refers to processes that result as sequelae from direct toxicity. Infection is the most common, often associated with leucopenia. Leucopenia occurs somewhere between 1 and 13% [41,42] of the time and significant infections up to 7% of the time [40]. Rare deaths have been reported attributable to leucopenia and sepsis in as high as 3/1000 patients in a large single-center experience [43] and also in a meta-analysis [8]. In addition to bacterial infections, viral infections have been reported more frequently in patients taking 6MP/AZA. The herpes viruses, specifically Epstein–Barr virus (EBV), cytomegalovirus (CMV), varicella zoster virus (VZV) and herpes simplex virus (HSV), usually cause self-limited disease, but have been associated with rare, life-threatening complications [44–47]. Recently, a higher incidence of cervical dysplasia has been reported in IBD patients taking immunomodulators [48,49], likely due to infection with human papilloma virus (HPV).

Table 47.2 Serious side effects of 6-mercaptopurine and azathioprine.

Event	Frequency (annual)
Allergic reactions	3% (3/100) [61]
Pancreatitis	3% (3/100) [61]
Severe infection	5% (5/100)*
Death (sepsis)	0.15% (15/10,000) [9]
Non-Hodgkins's lymphoma	0.04% (4/10,000)†
Death from lymphoma	0.01% (2/10,000) [7,9]

*Weighted average from Siegel and Sands [61].
†Calculation of the rate of NHL in Crohn's disease patients [50].

The risk of lymphoma has been a controversial topic. Much of this controversy lies in the baseline risk of lymphoma in IBD patients. Although some believe that IBD itself carries an increased risk of lymphoma, an analysis of a large United Kingdom database did not show a difference when compared with the general population [11]. Based on population-based data [7] and a meta-analysis [7], the risk of lymphoma [predominantly non-Hodgkin's lymphoma (NHL)] attributable to 6MP/AZA appears to be 3–4-fold higher than in the general population. Part of this risk may be related to the role of EBV-mediated lymphoma [51]. In absolute terms, the 1 year risk of lymphoma in the general population is approximately 2/10,000 [52]; therefore, the 1 year risk for IBD patients of developing lymphoma (Hodgkin's and non-Hodgkin's) while on 6MP/AZA is about 6–8/10,000. This calculates to an NNH (number of patients treated with 6MP/AZA to cause one additional lymphoma) of approximately 2000. For patients with Crohn's disease (excluding ulcerative colitis) and specifically looking at NHL, the rate appears to be 4/10,000 annually. An aggressive, usually fatal, form of lymphoma, HSTCL, has recently been of concern in patients treated concomitantly with immunomodulators with infliximab. Although the majority of IBD patients who have developed HSTCL have been on combination therapy, cases have also been reported in young patients on azathioprine monotherapy [53–56].

There has been some concern that 6MP/AZA can also contribute to other malignancies. However, in one study

with nearly 7000 patient-years of follow-up, this was not the case [57] (other than a slight increase in cervical cancer, likely related HPV infection). An increased risk of non-melanoma skin cancer is well recognized in the immunosuppressed transplant population and has also been reported in IBD [58].

Methotrexate is overall a safe treatment for IBD. Although this medication is associated with hepatotoxicity and lung disease (hypersensitivity pneumonitis), this is far more common in patients with psoriasis and rheumatoid arthritis, respectively. In patients with psoriasis, nearly one-quarter of patients treated with methotrexate had evidence of either active hepatitis or cirrhosis on follow-up liver biopsies [59]. A collaborative study by the University of Chicago and Mount Sinai Hospital in New York performed liver biopsies on 20 IBD patients who had received a mean cumulative dose of 2.6 g of methotrexate over about 2 years [60]. The majority of patients had either normal biopsies or only mild steatosis/inflammation and one patient (who was obese and had diabetes) had moderate to severe fibrosis. Routine baseline liver biopsies for IBD patients are not recommended (unless patients have risk factors for underlying liver disease) and surveillance biopsy is only recommended for persistent elevations in transaminases or dropping albumin (in the setting of well-controlled IBD) [61].

Hypersensitivity pneumonitis is reported to occur in approximately 1% of patients treated with methotrexate (with rheumatoid lung disease as a major risk factor) [62], but reports of this occurring in IBD patients are very limited [63,64]. Pretreatment chest radiographs or pulmonary function tests are not routinely ordered, but if pulmonary symptoms develop during treatment, further evaluation is important. Nausea and fatigue are common complaints of patients taking methotrexate. These can usually be minimized by changing the time of dosing (before bedtime), increasing folic acid intake from 1 to 2 mg daily or using anti-emetics around the time of the dose. Leucopenia appears to be uncommon [36,37], but has been reported and can be life-threatening [65]. Methotrexate is both teratogenic and a known abortifacient, so women need to use adequate birth control. In addition, methotrexate may be toxic to sperm, so men should stop taking it for at least 3 months before trying to conceive [66].

Lymphoma related to methotrexate use is well reported in the rheumatoid arthritis literature [67–70], but only rarely in IBD patients [71]. To date, no cases of HSTCL associated with methotrexate have been published.

Risks associated with biologic therapy

Despite the widespread use of immunomodulators over the past three decades, it does not appear that we have been able to alter significantly the natural history of Crohn's disease. In 1975, the cumulative rate of surgery for patients with Crohn's disease over a 5 year period was 35%, and it remained stable up to 2002 even when controlling for the increased use of immunomodulators [1]. Anti-tumor necrosis factor (TNF) therapy was not included in that analysis, but there is some suggestion in *post hoc* analyses that infliximab may decrease the need for hospitalizations and surgery [72].

Although anti-TNF drugs have revolutionized the treatment of patients with IBD, they come with potential costs. These potent drugs have a long list of side effects, but most of the concern has focused on the black-box warnings regarding severe infections and lymphoma [73]. The frequencies of these adverse reactions are of significant debate. As there is no mechanism to capture all users of anti-TNF drugs and all adverse events, we have to turn to our best alternatives, which include large single-center experiences, voluntary prospective registries and systematic analyses. Most of the data on anti-TNF treatment presented below are with regard to infliximab. This is primarily because infliximab has been on the market since 1998, whereas adalimumab was approved for Crohn's disease in 2007 and certolizumab pegol in 2008. Although it is impossible to compare safety between these agents, most likely the adverse events presented here are a class effect and these three medications have a very similar safety profile.

In the largest single-center experience, the Mayo clinic reported on 500 patients with Crohn's disease who were treated with infliximab [5]. In this series, one patient developed NHL and four patients died from sepsis-related complications. This translates to a 0.2% (2/1000) rate of lymphoma and a 0.8% (8/1000) rate of sepsis-related death. Age and co-morbidity are likely important risk factors as three of the patients who died of sepsis were in their 70s, one of whom had diabetes and another had diabetes and cirrhosis. In contrast to the Mayo clinic report, the TREAT registry [12], which at the time of a recent update included over 3000 IBD patients treated with infliximab, found a non-significantly increased risk of lymphoma [relative risk (RR) 1.3, 95% CI 0.36–5.03] and an increased risk of significant infections (OR 1.9, 95% CI 1.34–2.70). In multivariate analysis, the infection risk and associated mortality were dependent only on the concurrent use of corticosteroids or narcotics. Therefore, conclusions from TREAT were that infliximab does not directly contribute to lymphoma or death related to sepsis.

A systematic analysis of the literature examined significant adverse effects in 1711 patients treated with infliximab over the period of 1 year [9]. In the six studies included in this analysis, five patients developed non-Hodgkin's lymphoma and nine patients died from severe infections thought attributable to infliximab. Annualized, this calculates to a 0.4% (4/1000) risk of dying from sepsis (Table 47.3). As seen in the Mayo Clinic experience, age is probably an important risk factor as the nine

Table 47.3 Serious side effects of anti-TNF agents.

Event*	Frequency (annual)
Lymphoma (NHL)†	0.06% (6/10,000) [74]
Death from lymphoma	0.02% (2/10,000) [9]
HSTCL	Unknown
Stop treatment due to AE	9.6% (1/10) [9]
Death from sepsis‡	0.4% (4/1000) [9]
Tuberculosis	0.05% (5/10,000) [79]

†Combination therapy with anti-TNF and immunomodulators.
‡Typically older patients with co-morbidities, likely lower in younger patients.
*NHL, non-Hodgkin's lymphoma; HSTCL, hepatosplenic T cell lymphoma; AE, adverse event.

patients who died of sepsis had an average age of 73 years. Four of the nine were taking corticosteroids and none were taking concomitant immunomodulators (C.A. Siegel, unpublished data).

The results from this analysis, in addition to data from previously published literature, were applied to a decision analysis evaluating the risks and benefits of infliximab [9]. The decision analysis modeled patients with moderately active Crohn's disease. In the model, a patient could either be treated with standard therapy (including immunomodulators and prednisone) or add infliximab to their standard treatment regimen. A total of 100,000 hypothetical patients were included in each group. Although the model allows for multiple outcomes, the bottom-line results report how many patients respond to treatment versus the number of patients who developed lymphoma or died as a result of sepsis. The results show that at the end of one year, 12,216 more patients in the infliximab group went into remission and 4251 fewer patients in the infliximab group required surgery. However, this was at the expense of 142 more patients in the infliximab group who developed lymphoma and 271 more deaths in patients who were treated with infliximab (most of which were sepsis-related deaths). Despite the concerning increase in lymphomas and deaths in the infliximab group, due to its significant improvement in clinical status in the large number of patients, at the end of one year, patients in the infliximab group overall had a better quality of life. How patients might accept this risk–benefit tradeoff is complex and is discussed further below in the section Patient perceptions of risk.

A more recent study directly explored the question of NHL in patients with Crohn's disease treated with anti-TNF agents [74]. This meta-analysis included 26 publications, consisting of 9 randomized controlled trials (RCTs), 3 cohort studies and 14 case series of consecutive patients. On average, 66% of subjects were concomitantly taking immunomodulators. There were 13 NHL identified within the 8905 patients treated with anti-TNF drugs with 21,178 patient-years of follow-up. At least 10 of these 13 patients were also exposed to immunomodulators. The calculated absolute rate of NHL was 6.1 per 10,000 patient-years (Table 47.3). This rate was then compared with the expected rate in the SEER database. Overall, SEER has a rate of NHL of 1.9 per 10,000 patient-years. Therefore, the standardized incidence ratio (SIR) of anti-TNF-treated patients compared with SEER was 3.23 (95% CI 1.5–6.9). The pooled estimate was also compared with Kandiel *et al.*'s summary estimate from a meta-analysis of IBD patients treated with immunomodulators without anti-TNF exposure [50]. The rate from the Kandiel study was 3.6 per 10,000 patient-years by using only Crohn's patients and only NHL (they combined Crohn's and ulcerative colitis and NHL and Hodgkin's). Although the rate with anti-TNFs was higher than this rate on immunomodulators, the SIR of 1.7 was not statistically significant (95% CI 0.5–7.1). Since NHL appears to be age and gender sensitive, further analyses were performed to develop age- and gender-specific rates of NHL. The rate of NHL in anti-TNF exposure patients increased with increasing age; however, since it also increases in SEER, the only statistically significant subgroup were men aged 20–54 years (SIR 5.4, 95% CI 1.3–18.1). The conclusion of this meta-analysis was that the rate of NHL is increased in Crohn's disease patients treated with anti-TNF agents in combination with immunomodulators, but this absolute rate is still very low.

Since most Crohn's disease patients treated with anti-TNF agents have also been exposed to immunomodulators, there are few anti-TNF-only exposed patients available to study. Therefore, it is currently impossible to know if anti-TNF agents on their own are associated with an increased rate of NHL or HSTCL. A best guess would be that these lymphomas are related to immune suppression (not necessarily a particular agent) and that individually the agents may be associated with higher rates of lymphoma, which is slightly more frequent when using the drugs in combination.

The concern over HSTCL only came in 2006 after addition of a new black box warning for infliximab. HSTCL was first described only in 1990 and is a result of a clonal expansion of $\gamma\delta$ or $\alpha\beta$ T cells [75]. Patients tend to be young, but it has been reported in those aged 12–58 years. Most patients developing this type of lymphoma have been chronically immune suppressed (many reports are in the transplant literature). The clinical presentation includes hepatosplenomegaly, cytopenias, "B" symptoms and, characteristically, no lymphadenopathy. Diagnosis is made based on biopsy of the liver, spleen or bone marrow. Treatment involves multi-agent chemotherapy (CHOP-like). Unfortunately, this disease is almost universally fatal with median survival of just 16 months [76].

As of August 2009, 18 cases of HSTCL have been reported in IBD patients who have received infliximab (Data

on file, Centocor Inc.). All of these patients had received concomitant immunomodulators (6MP or AZA). As noted above, there have also been reported cases in the literature of Crohn's disease patients who developed HSTCL after receiving immunomodulators but no anti-TNF therapy [53–56]. Of the 18 reported cases, ages ranged from 12 to 58 years and they received from 1 to 24 doses of infliximab. Of the 18 patients, 16 had Crohn's disease (one had ulcerative colitis and another indeterminate colitis, thought most likely to be ulcerative colitis). Although initial concerns were related only to pediatric patients, there have been five patients older than 30 years of age, with an average age of 26 years. Interestingly, 17 of the 18 patients were male, also the pattern in the previously reported patients on AZA alone. A critical question is how much anti-TNF therapy alone plays a role versus the combination with immunomodulators? As most patients treated with anti-TNF therapy have been on concomitant immunomodulators at some point, there may not be enough anti-TNF monotherapy patients treated to draw conclusions. Clearly, there is cause for concern and further evaluation is required.

Malignancies other than lymphoma have been a concern and have been most carefully evaluated in the rheumatology literature. Most recently, a meta-analysis showed a dose-dependent increased risk of malignancies in rheumatoid arthritis patients treated with anti-TNF therapy [77]. They found an OR for malignancy of 3.3 (95% CI 1.3–3.1) and an NNH of 154 (for one additional malignancy within 1 year of anti-TNF treatment). A wide-range of severity of malignancy were reported, with many less serious (10 non-melanoma skin cancers) and others of much greater concern (10 lymphomas, 7 GI malignancies, 2 breast cancers, 2 lung cancers, 1 seminoma, 1 melanoma, 1 endometrial cancer and 1 leukemia). Patients were treated with either infliximab or adalimumab. How these results translate to IBD patients is unclear, but the rate of lymphoma [10 cases of lymphoma out of 3493 patients (0.29%)] is similar to that seen in the Mayo Clinic series [5].

In addition to sepsis and neoplasm, a number of other infectious and non-infectious processes have been reported with infliximab and adalimumab. Some data are available for IBD patients treated with anti-TNF medications, but significantly more information is available in the rheumatology and dermatology literature. Tuberculosis has been consistently reported as a problem related to anti-TNF therapy and was the first added black-box warning for infliximab. The Spanish Society of Rheumatology maintains a safety registry that included over 1500 patients who were treated with anti-TNF therapy [78]. In this registry, the annual rate of tuberculosis (TB) after starting infliximab was 1%. This rate is approximately 50–90 times higher than expected in their general population. A registry of over 10,000 patients in the United States with rheumatoid arthritis also showed an increased rate of TB after treatment with infliximab, but it was much lower than in the Spanish population with an eight-fold increased risk [79]. This translates to an absolute risk of approximately 5 out of 10,000 anti-TNF treated patients in 1 year. Due to the paucity of data, it is difficult to estimate how this risk translates into a North American population of IBD patients. Post-marketing data through 2006 estimated the risk of TB of approximately 1 per 10,000 United States patients treated with infliximab (Data on file, Centocor Inc.). A now outdated MedWatch report from 2001 reviewed 70 cases of TB associated with anti-TNF therapy [80]. Only 18 of these patients had Crohn's disease (the majority had rheumatoid arthritis) and less than one-quarter of the reports originated from the United States. Despite the suboptimal information regarding IBD, data from these 70 patients taught us important information about TB after anti-TNF treatment. The average time to presenting with TB was 12 weeks after initiating infliximab. The majority of patients presented with extrapulmonary disease and most patients were from countries where the incidence of TB is fairly low. The timing of infection suggests that most likely a majority of these patients developed recrudescent TB as opposed to a primary infection. The exact mechanism behind the increased risk of TB is not entirely clear, but likely includes the interruption of the normal host's TNF-α-induced macrophage apoptosis after bacillary infection [80]. Since infliximab has been available the longest, most data are focused on this medication; however, TB has also been reported in association with adalimumab [81,82] and is included as a black-box warning in the adalimumab package insert [83].

Steps can be taken to minimize, but not eliminate. the risk of TB in patients treated with anti-TNF agents. The Centers for Disease Control and Prevention (CDC) have offered guidelines for the screening, diagnosis and treatment of latent and active TB infection in candidates for anti-TNF therapy [84]. They suggest that all candidates for anti-TNF therapy are screened for risk factors which include birth in a country where TB is prevalent, residence in a congregate setting (jail or prison, homeless shelter, chronic care facility), substance abuse history and healthcare employment in setting with TB patients. Tuberculin skin testing (TST) is considered standard of care in the United States, but has limited value because Crohn's disease patients are often anergic [85] (no data on ulcerative colitis patients) and even those IBD patients who can respond to candida or mumps may have selective anergy for mycobacterial antigen skin testing [86]. A TST $\geq$5 mm should be considered positive, but $<$5 mm or negative should not be considered an exclusion of possible latent infection (control agents are usually not planted). Chest radiographs can be helpful, but a number of cases of TB have been reported in those with normal baseline radiographs [81,82].

British guidelines clearly separate how patients taking or not taking immunosuppressive medications should be handled [87]. They recommend starting with routine history and a chest radiograph in all patients. In those without evidence of prior TB exposure, a TST can be useful in guidance, but only in patients not taking immunosuppressive medications. In those on immunosuppressive medications, a TST is considered unreliable and a decision should be made based on balancing the individual annual risk of TB versus that of drug-induced hepatitis from INH (approximately 278 per 100,000 patients) [87].

This author's recommendation is to perform TST in all patients prior to starting anti-TNF therapy. In addition, chest radiographs should be obtained in any patient with a question of prior exposure, risk factor for TB or from a region of high TB prevalence. If a patient with a negative evaluation is on immunosuppressive medications (as many are when starting anti-TNF therapy) and considered at high risk for TB, empiric treatment can be considered in selected patients. The most important procedure is to educate patients carefully to be aware of signs of pulmonary or extra-pulmonary tuberculosis (e.g., fevers, night sweats, weight loss). If a patient has evidence of latent TB, treatment with INH should be started. Ideally, patients with evidence of latent TB should complete 9 months of INH therapy prior to starting anti-TNF therapy. This is not always practical, so treatment with infliximab can be started sooner with careful monitoring. Active TB based on evaluation should be treated according to American Thoracic Society (ATS) guidelines [88] and anti-TNF therapy should be delayed.

Other potentially serious infections related to treatment with anti-TNF therapy (and likely immunosuppression in general) include listeriosis, invasive fungal infections (histoplasmosis, coccidiomycosis, systemic candidiasis, *Pneumocystis carinii* pneumonia (PCP), aspergillosis, sporotrichosis, nocardiasis and cryptococcis [89,90]. In reports to the FDA, the majority of these infections occurred within 3 months of initiating therapy and were thought most likely related to reactivation of latent infection [90]. There have been no recommendations to perform testing with available serologies in patients from high-risk regions, but as above with TB, patients should be educated about early manifestations of these infections [89].

As noted for 6MP/AZA above, viral infections including herpes viruses and CMV are increased with immune suppression. These have also been reported with anti-TNF agents and rarely can be life-threatening [91,92]. Guidelines suggest that all IBD patients without a clear history of chickenpox should have serologic testing for varicella. Nonimmune individuals should receive varicella vaccination [93]. Varicella virus vaccine is a live vaccine, so this should only be given to patients who are not immune compromised (severe malnutrition or treatment with prednisone >20 mg daily, 6MP/AZA or anti-TNF agents). As many patients receive immune suppression over the course of their disease, it is logical to ascertain their varicella zoster status soon after diagnosis to allow for immunization before they are started on life-long immunosuppressive therapy.

Infectious complications may occur more often when anti-TNF agents are used in combination with corticosteroids or immunomodulators, but this association is not clear and was not seen in a recent clinical trial [94–96]. Since the clinical benefit of using anti-TNF agents with immunomodulators may outweigh the small increased infectious risk, risk–benefit decisions need to be made on an individual patient basis [96].

In addition to lymphoma and infectious processes, other adverse events have been associated with infliximab. Multiple sclerosis (MS) was first noted as a problem with the anti-TNF agent lenercept. During an RCT for the treatment of multiple sclerosis (MS), patients treated with lenercept had an increased rate of MS exacerbations [97]. MS has been reported in patients taking infliximab [98,99] and adalimumab [100]. The rate of MS while taking anti-TNF therapy for rheumatoid arthritis has been estimated at approximately 1 per 1000 patient-years [100]. The rate of MS occurrence attributable to anti-TNF therapy in IBD is difficult to determine, as patients with IBD at baseline appear to develop demyelinating disease more frequently than those in the general population [101]. Progressive multifocal leukoencephalopathy (PML), which can initially mimic MS, has been clearly reported in patients receiving natalizumab and there has been concern that it may also be an under-reported problem in those treated with anti-TNF therapy [102].

Anti-TNF therapy is associated with exacerbation of congestive heart failure. These agents should only be used in patients with moderate to severe CHF after careful consideration of other options and with great caution. Based on an early clinical trial using infliximab for the treatment of congestive heart failure, this appears to be a dose-dependent phenomenon [103]. Therefore, if anti-TNF agents are used in patients at risk for CHF, the dose should not exceed 5mg/kg [73].

Autoimmunity, specifically drug-induced lupus, has been implicated as a result of anti-TNF therapy. Although positive anti-nuclear antibodies (ANA) and even anti-double-stranded DNA are relatively common, actual drug-induced lupus appears to be fairly rare [104]. Routine evaluation of these antibodies is not warranted unless specific symptoms suggest lupus, and even if positive, further expert rheumatologic evaluation is warranted before abandoning anti-TNF therapy.

Uncommon hepatic complications related to anti-TNF therapy include acute liver failure [73] and reactivation of hepatitis B [105]. Screening for hepatitis B prior to therapy is important in at-risk patients and ideally all IBD

patients at any risk for hepatitis B should be vaccinated prior to immune suppression. Once immune suppressed, when evaluated in children post-liver transplant, hepatitis B vaccination appears to be safe and effective [106].

Rare reports of pancytopenia [107], non-infectious pulmonary complications [108], psoriaform eruptions [109], ocular processes (Data on file, Centocor Inc.) and others have been reported. At the present time, not enough data exist to confirm frequency or causality.

Natalizumab, an antibody against alpha-4 integrin, is a different class of biologic therapy that has been studied for both ulcerative colitis and Crohn's disease, in addition to the treatment of MS. In 2005, natalizumab was removed from the market for the treatment of MS and IBD clinical trials were suspended after the report of three patients who developed progressive multifocal leukoencephalopathy (PML) [110–112]. One of these patients had Crohn's disease [112]. Natalizumab has since returned to the market for the treatment of MS and it was also approved for the treatment of Crohn's disease through a special distribution prescribing program [113]. Only physicians, infusion centers and pharmacies associated with the infusion centers that are registered with the program can prescribe or deliver the medication. Since its return, as of August 2009, 11 further cases of PML have been reported, all in patients with MS. To date, over 56,000 patients have received natalizumab worldwide, with approximately 10,000 patients who have been treated for longer than 2 years (Data on file, Elan pharmaceuticals, 2009). Therefore, using a denominator of 20,000 patient-years (10,000 patients $\times$ 2 years' exposure), an estimated rate of PML in exposed patients is about 7–10/10,000 patient-years.

Communicating risks of IBD therapy to patients

Putting risk in perspective

It is important to be able to put risks of pharmacologic therapy into perspective. Life is full of everyday risks that are significantly higher than those related to IBD treatments. For example, the lifetime risk of dying in a motor vehicle accident is 1 out of 80 and the lifetime risk of dying from any cancer is 1 out of 8 [114]. The lifetime risk of lymphoma in the general population is 2% (1 out of 50) and the annual risk of developing lymphoma is approximately 2 out of 10,000 [52]. This helps keep the annual risk of NHL associated with immunomodulators of 4/10,000 or combination anti-TNF with immunomodulators of 6/10,000 in perspective. Furthermore, as above (see the section Risks of the disease), the risks of the disease itself are high. When medication risks are stated in these absolute and comparative terms, to most patients (and probably physicians), the risks of therapy are likely more acceptable.

Patient perceptions of risk

With uncertainty about the amount of risk that these medications carry, there is concern over the message that patients are receiving. Patients receive information from a number of sources, including their physicians, friends, family and the Internet. In fact, a recent survey showed that over half of IBD patients obtain disease-specific information from the Internet [115]. Although excellent "neutral" educational websites exist, there are a growing number of sites managed by law offices and pharmaceutical companies, which raises concern over bias of the information provided.

In one study, IBD patients from the United States were asked their perceptions about the risks and benefits of anti-TNF therapy, specifically infliximab [14]. Results showed that a majority of patients believed that infliximab would induce and maintain remission greater than 50–70% of the time. This is nearly double or triple the remission rates seen in induction and maintenance trials for any of the anti-TNF agents. Furthermore, when asked about the risks of lymphoma or death from sepsis, most underestimated the risk by nearly 10-fold. Finally, patients were given the description of a hypothetical "new" drug for the treatment of IBD with associated risks of lymphoma and death (that unbeknown to the patients were equal to risks estimated for infliximab). They were then asked how effective this new treatment would have to be before they would accept taking this "new" medication (the minimal demanded benefit). Interestingly, over 60% of patients responded that they would not take the medication at all, as it was too risky. Amazingly, over one-third of these patients were already taking (or had taken) infliximab. The same survey was given to a group of patients from The Netherlands (where top-down therapy was first studied) [116]. Nearly identical results were found, except for the fact that United States patients demanded more benefit than their Dutch counterparts. Results from these studies confirm the concern that patients have misconceptions over how effective and risky these medications are and that patient decisions regarding appropriate treatments for IBD are being made without proper information.

One way to approach the topic of informed medical decision making is to ask patients how much risk they are willing to tolerate regarding the treatment of their disease (maximal acceptable risk). This was addressed in a web-based survey of 580 patients with Crohn's disease [13]. To determine the subjects' maximal acceptable risk of treatment, they were given a series of treatment options as choice-format conjoint-tradeoff tasks. The options posed one treatment that would give significant clinical benefit, but at the cost of a higher chance of a serious side effect, against a second safer, but less effective medical treatment. Specific adverse reactions included in the choices were lymphoma, sepsis and PML. As might be expected, patients with more severe disease were willing

to take higher risks, but only if the clinical benefit was substantial (e.g., leading to remission). For each of the above adverse outcomes, the majority of patients were willing to accept risks significantly higher than what have been seen in the literature. For instance, in scenarios with severely active Crohn's disease where treatment would induce remission, patients were willing to accept annual risks of dying from lymphoma, sepsis and PML of 0.73, 0.82 and 0.70%, respectively. On the other hand, patients with moderately active disease where treatment would improve (but not resolve) symptoms were significantly less risk taking. Interestingly, when examining how individual patient demographics influenced decision making, it was the patients with more mild overall disease who were the most risk taking. This may be because patients who are very ill adapt to their symptoms and that those with mild disease have more to lose and are therefore more risk taking. Length of disease also influenced decision making, with patients with longer-standing disease being more risk taking. The implication from this finding is that early aggressive or "top-down" therapy may not be appropriate for some with newly diagnosed disease, as they are simply not ready to take the risks. Conclusions from this work primarily reflect that patients are willing to accept higher levels of risk than have been reported and that decisions to take risks are dependent on a number of individual factors.

Decision making becomes even more complex when decisions are being made for others (surrogate decision making). Although in IBD this may occasionally come up in the elderly or for adults incapable of making their own decisions, this is most common in the pediatric population. Parents are asked to make difficult decisions for their children regarding procedures, use of medications and timing of surgery. Using the same choice-format conjoint-tradeoff tasks as in the above project asking adult patients to define their maximal acceptable risk, parents were asked how much risk they were willing to tolerate for their children [117]. Similarly to the adult patients, parents were willing to take increased risks for their children with increasing severity of disease and higher promised benefit. When pediatric patients were only moderately sick and promised disease improvement (but not remission), parents were conservative and were willing to take only a small amount of risk (0.13% risk of dying from lymphoma versus 0.42% for adults in the same scenario). However, when children were sicker (severe disease) and promised disease remission with treatment, parents took even higher risks for their children than did adults for themselves (1.05% risk of dying from lymphoma versus 0.81% for adults). The 1/100 risk that parents were willing to take is significantly higher than any estimates of non-Hodgkin's lymphoma or HSTCL. Therefore, it appears that if children are sick enough, parents will accept the above-proposed risks of biologic therapy.

Risk taking behavior and decision making are probably very different for patients with ulcerative colitis as opposed to Crohn's disease. In ulcerative colitis, a "curative" colectomy is a legitimate treatment option at nearly any point in disease. When posing risks of immunomodulators and biologic therapies to patients with ulcerative colitis, the option of colectomy needs to be proposed. Arseneau *et al.* explored how patients value colectomy versus aggressive medical therapy for ulcerative colitis [15]. They used utility assessments to determine how individual patients valued different states of disease (colectomy versus medical therapy with infliximab or cyclosporin). The results showed that approximately one-third of patients with active ulcerative colitis would prefer immediate colectomy to trying further medical therapy with infliximab or cyclosporin. Furthermore, the overall QALYs (quality-adjusted life-years) were better for colectomy, unless the efficacy of infliximab was greater than 65%. As long-term remission rates are significantly lower than 60%, these results beg the question of how many ulcerative colitis patients prefer medical therapy over surgery.

Risk communication and shared decision making

Even if physicians could agree on the absolute risks and benefits of immunomodulator and biologic therapy, a significant hurdle still exists. Notoriously, risk communication is very difficult. An inherent problem in medical decision making is that of an informational asymmetry. As Hurley *et al.* note, "The crux of the information problem is that while the health care provider possesses better knowledge regarding the expected effectiveness of health care in improving health status, the individual [patient] knows best how improvements in health status affect his or her well-being" [118]. Ideas such as this have led to an attempt to have patients more involved in their medical decisions. Patients can be involved in a number of ways. Although there is a broad spectrum, the extremes are the passive decision makers where patients want physicians to make all of the decisions (paternalism) and the active decision makers (patient autonomy) where patients want to review the data themselves so that they can make a decision on their own [119]. Most patients lie somewhere in between and will choose to take a collaborative approach. This collaborative approach is called shared decision making.

Shared decision making (also called decision support) is defined as the process of interaction with patients who wish to be involved with their healthcare providers in making medical decisions [120]. In this model, physicians have the responsibility of informing and recommending treatments to patients, but the process of deciding on how to act on this is shared. The goal is to enhance patient involvement and, on the basis of the available evidence, facilitate "evidence-based patient choice"(121]. The fairly new and increased attention to shared decision making

derives from a number of different factors [120], which include a move from the idea of informed consent to "informed choice," the consumer (patient) rights movement and the changing nature of medical practice (long-term management of chronic disease) [120]. This recent interest has led to over 500 academic publications on the topic of shared decision making since 2000 (PubMed Search, 10 September 2006: Search term "Shared Decision Making").

A critical step in involving patients in the decision making process is the effective communication of the harms and benefits of treatment. Data can be presented in a number of ways, but in most cases, appropriate interpretation requires a high level of numeracy (quantitative literacy). This includes the ability not only to comprehend percentages, but also to grasp very small numbers (i.e., <1%) and to have a basic understanding of probabilistic language, all of which can be barriers to patient comprehension [122]. When compounded by the emotional aspect of being sick and the need to make difficult judgments under uncertainty, decisions can simply become overwhelming for patients.

Physicians have become familiar with numerical concepts to help us comprehend, compare and interpret risk. These include the statistical terms relative risk, absolute risk, absolute risk reduction, number needed to treat, odds ratios and *p*-values. However, these concepts may not be easily comprehensible by patients. Furthermore, patients are susceptible to unintentional "framing" of the data, which can be inadvertently misleading [123,124]. Framing relates to the fact that patients will interpret information differently, depending on how it is presented. For example, it has been shown that presenting benefits of anti-hypertensive therapy as relative risk reduction (e.g., 34% reduction in heart attacks) is far more acceptable (albeit misleading) to patients when compared with the same statistical absolute risk reduction of 1.4% or the number needed to treat of 71 persons for 5 years to prevent one heart attack [123]. These results are also consistent across other studies [124]. Conversely, if risk is presented in this same manner, patients would unnecessarily be frightened. Relating this to Crohn's disease, if the risk of NHL with immunomodulators is presented as a 200% increased relative risk from baseline, most likely few patients would be comfortable with this treatment. However, when described as an absolute risk increase of 0.02% (or an extra 2 out of 10,000 patients), this becomes less frightening and far more acceptable. In general, presenting benefit/harm data in absolute terms leads to improved comprehension. Furthermore, presenting risk in context with other life risks (as above) puts things in perspective and allows patients to see that life is full of risks and, when necessary, these risks may be worth taking.

Decision aids are interventions developed for preparing patients for decision making about specific treatment choices [125,126]. These are not patient education materials given to patients after a prescription has been written or a surgery is scheduled, but a presentation of information before a choice of therapy has been made. Decision aids are essentially the presentation of evidence-based information in a patient-friendly format. Successfully developed and evaluated decision aids include treatment options for atrial fibrillation [127], management of breast cancer [128] and prostate cancer [128] and treatment choices for coronary artery disease [130]. Decision aids are available in various media, ranging from simple pamphlets to elaborate videos. Web-based decision aids are popular due to low production cost and ease of being rapidly updated as new data become available. Examples of web-based decision aids include one on prostate cancer developed by the CDC and another on the management of breast cancer produced by the Canadian Cancer Society [130,132].

Decision aids are becoming well studied and data are accumulating on their effectiveness. A Cochrane analysis identified over 200 patient decision aids, of which 30 were evaluated in RCTs (including the breast cancer decision aid described above) [133]. Outcome measures in the RCTs included patient knowledge, satisfaction in the decision making process, impact on decision making and decisional conflict. Decisional conflict was evaluated using the decisional conflict scale, a validated questionnaire [134] that has been used in multiple studies on this topic [127,128,135,136]. The authors of the Cochrane analysis concluded that decision aids were effective in improving patient knowledge and realistic expectations, enhancing active participation in decision making, lowering decisional conflict, decreasing the proportion of people remaining undecided and improving agreement between individual patient values and choice of therapy. A guideline on how to develop a decision aid properly is defined by the Ottawa Decision Support Framework [137]. This evidence-based framework is based on concepts from general psychology, decision analysis, decisional conflict, social support and economic concepts of expectations and values [138,139]. It has been used to guide the development and evaluation of over 30 patient decision aids. To measure the quality of a decision aid, the International Patient Decision Aids Standards (IPDAS) collaboration has recently developed and published criteria by which all decision aids can be measured [140]. There is one decision aid for IBD regarding surgery for ulcerative colitis, but it does not review benefits and harms of medical therapy and has not been studied systematically [141].

As treatment algorithms evolve, new biologics become available and new side effects are described, the benefit/harm profiles of treatment will become even more difficult to interpret. With the complexities of interpreting and presenting data, increasingly limited time with which to do this effectively and continued importance of proper

informed consent, it will be important to embrace the process of shared medical decision making for the management of patients with IBD.

Conclusion

Risk assessment and communication in IBD are complicated. The uncertainty of the data, technical and emotional aspects of decision making and quickly evolving information make the "right choice" a moving target. What appears to be clear is that immunomodulators and biologic therapy do carry measurable risks. Although rare, potentially devastating and life-threatening side effects do occur, even in young, otherwise healthy patients. We have a long way to go in developing more effective and safer medications for the treatment of IBD. For now, to maximize the benefit/risk ratio we need to focus on how to (1) target the patients most appropriate for these therapies, (2) take measures to decrease the risks and (3) optimize the use of existing therapies. Of critical importance is proper communication with patients and their families. Patients appear to be willing to accept the risk of existing therapies; however, they need to understand their choices to ensure the opportunity for informed medical decision making.

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Chapter 48
Complementary Medicine

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Summary
- Complementary and alternative therapies are widely used by patients with IBD.
- There are limited controlled data indicating clinical efficacy for some traditional Chinese medicines, *Aloe vera* gel, wheat grass juice, *Boswellia serrata*, curcumin, soya extract and bovine colostrum enemas in ulcerative colitis and for wormwood in Crohn's disease.
- After formal evaluation, some treatments previously classed as complementary or alternative have become part of the conventional armory: probiotic therapy for remission maintenance in ulcerative colitis is the best example to date.
- Contrary to popular belief, natural therapies are not necessarily safe: fatal and irreversible hepatic and renal damage have occurred in patients given some herbal therapies.
- There is an urgent need for further research to evaluate the mechanisms of action of complementary and alternative approaches, for more controlled trials of their efficacy in IBD and for enhanced legislation to maximize their quality and safety.

Introduction

The terms complementary medicine and alternative medicine denote theories and practices of medicine which deviate from the conventional. The combined term, complementary and alternative medicine (CAM), encompasses a wide and heterogeneous range of diagnostic and therapeutic procedures and of concepts of health and disease; these include not only traditional practices such as traditional Chinese medicine, acupuncture, Ayurvedic medicine, homeopathy and herbal medicine, but also more modern modalities such as aromatherapy and reflexology (Table 48.1). The denominator common to all types of CAMs is their exclusion from the realms of scientific medicine and consequently their under-representation in research and teaching at universities. The barrier between complementary and conventional medicine is not, however, immutable: in relation to inflammatory bowel disease (IBD) at least, certain modalities previously labeled as alternative, of which probiotic therapy is a recent example, have passed, as a result of formal scientific study both in the laboratory and in clinical trials, into the conventional therapeutic armamentarium.

Alternative medicine practices are often based on ideas or beliefs which ignore modern pathophysiological and pharmacological mechanisms, relying more on ancient practices and on "natural" remedies, which are perceived by the public as being less toxic than conventional drugs. CAM differs further from much conventional medicine by taking a holistic approach to patient care, calling on self-healing by the body and being applied in an individualized way. Although increasing numbers of controlled trials and meta-analyses are being reported (for reviews, see [1–4]), much information relating to the possible effectiveness of CAM remains anecdotal or historical.

Use of CAM

Over 30% of the Western population now uses some form of CAM. In most studies, the single most commonly used modality in most surveys is herbal therapy [5–7]. Indeed, annual spending on herbal products by the general population 10 years ago already exceeded £40 million per year in the United Kingdom [7] and $5 billion per year in the United States [5]. These are extraordinary figures given the dearth of scientific evidence about the efficacy or safety of herbal therapies in almost all the contexts in which they are used.

Surveys of use of CAM by patients with gastrointestinal complaints have reported rates of usage ranging from 9% [8] to over 50% [9–12]. CAM for all digestive indications appears to be more popular in North America than Europe, although the growth of the industry in Europe is

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Table 48.1 Types of complementary and alternative therapy potentially relevant to IBD (derived from nccam.nih.gov.health.whatiscam).

Alternative medical systems – Complete systems of theory and practice
- Homeopathy
- Naturopathy
- Traditional Chinese medicine, including acupuncture
- Ayurvedic medicine

Mind-body interventions – Techniques to enhance the mind's capacity to affect bodily function
- Meditation
- Prayer
- Hypnotherapy
- Creative therapies, e.g. art, music, dance

Biologically based therapies – Use of naturally occurring substances
- Herbalism
- Dietary manipulation and supplements
- Vitamins

Manipulative and body-based – Based on movement or manipulation of one or more parts of the body
- Chiropractice
- Osteopathy
- Reflexology
- Massage

Energy therapies – Unconventional use of magnetic and electromagnetic fields
- Biofield, e.g. Reiki
- Bioelectromagnetic field therapy

now probably faster. As in other contexts, the single most used type of CAM for gastrointestinal disorders is herbal therapy [13,14].

Usage appears to be most common in patients with IBD [9,12–14] and with irritable bowel syndrome [12,15]. At least as many children and adolescents, presumably prompted by their parents, use CAM [16–20], although in these age groups probiotics, vitamins and dietary supplements are more often used than herbal therapies.

The widespread use of CAM by patients with IBD may be related to the chronic and refractory nature of these disorders [14,21] and also to psychological factors [21]. Indeed, it has been shown that the use of CAM by both adults and children with IBD is most common in those with perceived stress and a poor quality of life [18,22–24], a finding analogous to that occurring in patients with breast cancer [25]. In a national survey from Germany, 51% of IBD patients had experience with CAM, with homeopathy and herbal therapy being the most popular. Patients' total systemic steroid intake, suggesting poorly controlled disease, was a strong predictor of the use of CAM [10]. Other factors appearing to predispose to use of CAM by patients with IBD include female gender [12,26] and higher educational achievement [11]; some, such as duration of disease and a history of hospital admissions, are recorded inconsistently, even within a single country [14,27].

Given its widespread usage, doctors in general, and gastroenterologists in particular, can no longer ignore the potential benefits and dangers of CAM. Indeed, given that many patients do not disclose their use of CAM to their conventional doctors [26], it is important that consultations include specific enquiry about this possibility.

Review of efficacy of CAM in human IBD

The literature review that we provide here was compiled using a systematic search of Medline database 1966–2009. Reports published either in English or with English abstracts available were used; only exceptionally do we include data published only in abstract form, however. Search headings and keywords used were combinations of complementary, alternative, herbal, acupuncture, hypnosis, hypnotherapy, reflexology, aromatherapy, remedies, homeopathy, osteopathy, chiropractic, naturopathy, traditional Chinese medicine, prayer and inflammatory bowel disease, colitis, Crohn's, ulcerative colitis and proctitis. The review is primarily restricted to human studies: results in experimental IBD in animal models are alluded to briefly in the ensuing section discussing possible mechanisms of action of CAM.

Evaluating the efficacy of CAM in IBD

The difficulties associated with designing, executing and interpreting trials of new conventional therapies in IBD are numerous [28]; they include the heterogeneity of ulcerative colitis and particularly Crohn's disease, the definition of indications for treatment and the selection of appropriate therapeutic endpoints. Such problems are compounded in relation to trials involving alternative therapies. Indeed, some authors believe that attempts to resolve questions of effectiveness of CAM using randomized controlled trials (RCTs) are misguided in view of their exclusion from the realms of scientific hypotheses [29,30].

Trial design and execution

The huge variety of herbal products available and the lack of standardization of their manufacture, content and directions for use reduce the likelihood of different trials even of the same remedy giving reproducible results. Similar comments apply to physical treatments, where standard protocols are often lacking. The widely used CAM practice of individualized therapy is also difficult to incorporate into conventional clinical trial design, although meaningful results can be obtained by using, for example, a crossover design and multiple groups [31]. Devising appropriate control arms, for example sham acupuncture points, for

physically or psychologically based therapies often raises arguments about blinding and the placebo response.

Funding issues tend to exceed those relating to trials involving standard agents. For example, analytical dossiers are difficult to assemble for complementary and particularly herbal medicines. The costs of analysis are high and place a significant financial burden on researchers or companies wishing to carry out clinical trials. Given the expense, it is unlikely that commercial companies will fund clinical trials for existing remedies which already have a successful market [32].

Lastly, many grant-giving bodies remain wary, at best, of research involving CAM, so that funding from such sources for trials (or laboratory research) is even harder than usual to obtain.

Trial interpretation

A number of studies of CAM for the treatment of IBD have been reported (Table 48.2), some claiming at least equivalence to conventional therapies. In many instances trial design has been insufficiently rigorous to permit reliable conclusions to be drawn. In particular, many studies have been non-randomized, inadequately or totally uncontrolled and unblinded; many have contained small numbers of patients and are underpowered. There is also likely to have been bias introduced by failure to publish negative trials. Lastly, trials published in languages other than English are not always easy to obtain or interpret.

Herbal therapies

Traditional Chinese medicine

Although there are numerous reports in the Chinese literature about the treatment of ulcerative colitis with herbal remedies, often only the abstracts are available in English.

In an RCT, 153 patients with ulcerative colitis were given either *Jian Pi Ling* tablets and *Radix sophorae flavescentis* and *Flos sophorae* (*RSF–FS*) concoction enemas, conventional treatment with oral 5-aminosalicylate (5-ASA) and prednisolone enemas or oral placebo and RSF–FS enemas [33]. Remission rates in the first group were reported to be significantly higher (53%) than in the other two (28 and 19%, respectively), but the very low success rate of conventional therapy makes this study hard to interpret.

In another study, the traditional Chinese remedy *Kui jie qing* (*KJQ*) was given as a four times daily enema to 95 patients with active ulcerative colitis [34]. Eleven patients given sulfasalazine 1.5 g three times daily, oral prednisolone 30 mg daily and prednisolone enemas 20 mg four times daily for 20 days were used as controls. Effective "cure" was reported in 72% of KJQ-treated patients but in only 9% of controls ($p < 0.001$). A further 23% of patients using KJQ enemas improved compared with 53% of the controls, leading the authors to conclude a 95% effectiveness rate for KJQ, as against 62% for conventional western treatment. This study does not provide definitions for "cure" or "improvement" but the conventionally treated patients had a less good response rate than would usually be expected.

In a similar trial, 118 patients with active ulcerative colitis were treated with *Yukui tang* ("decoction for ulcer healing") orally and herbal decoction enemas, plus oral prednisolone 15 mg daily, neomycin and vitamin B for 40 days [35]. The overall effectiveness rate was 84% for the herbal therapy group (33% "cured", 51% improved) and 60% for 86 control patients (17% "cured", 43% improved) ($p < 0.01$) who were given a low dose of prednisolone (15 mg), neomycin and vitamin B only.

Interpretation of the results of these comparative studies is compromised by a lack of randomization and blinding and the rather unusual combinations of the "conventional" therapies used in the comparator groups.

Open-label studies are, of course, of very limited value in IBD. With this proviso, an uncontrolled trial of the *T2 extract* of the root of a traditional Chinese anti-inflammatory herb, *Tripterygium wilfordii* Hook F, also known as "thunder god vine," improved Crohn's disease activity index (CDAI), C-reactive protein (CRP) and endoscopic appearance after 12 weeks in 16 patients with active Crohn's disease [36]. Diarrhea was a side effect in three patients.

Other herbal therapies

A randomized, double-blind, controlled study showed that *Aloe vera gel*, given for 4 weeks to 44 patients with moderately active ulcerative colitis, produced a clinical response in significantly more patients than did placebo. Clinical remission, improvement and response occurred in 9 (30%), 11 (37%) and 14 (47%), respectively, of 30 patients given *Aloe vera*, compared with 1 (7%), 1 (7%) and 2 (14%) ($p < 0.05$), respectively, of 14 patients taking placebo (using a 2:1 *Aloe vera*: placebo randomization schedule). The Simple Clinical Colitis Activity Index and histological scores decreased significantly during treatment with *Aloe vera* but not with placebo [37].

In a randomized, double-blind, controlled trial, 23 patients with active distal ulcerative colitis were given oral *wheat grass juice* or placebo for 4 weeks [38]. Treatment with wheat grass juice was associated with greater reductions in a composite clinical disease activity index, in the severity of rectal bleeding and in the physician's global assessment than occurred in the placebo group. No side effects were reported.

Two open-label Japanese trials suggested efficacy in ulcerative colitis for a *germinated barley foodstuff* (GBF) [39,40]. In the first report, 11 patients given GBF for 4 weeks as adjunctive treatment showed a greater fall in clinical disease activity than nine patients given conventional therapy alone. In a follow-up study, 24 weeks of treatment of 21 patients with GBF together with

Table 48.2 Trials of complementary and alternative therapy in active IBD*.

Approach	Disease	Trial design	*n*	Comparator	Duration of treatment	Remission on CAM (%)	Remission on comparator (%)	Ref.
Jian Pi Ling tablets, RSF–FS enemas	UC	Randomized controlled	153	Oral sulfasalazine, dexamethasone enemas	90 days	53†	28	Chen *et al.* 1994 [33] (in Chinese)
Kui jie qing enemas	UC	Controlled	106	Oral sulfasalazine, oral prednisolone, prednisolone enema	20 days	72†	9	Wang *et al.* 1997 [34]
Yukui tang tablets, herbal decoction enemas	UC	Controlled	118	Oral prednisolone, neomycin and vitamin B	40 days	33†	17	Chen and Zhang 1999 [35]
Tripterygium wilfordii Hook F extract (T2)	CD	Open	16	–	12 weeks	56	–	Ren *et al.* [36]
Aloe vera gel	UC	Randomized, double-blind, controlled	44	Placebo	4 weeks	30†	7	Langmead *et al.* 2004 [37]
Wheat grass juice	UC	Randomized, double-blind, controlled	23	Placebo	4 weeks	Not stated, but wheat grass improved symptoms and bleeding more than placebo	Not stated	Ben-Arye *et al.* 2002 [38]
Germinated barley	UC	Open	21	–	24 weeks	Not stated, but bleeding and nocturnal diarrhea improved	–	Kanauchi *et al.* 2003 [39,40]
Boswellia serrata gum resin	UC	Controlled	30 [36]	Sulfasalazine	6 weeks	82 [ref 42] 70 [ref 43]	75 [42] 40 [43]	Gupta *et al.* 1997 and 2001 [42,43]
Boswellia serrata extract H15	CD	Controlled	102	Mesalazine	8 weeks	36	31	Gerhardt 2001 [44] (in German)
Curcumin	UC and CD	Open	10		12 weeks	Not stated, but 9/10 patients improved	–	Holt *et al.* 2005 [46]
Bowman–Birk inhibitor concentrate from soy	UC	Randomized, double-blind, controlled	28	Placebo	12 weeks	36	7	Lichtenstein *et al.* 2008 [48]
Tormentil	UC	Open	16	–	12 weeks	Improved clinical activity index	–	Huber *et al.* 2007 [49]
Mastic	CD	Open	10	–	4 weeks	Improved CDAI, CRP	–	Kaliora *et al.* 2007 [50]
Wormwood	CD	Randomized, double-blind, controlled	40	Placebo	10 weeks	60†	0	Omer *et al.* 2007 [51]
Acupuncture with moxibustion	CD	Randomized, single-blind, controlled	51	Sham acupuncture	10 sessions in 4 weeks	41	33	Joos *et al.* 2004 [52]
	UC	As above	29	As above	10 sessions in 5 weeks	Not stated	Not stated	Joos *et al.* 2006 [53]

(*Continued*)

Table 48.2 (*Continued*)

Approach	Disease	Trial design	*n*	Comparator	Duration of treatment	Remission on CAM (%)	Remission on comparator (%)	Ref.
Acupuncture with moxibustion	UC	Comparative	62	Sulfasalazine	Not stated	62	33	Yang and Yan [54]
Hypnotherapy	UC and CD	Open	12	–	6 weeks	Not stated	–	Shetty *et al.* 2004 [55]
Hypnotherapy	UC and CD	Open	15	–	Variable	Not stated, but IBDQ improved	–	Keefer *et al.* 2007 [56]
Hypnotherapy	UC and CD	Open	15	–	Median 5 years	27	–	Miller and Whorwell 2008 [57]
Bovine colostrum enemas	UC	Randomized, double-blind, controlled	14	Placebo	4 weeks	Not stated, but colostrum improved symptoms and histology more than placebo	Not stated	Khan *et al.* 2002 [58]

*UC, ulcerative colitis; CD, Crohn's disease; RSF–FS, *Radix sophorae flavescentis* and *Flos sophorae*; CDAI, Crohn's disease activity index; CRP, C-reactive protein; IBDQ, Inflammatory Bowel Disease Questionnaire.
†denotes significantly higher than comparator

continuing 5-ASA and steroid therapy reduced rectal bleeding and nocturnal diarrhea. Adjunctive GBF also produced a lower relapse rate over 12 months when given to 22 patients with ulcerative colitis in remission than did conventional therapy in 37 such patients [41]. GBF was well tolerated and appeared to be safe in all three reports.

Boswellia serrata (*frankincense*) is a traditional Ayurvedic remedy and a component of incense. In India, the effect of the gum resin from *Boswellia serrata* in moderately active ulcerative colitis was compared with sulfasalazine: the remission rate in the *Boswellia* group (82%) resembled that occurring in patients given conventional therapy (75%) [42]. The same authors reported a similar study in 2001 resulting in a 70% remission rate in 20 patients taking *Boswellia* for 6 weeks compared with 40% in 10 patients on sulfasalazine [43]. In a randomized, double-blind, controlled 8 week trial, the *Boswellia serrata extract H15* was compared with mesalazine for active Crohn's disease [44]. The study included 102 patients and was powered to show non-inferiority. The mean CDAI fell in both groups and H15 was well tolerated. This result was interpreted by the authors as evidence for efficacy of H15 in the treatment of active Crohn's disease, but the clinical remission rates on both therapies, as in previous trials with 5-ASA preparations [45], were only moderate [44].

Curcumin is the yellow pigment of turmeric (*Curcuma longa*), a major ingredient of curry: in animal and *in vitro* studies it has a range of anti-inflammatory effects. In a pilot study, curcumin, when given orally, was reported to benefit five patients with proctitis and five with Crohn's disease [46]. Subsequently, in a 6 month controlled maintenance study of 89 patients on a 5-ASA for ulcerative colitis, the relapse rate was 5% in those taking, in addition, curcumin 1 g twice daily, as compared with 21% in those on placebo ($p = 0.04$) [47]. Patients on curcumin in this study also showed significant improvements in clinical activity index and endoscopic score.

In a recent randomized, double-blind, placebo-controlled trial in 28 patients with active ulcerative colitis, *Bowman–Birk inhibitor concentrate* (*BBIC*), a soy extract, given for 12 weeks, appeared to produce a greater fall in disease activity index and a higher remission rate than did placebo, but neither effect reached statistical significance [48].

Tormentil has antioxidant properties and has been used anecdotally by patients with IBD. In a dose-escalating open-label study in 16 patients with active ulcerative colitis, higher doses were associated with reductions in clinical activity index and CRP: there were no overt side effects [49]. *Mastic* is a resin from the Mediterranean evergreen shrub *Pistacia lentiscus*, which has been reported to have both antibacterial and antioxidant actions. In a recent pilot study in 10 patients with active Crohn's disease, mastic prepared as a powder packed in capsules improved CDAI and CRP after 4 weeks [50]. Controlled trials are required to evaluate further both of these preparations.

In a multicenter, double-blind, placebo-controlled trial from Germany, *wormwood* (*Artemisia absinthium*) was assessed as a steroid-sparing herbal remedy in 40 patients with active Crohn's disease whose dose of prednisolone was tapered to zero over a 10 week period [51]. After 8 weeks, 65% of the wormwood-treated patients were reported to be in remission (on CDAI), compared with none given placebo. After stopping steroids, only 10% of the

subjects given wormwood needed prednisolone restarted, whereas 80% of those on placebo did so. These results point strongly to the need for further clinical trials, perhaps using a less complex study design.

Other CAM modalities

Acupuncture and moxibustion

In a single-blind controlled trial of 51 patients with mild to moderately active Crohn's disease, acupuncture and moxibustion (in which heat is added by burning herbs over the acupuncture site) reduced CDAI and α_1-acid glycoprotein and improved general well-being. CDAI fell to a significantly greater degree (87 points) than occurred in the control group in whom needles were inserted into non-acupuncture points (39 points) ($p = 0.003$), but there was no difference in the remission rates achieved in the two groups [52]. An analogous study by the same group in 29 patients with ulcerative colitis again showed that active treatment twice a week for 10 weeks produced a slightly larger fall in Colitis Activity Index than did sham acupuncture, but the two interventions caused similar improvements in general well-being and quality of life scores [53]. Furthermore, a comparative study from China suggested that acupuncture with moxibustion was as effective as conventional Western therapy in 62 patients with ulcerative colitis [54].

Hypnotherapy

There are anecdotal reports but no adequate trial data to support the proposal that hypnotherapy might be beneficial in patients with IBD: at present its routine use cannot be recommended. In one unblinded study of 12 patients with active ulcerative colitis and Crohn's disease, after 6 weeks of gut-focused hypnotherapy, there was a non-significant trend to improvement in Inflammatory Bowel Disease Questionnaire (IBDQ) [55]. More recently, similar findings were reported in one study of eight women with inactive IBD [56] and in another of 15 IBD patients with either severe disease or disease refractory to corticosteroids [57]. Two controlled studies are in progress to evaluate the possibility that hypnotherapy might reduce relapse rate in patients with inactive ulcerative colitis.

Bovine colostrum enemas

Fourteen patients with mild to moderately severe distal colitis received bovine colostrum enemas (100 ml of 10% solution) or placebo (albumin solution) twice daily for 4 weeks. Both groups also received mesalazine (1.6 g per day) or, if already taking it, had a dose increment of 1.6 g per day. After 4 weeks, the colostrum group showed a mean reduction in symptom score of –2.9 [95% confidence interval (CI) –5.4 to –0.3], whereas the placebo group showed a mean response of +0.5 (95% CI –2.4 to +3.4). The histological score improved in five of the eight patients in the colostrum group [58].

Possible modes of action of CAM

One barrier to the acceptance of CAM by conventional doctors has been the apparent lack of any scientific explanation for their possible efficacy. Indeed, types of CAM such as acupuncture have been based on historical and cultural constructs entirely unfamiliar to the majority of Western clinicians. Recently, however, mechanisms by which some of these modalities may work have become apparent: we shall restrict our review to approaches that have been reported to be of clinical benefit in IBD (Table 48.2).

Herbal therapies and colostrums

Unpurified herbal preparations contain a huge range of biologically active compounds [59], some of which may have beneficial and others adverse effects. Extensive work of varying quality, clinical relevance and accessibility has suggested that, *in vitro* at least, individual chemicals derived from a variety of plants may have antibacterial, antioxidant, anticytokine, antispasmodic and neuromodulatory actions [59]. *In vivo*, the polysaccharide content of plant preparations means that they may also act as prebiotics [60]. It is clearly difficult, however, to extrapolate from a knowledge of the chemical composition and activities *in vitro* of an extract from a given plant to its possible efficacy (or safety) *in vivo*. This will depend on a number of factors, including the amounts of individual constituents in the extract (which may vary with the plant's geographical origin and the method of preparation of the extract), interactions between individual constituents and their pharmacokinetics, of which little is known in most instances.

Traditional Chinese medicines (TCMs)

As indicated earlier, much of the literature relating to TCMs is relatively inaccessible to Western readers. Furthermore, the range of traditional Chinese herbal products used in IBD and other human inflammatory disorders is wide and the individual components of these are innumerable. In brief, it appears that several different TCMs have beneficial effects in experimental colitis in animals, possible mechanisms of action of different preparations including inhibition of mucosal expression of tumor necrosis factor alpha (TNFα) [61,62], interleukin (IL)-6 and interferon gamma (IFN-γ) [61], antioxidant effects [63] and down-regulation of intercellular adhesion molecule and iNOS expression [63]. In other models, anti-inflammatory TCMs have been reported to inhibit chemokines and chemokine receptors [64].

Aloe vera gel

Results from our own laboratory [65] and others [66–69] have shown that various fractions of *Aloe vera*, and also the unfractionated whole gel, are antioxidants *in vitro*. Indeed, *Aloe vera* gel contains glutathione peroxidase activity [70,71], seven superoxide dismutase enzymes [72] and a phenolic antioxidant [67]. Aqueous and glycoprotein extracts, and the whole gel itself, also have inhibitory effects on cyclooxygenase and thromboxane synthase activity [69–73]. Other reported results include inhibition by *Aloe vera* gel of IL-8 production by cultured colonic epithelial cells [65].

Germinated barley

This consists mainly of dietary fiber and glutamine-rich protein. Several reports from the same group in Japan suggest that it acts primarily as a prebiotic stimulating, in experimental colitis, production of butyrate from *Eubacteria* and *Bifidobacteria* with consequent reductions in mucosal STAT3 expression and possible inhibition of NFκB activity [74,75].

Wheat grass

There appear to be no experimental data relating to the mechanism of action of wheat grass in ulcerative colitis, but it seems likely that it could act at least in part as a prebiotic.

Boswellia serrata

Boswellic acids are thought to be the active component of this preparation and have a range of potentially beneficial actions. *In vitro* and animal experiments indicate that these include inhibition of 5-lipoxygenase [76], a switch from TH1 to TH2 cytokine production [77], inhibition of P-selectin-mediated leukocyte and platelet–endothelial cell adherence [78,79], prevention of fibrosis by inhibition of the TGFβ1/Smad3 pathway [80] and reduction of matrix metalloproteinase (MMP) expression by human microvascular endothelial cells [81]. It should be noted that not all reports indicate a beneficial effect of *Boswellia* extracts in experimental colitis, one recently suggesting a lack of efficacy in both dextran sulfate sodium (DSS)- and trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice [82]; in this study, *Boswellia* increased intestinal epithelial cell NFκB activity and caused hepatic steatosis.

Curcumin

There is a rapidly growing literature on the mechanisms by which curcumin exerts its anti-inflammatory actions in a range of animal models (for reviews, see [83–85]). Several groups have reported specifically on the beneficial effects of curcumin in experimental rodent colitis, proposed modes of action identified in these settings being an antioxidant action [86,87], inhibition of NFκB activation and expression of pro-inflammatory cytokine mRNA [88], reduction in p38 MAPK activity [87,89], reduced nitric oxide synthesis [86,87], a shift from TH1 to TH2 cytokine profiles [90], activation of retinoid X receptor [91] and inhibition of COX-2 [87,92].

Most of these anti-inflammatory actions of curcumin have been confirmed *in vitro*. Additional *in vitro* actions reported include inhibition of PAR2- and PAR4-mediated activation of mast cells [93], inhibition of a range of immunostimulatory actions of murine bone marrow-derived dendritic cells [94], regulation of STAT-3 and -5 [95], inhibition of 5- and 12-lipoxygenase [76], reduction in MMP-9 activity [96] and inhibition of expression of vascular cell adhesion molecules [97].

Which of these numerous effects predominates in the apparent anti-inflammatory actions of curcumin in human and experimental colitis is not yet clear. In relation to human IBD, it is also interesting that dietary supplementation with curcumin reportedly opposes the adverse effect of TNBS-induced colitis in mice on expression by osteoblasts of the *Phex* gene, disruption of which impairs bone mineralization: this effect could conceivably be utilized in the prevention of osteoporosis in IBD [98].

Bovine colostrum

Colostrum contains a range of potentially beneficial constituents, which include immunoglobulins, antimicrobial peptides and growth factors [99]. In active human ulcerative colitis, enemas containing epidermal growth factor (EGF) [100], but not trefoil factor 3 [101], have been shown to be effective, while in experimental animal studies, EGF, platelet-derived growth factor, transforming growth factor-β and insulin-like growth factor-1 have each been shown to have benefit when given immediately before the damaging agent [58,102]. However, it is not known which of the many constituents of colostrums, acting alone or in combination, accounts for its apparently ameliorative effects in ulcerative colitis [58].

Physical and psychological therapies

Acupuncture

It is now clear from the emerging field of psychoneuroimmunology that neuronal connections between the brain and the enteric nervous system, and in turn with immune and inflammatory cells in the lamina propria, could mediate any anti-inflammatory gastrointestinal effects of modalities such as acupuncture and hypnotherapy [103,104]. While most evidence has been derived from animal experiments (for references, see [105]), data from human studies are beginning to be reported (for a review, see [106]). It now appears possible that afferent stimulation by acupuncture and related techniques of the hypothalamic–pituitary–adrenal axis [103] could have down-regulatory anti-inflammatory effects in the gut and elsewhere through efferent routes which include

changes in neuropeptide release from efferent neurons of the enteric nervous system, in adrenal production of catecholamines and cortisol and activation of the vagal cholinergic anti-inflammatory pathway [107–111].

Hypnosis

Several reports have addressed the effects of hypnotherapy on the immune system in normal volunteers and again illustrate the potential relevance of psychoneuroimmunologic pathways [103,104]. Two studies found that self-hypnosis of students antagonized decreases in NK cell T cell counts induced by the stress of examinations [112,113]; changes in $CD3^+$, $CD4^+$, $CD8^+$ lymphocyte counts before examinations were also reduced by self-hypnosis [112–114]. In contrast, in two other studies unassociated with stressful stimuli, hypnosis was found to reduce NK cell activity quickly but transiently [115,116]; reductions in lymphocyte proliferative responses to mitogens [115] and in proportions of T cells expressing IFN-γ and IL-2 [116] also occurred. Our own work showed that a 45 min period of gut-directed hypnotherapy (unlike a rest period of the same duration) reduced serum IL-6 concentration and rectal mucosal production of substance P and IL-13 in patients with active ulcerative colitis [117].

Placebo response

It has been suggested that the clinical response to CAM relates largely to its placebo effect [118] and that placebo responses to alternative remedies will be high in individuals strongly committed to the concept that such therapy will work [119]. Discussion of the mechanism of the placebo effect and the extent to which it related to the quality of the therapist [120] is beyond the scope of this review.

Side effects of CAM

Direct toxicity

Contrary to the widely held popular view that because it is "natural" it is safe, herbal therapy is likely to carry more risks and produce more serious side effects than any other forms of alternative therapy [7,121]. Indeed, toxicity from herbal therapies has included fatal liver and renal failure [59,122]. Unfortunately, there are limited formal data on the incidence even of acute severe side effects such as these and knowledge of possible longer term sequelae such as mutagenicity and carcinogenicity is even more scanty.

Toxic effects have also been associated with the deliberate inclusion of prescription medicines in some herbal preparations: these have included corticosteroids, fenfluramine and glibenclamide [123]. Other toxic products found in some preparations have included mercury, arsenic, lead, human placenta (with a risk of transmitting hepatitis C or HIV) and bat excreta.

Reports of injuries during manipulative therapies such as osteopathy are infrequent. Injuries from acupuncture needles such as pneumothorax are also seldom reported, but hepatitis B and C have occurred [124] and, in one notorious incident in 1998, contaminated blood led to a major outbreak of hepatitis B in patients treated in north London, UK, by a variant of acupuncture known as autohemotherapy [125].

Drug interactions

Potentially and actually dangerous interactions between herbal therapies and conventional drugs have been widely reported (for a review, see [126]) but are insufficiently widely recognized or even suspected by many conventional practitioners. In the context of ulcerative colitis, St John's wort reduces blood levels of ciclosporine by enhancing the activity of cytochrome P450 enzymes [59]. Indeed, in a systematic review, 17 studies reported a decrease in systemic bioavailability of conventional drugs when used in conjunction with St John's wort [127].

Indirect adverse effects

Perhaps more important than direct toxicity or drug interactions, use of CAM may be complicated by indirect adverse effects. For example, patients with IBD initially consulting alternative practitioners may be wrongly diagnosed, for example, with irritable bowel syndrome. Others may delay or forego appropriate conventional options in favor of ineffective unconventional ones; this may lead to late presentation to a gastroenterologist with severe or complicated IBD. Lastly, patients may undergo inappropriate further investigation or even treatment as a result of abnormalities in laboratory tests induced by herbal preparations [128]: the most important example in the context of IBD is the reduction in blood ciclosporine levels produced by St John's wort (see above).

Regulation

Until recently, there was little or no legislation in most countries controlling the application of conventional and alternative therapy, but changes are afoot in most Western countries at least. Itemization of regulatory systems introduced across the world would not be of interest here. Suffice it to say that in most countries further legislation is needed to regulate both the modalities available and the training, accreditation and practice of complementary healthcare professionals.

In relation to herbal therapy used in Europe, the European Union Traditional Use Directive [32] suggested that evidence of 30 years' use, of which 15 years must be in the Community, provides confirmation of efficacy. For many, it is difficult to accept the logic of this approach, which of course contrasts strongly with that taken for

conventional drugs: it appears that decisions about efficacy of many herbal remedies will be based on tradition and even folklore [129] rather than rigorous clinical trials.

The frequency with which adverse responses to CAM are reported to the World Health Organization's monitoring center is small compared with that of conventional drugs [130,131]. A mandatory national and/or international systematic reporting scheme for the collection of adverse responses to herbs is highly desirable, if difficult to implement [7,132]. Any new regulatory framework needs descriptions of Good Agricultural Practice, Good Laboratory Practice, Good Manufacturing Practice and Good Clinical Practice. Analytical techniques which provide reproducible fingerprinting to verify the chemical components of products, should help prevent the adulteration of preparations, either by similar but toxic plant species such as *Aristolochia* or by chemicals such as heavy metals or steroids.

Conclusion

Up to 50% of patients with IBD have tried some form of CAM. Although there is a wide range of therapies available, there is a lack of reliable data about the efficacy and safety of most remedies. This is in part a consequence of the problems associated with designing and funding clinical trials involving CAM modalities. Since patients with IBD are frequently and increasingly resorting to alternative and complementary therapies, it is imperative that efforts are accelerated to assess their therapeutic efficacy and safety and to regulate more closely their quality and marketing. Lastly, further education of doctors and other healthcare workers about the potential benefits and dangers of CAM is essential if we are to give well-informed advice to patients who are considering or already using CAM for their IBD.

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Chapter 49
Legal Pitfalls in Treating Inflammatory Bowel Disease Patients

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Summary
- The two areas of greatest medico-legal hazard in IBD are drug prescribing and management of acute colitis.
- Patients commencing azathioprine/mercaptopurine should have their thiopurine methyltransferase (TPMT) levels measured before commencing the drug.
- Responsibility for monitoring of immunosuppressive medications should be clearly assigned.
- Patients with acute colitis should be managed jointly by a gastroenterologist and specialist colorectal surgeon: general physicians and surgeons should not manage these patients.
- IBD physicians should comply with local/national guidelines on cancer surveillance in ulcerative colitis and screening for osteoporosis in IBD patients.
- Patients commencing colonoscopic cancer surveillance should be advised that surveillance has inherent limitations and that interval cancers may occur.

Introduction

There is a widespread perception in Western countries that negligence claims against doctors have risen dramatically in number over the last two decades. Medico-legal risk varies from country to country; in the UK, for example, the number of claims may have fallen following restrictions on government-funded Legal Aid (G. Neale, personal communication). Contributing factors include perceived poor communication on the part of doctors (although there is no evidence that doctors' communication skills have declined – the reverse is probably true), higher expectations of patients (fuelled in part by the easy access to medical information via the Internet) and the rising costs of medical care. Some lawyers have contributed in part by driving consumer demand for redress in the event of injury (and by advertising "no win, no fee" schemes). The most important factor, in my view, is the broad cultural shift which has occurred in developed countries. The last two decades have witnessed a profound shift in the relationship between doctors and patients. Paternalism (or what was perceived as paternalism) has died out, replaced by the concept of the patient as an equal partner in the decision-making process. The Internet has democratized access to information, both good and bad, for better and for worse. The patient is now primarily a consumer and consumerism now drives medical care.

As society has changed, so too has medicine. Sir Cyril Chantler remarked that "Medicine used to be simple, ineffective and relatively safe. Now it is complex, effective and potentially dangerous" [1]. The rise in medical litigation has led to the practice of so-called "defensive medicine", that is, the ordering of investigations and treatments which may not be clinically indicated, but which may protect the doctor from litigation. Unnecessary tests and treatments can never be ethically justified, even if they are perceived to offer some protection against litigation for the doctor. A more subtle form of defensive medicine is the avoidance of high-risk patients and high-risk procedures. Again, this strategy cannot be ethically justified, as it denies patients access to potentially life-saving treatments.

The climate of medical litigation varies enormously from country to country. For example, Japanese doctors are rarely sued [2], whereas the risk of a malpractice suit is part of daily life for the American physician. Medical litigation in the United States has reached the stage of crisis: insurance costs in some specialities are so high that parts of the country are without neurosurgeons, obstetricians and orthopedic surgeons [3]. The former Democratic Senators Hillary Clinton and Barack Obama introduced legislation, the National Error Disclosure and Compensation (MEDiC) Bill [4]: this model promotes disclosure of medical error to patients, with negotiation of compensation

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at the time of disclosure. In Norway, a compensation scheme was established in 1988, but litigants still have access to the courts and the traditional adversarial system [5]. Mexico has a similar system: in 1996, the Mexican government established the National Commission for Medical Arbitration (Conamed); about two-thirds of negligence cases are resolved by this agency without recourse to the courts [6]. Medical litigation in Italy is complex and frequently involves the criminal justice system; one Italian commentator has described the process faced by the doctor as the "*via crucis*" [7]. New Zealand has had a "no fault" system of compensation for many years [8]. In many other countries, such as the United States, the United Kingdom and Ireland, the traditional adversarial system or law of tort applies, with the patient as plaintiff and doctor as defendant. In the United Kingdom, the National Health Service (NHS) Redress Act was passed in November 2006. This Act introduces a scheme for redress without recourse to civil law. This scheme provides an alternative to (but not a substitute for) the traditional civil court system [9]. Complaints are investigated, liability assessed and a remedy offered to the complainant: the remedy may be an apology, explanation or financial compensation up to a maximum of £20,000.

There is general consensus that the law of tort, particularly in the United States, is in need of reform [3]. Although the tort system is widely regarded as capricious and unpredictable, a recent study from the United States showed that although claims that lack evidence are not uncommon, most were denied compensation [10]. The same study concluded, however, that the overhead costs of malpractice litigation are exorbitant.

As closer scrutiny of the medical profession continues apace, being sued is now merely one of a number of potential medico-legal adversities which may face the physician. (Some would say that being sued is a relatively benign process when compared with these disciplinary processes.) In the United Kingdom, doctors (particularly surgeons) may be suspended from clinical duties if a question has been raised about their competence: it can sometimes take years for the doctor to be reinstated, even if the allegations are subsequently shown to be groundless. (Suspended doctors have a substantial risk of suicide and may become de-skilled and unemployable.) Patients may make a formal complaint to professional regulatory bodies. such as the UK General Medical Council (GMC), and all such complaints, however trivial or vexatious, must be scrupulously investigated. Non-clinical activity may also lead to litigation or disciplinary action: examples of such non-clinical activities which have led to disciplinary or legal action include giving evidence in court as an expert witness, acting as part of an external review board to investigate doctors whose performance has caused concern and even writing a reference.

Clearly, medico-legal risk varies greatly from country to country, with cultural differences and variations in the judicial process and legal code. I do not intend to deal with medico-legal issues specific to certain countries. My remarks in this chapter are of a general nature and should be applicable to all doctors treating patients with inflammatory bowel disease (IBD).

The review in this chapter is not designed to be exhaustive; I have chosen a number of areas where medico-legal difficulties commonly arise and where simple preventive measures are easily applied. In researching this review, two recurring themes emerged as the main medico-legal pitfalls in IBD: management of acute colitis and the use of immunosuppressive medications.

Long-term management of the IBD patient

Most IBD patients are diagnosed initially as teenagers and young adults and thus face a lifetime of specialist care. In developed countries, the patient is likely to attend a gastroenterologist regularly and, increasingly, that gastroenterologist is likely to have a special interest in IBD. Outpatient care for IBD is increasingly delivered via specialist clinics and the gastroenterologist may be supported by a nurse or nurses, with a special expertise in IBD. Care may be shared with a primary-care physician (general practitioner): responsibility for monitoring immunosuppressive agents should be clearly assigned. Regular combined medical–surgical clinics facilitate decisions regarding surgical intervention in IBD patients. Over the many years of specialist care, the patient and the doctor develop a relationship: the healthier and more open this relationship, the fewer are the risks of litigation. At the time of initial diagnosis, patients with IBD require a careful explanation of the etiology, treatment and long-term prognosis of the condition. The IBD nurse specialist is of particular use in this aspect of management. Some units advocate "guided self-management with follow-up on request" for ulcerative colitis patients, and there is evidence that such an approach leads to quicker treatment of relapses [11]. More importantly, this form of long-term care is much preferred by patients compared with the traditional model [11]. This form of care has some medico-legal implications: it may not be suitable for patients with more severe disease, particularly those taking immunosuppressants.

Patients with IBD should have rapid access to the clinic in the event of a flare-up. A flexible appointments system and a telephone "hot line" should be available.

The role of the IBD nurse specialist is one which has developed in recent years, particularly in the United Kingdom. Typically, the nurse specialist is attached to a large, tertiary IBD center and carries out the following

duties: (1) advice and support for IBD patients; (2) manning a telephone helpline; (3) monitoring of immunosuppressive medications; (4) nursing support for clinical trials in IBD; and (5) liaison between primary care and the specialist clinic. A recent study from Cambridge found that the introduction of an IBD nurse specialist improved patient satisfaction and reduced hospital visits and inpatient bed utilization [12]. Hospitals employing IBD nurse specialists need to be clear about accountability and responsibility [13].

"Multidisciplinary care" and "clinical teams" have become the mantras of the age, with care delivered by physicians, surgeons, radiologists, specialist nurses and dietitians. The wise physician, however, should bear in mind that "we work in teams, but are blamed as individuals" [14].

Clinical guidelines

The rise in medical litigation over the last decade has been paralleled by the proliferation of clinical guidelines. Guidelines may be produced by specialist societies, Colleges (Royal or otherwise) and government agencies [such as the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom]. Hospitals may produce their own "in-house" guidelines. As we shall see later in this chapter, these various agencies may dispense conflicting advice, as in the case of cancer surveillance in ulcerative colitis. Guidelines have been defined as "evidence filtered through opinion" [15]. Some have expressed grave doubts as to the true evidence base of clinical guidelines and also concern that slavish adherence to guidelines may not always be in the patient's best interest. Grade A evidence (based on meta-analyses of randomized controlled trials) may not always be available for any given intervention: cancer surveillance in ulcerative colitis being a good example. There is increasing concern that failure to follow clinical guidelines may result in negligence claims. A recent review concluded that "guidelines do not actually set legal standards for clinical care but they do provide the courts with a benchmark by which to judge clinical conduct" [16]. Compliance with guidelines is likely to exonerate, but deviation from guidelines may be defensible.

Despite their widespread propagation, clinical guidelines have had a limited effect on altering doctors' everyday practice. There are many potential barriers to the successful implementation of guidelines, ranging from lack of awareness to inability to overcome the inertia of previous practice [17].

I believe that, in general, guidelines fulfill their dual roles of education and guiding clinical practice. Those guidelines which may not be based on the best evidence at least provide some practical direction for the doctor in difficult and controversial areas of care.

Drugs

Aminosalicylates

In an analysis of personal experience as an expert witness in 85 malpractice claims against gastroenterologists, Neale [18] identified 37 cases which arose from adverse events following endoscopy and 48 cases from clinical practice. Of these 48 cases, six were related to adverse reactions to drugs commonly used in IBD: four following treatment with sulfasalazine and two following treatment with steroids. The adverse reactions to sulfasalazine included thrombocytopenia, interstitial nephritis, peripheral neuropathy and toxic epidermal necrolysis. Adverse reactions to steroids included osteoporosis and avascular necrosis of head of femur. The majority of IBD patients will be on maintenance 5-aminosalicylic acid (5-ASA) therapy, although the evidence for this practice in Crohn's disease patients whose disease is predominantly small intestinal is controversial. IBD patients are now rarely commenced on sulfasalazine as first-line 5-ASA therapy, as the drug is more likely to cause side effects than the newer 5-ASA drugs. Side effects are generally related to the sulfapyridine moiety, although 5-ASA may rarely cause interstitial nephritis and blood disorders. It is therefore hard to justify using sulfasalazine as a first-choice 5-ASA drug in IBD. Nevertheless, if the patient has been taking the medication for some time without adverse effects, it would seem meddlesome to change it.

Patients starting any of the 5-ASA drugs should have their renal function checked before commencement. All patients commencing a 5-ASA drug (including sulfasalazine) should be advised to report any unexplained bleeding, bruising, purpura, sore throat, fever or malaise that occurs during treatment. A blood count should be performed and the drug stopped immediately if there is suspicion of blood dyscrasia.

In general, 5-ASAs are remarkably well tolerated and side effects are rare. A recent study of 5-ASA-associated nephrotoxicity reported an incidence of one in 4000 patients per year [19]. The risk of nephrotoxicity is unrelated to the dose or type of 5-ASA.

Corticosteroids

Steroids are the mainstay in the management of moderate to severe exacerbations of IBD. Unlike 5-ASA drugs, where side effects are rare and idiosyncratic, adverse reactions to steroids are common, dose related and predictable. Patients with IBD generally dislike taking steroids, but recognize their effectiveness. The standard steroid regimen used in our IBD clinic is of 12 weeks' duration, commencing at a dose of 40 mg daily. We use a standard tapering regimen (taper by 5 mg weekly to 20 mg and by 2.5 mg weekly thereafter), although there is no evidence to favor one regime over an other. I co-prescribe a calcium and

vitamin D preparation (Calcichew D3) along with cod liver oil. Oral budesonide may be an alternative to prednisolone in some Crohn's disease patients; it is generally better tolerated, but is not entirely free of corticosteroid-related side effects. Steroids are ineffective in maintenance of remission and patients requiring frequent courses of steroids should be commenced on second-line immunosuppressive therapy.

Thiopurines

These drugs are the most commonly used immunosuppressants in IBD. As patients are likely to be taking these medications for many years, careful counseling before commencing the drug is vital. Azathioprine is predominantly used in Europe, whereas 6-mercaptopurine (6-MP) is preferred in the United States. The main medico-legal issue with this family of drugs relates to the issue of whether thiopurine methyltransferase (TPMT) should be measured before commencing the drug. TPMT is one of a number of enzymes involved in the metabolism of 6-MP and azathioprine and deficiency of this enzyme may increase the risk of marrow suppression. TPMT controls the production of the 6-thioguanine nucleosides (6-TGN), which are responsible for thiopurine-induced myelotoxicity. The TPMT gene is polymorphic, resulting in interindividual variation of the enzyme's activity. Patients who are heterozygous for these alleles are at increased risk of myelotoxicity. Homozygotes almost invariably develop marrow toxicity when exposed to 6-MP/azathioprine. A recent study of TPMT variations reported normal levels in 89.5% of a European population, low levels in 9.9% (heterozygotes) and absent levels in 0.6% (homozygotes) [20]. The concordance rate between genotype and phenotype was 98.4%. Heterozygotes with intermediate activity can be treated safely by reducing the dose of thiopurine [21]. Should all patients commencing 6-MP/azathioprine have their TPMT status assessed? The question is a controversial one: the European Crohn's and Colitis Organization (ECCO) Consensus document on the management of Crohn's disease states [22]: "It [measurement of TPMT activity] cannot yet be recommended as a prerequisite to therapy, as decades of experience has shown AZA to be safe in clinical practice." Several other studies [23,24] have taken a differing view, with which I would concur. It should be borne in mind that TPMT accounts for only a minority of thiopurine-induced myelotoxicity [25], suggesting that other factors may also be responsible, which is why standard full blood count monitoring is still necessary even when TPMT levels are normal. Nevertheless, I believe that measurement of TPMT activity is reassuring for doctor and patient: 10% of patients will have intermediate activity and will do better on a lower dose; the very occasional patient (between 1 in 200 and 1 in 300) will have absent activity and is at risk of profound myelosuppression. Such cases are, it is true, rare, but I believe that identification of such patients is worthwhile. Should any IBD patient develop serious marrow suppression and the TPMT status has not been assessed, the doctor may be vulnerable.

Methotrexate (MTX)

Methotrexate (MTX) is the preferred second-line immunosuppressive agent in Crohn's disease. Evidence for its efficacy in ulcerative colitis is less convincing. Although the main studies in this area assessed intramuscular MTX, rather than the oral preparation, MTX is commonly given orally, for ease of administration. MTX is given once weekly and the greatest medico-legal risk with this medication is that it may inadvertently be given daily, leading to marrow suppression. This mistake is easier to commit than one might think: a guideline document on the management of Crohn's disease produced in 2006 by ECCO contained a printing error advising *daily* administration of MTX [22]: the error was quickly rectified. The NHS National Patient Safety Agency published and circulated a safety alert on MTX in 2004 [26], after it became apparent that over a 10 year period in England, 25 patients died, with a further 26 suffering harm, because the medicine had either not been used or monitored properly. The most common mistakes were: (1) the patient did not understand the correct dosage, perhaps because of lost or unclear instructions; (2) the drug was taken more frequently because it had been confused with other medication; (3) the wrong dose was given because of unclear packaging; and (4) confusion existed between healthcare providers as to who is responsible for monitoring of the drug. Pfizer and Mayne Pharma have already changed the shape of the 10 mg tablet to distinguish it from the 2.5 mg tablet. All patients taking MTX should be given folic acid.

Biological agents

Infliximab has been used for some years in the management of Crohn's disease and more recently in ulcerative colitis. Newer biologicals being assessed in IBD include natalizumab and adalumibab. Many more biological agents are in development. These agents constitute an entirely new area of medico-legal risk. Side effects are common and the overall mortality in Crohn's patients exposed to infliximab may be as high as 1%. A study from the Mayo Clinic of 500 patients treated with infliximab reported a 6% risk of serious adverse reactions and a 1% mortality [27]. The TREAT registry has been established in the United States to provide long-term safety data. This registry was established by the manufacturer, Centocor. A recent report concluded that mortality was not higher in infliximab-treated patients and that the increased risk of serious infection observed with infliximab was probably

due to disease severity and steroid use [28]. The principal risks with biologicals are sepsis and allergic reactions. Patients must be advised about these risks. Reactivation of tuberculosis is a particularly worrying adverse event. Until recently, it was standard practice to carry out chest X-ray and tuberculin testing on all patients. The issue of tuberculosis prevention in patients starting anti-tumor necrosis factor (TNF) treatment was addressed recently by the British Thoracic Society, along with representatives of the British Society of Gastroenterology and British Society of Rheumatology [29]. These UK guidelines advise chest X-ray, but not tuberculin testing. Tuberculin skin tests may be falsely negative in patients with Crohn's disease, particularly in severely ill and immunocompromised patients [30]. If the chest X-ray shows old tuberculosis changes or if there is a previous history of tuberculosis, 6 months' therapy with isoniazid is recommended; more significantly, the guidelines advise prophylactic isoniazid therapy in all patients from Sub-Saharan Africa and the Indian subcontinent, as well as other ethnic groups resident in the United Kingdom for less than 5 years.

I believe that biological agents pose a potentially huge medico-legal risk. IBD specialists should, I believe, adopt a more conservative approach to using these agents until their long-term effects are better known.

Complementary/alternative therapies

Many IBD patients use complementary and alternative medicine (CAM): a survey in 2003 reported that 60% of 150 IBD patients attending a tertiary center used CAM [31]. A recent review of CAM in IBD [32] found some limited evidence for the use of Chinese medicines, *aloe vera* gel, wheat grass juice, *Boswellia serrata* and bovine colostrum enemas in ulcerative colitis. Annual spending on herbal medicines in the United States is thought to exceed $5 billion annually. Although herbal medicines are regarded by patients as "natural" and therefore, safer, fatal renal and hepatic toxicity has been described with their use. There is no system in place for reporting adverse effects of CAM and use of these therapies is unregulated in most countries. More worryingly, a number of so-called herbal medications have been found to contain prescription medications such as corticosteroids, fenfluramine and glibenclamide.

IBD specialists are frequently asked to give advice on CAM; it is useful, therefore, to be familiar with the literature in this area, which has been well reviewed by Langmead and Rampton [32]. Patients should be advised that herbal medications are unregulated and may have serious side effects. IBD specialists should not take responsibility for monitoring these therapies and it must be made clear to the patient that the IBD specialist cannot take responsibility for adverse events associated with their use.

Acute colitis

Four of the cases documented by Neale [18] related to acute ulcerative colitis. In one patient there was delayed diagnosis of perforation and another with an unrecognized toxic dilatation: both patients died. In another unpublished series from a gastroenterologist with wide experience of medico-legal problems in gastroenterology, acute colitis was one of the most common areas of practice leading to negligence claims (A. Axon, personal communication): "Most people have come to grief because they have not monitored the patients properly, there has been inadequate discussion between physician and surgeon and lack of appreciation of the importance of toxic dilatation, too few X-rays being taken, people hanging on for a long time because the bowel habit has settled but the parameters (albumin, hemoglobin, etc.) have been worsening, lack of senior involvement in the management of the case and delayed diagnosis after admission." Stool culture is mandatory in all patients, even in those with known ulcerative colitis, as infective or amebic colitis can mimic an acute exacerbation of ulcerative colitis. Early sigmoidoscopy/colonoscopy with biopsy is mandatory; unexpected or unusual colitides, such as CMV colitis, will otherwise be missed. (I was once asked to give an expert opinion on a negligence claim involving a patient with colitis who required an emergency colectomy: histologic examination of the resected colon showed features of CMV colitis; preoperative sigmoidoscopy was performed but no biopsies were taken.)

A recent UK audit of the outcome of severe colitis admitted under non-specialist general physicians at a District General Hospital (DGH) reported a truly frightening mortality rate of 24% [33]. The British Society of Gastroenterology (BSG) operates a "Blue Card" scheme of voluntary reporting by gastroenterologists and recently evaluated 44 deaths in patients with ulcerative colitis [34]. About 47% of deaths occurred around the time of surgery and young people were disproportionately represented. Patients with severe ulcerative colitis should not be managed by general physicians and general surgeons.

For those patients with acute, severe ulcerative colitis who do not respond to steroid therapy, there is a choice between cyclosporin and infliximab therapy. The decision to use these agents should not be delayed. The Oxford group, for example, recommend starting second-line medication at day 3 [35]: at this stage, objective parameters [more than five bloody stools per day, C-reactive protein (CRP) >45] predict those patients who are likely to require colectomy. The choice of cyclosporin or infliximab is beyond the scope of this chapter. If cyclosporin is used, the lower dose of 2 mg kg^{-1} per day is as effective as the higher dose of 4 mg kg^{-1} per day with fewer side effects. Patients should have cholesterol levels checked to identify those patients

at risk of seizure. Renal function and blood pressure must be carefully monitored.

The advent of these newer, second-line therapies may paradoxically be a factor in the reported poor outcome in severe ulcerative colitis: patients are understandably reluctant to lose their colons and will be anxious to try any alternative drug therapy. This can lead to further delay in proceeding to colectomy. The physician may be persuaded by a frightened patient to persist with medical therapy when surgery would be in the individual's best interest.

Surgical intervention is frequently very late in acute colitis: the patient may be treated with steroids for up to 2 weeks, followed by a further week or more on cyclosporin. By the time colectomy is decided on, the patient is nutritionally and immunologically in a poor state and postoperative complications are common.

It is well recognized that IBD is a pro-thrombotic state, with a significant risk of venous thromboembolism, particularly in hospitalized patients [36]. For this reason, all hospitalized IBD patients, but particularly those with acute/fulminant colitis, should receive prophylactic low-dose heparin therapy.

In summary:

- Always do a stool culture on admission.
- Do early colonoscopy and biopsy (sigmoidoscopy is usually adequate – full colonoscopy carries a higher risk of perforation in acute colitis).
- Do daily plain X-rays (abdominal and chest).
- Involve the surgeon early.
- Make an early decision (day 3) on second-line medical therapy (infliximab, cyclosporin).
- Do not persist for too long with medical therapy: in particular, do not persist with intravenous steroids after 1 week.
- Patients are best managed jointly by a gastroenterologist with a special interest in IBD and a colorectal surgeon.

Delayed diagnosis of Crohn's disease

Crohn's disease may mimic a variety of other gastrointestinal disorders, such as celiac disease and irritable bowel syndrome. Indeed, these common disorders may coexist with Crohn's disease. In three such cases described by Neale [18], small bowel barium radiology had not been requested. The following may help avoid delayed diagnosis:

- Do ileoscopy routinely at colonoscopy.
- Always check inflammatory markers (particularly CRP) in patients with abdominal pain/diarrhea.
- Beware of diagnosing irritable bowel syndrome in older patients and do not make the diagnosis on the basis of history and physical examination alone.

Cancer surveillance

Guidelines for cancer surveillance in IBD have been produced by a number of bodies, including the British Society of Gastroenterology (BSG), American Society for Gastrointestinal Endoscopy (ASGE), the American Gastroenterology Association (AGA), the American College of Gastroenterology (ACG) and the Crohn's and Colitis Foundation of America Colon Cancer in IBD Study Group [37]. It has been pointed out that these various guidelines differ in their surveillance protocols *vis-à-vis* commencement and frequency of surveillance [37]. For example, the ACG Guidelines recommend annual or biannual surveillance after 8–10 years of colitis [38]. The guidelines do not differentiate between left-sided and total colitis. The BSG guidelines [39], on the other hand, recommend commencement of surveillance at 8–10 years in patients with pancolitis and at 15–20 years in patients with left-sided colitis. These guidelines recommend a decrease in the screening interval with increasing disease duration, with screening every 3 years in the second decade of disease, every 2 years in the third decade and annually after the fourth decade. A patient with pancolitis living in London might therefore have surveillance colonoscopy every 3 years after 10 years of disease, whereas a similar patient living in New York, might have surveillance done *six* times more frequently, at 6 monthly intervals. Is the New York patient better managed than the London patient? There are no data to guide us: indeed, there is no evidence that surveillance is a worthwhile exercise. Although some have cast considerable doubt on the usefulness of surveillance programs [40], it would be a very brave physician who would not carry out surveillance. As there is no evidence to favor one surveillance protocol over another, it would seem sensible to follow local guidelines: When in Rome, do as the Romans! In the United Kingdom, the BSG guidelines have attracted controversy and a recent audit demonstrated significant divergence from these guidelines [37].

The principal medico-legal risk relating to cancer surveillance in IBD is the issue of interval cancer. Patients need to be made aware that colonoscopy has inherent limitations: these limitations may be related to the procedure itself and to the biology of colon cancer complicating IBD. "Tandem" colonoscopy studies have demonstrated that lesions of less than 1 cm diameter are commonly missed [41]; over-rapid withdrawal and poor bowel preparation may also increase the risk of missing lesions. Compared with sporadic colon cancer, cancers arising on a background of IBD are more likely to be multiple, anaplastic and more likely to arise from flat mucosa [38].

Multiple biopsies should be taken every 10 cm, taking a minimum of 30 biopsies [42]. High-grade dysplasia or cancer is a clear indication for colectomy, but there is controversy regarding low-grade dysplasia. The ACG guidelines [38] recommend surgery for low-grade dysplasia arising

from flat mucosa or a DALM (dysplasia associated with lesion or mass), whereas low-grade dysplasia associated with a sporadic adenoma may be managed by continued endoscopic surveillance.

Patients with IBD commencing colonoscopic surveillance need to be carefully counseled. The patient should be advised that surveillance is now uniformly practiced, although there is no evidence that this practice reduces the risk of death from colon cancer in IBD. The patient should be advised that surveillance has inherent limitations, related to both technical aspects of the procedure itself and to the biology of cancer in IBD. Rex *et al.* [43] have reviewed the medico-legal aspects of interval cancers in colonoscopic surveillance. Potential explanations for incident cancers following a "clear" colonoscopy include (1) the inherent miss rate of colonoscopy, (2) variable growth of colorectal adenomas and cancers and (3) the phenomenon of flat neoplasms. They recommend the following measures to reduce medico-legal risk: (1) informed consent regarding not just the potential complications of colonoscopy, but also the inherent limitations of the procedure for cancer surveillance in IBD; (2) documentation of cecal intubation (preferably by photography); (3) description of bowel preparation; (4) documentation of examination time; (5) use of careful withdrawal technique; and (6) low threshold for biopsy of subtle lesions.

It should not be forgotten that Crohn's colitis carries a similar risk of colon cancer as ulcerative colitis and these patients should also undergo surveillance [39].

Surgical issues

It is vitally important to carry out a careful histological assessment of the resected colectomy specimen in patients who are being considered for an ileo-anal pouch. If there is any question that the diagnosis may be Crohn's disease, rather than ulcerative colitis, pouch surgery should not be carried out. Even if the histology is consistent with ulcerative colitis, it is possible that a diagnosis of Crohn's disease may declare itself some time after pouch surgery and patients need to be made aware of this.

The most important aspect of surgical practice in IBD from a medico-legal point of view is specialization. Surgery in IBD patients is often complex and hazardous and should be carried out by a gastrointestinal surgeon with expertise in IBD.

Osteoporosis

There are several international guidelines for osteoporosis screening and treatment in IBD patients [44,45]. The increase in fracture risk in IBD patients is modest, with a relative risk of 1.3 for Crohn's disease and 1.2 for ulcerative colitis [44]. The new BSG guidelines [44] now recommend bisphosphonate therapy in older (over 65 years) IBD patients commencing a course of systemic steroids.

Avascular necrosis

Avascular necrosis (osteonecrosis) typically involves the head of the femur and is a well-recognized, if rare, complication of IBD. Historically, this complication has been linked to corticosteroid use, but a Canadian study found no link with steroid use [46]. Carter has reported on two cases of avascular necrosis complication IBD which led to negligence claims, with awards in excess of $1 million [47].

Intestinal tuberculosis

Intestinal tuberculosis may mimic Crohn's disease and clinicians should be aware of this as a possible diagnosis. We have reported a series of five cases of intestinal tuberculosis referred to our IBD clinic with a diagnosis of Crohn's disease [48]. Three of the patients were from India and South-East Asia. Misdiagnosis of intestinal tuberculosis as Crohn's disease can have disastrous consequences if steroids or biological agents are given.

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Chapter 50
The Present and Future of Research and Treatment of Inflammatory Bowel Disease

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The foregoing chapters describe the current state of research and clinical practice in inflammatory bowel diseases. Despite spectacular progress and pivotal improvements in the techniques available to researchers and physicians studying and treating these diseases, we have not fully defined the pathogeneses, our treatments remain imperfect and prevention remains a very distant goal. As we move forward, the primary considerations become: How do we build on this solid platform of discovery? How best do we approach the future and achieve our objectives?

A decade ago, we saw the beginning of the 21st century as the dawn of the "Age of Translationalism" [1]. We projected then that the keys to advancement in IBD lay in four areas: (1) bidirectional translation of rodent and genetic research findings to identify variants with relevance to human disease; (2) definition of relevant bacterial antigens for a better understanding of environmental triggers; (3) carefully designed clinical trials that would lead to more efficacious, targeted therapies; and (4) "reagent grade" populations to allow personalization of diagnosis and prognosis, to indicate therapeutic targets and individualized treatment plans.

Bidirectional translation of rodent and genetic research

In keeping with our predictions, this decade started with the discovery of an association between NOD2 variants and Crohn's disease that was made using a series of randomly defined candidate genes, generally in small numbers of patients. In terms of technology available to support our goals, the last decade saw the advent of "affordable" high-throughput molecular technologies that have yielded highly significant findings about which genes are relevant to which molecules and pathways and which may be most relevant to inflammatory bowel diseases. Currently, researchers are finalizing complete characterization of the "IBD genome" and identifying the molecules, pathways and processes unique to certain subsets of patients. As discussed in Chapter 2, with the important molecules defined, we must return to the laboratory to understand precisely how these are related to immune pathogenesis and focus investigation on abnormalities that are known to be relevant to disease. As scientists, our task is two-fold. First, we must perform scientifically informed *ex vivo* biology on tissues collected from well-characterized subjects. Second, we must generate very precise *in vivo* correlates to understand the pathogenic pathways.

Definition of relevant bacterial antigens

We have also made progress in understanding the microenvironment and increased focus on the role of the "microbiome." Substantial advances have been made in understanding the mechanisms by which specific antigens and commensal associated molecular patterns are sensed and responded to by the host. Several antigens have been defined which appear to be sources of immunoreactivity by patients with inflammatory bowel disease patients. We now know that the inflammatory response is underpinned by genetic variation in both innate and adaptive immune mechanisms. High-throughput genetic technology that has been used to unravel the complex human genome can be modified to aid in understanding the complex bacterial microbiome related to inflammatory bowel disease. Further investigation is needed to clarify the role played by the microbiome in altering expression of the important regulatory immune molecules and the role

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played by inflammation in altering the microbiome. This integrative science will identify the microenvironmental consequences of the gene variants common to groups of patients with IBD.

Intelligently designed clinical trials

The cooperation of academic institutions and the pharmaceutical industry is resulting in targeted therapeutic efficacy. A decade ago, there appeared to be too many potential therapeutic targets and too few patients to evaluate efficacy if the standard approaches were applied to clinical trials. Almost a decade later, there are even more targets, making established clinical research techniques obsolete and wasteful. Careful analysis of clinical trial results continues to reveal that intelligent design is needed to identify subpopulations in which certain treatments are most beneficial and, in the coming year, the first drug trial in which the treatment groups will be selected by genotypes reflecting the target molecule will begin.

"Reagent grade" populations

Our efforts to define IBD phenotypes are now combining genetic, clinical and immunologic information to predict disease course. The goal of defining "reagents grade" populations is customization of diagnosis, prognosis and treatment plans – what is now known as "personalized medicine." Another goal is the acceleration of drug development by characterizing patients who are most likely to benefit from early intervention and thus most likely to respond to manipulation of a population-specific therapeutic target. Drug toxicity has emerged as a significant issue in acceptance of novel therapeutics. Therefore, analyzing the reasons why some patients will not respond will advance the field and, more importantly, eliminate undue exposure to potent therapies with little potential for working. Further understanding of the reasons for response or lack thereof will allow treatment of patients with the most effective therapy much sooner after diagnosis and spare patients from undergoing therapy with ineffective, non-specific treatment and preventing their disease from progressing to a more severe state with more complications. The challenge in the next decade is to define the reagent grade patients by combining a deeper understanding of the microbiome with further delineation of the IBD genome in order to stratify patients appropriately for such approaches.

The need for multidisciplinary investigations in inflammatory bowel diseases has long been and will remain a part of the scientific rhetoric; however, operationalization of this concept must now be refined. The data generated at an unprecedented pace by spectacular advances in genetic research now inform biologic research in such a way that ever more precise findings are yielding quickly to clinical application. It is rewarding to review this century's first decade in the context of progress that is the building block of a platform from which to start what we hope will be our final charge toward personalized therapy for inflammatory bowel disease patients and ultimately, to prevent onset altogether. Is prevention on the horizon? We believe so. There is much work to be done, but the groundwork can be laid now. Complete characterization of the "IBD genome" will set the stage for thorough micro- and macro-epidemiologic studies that can define the natural risk and natural history of IBD, first in families and then in the general population. The results of these studies will show how early and later life environmental alterations could be blocked to interfere with the host interface with certain pathogens. Ultimately, IBD researchers should be able to harness stem cell technology to "design" multi-capacity cells to target alteration of specific genomic profiles. A key element for success is the establishment of natural history defined cohorts in which studies can be performed as the state of the art advances.

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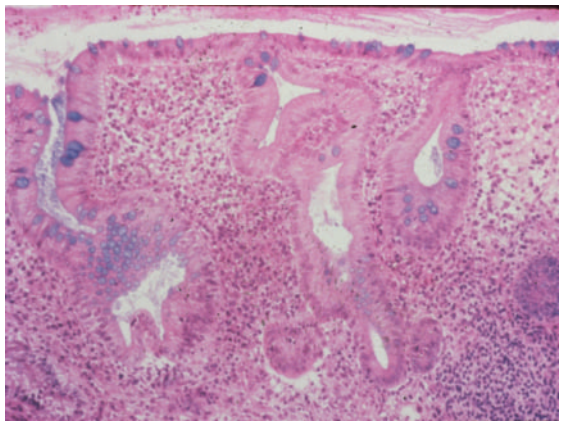


Plate 21.1 Rectal biopsy from a patient with active ulcerative colitis shows distorted crypt architecture and diffuse lamina propria inflammation with acute and chronic inflammatory cells. Reprinted with permission from Surawicz CM. Diagnosing colitis. *Contemp Intern Med* 1991; **3**:19.

Plate 21.2 Rectal biopsy from a patient with *Campylobacter* colitis. Note normal architecture, predominately acute inflammatory colitis in the lamina propria and crypt abscesses. Right string of pearls crypt abscess (higher power).

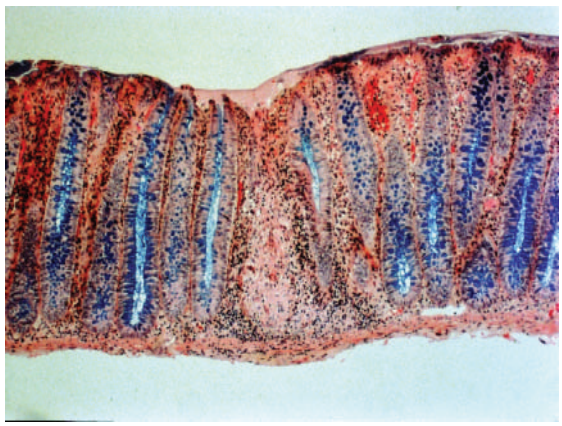


Plate 21.3 Rectal biopsy from a patient with Crohn's disease shows a typical epithelioid granuloma in otherwise normal mucosa. Reprinted with permission from Surawicz CM. Diagnosing colitis. *Contemp Intern Med* 1991; **3**:20.

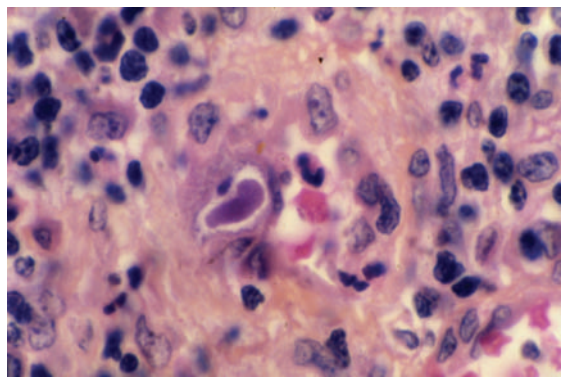
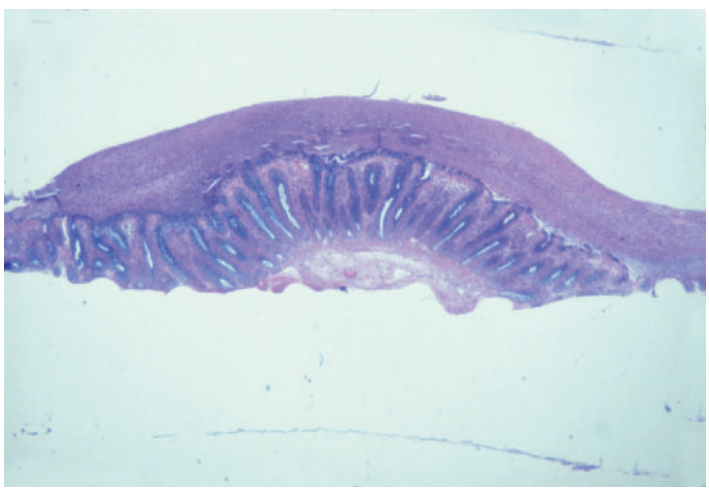
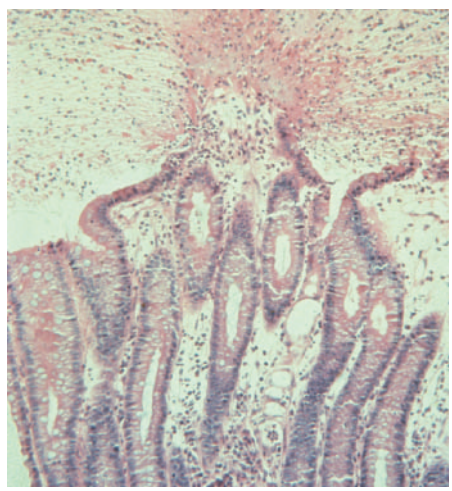


Plate 21.4 Cytomegalovirus inclusion in an endothelial cell in a colonic biopsy from a woman with a self-limited colitis due to cytomegalovirus. Note the typical cytomegalovirus intranuclear inclusion surrounded by a clear halo; the cytoplasm is also enlarged. This cell is diagnostic of cytomegalovirus. Reprinted with permission from Surawicz CM, Myerson D. Self limiting cytomegalovirus colitis in immunocompetent individuals. *Gastroenterology* 1988; **94**:194–9.



(a)



(b)

Plate 21.5 (a) Rectal biopsy from a woman with pseudomembranous colitis. (b) Note the pseudomembrane, composed of fibrin, polymorphonuclear cells and debris, eruption form the surface in a "volcanic" manner.

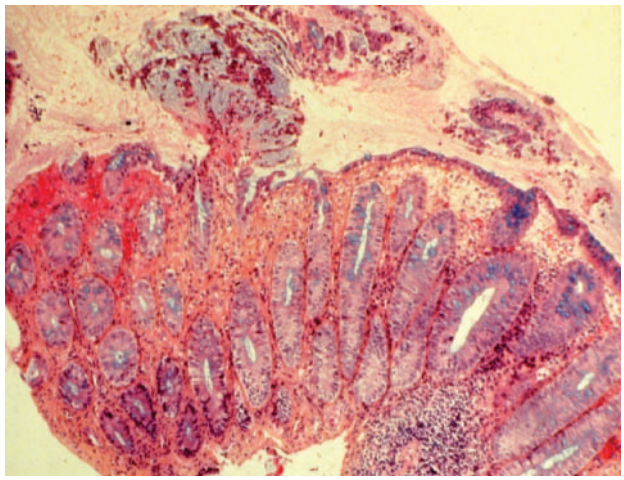


Plate 21.6 Rectal biopsy from a young woman with *E. coli* 0157:H7 colitis, who developed hemolytic uremic syndrome. Crypt architecture is normal; there is superficial necrosis and a pseudomembrane. Histologically, these features can suggest ischemia or pseudomembranous colitis.

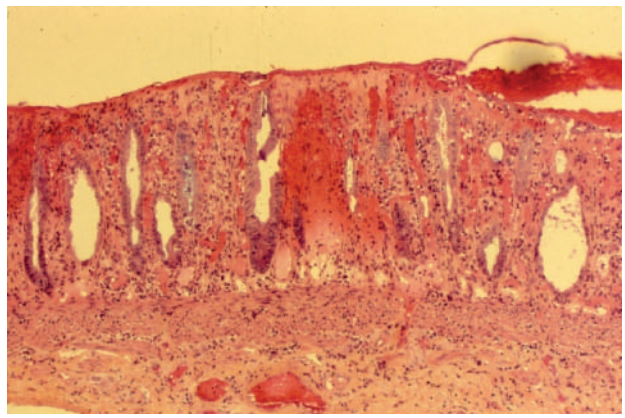


Plate 21.7 Colorectal biopsy from a patient with ischemic colitis shows superficial necrosis and “erased" crypts without an inflammatory response. Reproduced with permission from Dr Cyrus E. Rubin, University of Washington, Seattle, WA.

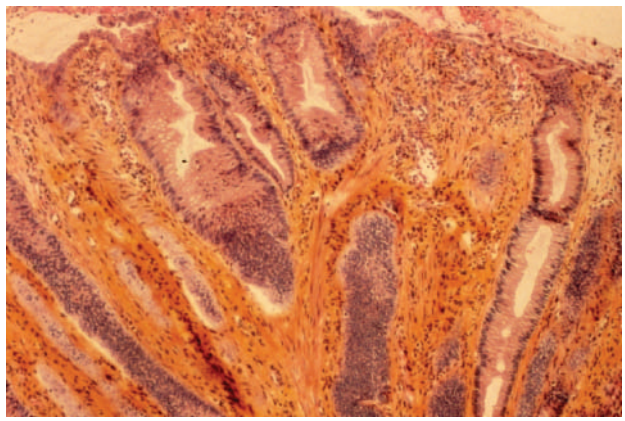
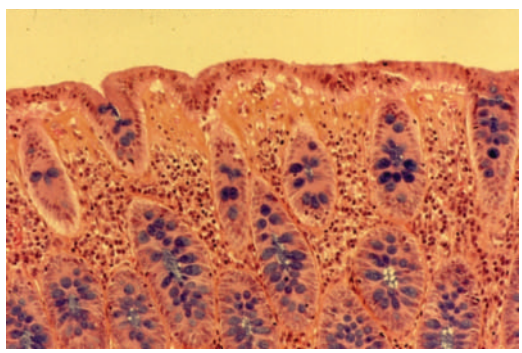
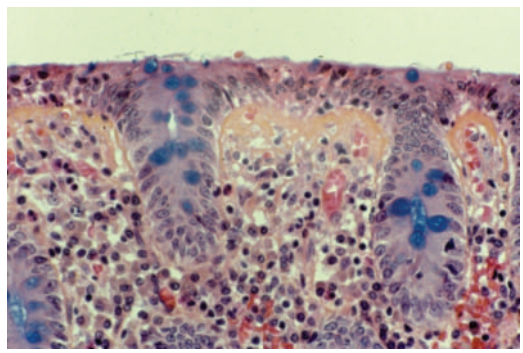


Plate 21.8 Rectal biopsy from a patient with solitary rectal ulcer syndrome shows classic pathological findings of hyperplastic crypts and mucosal fibrosis. The excess diffuse mucosal collagen in the lamina propria is easily seen when stained yellow with saffron. Reproduced with permission from Dr Douglas Levine, Seattle, WA.



(a)



(b)

Plate 21.9 Two biopsies from a patient with collagenous colitis, (a) collagenous colitis and (b) collagenous colitis – patch, showing the thickened subepithelial band of collagen (here stained yellow with saffron). Note that the collagen band is focal; hence multiple biopsies should be taken to exclude the diagnosis.

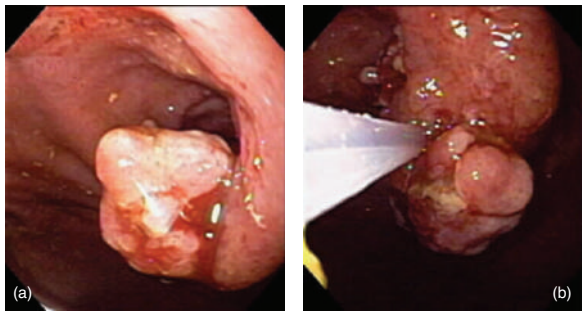


Plate 30.1 Chronic pouchitis with a large inflammatory polyp: endoscopic snare polypectomy was performed.

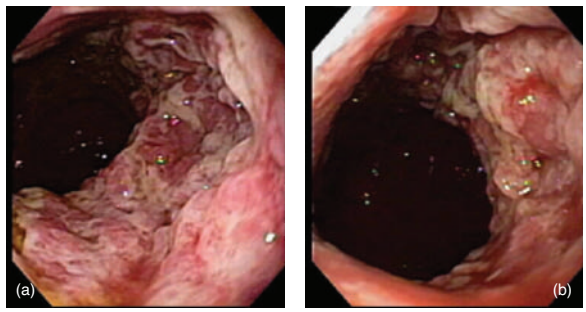


Plate 30.2 Concurrent severe pouchitis (a) and cuffitis (b) in a pregnant woman.

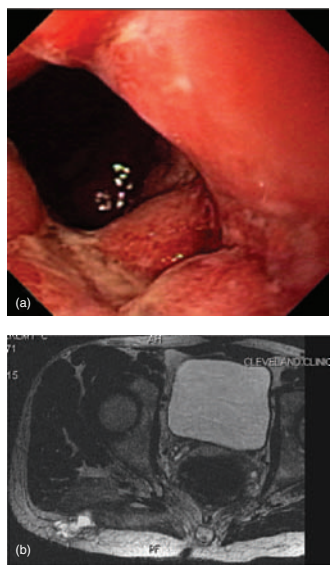


Plate 30.3 Fistulizing CD of the pouch. Nodularity of rectal cuff on pouch endoscopy (a); multiseptated fluid collection is seen in the subcutaneous region of the right buttock measuring 3.2 × 1.7 cm in axial images of MRI (b).

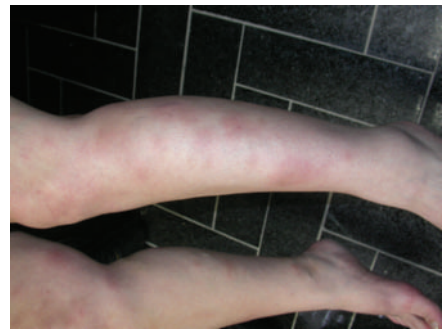


Plate 38.1 The typical erythematous, raised, tender nodules of erythema nodosum. Photograph courtesy of Dr Shane Silver.

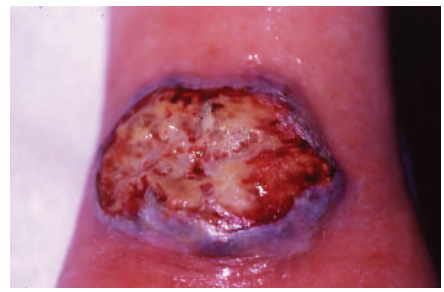


Plate 38.2 The centrally ulcerated, necrotic ulcer with a classic violaceous border, typical of pyoderma gangrenosum. Photograph courtesy of Dr Shane Silver.

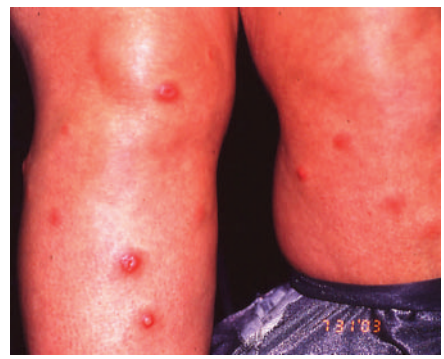


Plate 38.3 The urticarial lesions with central umbilication of Sweet's syndrome. Photograph courtesy of Dr Shane Silver.

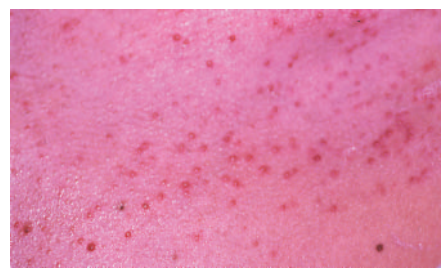


Plate 38.4 Corticosteroid-induced acne. Photograph courtesy of Dr Shane Silver.

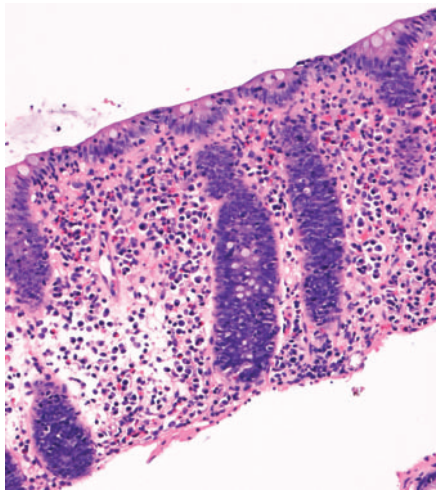


Plate 41.1 Diffuse intra-epithelial lymphocytosis and cuboidal flattening of surface colonocytes in lymphocytic colitis (H&E, ×200).

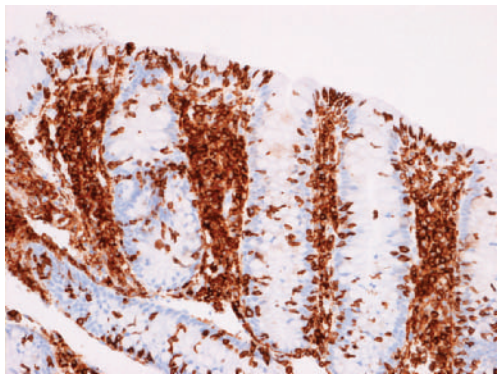


Plate 41.2 Common leukocyte antigen (CLA) immunostaining confirming the diagnosis of lymphocytic colitis (×400).

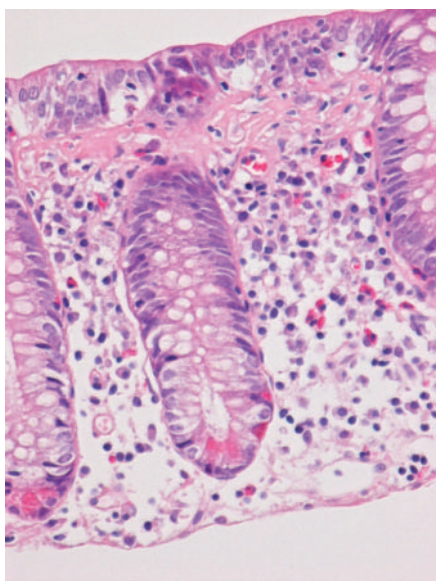


Plate 41.3 Lacy infiltration of superficial lamina propria by collagen (H&E, ×400).

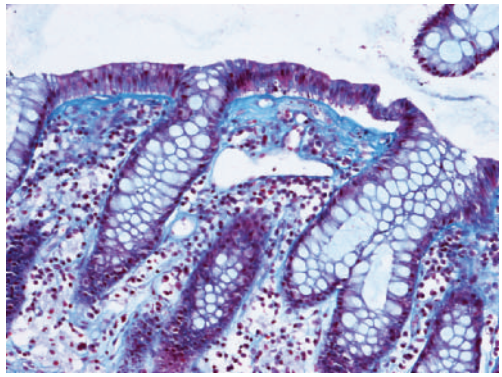


Plate 41.4 Increased subepithelial collagen stains blue (Masson Trichrome, ×400).

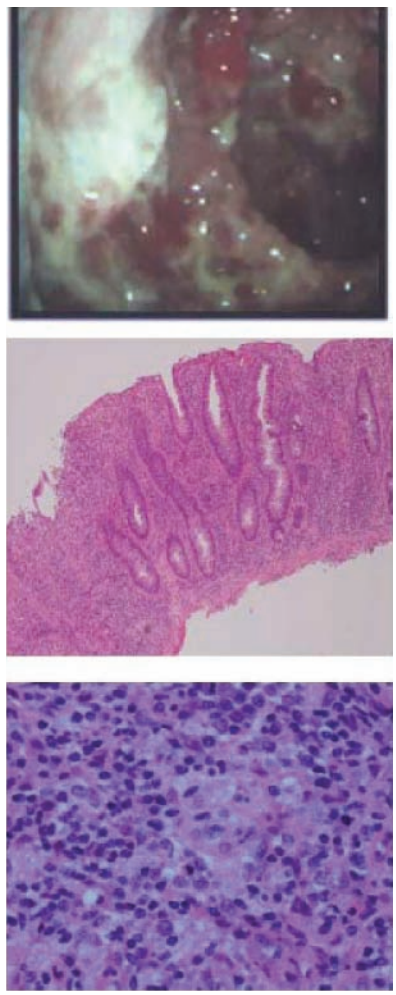


Plate 45.1 Endoscopic and histologic appearances of a patient with lymphogranuloma venereum (LGV)-associated proctitis