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Mini Review

A porous defense: the leaky epithelial barrier in intestinal disease

Daniel R Clayburgh*, Le Shen* and Jerrold R Turner

Department of Pathology, The University of Chicago, Chicago, IL, USA

A critical function of the intestinal mucosa is to form a barrier that separates luminal contents from the interstitium. This intestinal barrier is compromised in a number of intestinal diseases, most notably inflammatory bowel disease. *In vitro* studies have demonstrated that cytokines elaborated by immune cells can cause the mucosal barrier to become leaky; these cytokines are known to be increased in intestinal mucosa involved in inflammatory bowel disease. Detailed information describing the mechanisms by which altered cytokine signaling occurs is not available, but recent data implicate the cytoskeleton within epithelial cells as a critical regulator of the mucosal barrier under physiological and pathophysiological conditions. Using available data, we describe a model of intestinal disease where an initial insult to the epithelial barrier may trigger a self-amplifying cycle of immune activation, cytokine release, and further barrier dysfunction. This model is supported by the observation that pharmacological abrogation of cytokine signaling corrects both barrier defects and clinical disease in animal models and human patients, although such therapy clearly has multiple mechanisms. Other therapeutic targets that represent strategies to prevent or reverse disease processes are also considered. The overarching hypothesis is that modulation of the mucosal epithelial barrier plays a critical role in the initiation and propogation of inflammatory intestinal diseases.
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Crohn's disease and ulcerative colitis are chronic disorders of the intestines, collectively known as inflammatory bowel disease. It is estimated that up to one million Americans suffer from inflammatory bowel disease, with approximately 30 000 new cases diagnosed each year. The peak age of diagnosis is between 15 and 35 years, and more than 10% of new presentations occur in patients less than 18 years of age. Given the young age of presentation of these chronic illnesses, the subsequent lifetime morbidity is substantial. For example, the direct and indirect costs of inflammatory bowel disease in 2000 have been estimated to be in excess of $1.2 billion.[1] Inflammatory bowel disease has a familial link; approximately 20% of patients have a relative with either Crohn's disease or ulcerative colitis and several inflammatory bowel disease-related genes have been identified.[2–4] Thus, although the causes of inflammatory bowel disease remain unknown, a variety of epidemiologic, genetic, morphologic, and biochemical data provide some clues as to mechanisms and pathogenesis.

While these diseases are often viewed as a single entity with common symptoms, differing morphologies and disease courses demonstrate that these are distinct diseases that can be separately classified in the majority of patients. For example, while both diseases show characteristic features of chronic mucosal damage, including epithelial metaplasia, glandular atrophy, and architectural distortion, the presence of fissuring ulcers or fistula tracts, strictures, deep granulomas, skip lesions, or small intestinal disease all result in the classification of a patient as having Crohn's disease. In contrast, while backwash ileitis may occasionally be present, the small intestine is typically normal in ulcerative colitis, disease is continuous from the rectum to a proximal sharp demarcation of disease, and strictures, fistulae, and granulomas should not be present. However, classification is not always straightforward. For example, cases of inflammatory bowel disease with left-sided disease and involvement of cecal/periappendiceal mucosa or the appendix itself are recognized to be ulcerative colitis, despite the potential for interpretation as a skip lesion.[5–7] The subset of approximately 10% of

Correspondence: JR Turner, MD, PhD, Department of Pathology, The University of Chicago, 5841 South Maryland Avenue, MC 1089, Chicago, IL 60637, USA.
E-mail: jturner@bsd.uchicago.edu
*Both authors contributed equally to this work.
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inflammatory bowel disease cases that defy absolute classification as either Crohn's disease or ulcerative colitis further highlights the tremendous clinical, morphological, and biological overlap between Crohn's disease and ulcerative colitis.

A role for the immune system in inflammatory bowel disease is obvious. Indeed, in murine models, adoptive transfer of CD45RB high T cells induces an inflammatory intestinal disease with many features of inflammatory bowel disease, including granulomas, mucosal architectural distortion, crypt abscesses, and lamina propria mononuclear infiltrates.[8] However, the available data suggest that epithelial dysfunction may have an equally important role in the disease process. For example, numerous *in vivo* human studies have shown that disruption of intestinal epithelial barrier function closely mirrors disease activity and actually correlates with CD45RO expression on circulating CD19 positive B cells.[9–11] Even more intriguing is the observation that, in clinically asymptomatic Crohn's disease patients, increased intestinal epithelial permeability precedes clinical relapse by as much as 1 year,[12–14] indicating that a permeability defect may be an early event in disease reactivation. Further evidence supporting the hypothesis that abnormal intestinal barrier function occurs early in the pathogenesis of Crohn's disease comes from numerous studies showing that a subset of clinically healthy first-degree relatives of Crohn's disease patients have abnormally increased intestinal permeability.[10,15–20] Notably, the presence of this permeability defect in genetically unrelated relatives, for example, spouses, suggests that this abnormal permeability may be secondary to environmental as well as genetic factors.[17] Nonetheless, the potential importance of this permeability defect is emphasized by a case report of a healthy first-degree relative of Crohn's disease patients who exhibited increased intestinal permeability at the age of 13 years. She was evaluated thoroughly, including biopsies, small bowel followthrough, and ^{111}Indium-labeled white cell scan and showed no clinical features of intestinal disease.[21] However, 8 years later, at the age of 21 years, the subject returned with stricturing and ulcerated ileocolonic Crohn's disease.[21] Although a single case report, this patient shows that a permeability defect can exist long before the onset of full-blown disease, indicating that, at least in this case, an intestinal permeability defect may have been an early event in disease pathogenesis.

Increased intestinal permeability is not unique to inflammatory bowel disease; several other diseases, not all of which include a significant inflammatory component, also exhibit altered permeability. Graft *vs* host disease, as occurs following bone marrow transplantation, may be the best example of this. Donor T cells react to host tissues and produce diseases ranging from severe, widespread tissue destruction to diarrhea. This diarrhea is associated with acute epithelial damage, including pronounced crypt cell apoptosis, and can proceed to a chronic injury pattern with glandular atrophy. Active graft *vs* host disease is also associated with a significant intestinal permeability defect.[22] Although this permeability defect could conceivably be due to the marked apoptosis that occurs, numerous studies have now clearly shown that epithelial cell apoptosis alone is insufficient to cause permeability deficits.[23–26]

Permeability defects associated with malabsorption are also prominent in celiac sprue along with the classic histological features of villous blunting and increased numbers of intraepithelial lymphocytes.[27–29] At first this seems counterintuitive, since villous atrophy leads to decreased epithelial surface area and should therefore reduce paracellular permeability. Moreover, as ulceration and erosion are distinctly unusual in celiac disease, the increases in permeability cannot be explained as a result of gross epithelial loss. Thus, the permeability defects present in sprue are best understood as the result of epithelial dysfunction rather than epithelial destruction. Similarly, enteric bacterial and parasitic infections are known to result in barrier defects.[30,31] These have been studied in great detail using *in vitro* systems and, in the case of enteropathogenic *Escherichia coli*, depend on the presence of specific bacterial proteins that communicate with the epithelial cell.[32] In the cases of *Giardia lamblia* infection, the increases in permeability are also not due to direct tissue damage or tissue invasion by microorganisms, since neither is evident histologically. However, as discussed below, the intestinal permeability defect induced by *Giardia lamblia* infection does appear to require the activation of specific signal transduction pathways within host intestinal epithelial cells.[33,34] Thus, an understanding of the regulation of the epithelial barrier and its interaction with a variety of host- and pathogen-derived extracellular signals may yield insight into a wide array of intestinal diseases.

Paracellular permeability and barrier function are primarily determined by the epithelial tight junction

As implied above, the gastrointestinal epithelium forms a barrier that separates the finely regulated homeostasis of the body interstitium from the harsh environment of the intestinal lumen. The intestinal epithelial cell plasma membrane serves as an effective barrier to most hydrophilic solutes. However, the paracellular space must also be sealed to form an intact epithelial barrier. This seal is provided by the tight junction.[35] Tight junctions cannot be well visualized by light microscopy. However, their location is easily resolved on hematoxylin and eosin-stained slides as the terminal bar, the refractile area of membrane and

cytoplasm just subapical to the brush border. Transmission electron microscopy demonstrates the tight junction to be a discrete region of membrane apposition between adjacent epithelial cells at the luminal aspect of the apical junction complex. At this site, the adjacent plasma membranes appear to fuse.[36–38] This led to the initial misinterpretation of the tight junction as an impermeable barrier.[39]

As further studies showed the tight junction to be the rate-limiting step in the paracellular pathway, it became apparent that the tight junction forms a selectively permeable barrier. The barrier exhibits both size and charge selectivity, a vital attribute for the regulation of fluid and solute movement. Indeed, these barrier functions differ remarkably between tissues. For example, the mammalian small intestinal paracellular pathway is at least four-fold more permeable to K^+ than to Cl^- ions, while this selectivity is altered significantly in other areas of the gastrointestinal tract. The overall permeability also varies more than 30-fold, depending on the tissue type studied.[40,41] Freeze fracture electron microscopy shows the tight junction to be a series of anastomosing strands. Mathematical analyses suggested that the strands did not function as resistors in series, but housed a series of channels with individual open and closed probabilities.[42]

The precise roles of many tight junction proteins remain unknown. Numerous transmembrane proteins, such as claudins and occludin,[43,44] and cytoplasmic peripheral membrane proteins, including ZO-1, -2, and -3, and cingulin,[45–49] have been described. Although recent work has clarified the roles of the claudin family of proteins, the specific roles of others remains enigmatic. It appears that claudins form the actual pores that determine the charge selectivity of the paracellular pathway.[50–52] For example, human mutations in paracellin-1/claudin-16 result in defective paracellular reabsorption of Mg^{+2} across the renal proximal tubule.[53] This results in renal Mg^{+2} wasting and a familial hypomagnesaemia syndrome that cannot be corrected by Mg^{+2} supplementation.[53,54] Thus, mutation of a single protein can have enormous consequences for the permeability of the tight junction complex.

The tight junction protein complex is intimately related to the apical perijunctional actomyosin ring: functionally, structurally, and biochemically (Figure 1). Many tight junction proteins interact with both F-actin and myosin.[45,46,55,56] These interconnections between the various tight junction proteins and cytoskeletal elements are believed to stabilize the tight junction and to be critical to its regulation.[57]

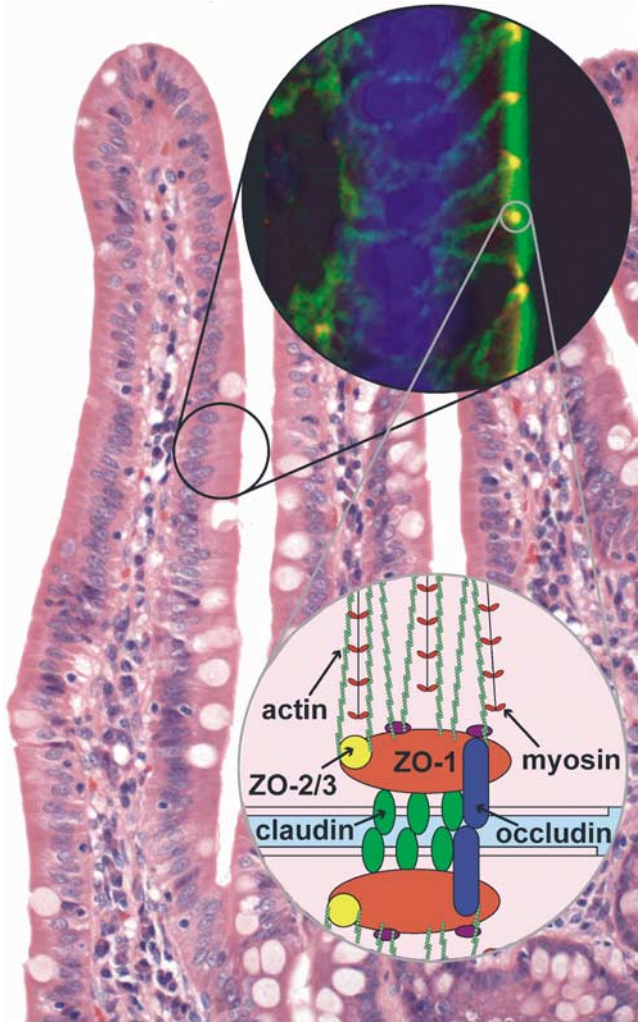

Figure 1 The tight junction. Although the tight junction cannot be seen on hematoxylin- and eosin-stained sections, immunofluorescence demonstrates the restricted location of the tight junction (top inset). Here, nuclei are stained blue, actin is green, and ZO-1, a tight junction protein, is red. Virtually all of the ZO-1 colocalizes with perijunctional actin, producing a yellow color at the tight junction. A simplified schematic of the tight junction is also shown (bottom inset). The claudin family of proteins forms the actual paracellular pore within the tight junction and is associated with another transmembrane protein, occludin. ZO-1 and other cytoplasmic proteins, such as ZO-2 and ZO-3, attach to this complex. Several of these proteins, including occludin and ZO-1, interact directly with F-actin.

Tight junction permeability is plastic

Although previous models of the tight junction considered it to have static permeability properties, more recent research has shown it to be a dynamic structure with the ability to alter its permeability in response to extracellular stimuli. Physiologically, the response of the tight junction to luminal glucose is particularly well studied. Transcellular transport of glucose, predominantly under the control of the Na^+–glucose cotransporter SGLT1, is a saturable system. However, the transport of glucose across the epithelial barrier is not saturable; continued increases in luminal glucose concentration result in continued increases in glucose absorption.[58] These data suggest that an alternative diffusion-driven pathway for glucose transport exists. The existence

of this pathway has been confirmed in studies in humans and experimental animals,[59,60] isolated human or rodent small intestinal mucosa,[25,61–63] and intestinal epithelial cell lines.[62,64] These studies all confirm an increase in paracellular permeability in response to Na^+–glucose cotransport. In isolated tissues and cell lines, this corresponds to a decrease in transepithelial resistance, an inverse measure of paracellular permeability. Transmission electron microscopy of isolated rodent mucosa showed that Na^+–glucose cotransport was accompanied by condensation of microfilaments within the perijunctional actomyosin ring, suggesting actomyosin contraction. Subsequent analyses using cell lines and isolated human and rodent small intestine showed that this condensation was driven by myosin light chain kinase-mediated phosphorylation of myosin II regulatory light chain.[62,63] This myosin light chain phosphorylation occurs within the perijunctional actomyosin ring and colocalizes with the tight junction.[63] Moreover, inhibition of myosin light chain kinase blocks both Na^+–glucose cotransport-induced myosin light chain phosphorylation and tight junction regulation in isolated mucosa and intestinal epithelial cell lines.[62,63,65] Consistent with this central role of myosin light chain phosphorylation in regulating paracellular permeability, studies using an inducible constitutively active myosin light chain kinase have shown that this activity is sufficient to activate the downstream events necessary for tight junction regulation.[57] Although this is only one physiological mechanism of tight junction regulation, accumulating evidence suggests that myosin light chain kinase-mediated regulation of tight junction permeability is a common intermediate in a variety of physiological and pathophysiological pathways related to altered paracellular permeability *in vitro* and *in vivo*.[57,66,67]

Bugs, cytokines, and drugs: factors that influence the tight junction barrier

The spectrum of barrier dysfunction that occurs in patients with intestinal disease suggests that pathophysiological factors may hijack the normal physiological pathways that regulate tight junction permeability. Consistent with this hypothesis, enteropathogenic *E. coli* (EPEC) infection causes diarrhea in pediatric patients and an *in vitro* model of this infection using intestinal epithelial cell lines demonstrates that EPEC induce a large increase in tight junction permeability. This is accompanied by disruption of tight junction morphology, including reorganization of the actin cytoskeleton, redistribution of tight junction proteins, and increased myosin light chain phosphorylation and can be reversed by inhibition of myosin light chain kinase.[24,68] Thus, the data suggest that EPEC utilizes an intrinsic pathway of tight junction regulation through myosin light chain kinase to affect barrier dysfunction. Similarly, the barrier disruption induced by *Giardia* infection can also be reversed by myosin light chain kinase inhibition, suggesting that, like EPEC, *Giardia* also disrupts tight junction permeability via myosin light chain phosphorylation.[34]

Increased epithelial permeability is not only caused by exogenous factors such as infection; a growing body of evidence suggests that the immune system plays an important role in modulating intestinal permeability. Two cytokines, interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα), are found in high levels in intestinal mucosa involved in inflammatory bowel disease.[69,70] These same two cytokines have also been found to decrease barrier function of cultured intestinal epithelial monolayers.[24,71–73] Incubation of intestinal epithelial cell monolayers with both IFNγ and TNFα leads to reorganization of many tight junction proteins, including ZO-1, junctional adhesion molecule 1, occludin, claudin-1, and claudin-4.[23] The changes in paracellular permeability caused by IFNγ and TNFα are associated with marked increases in myosin light chain phosphorylation and can be reversed using a specific membrane permeant inhibitor of myosin light chain kinase, indicating that these cytokines also utilize the myosin light chain kinase-driven pathway to increase tight junction permeability.[24] Thus, a key step in the pathogenesis of inflammatory bowel disease may be myosin light chain kinase activation by IFNγ and TNFα, leading to intestinal barrier dysfunction. Experiments using animal models of inflammatory bowel disease can provide an insight into the origin of these cytokines in the disease process as well as further elucidate the interactions of the immune system with the intestinal epithelium.

What can animal models teach us about human disease?

Over 60 different animal models of inflammatory bowel disease have been reported[74] and there are numerous excellent models of graft *vs* host disease. While no single animal model is a perfect replica of human disease, certain features are common to many models, indicating that these principles may also apply to human disease. For example, almost all animal inflammatory bowel disease models require the presence of intestinal flora; animals raised in a germ-free environment are rarely affected. This highlights the likely importance of exogenous stimuli in the development and persistence of the abnormal immune response observed in intestinal disease. While the exact factor(s) involved in this process are unknown, studies of a murine graft *vs* host disease model have shown that lipopolysaccharide sensitivity can predict disease severity and that lipopolysaccharide antagonism can inhibit disease progression.[75,76]

The IL-10 knockout mouse model of intestinal disease demonstrates two other commonalities of animal intestinal disease models: the importance of the genetic background of the animal and the necessity of T cells and their interactions with macrophages in the T_H1 immune response. The loss of IL-10 removes a key mechanism downregulating macrophage activation by T cells, leading to an unchecked T_H1 inflammatory response.[77] This response is associated with an intestinal permeability defect upon exposure to intestinal flora.[78] Along with other genetic inflammatory bowel disease models, this demonstrates the necessity of activated macrophages, T cells, and cytokines associated with the T_H1 response in promoting inflammatory changes and altering the intestinal barrier. This inflammation is also subject to genetic modulation. For example, the IL-10 knockout produces a severe colitis on a C3H genetic background, while C57Bl/6J mice lacking IL-10 only develop mild disease.[79] Thus, although the exact genes involved are unknown, other genetic differences between inbred laboratory mouse strains must contribute to the development and severity of intestinal disease.

Almost all animal models of inflammatory bowel disease involve disruption or abnormal stimulation of the immune system, such as T-cell transfer, genetic disruption of the immune system, or chemical stimulation of inflammation. However, one model exists in which a selective disruption of epithelial function leads to an inflammatory disease involving the intestines.[80] In this model, disruption of the adhesion molecule E-cadherin by tissue-specific expression of a dominant negative cadherin construct in small intestinal epithelial cells throughout the crypt-villus axis resulted in disruption of adherens junctions. When chimeric mice were created, it was evident that a defect in cell migration and proliferation only occurred in epithelial units expressing the dominant negative cadherin.[80] By 3 months of age, the mice developed typical histological features of inflammatory bowel disease, including architectural distortion, crypt abscesses, and both aphthous and linear ulcers.[80] Strikingly, in chimeric mice, these changes were limited to epithelia expressing dominant negative cadherin.[80] Thus, this model indicates that epithelial dysfunction alone may be sufficient to initiate the disease process in the absence of systemic inflammatory disease.

Interdependence of inflammation and barrier function in intestinal disease

The accumulated evidence from studies of patients, animals, and cultured cell models is sufficient to begin to construct a model of the pathogenesis of inflammatory diseases of the intestine (Table 1). Three key components appear to be necessary in the progression of disease: (i) disruption of the epithelial barrier, (ii) access of luminal contents to the lamina propria, that is, immune cells, and (iii) an abnormal immune response (Figure 2). According to this model, a defect in the intestinal barrier allows contents of the intestinal lumen to mix freely with the contents of the lamina propria. Most importantly, these luminal contents include bacteria, bacterial products, food antigens, and other immunostimulatory antigens. Antigen presenting cells in the lamina propria process and present these antigens to T cells and also secrete IL-12, thereby directing the T cells to initiate a T_H1 immune response. Central to the T_H1 response is the secretion of IFNγ from the T cell, which activates macrophages to respond to the stimulus. During a normal T_H1 response, antigen presenting cells also secrete IL-10, which acts to limit the T_H1 response. This and other regulatory mechanisms may be disrupted in inflammatory bowel disease, allowing an abnormally robust inflammatory response. A central downstream event in this immune cascade is the secretion of TNFα from the activated macrophage. Two major cytokines, IFNγ and TNFα, then

Table 1 Relationship between barrier dysfunction and TNFα in selected diseases

Disease/model	*Species*	*Intestinal permeability*	*Antigen*	*TNFα level*	*Effect of TNFα antagonism*
Crohn's disease	Human	Increased[90]	Gut flora ?	Increased[91]	Restores barrier function[84]
Ulcerative colitis	Human	Increased[92]	?	Increased[69,91]	Variable results[93,94]
IL-10 knockout	Mouse	Increased[78]	Gut flora ?	Increased[78]	Slows progression[95]
Dominant negative cadherin transgene	Mouse	?	?	?	?
Systematic T-cell activation	Mouse	Increased[96,97]	None	Increased[96,97]	Decreases diarrhea[96]
Graft *vs* host disease	Human	Increased[98]	Host	Increased[99]	Variable results[100–102]
Graft *vs* host disease	Mouse	Increased[75]	Host	Increased	Decreases severity, corrects barrier defect[75,85]
Celiac sprue	Human	Increased[27,29]	Gluten	Increased[103]	Clinical-histological regression (case report)[104]
Enteropathogenic *E. coli* infection	Human	Increased[30,32]	?	?	?
C. difficile infection	Human	Increased[105–107]	?	?	?

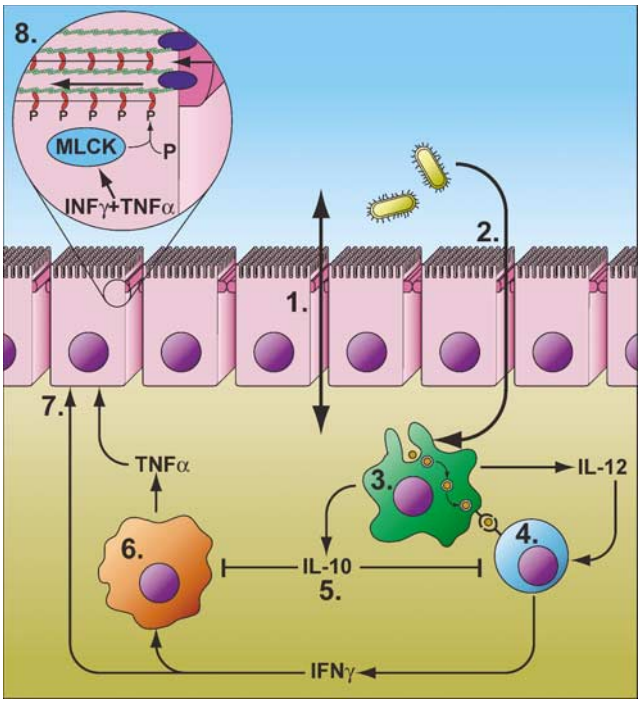

Figure 2 A model for the pathogenesis of inflammatory bowel disease. (1) Initial barrier disruption may be caused by injury (ischemia, infection), genetic predisposition, or by underlying inflammation. This leads to a mixing (2) of luminal contents, including bacteria and other pathogens, with lamina propria contents, notably antigen presenting cells. These antigen presenting cells (3) process and present antigens in association with MHC class II molecules while simultaneously secreting cytokines such as IL-12 that promote a T_H1 response. T cells (4) recognize the presented antigens and respond to the cytokine stimulus by secreting IFNγ to initiate the T_H1 response. Simultaneously, loss or downregulation of anti-T_H1 cytokines such as IL-10 (5) allows the inflammatory reaction to grow large and persist longer than usual. The IFNγ secreted by T cells promotes macrophage activation (6); these activated macrophages in turn secrete TNFα to promote the inflammatory reaction. TNFα and IFNγ also influence the epithelial barrier (7). Through unknown mechanisms, these cytokines activate MLCK (8), leading to MLC phosphorylation, actomyosin contraction, and opening of the tight junction. This leads to further loss of barrier function, continuing the cycle of disease progression.

act on the epithelium, further disrupting the barrier and increasing permeability. In this manner, a vicious cycle is created in which barrier dysfunction allows further leakage of luminal contents, thereby triggering an immune response that can in turn feed back on the intestinal barrier to promote further leakiness. As various mouse models so clearly demonstrate, any one of these disease components, barrier dysfunction, abnormal immune stimulation, or an abnormal immune response, may initiate the cycle. This may occur as a genetic predisposition to a leaky barrier, an abnormal immune response, or environmental factors, such as infection, that cause immune stimulation. However, human inflammatory bowel disease shows abnormalities of all three components. Thus, effective therapy that blocks this cycle at one or more points may be effective in allowing the intestine to return to normal function.

Breaking the cycle: current and potential therapies for inflammatory bowel disease

For many years, the mainstay of therapy for active inflammatory bowel disease was corticosteroids. As a potent immunosuppressive, this therapy was effective in controlling disease; however, the severe side effects associated with corticosteroids drove the search for better therapies. Unfortunately, immunosuppression remains the core approach for medical treatment of active ulcerative colitis. However, the arrival of infliximab, the first therapy targeted towards a specific part of the disease cycle, has revolutionized the treatment of Crohn's disease. Continued advances in our understanding of inflammatory bowel disease offers several potential targets for further therapeutic breakthroughs.

TNFα: A current mainstay in the treatment of Crohn's disease is the monoclonal antibody infliximab. This anti-TNFα antibody downregulates the inflammatory process in Crohn's disease.[81] Infliximab also induces apoptosis of lamina propria lymphocytes, decreases mucosal IFNγ production, and restores intestinal barrier function.[82–84] Perhaps most striking is the observation that, in addition to reducing mucosal lymphocytic and neutrophilic infiltrates, mucosal architecture is restored in a subset of patients.[81] Thus, in conjunction with its remarkable clinical therapeutic effect, infliximab normalizes histopathology, mucosal inflammation, cytokine production, and intestinal permeability. In a murine model of graft *versus* host disease, anti-TNFα therapy also normalizes mucosal histology, restores the mucosal barrier, and reduces serum lipopolysaccharide levels.[75,85] Thus, by potentially blocking several critical steps, including signaling between immune cells and from immune cells to the epithelium, inhibition of TNFα signaling corrects multiple aspects of intestinal disease.

IL-10: The presence of such striking intestinal disease in IL-10 knockout mice has spurred research into the possibility of using this cytokine as a therapy in human inflammatory bowel disease. Two studies have shown IL-10 to be moderately effective in the treatment of Crohn's disease.[86,87] In addition to its role in suppressing the T_H1 immune response, IL-10 also appears to have a role in preserving the epithelial barrier of cultured cells in the face of IFNγ treatment, indicating that, like infliximab, the effects of IL-10 may extend beyond T_H1 cells and macrophages.[88]

Myosin light chain kinase: As described above, myosin light chain kinase-mediated phosphorylation of myosin light chain is a central event in one pathway of tight junction regulation.[24,34,55,62,63,89] Moreover, myosin light chain kinase inhibition can correct barrier disruption induced in model intestinal epithelia by IFNγ and TNFα.[24] Thus, myosin light chain kinase inhibition may provide a novel mechanism for restoration of intestinal barrier function that would stop the cycle of disease

progression. While only a hypothesis at present, the success of myosin light chain kinase inhibitors in this and other *in vitro* and *in vivo* models suggests that this may be a therapy for the future.[24,34,67]

Unanswered questions

Despite significant progress in our understanding and treatment of inflammatory bowel disease and related intestinal disorders, numerous gaps in our knowledge remain. The initial events leading to the development of intestinal inflammatory diseases remain unclear. The identification of disease-associated mutations represents and an important first step in identifying the genetic factors that predispose individuals to the development of inflammatory bowel disease. However, it is likely that further mutations in proteins or regulatory pathways that control epithelial barrier function and the immune response remain to be discovered. In addition, evidence for environmental factors in disease pathogenesis suggests that further research into pathogens and other environmental factors may yield new insights into the development of inflammatory bowel disease. The factors that drive the continued abnormal immune responses are also undefined. Finally, the mechanisms by which the immune system leads to barrier dysfunction may be a particularly fertile area of future exploration. Although both TNFα and IFNγ can cause barrier dysfunction, the signaling pathways by which they increase paracellular permeability remain unknown. Elucidation of these pathways may yield further treatments for inflammatory bowel disease as well as related disorders, including graft *vs* host disease and enteric infections.

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