The Pathogenesis of Inflammatory Bowel Disease: Translational Implications for Clinicians

Maria T. Abreu, MD

Department of Medicine, Division of Gastroenterology, Inflammatory Bowel Disease Center, Cedars-Sinai Medical Center, 8631 West 3rd Street, Suite 245E, Los Angeles, CA 90048, USA.
E-mail: Maria.Abreu@cshs.org

Current Gastroenterology Reports 2002, **4:**481–489
Current Science Inc. ISSN 1522-8037

Research in the pathogenesis of inflammatory bowel disease (IBD) has dramatically broadened our understanding of these complex disorders. These clinical manifestations result from a dysregulated immune response in the presence of luminal bacteria. Recent identification of mutations in the *NOD2* gene, a protein involved in the sensing of bacteria, offers genetic support for the model of perturbed host-microbial interactions in Crohn's disease. Several immunologic pathways have been identified that play a role in maintaining gut immune homeostasis. Abnormal expression of proinflammatory, deleterious cytokines such as tumor necrosis factor-α and interferon-γ results in direct and indirect tissue damage. The search for specific causative microbial agents in IBD continues to be intense. This paper describes the advances in our understanding of IBD pathogenesis, with an emphasis on how this information is translated into patient care. The next stage of research will take advantage of such molecular biologic techniques to identify new pathogenetic mechanisms and targets for therapy tailored to individual patients.

Introduction

Since the first description of inflammatory bowel disease (IBD) and its successful treatment with corticosteroids, clinicians and scientists have been studying IBD pathogenesis with the hope of improving therapy for these diseases. Until relatively recently, corticosteroids and surgery dominated the treatment of IBD, often with devastating consequences. This paper distills the progress that has been made in understanding the pathogenesis of IBD. Therapies that we use currently for patients with IBD, as well as those on the horizon, target some aspect of the pathogenetic process. This review is intended to help clinicians to understand how observations made at the bench are translated into the clinical setting.

Translational Research in Inflammatory Bowel Disease

Modern molecular biology and high-throughput drug development offer the promise of translating the work of basic scientists into therapies that can be used in the care of patients with IBD. The relationship between clinicians and basic scientists should be a symbiotic one. Clinicians make observations about disease behavior, familial patterns of inheritance, and responses to therapy. These observations in turn guide scientists to identify pathogenetic mechanisms and direct drug development. Occasionally, a therapy works better than anticipated in the clinical arena, as with anti–tumor necrosis factor (TNF)-α, and prompts scientists to revisit the bench to identify additional mechanisms that may be at play. With more precise identification of the mechanisms causing IBD, the therapies we use to treat IBD have become more sophisticated and specific. Clinicians at the front lines of treating patients with IBD should therefore have an understanding of IBD pathogenesis to administer therapy rationally and deal with its consequences, both good and bad. Thus, modern research in IBD is characterized by its continuous shift from the bench to the bedside and back to the bench.

Understanding the Pathogenesis of Inflammatory Bowel Diseases

A combination of clinical and basic research has provided a picture of the pathogenetic mechanisms that culminate in IBD (Fig. 1). The principal hallmark of these diseases is the presence of intestinal inflammation. This inflammation results in perturbed barrier function, which fuels the flame further by exposing the mucosal immune system directly to luminal bacterial products. Although patients may have extraintestinal manifestations, the primary inflammatory problem resides in the gut and is not part of a generalized autoimmune syndrome. Clinicians have long recognized that there must be a genetic component given that these diseases are often present in family members.

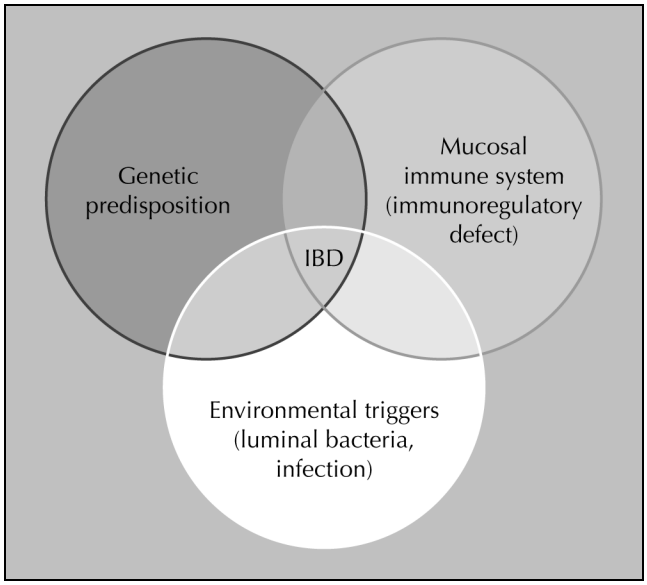

Figure 1. Pathogenesis of inflammatory bowel disease (IBD). The interplay of genes and the environment culminates in chronic intestinal inflammation.

Even in the genetically predisposed host, environmental triggers are required to unleash the intestinal inflammation. These environmental triggers may be bacterial, viral, or dietary. In general, we classify patients with IBD as those with Crohn's disease and those with ulcerative colitis. Clinicians and researchers recognize, however, that there is great diversity within Crohn's disease and ulcerative colitis. This clinical diversity suggests that the underlying pathogenetic mechanisms may also be distinct. An understanding of the specific genetic, immunologic, and microbial mechanisms resulting in chronic, debilitating intestinal inflammation will result in improved therapy for IBD.

How do we identify IBD genes?

With the sequence of the human genome nearly complete, it may seem that the job of geneticists would be finished. Although an improved road map has made research in many fields easier, the task of identifying specific genetic associations in complex diseases remains a difficult one. The imperfections in genes that result in disease susceptibility may be quite subtle and require the input of the environment for their full expression. The other caveat for clinicians trying to interpret the genetic literature is that different ethnic groups may carry distinct genetic mutations that result in disease and, therefore, different research groups may obtain different results depending on the population they are studying. In spite of these intricacies, enormous progress has been made in the identification of genes associated with IBD [1•].

Geneticists have used two approaches to identify genetic associations in complex diseases such as IBD. The first is termed the "candidate gene" approach. In this strategy, researchers begin with a hypothesis about which gene is responsible for IBD and then examine whether there are consistent differences between patients and unaffected control subjects at that gene locus. An example of the information gleaned using this approach is the association of major histocompatibility complex (MHC) genes in IBD. Because MHC genes are intimately involved in the ability of the immune system to distinguish self from non-self, investigators reasoned that certain MHC genes may predispose an individual to the aberrant recognition of self in IBD. The findings of multiple groups support an association in this region, and the following section describes the clinical implications of these findings.

How do we use this information in the clinic?

The alternative strategy to the candidate gene approach is "genome-wide scanning." In this approach, scientists take an unbiased view of the genes that may be associated with a particular disease by examining the entire genome for differences between IBD patients and ethnically matched control subjects. Once a general region on a chromosome is identified as containing a potential gene of interest, finer and finer mapping of this region eventually pinpoints the gene containing a mutation or variation that is associated with a disease. The greatest success using this approach is the identification of the *NOD2* gene and its association with Crohn's disease. Using genome-wide scanning, Hugot *et al*. [2•] first described a region on chromosome 16 associated with Crohn's disease. Ultimately, positional cloning and sequencing of a large region of chromosome 16 identified *NOD2* as the principal gene in this region associated with Crohn's disease. Simultaneously, Ogura *et al*. [3] had cloned the *NOD2* gene and found that it had similarities to disease-resistance genes in plants and was also located on chromosome 16. The latter authors reasoned that this gene might be involved in Crohn's disease based on its function and location. Thus, the *NOD2* gene was identified independently using both the candidate gene and genome-wide scanning approaches [3].

The promise of genetic research is to identify the underlying causes of IBD and thereby attain improved therapy. Because the inflammatory bowel diseases are multigenic, gene therapies being explored for single-gene diseases, including cystic fibrosis and hemophilia, are unlikely to provide a cure. The true benefit of gene identification is to focus research on the pathogenetic mechanisms that lie distal to the function of that gene. For example, mutations in *NOD2* result in diminished immune cell activation in the presence of lipopolysaccharide (LPS) (Fig. 2). At first blush, this may seem counterintuitive, given that we believe IBD patients have abnormally increased immunologic responsiveness to enteric flora. A provocative hypothesis to reconcile these observations is that patients with *NOD2* mutations may be susceptible to a chronic intracellular infection or may not develop a tolerizing immune response in the presence of commensal flora. By studying the normal and abnormal function of *NOD2*, we may identify a therapy that corrects

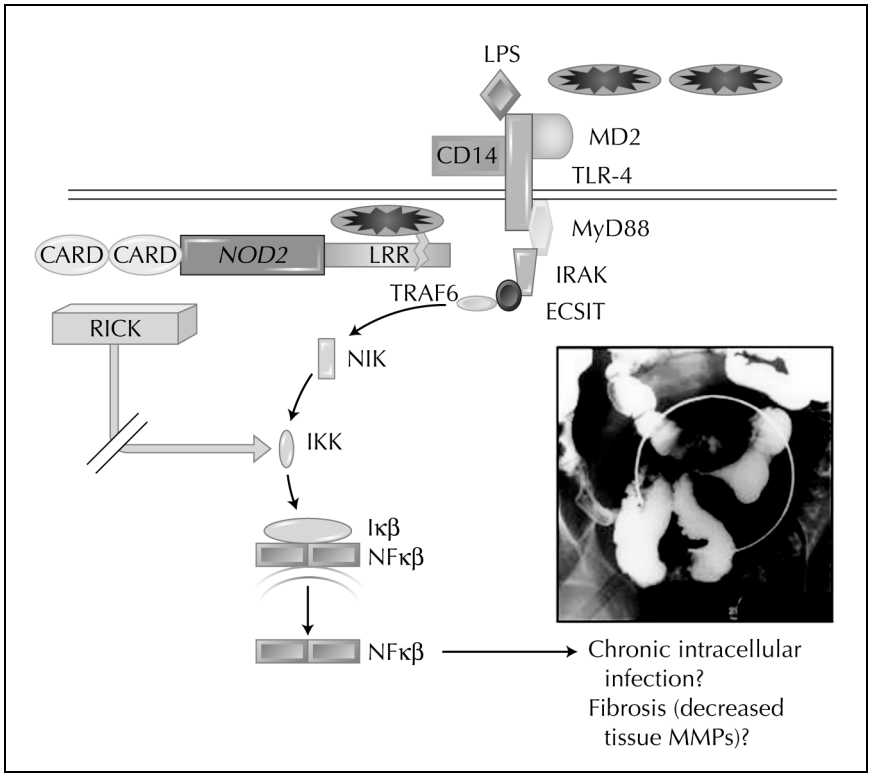

Figure 2. Model of *NOD2* mutation in the pathogenesis of Crohn's disease. Mutations in *NOD2* result in diminished NFκβ activation in lipopolysaccharide (LPS). Patients with *NOD2* mutations are more likely to develop fibrostenotic complications. The link between the molecular phenotype and the clinical phenotype is unclear.

or ameliorates the deficient function downstream of *NOD2* in patients with these mutations.

Perhaps a more tangible application of genetic information is for prediction of who will develop IBD and what the characteristics of the disease will be. In spite of the multiple genetic associations described in IBD, no genetic mutation or combination of genetic mutations is sufficiently sensitive or specific to identify patients who will develop IBD. Even for people who inherit two mutations in *NOD2* (one from each parent), the predicted absolute risk for developing Crohn's disease is only about 3%. Without a known way to prevent the development of IBD in patients at high risk for the disease, this type of genetic information can result in unwarranted anxiety.

We and others have described the clinical phenotype of patients carrying *NOD2* mutations and found that they are more likely to develop fibrostenosing complications of Crohn's disease, compared with patients who do not have *NOD2* mutations [4]. Whereas patients who were not carriers of *NOD2* mutations demonstrated fibrostenosing disease 43% of the time, patients who were carriers of one mutation demonstrated fibrostenosing 64% of the time, and patients with two mutations demonstrated fibrostenosing 85% of the time. These data suggest that carriage of *NOD2* mutations may predict who will develop fibrostenotic complications. In practice, however, the frequency of fibrostenosis is so high even in patients without *NOD2* mutations that the positive predictive value of having *NOD2* mutations is unacceptable for clinical decision making. The combination of *NOD2* mutations with other available serologic tests may improve the diagnostic value of the former.

Another dimension in which genetic information may be valuable is to predict response to therapy. Polymorphisms (*ie*, genetic variations) within the TNF-α promoter have been associated with steroid-dependent Crohn's disease, and polymorphisms at the MHC class I chain gene A have been associated with peripheral arthropathy in ulcerative colitis. The knowledge we have gained from genetic research has not yet made the transition to the bedside but soon will. Combined with knowledge of disease pathogenesis, genetic information may some day be useful to prevent the development of IBD in patients at high risk and to predict response to therapy.

Navigating the alphabet soup of immunology: What does it mean to the clinician and the patient?

Conceptually, the immune system is designed around protection of the host from danger. Whereas bacteria in contact with any other epithelia, such as those in the lung or bladder, would trigger a potent inflammatory response, the epithelium and the associated mucosal immune system of the intestine have developed ways to protect against chronic inflammation (Fig. 3). The epithelial cells themselves represent the first layer of protection. The intestinal epithelial cells form a relatively impermeable barrier that does not permit the passage of LPS or bacteria unless the bacteria are themselves pathogenetic and invasive. We and others have also demonstrated that colonic epithelial cells express low levels of the receptors that recognize LPS and are not LPS responsive [5]. The design of this epithelial system, therefore, is to limit proinflammatory signaling in response to normal commensal flora. Sampling of bacteria and bacterial products is

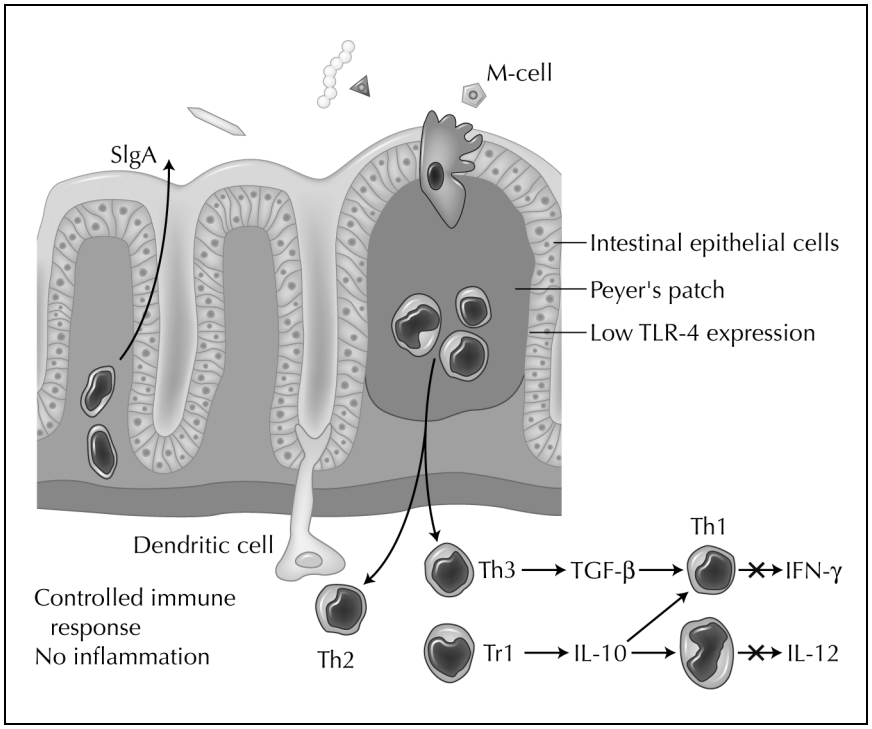

Figure 3. The intestinal epithelium forms an intact barrier that prevents the passage of bacteria and bacterial products. Sampling of the luminal contents occurs constantly through the action of specialized epithelial cells, M cells, which transport luminal antigens to the lymphoid follicles of Peyer's patches. In addition, dendritic cells inserted in the epithelial monolayer perform a similar function to sample the luminal contents. This system of sampling and controlled antigen presentation permits the mucosal immune system to develop a tolerizing or "anti-inflammatory" response to commensal flora characterized by production of transforming growth factor (TGF)-β and interleukin (IL)-10. TLR—toll-like receptor.

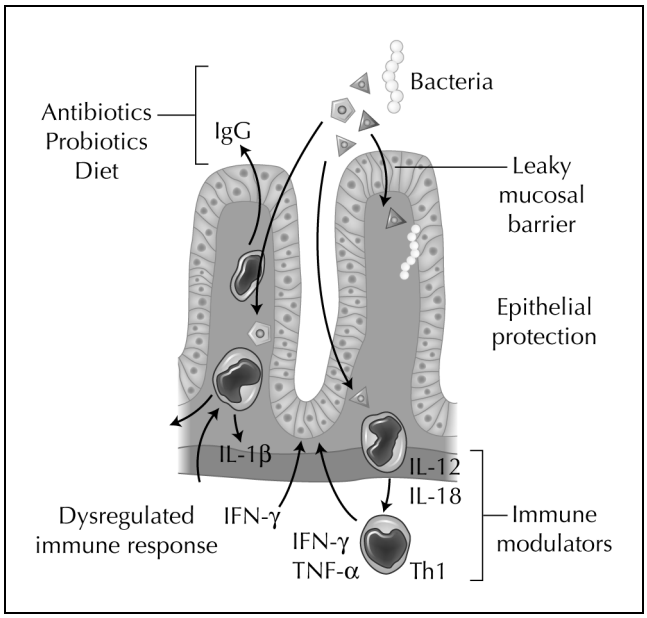

Figure 4. Targeting of therapy to pathogenesis is depicted. There are multiple approaches to treatment of IBD. Ideally, therapies directed at each of the principal pathogenetic mechanisms should be used.

performed by specialized epithelial cells, called M cells, which transport luminal antigens to the lymphoid follicles or Peyer's patches. In addition, recent studies have found that mucosal dendritic cells can insert projections between intestinal epithelial cells and act as periscopes to sample the luminal contents [6]. In vitro, these dendritic cells withdraw from the epithelial monolayer when they confront pathogenetic bacteria, such as *Salmonella* species, but remain inserted in the presence of nonpathogenetic *Escherichia coli*. This system of sampling and controlled antigen presentation permits the mucosal immune system to develop a tolerizing or "anti-inflammatory" response to commensal flora characterized by production of transforming growth factor (TGF)-β and interleukin (IL)-10 (described later under this heading). Because IBD is characterized by damage or destruction of the lining epithelium, bacterial and food antigens gain direct access to the mucosal immune system and perpetuate the inflammatory response. Therapies directed at epithelial protection may therefore be beneficial (Fig. 4). Trophic growth factors such as growth hormone, keratinocyte growth factor, or epidermal growth factor may aid in epithelial restitution and are being evaluated in clinical trials.

The most intense and fruitful investigations with respect to IBD pathogenesis have been directed at the layer beneath the intestinal epithelial cells, the mucosal immune system. Naïve, undifferentiated T cells await stimulation from other cells, such as antigen-presenting cells, to activate them and provide guidance with respect to the type of T cell they will become (Figs. 3 and 5). This process of activation and differentiation has several steps. First, the T cell recognizes the antigen in the context of the MHC molecule. By itself, this interaction is insufficient to result in T-cell activation and proliferation. Interaction between costimulatory molecules on the surface of such T cells as CD40 ligand (CD40L or CD154), and their cognate receptors on antigen-presenting cells, such as CD40, are required for full T-cell activation. Because T cells are considered the principal conductors of the immune response, characterization of the immune response is based on the

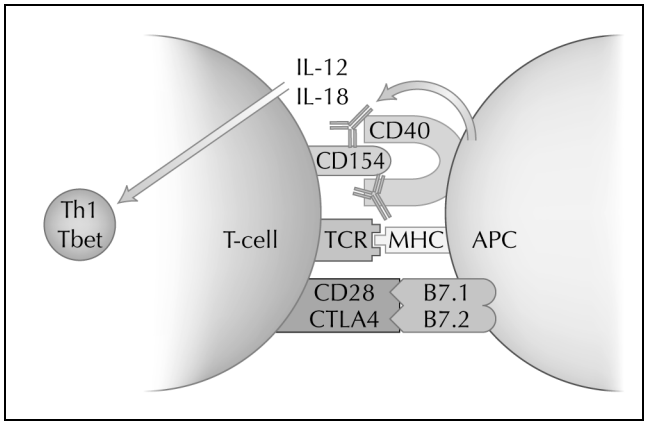

Figure 5. T-cell activation and differentiation are shown. T cells interact with antigen-presenting cells (APC) through the T-cell receptor (TCR) as well as several costimulatory molecules. Cytokines secreted by APCs in turn guide the differentiation of T cells toward Th1 development. Antibodies can block these costimulatory molecules. The transcription factor Tbet guides differentiation toward Th1. MHC—major histocompatibility complex.

pattern of cytokines or the surface markers expressed by mature T cells, such as the T-helper cells Th1, Th2, and Th3, or T regulatory 1 (Tr1) [7•]. Antigen-presenting cells (macrophages and dendritic cells) play a critical role in shaping the immune response through secretion of cytokines that guide the differentiation of T cells (Fig. 5). IL-12, a macrophage-derived cytokine, and IL-18, a macrophage- and epithelial-derived cytokine, shift the immune response toward a Th1-type response and are elevated in the mucosa of patients with Crohn's disease (Fig. 4). Th1 cytokines such as interferon (IFN)-γ, IL-2, and TNF-α are frequently elevated in the mucosa of patients with Crohn's disease. TNF-α is not formally considered a Th1 cytokine because it is usually a macrophage-derived cytokine, but evidence suggests that T cells are an important source of TNF-α in the lamina propria. In animal models, absence of IL-10 (IL-10 knockout mice) results in a Crohn's disease–like IBD, whereas absence of IL-2 (IL-2 knockout) results in an ulcerative colitis–like IBD. Oxalazone administration in mice results in ulcerative colitis–like inflammation and increased mucosal production of IL-4. For these reasons, Crohn's disease is characterized as a Th1-predominant disease and ulcerative colitis as a Th2-predominant disease. Clearly, however, there are areas of overlap between Crohn's disease and ulcerative colitis, and these descriptions oversimplify a complex situation.

Adding to the complexity of cytokine dynamics is the temporal relationship of cytokines to the underlying inflammatory disease. In IL-10 knockout mice, intestinal inflammation begins at about 10 weeks of age and continues thereafter. Initially, IFN-γ and IL-12 levels peak with the onset of the inflammation but decrease by week 20 at a time when the intestinal inflammation persists [8]. In Crohn's disease, biopsies taken at the anastomotic site within 3 months of surgery reveal a predominant Th2 pattern of cytokines rather than the characteristic Th1 inflammation in established Crohn's disease. These data suggest that distinct cytokines may exert their influence on the inflammatory cascade at different times. An understanding of these dynamics will ultimately be important for guidance of therapy.

Th1 and Th2 are effector cytokines that may have an important role in determining the clinical manifestations we associate with Crohn's disease or ulcerative colitis. Other cytokines act upstream to shape the immune response and dampen proinflammatory responses so that these do not become deleterious to the host (Fig. 3). TGF-β and IL-10 are secreted by Th3 and Tr1 cells, respectively. In several animal models, delivery of TGF-β or IL-10 can prevent or ameliorate colitis. Recent data demonstrate that TGF-β signaling is blocked in mucosal T cells isolated from patients with Crohn's disease by a cellular inhibitor of the TGF-β signaling pathway. Restoring the ability of these mucosal T cells to respond to TGF-β resulted in TGF-β–mediated inhibition of cytokine production. Because of the apparent beneficial effect of these regulatory cytokines, investigators have attempted to isolate Tr1 and Th3 cells from lamina propria to harness their power. These cells have been difficult to isolate until recently. Because TGF-β is associated with increased fibrosis, it is unlikely that systemic administration will be tolerated. As clinicians treating patients with IBD, we need to adjust to an ever-growing list of potential targets in the mucosal immune system.

In clinical practice, however, we do not use cytokine measurements to make decisions about patient care. At present, cytokine measurements are cumbersome and are not sufficiently specific because the range of normal and abnormal cytokine levels is broad. We also know that peripheral blood mononuclear cells, which are readily available, do not adequately reflect what is happening in the intestinal mucosal compartment. A potential alternative to characterizing the mucosal immune response of patients with IBD is the use of serologic markers. Clinicians are familiar with serologic markers as diagnostic tools, especially for patients with indeterminate colitis. In the context of describing the mucosal immune response in IBD, pANCA (perinuclear antineutrophil cytoplasmic antibodies) can be loosely considered a marker of Th2-like IBD, whereas ASCA (anti-*Saccharomyces cervisiae*) can be considered a marker of Th1-like IBD. Support for this model comes from the association of pANCA positivity in patients with ulcerative colitis or in patients with Crohn's disease with colonic involvement and ulcerative colitis–like symptoms, and positivity for ASCA in patients with small bowel and perforating Crohn's disease. As might be predicted based on this dichotomy, patients with pANCA-positive Crohn's disease tend to respond less well to anti–TNF-α therapy than do those who are serologically negative or ASCA positive [9]. Preliminary data from a randomized, controlled trial of infliximab in ulcerative colitis do not show a benefit. We may conclude from these data that expression of serologic markers reflects the underlying immune dysregu-

lation and, in the absence of direct cytokine measurements, that it may help to guide therapeutic decision making.

Are bugs good guys or bad guys in IBD?

Commensal bacteria are both good and bad in the pathogenesis of IBD. Animal studies have provided a wealth of information with respect to normal and abnormal host-microbial relationships. In particular, animal studies have taken advantage of the ability to breed mice under sterile conditions and reconstitute these mice with selective bacteria to examine the role of individual bacterial strains on inflammation. Normal mice, when reconstituted with *Bacteroides thetaiotaomicron*, a mouse and human commensal bacterium, increase the intestinal epithelial expression of genes involved in nutrient absorption and barrier function but not the expression of proinflammatory genes [10•]. Laser capture microdissection is then used to isolate intestinal epithelial cells, and microchip gene array technology is used to examine the pattern of gene expression in response to the presence of bacteria. These data suggest that the normal relationship between commensal bacteria and the host is a symbiotic one that does not result in an inflammatory response. In mice with a genetic predisposition to IBD, bacteria are required for the development of intestinal inflammation [11•]. Mono-association studies in which one strain of bacteria is introduced into germ-free mice or rats have demonstrated that the same bacterial strain (*eg*, *Bacteriodes vulgatus* or *Helicobacter hepaticus*) may elicit severe inflammation in one animal model but not in another. Mono-association studies with *Lactobacillus* species in several animal models have demonstrated that this strain of bacteria does not cause chronic inflammation. These findings support the hypothetical model suggesting that chronic intestinal inflammation requires both a genetic predisposition to immune dysregulation and the presence of enteric bacteria. The enteric bacteria required to unleash chronic inflammation may be different, depending on factors expressed by the host.

Several lines of evidence suggest a role for bacteria in the initiation and perpetuation of human IBD as well. First, patients with IBD demonstrate immunologic reactivity toward their own commensal flora, whereas healthy control subjects do not. In a study by D'Haens *et al*. [12•], fecal diversion was effective in modifying intestinal inflammation, and reinstallation of fecal contents into the diverted bowel resulted in recurrent inflammation within days. Patients who had a diverting ileostomy for Crohn's disease had infusion of the ileal contents into the diverted segment and then had biopsies taken of the distal segment. These investigators found a mononuclear cell infiltrate 8 days later. Antibiotic therapy is partially effective in treating Crohn's disease. Probiotics have been used successfully in patients with chronic pouchitis after an ileal pouch–anal anastomosis to prevent recurrent episodes of pouchitis [13]. Serologic markers expressed by patients with IBD, such as pANCA and ASCA, cross-react with bacteria and yeast, respectively. Theories regarding specific causative agents for Crohn's disease are abundant. Possible agents include *Mycobacterium paratuberculosis* and mumps virus. Support for these two agents comes from the demonstration of bacterial or viral DNA, respectively, in tissue samples from patients with Crohn's disease. Unfortunately, all of these are indirect lines of evidence and do not fulfill Koch's postulates for ascribing a causative role for bacteria in the pathogenesis of IBD.

Given the diversity of clinical manifestations in patients with IBD, it is likely that specific agents (bacteria and viruses) may be responsible for eliciting inflammation in a subset of patients, whereas normal commensal flora may be sufficient to trigger inflammation in others. One deficiency in our understanding of the normal host-microbial relationship in the gut is our relatively poor understanding of the microecology of the human intestine. An estimated 50% of normal human flora cannot be cultured. In lieu of culturing bacteria, molecular techniques have been used to type bacteria by the pattern of their ribosomal RNA. Recently, investigators using a variety of modern microbiologic techniques compared the density and diversity of colonic bacteria from patients with IBD with that of healthy control subjects [14]. These investigators found a dramatic increase in the number of bacteria adherent to the intestinal epithelium in IBD mucosa, even in mucosa that was not inflamed. It is not clear, however, whether this increase in adherence is a primary event or caused by the therapy used to treat the IBD. The adherent bacteria were diverse and were similar to those found in the healthy control subjects. Other investigators have attempted to find bacteria that are unique to IBD mucosa. To this end, Sutton *et al*. [15] reasoned that if a specific bacterium results in Crohn's disease, then its bacterial DNA should be present in lamina propria mononuclear cells from inflamed Crohn's disease mucosa but not in uninvolved Crohn's disease mucosa. Using a subtractive hybridization strategy, they identified a unique bacterial sequence, termed I2, associated with Crohn's colitis. Subsequent studies from this group have demonstrated that this bacterial sequence can act as a super-antigen in CD4+ T-cell activation. These clinical studies provide evidence that the precarious host-microbial relationship is disturbed in patients with IBD. Our knowledge of normal and abnormal flora and the interdependence of bacterial species in the intestine will need to improve before we can fully exploit manipulation of these flora for therapeutic effect.

Using Our Understanding of IBD Pathogenesis to Treat Patients

Given our understanding of mucosal immunology and the dysregulation that occurs in Crohn's disease and ulcerative colitis, the approach to treating IBD is to restore the delicate balance of competing cytokines and/or the cells that produce them. Simplistically, we wish to antagonize those

cytokines that are believed to be produced in excess or replace ones that are relatively deficient. Not only can downstream effectors like cytokines be blocked or replaced, but T-cell activation itself can also be interrupted by blocking the ability of costimulatory molecules on T cells to engage their cognate receptors on antigen-presenting cells (Fig. 5). The first round of biologic therapies has exploited antibody-based approaches to target certain cytokines or cell populations. Infliximab is the prototype of such a strategy. Antibodies are now available in clinical trials to antagonize a variety of Th1 cytokines, including IL-12, TNF-α, and IFN-γ, as well as such costimulatory molecules as anti-CD40L (CD154) that inhibit T-cell activation. Chemical compounds are under development to achieve similar endpoints. Recently, a small molecule inhibitor of a pathway leading to TNF-α gene expression was tested in patients with Crohn's disease [16]. Preliminary data suggest that this compound, CNI-1493, may be an effective therapy. It is important to note, however, that no cytokine is inherently "bad." Cytokines and other immune effectors exist to protect the host against specific pathogens. Antagonizing certain cytokines may therefore have a beneficial effect on the underlying IBD, as in anti–TNF-α therapy for Crohn's disease, but have a detrimental effect on host defense, such as tuberculosis.

In addition to targeting a specific cytokine, monoclonal antibodies are being used to block the ability of lymphocytes to traffic or "home" to the lamina propria of the intestine. The rationale for this type of therapy is that lymphocytes cannot do harm in patients with IBD if they cannot get to the intestine. Lymphocytes homing to the intestine usually express the α4 integrin in combination with β1 or β7. Examples of these therapeutic strategies are antibodies against the α4 integrin (natalizumab) or the α4β7 (LDP-02) integrin. Papadakis *et al*. [17] reported that lymphocytes expressing the CCR9 chemokine also home to the small intestine. Blockade of these chemokines may provide another avenue for alteration of lymphocyte trafficking.

In addition to modulating the immune system, treatment of IBD should aim to restore the physiologic host-microbial relationship. Identification of a specific causative agent such as a chronic intracellular pathogen would permit antibiotic treatment to eradicate this pathogen. It is not clear, however, that once a supposed pathogen is eradicated, the inflammatory process would likewise cease to exist. As primary therapy for Crohn's disease, antibiotics have fared about as well as aminosalycilates—which is not particularly well. The discouraging results may be caused in part by the lack of identification of patients who would be most likely to respond. Better markers of patients with "bacterially sensitive" disease are required to define the subpopulation who will benefit from antibiotics.

The complementary strategy is to repopulate the intestine with "healthy" bacteria through the use of probiotics. Although the use of probiotics is rampant, the science justifying their use lags behind. Randomized, controlled trials justify the use of probiotics in chronic pouchitis. A nonpathogenic strain of *E. coli* (Nissle 1917) studied in patients with ulcerative colitis was not effective for maintenance of remission. Preliminary data suggest that even the DNA from dead probiotic bacteria (VSL#3 cocktail) is effective at decreasing intestinal inflammation in vitro and in animal models. Clinical trials are ongoing to address the efficacy of probiotics in various settings such as postoperative recurrence of Crohn's disease and active Crohn's disease.

Pushing the Envelope Further: What Can New Technologies Offer in the Search for the Cause and Treatment of IBD?

Research in IBD is tapping into the latest technologies available to understand its pathogenesis and translate that understanding into therapy. The majority of research to date has investigated particular immunologic pathways and their role in IBD. However, newer methodology will take an unbiased approach to pathogenetic mechanisms. Examples of these approaches that apply to IBD include microchip array technology and mass spectrometry to identify genes or proteins expressed in IBD mucosa. Microchips are able to represent tens of thousands of genes on one chip. Messenger RNA isolated from clinical specimens of affected and healthy individuals is then applied to these chips, permitting simultaneous measurement of thousands of genes. Sophisticated computer programs can then identify clusters of genes that are over- or underexpressed in IBD mucosa compared with normal mucosa. This information can elucidate signaling pathways that may subsequently be targeted by small molecules or antibodies. Using similar technology, biotechnology companies will soon be able to offer cytokine profiles for patients with IBD. Mass spectrometry can analyze protein samples isolated from affected or unaffected mucosa and identify peptide sequences, including bacterial peptides, that are unique to IBD. These approaches offer the possibility to quickly identify pathways that can be targeted for therapy.

The rapid advances in our understanding of IBD pathogenesis will be paralleled by the rapid development of therapeutic agents. Identification of a gene or protein involved in perpetuation of intestinal inflammation can be used to model a specific small molecule that can antagonize the function of this deleterious protein. High-throughput drug testing will allow in vitro testing of the efficacy of thousands of compounds simultaneously. For example, small molecules can be tested for their ability to inhibit some aspect of TNF-α synthesis. Monoclonal antibodies that are entirely human can be made in vitro or in mice that express only human immunoglobulin genes. Another relatively inexpensive strategy that may be applied to IBD treatment is the use of genetically engineered bacteria as vehicles for delivery of recombinant proteins [18]. Bacteria expressing specific cytokines or other proteins (*eg*, trefoil factor) offer a means to deliver these proteins to the intesti-

nal lumen at a high local concentration. Although we do not know the exact cause of IBD, we now have many targets and effective delivery systems that should dramatically improve our ability to treat patients.

Taking a Good Idea and Designing a Clinical Trial

Perhaps not surprisingly, an entirely nonspecific therapy such as corticosteroids is effective in both Crohn's disease and ulcerative colitis and countless other inflammatory conditions. Compounds that block trafficking of lymphocytes to the intestine, such as those directed against the α4 integrin, would be predicted to be effective in both Crohn's disease and ulcerative colitis. As therapies become more specific, for example blockade of a particular cytokine, it is less likely that one therapy will be effective in a large group of patients. Anti–TNF-α is an example of such a therapy. Potent inhibition of TNF-α is effective in the majority of patients with Crohn's disease. Results of a randomized, placebo-controlled trial of infliximab in ulcerative colitis do not demonstrate a similar degree of efficacy. However, certain patients with ulcerative colitis probably will respond to infliximab or other similar agents. Identification of these patients prior to initiation of a large-scale clinical trial would be ideal. Conversely, when no data exist to predefine a subgroup that is most likely to respond, investigators should use the clinical trial as an opportunity to collect data for possible use in the design of future trials. Examples of such data are genetic, serologic, and immunologic information before and after therapy. Thus, clinical trials can be fruitful in many ways beyond the testing of a particular compound.

Not all therapies or targets will work when they are translated into the IBD clinic. Although this is unfortunate for some patients on whom these therapies are tested, these observations should be viewed as an opportunity for further research. In the past several years, clinical researchers and patients with IBD have experienced several disappointments in spite of sound and plentiful basic research justifying these trials. IL-10 is a critical regulatory cytokine produced by Th3 lymphocytes in the lamina propria. Evidence to support the important role of IL-10 in intestinal inflammation includes the development of colitis in animals deficient in IL-10 and the effective treatment of murine colitis with IL-10. Studies in which IL-10 was administered to patients with Crohn's disease have demonstrated marginal efficacy. Part of the reason for the lack of benefit may be too little IL-10 in the appropriate compartment (*ie*, the lamina propria). The systemic toxicity of IL-10 limits the dose that can be delivered. The overall strategy of IL-10 delivery may benefit from better targeting to the mucosal surface, as has been done in animal models using genetically engineered bacteria expressing IL-10 [18] or microspheres coated in IL-10 and delivered rectally. Another important consideration in the design of clinical trials for IBD is the relationship of the targeted pathway to the development or perpetuation of inflammation. Such cytokines as IL-12 may be important in the early stages of IBD but may not be required in established disease. Ultimately, the most promising therapies must be tested in patients with IBD. Only after well-designed studies are performed in humans can we reach conclusions about the efficacy of specific therapies for our patients.

Conclusions

Research into the pathogenesis of IBD has highlighted many potential therapeutic targets. Advances in drug and antibody development can translate these observations from the bench to the bedside more quickly than ever before. Because new therapies are more likely to target specific pathways, the selected therapy will need to be matched to the patient's disease. As clinicians, we are left with the task of designing appropriate clinical trials to determine which subset of patients with IBD will respond to a particular form of therapy. Future therapy may include manipulation of the microbial environment combined with targeting of an inflammatory pathway to reset the immunologic balance.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.• Watts DA, Satsangi J: **The genetic jigsaw of inflammatory bowel disease.** *Gut* 2002, **50:**31–36.

Well-written review of genetic research in IBD.

2.• Hugot JP, Chamaillard M, Zouali H, *et al.*: **Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease.** *Nature* 2001, **411:**599–603.

This article describes the association of *NOD2* mutations with Crohn's disease.

3. Ogura Y, Bonen DK, Inohara N, *et al.*: **A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease.** *Nature* 2001, **411:**603–606.

4. Abreu MT, Taylor KD, Lin YC, *et al.*: **Mutations in NOD2 are associated with fibrostenosing disease in patients with Crohn's disease.** *Gastroenterology* 2002, **123:**679–688.

5. Abreu MT, Arnold ET, Thomas LS, *et al.*: **TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells.** *J Biol Chem* 2002, **277:**20431–20437.

6. Rescigno M, Urbano M, Valzasina B, *et al.*: **Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria.** *Nature Immunol* 2001, **2:**361–367.

7.• Monteleone I, Vavassori P, Biancone L, *et al.*: **Immunoregulation in the gut: success and failures in human disease.** *Gut* 2002, **50:**60–64.

An up-to-date review of immunoregulation in the intestine.

8. Spencer DM, Veldman GM, Banerjee S, *et al.*: **Distinct inflammatory mechanisms mediate early versus late colitis in mice.** *Gastroenterology* 2002, **122:**94–105.

9. Taylor KD, Plevy SE, Yang H, *et al.*: **ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease.** *Gastroenterology* 2001, **120:**1347–1355.

10.• Hooper LV, Wong MH, Thelin A, *et al.*: **Molecular analysis of commensal host-microbial relationships in the intestine.** *Science* 2001, **291:**881–884.

These authors bred mice under sterile conditions and reconstituted them with *Bacteroides thetaiotaomicron*, a normal commensal in both mice and humans.

11.• Rath HC, Schultz M, Freitag R, *et al.*: **Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice.** *Infect Immun* 2001, **69:**2277–2285.

A continuation of the pioneering work of these investigators in understanding host-microbial interactions.

12.• D'Haens GR, Geboes K, Peeters M, *et al.*: **Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum.** *Gastroenterology* 1998, **114:**262–267.

This study provides a rare glimpse into the natural history of Crohn's disease lesions.

13. Gionchetti P, Rizzello F, Venturi A, *et al.*: **Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial.** *Gastroenterology* 2000, **119:**305–309.

14. Swidsinski A, Ladhoff A, Pernthaler A, *et al.*: **Mucosal flora in inflammatory bowel disease.** *Gastroenterology* 2002, **122:**44–54.

15. Sutton CL, Kim J, Yamane A, *et al.*: **Identification of a novel bacterial sequence associated with Crohn's disease.** *Gastroenterology* 2000, **119:**23–31.

16. Hommes D, van den Blink B, Plasse T, *et al.*: **Inhibition of stress-activated MAP kinases induces clinical improvement in moderate to severe Crohn's disease.** *Gastroenterology* 2002, **122:**7–14.

17. Papadakis KA, Prehn J, Moreno ST, *et al.*: **CCR9-positive lymphocytes and thymus-expressed chemokine distinguish small bowel from colonic Crohn's disease.** *Gastroenterology* 2001, **121:**246–254.

18. Steidler L, Hans W, Schotte L, *et al.*: **Treatment of murine colitis by Lactococcus lactis secreting interleukin-10.** *Science* 2000, **289:**1352–1355.