

# Mechanisms and Functional Implications of Intestinal Barrier Defects

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## Key Words

Tight junction · Crohn's disease · Inflammatory bowel disease · Occludin · Myosin light chain kinase · Claudin

## Abstract

Intestinal epithelial barrier defects, or increased paracellular permeability, were first reported in patients with Crohn's disease (CD) over 25 years ago. Although increased permeability may herald relapse to active disease, suggesting that impaired barrier function may contribute to progression, limited understanding of the mechanisms that create barrier defects in CD has made it impossible to determine whether increased permeability is a cause or effect of disease. It is now clear that inflammatory cytokines trigger intestinal barrier defects acutely, by cytoskeletal contraction, or chronically, via modulation of tight junction protein expression. Both mechanisms cause barrier dysfunction, but their effects on paracellular size and charge selectivity differ. The clinical ramifications of this distinction are not yet clear. Recent data using in vivo models demonstrate that cytoskeletally mediated barrier dysfunction is sufficient to activate innate and adaptive components of mucosal immunity. Consistent with the presence of increased permeability in some healthy first-degree relatives of CD patients, these barrier defects are insufficient to cause disease in the absence of other stimuli. However, cytoskeletally mediated barrier defects are sufficient to accelerate onset and increase severity

of experimental inflammatory bowel disease. Thus, inflammatory cytokines can cause barrier defects and, conversely, barrier defects can activate the mucosal immune system. This raises the possibility that restoration of barrier function may be therapeutic in CD. Consistent with this hypothesis, emerging data indicate that inhibition of cytoskeletally mediated barrier dysfunction may be able to prevent disease progression. Barrier restoration may, therefore, represent a non-immunosuppressive approach to achieving or maintaining disease remission. Copyright © 2009 S. Karger AG, Basel

The intestinal mucosa is charged with the complex tasks of absorbing nutrients and secreting waste products [1]. The successful completion of these tasks requires a semi-permeable barrier that limits back-diffusion of actively absorbed or secreted materials into the lumen or lamina propria, respectively. The barrier also supports paracellular transport, a passive process driven by concentration gradients, while preventing luminal microbes and their products from contaminating the internal milieu. Regulation and dysregulation of the barrier and the impact of these events on health and disease are the focus of this review.

## The Intestinal Mucosal Barrier

The barrier is composed of cellular as well as extracellular components. The latter include the presence of an aqueous unstirred layer of as much as 800  $\mu\text{m}$  in thickness and a layer of mucins that form a viscous hydrated gel. These extracellular barriers limit exposure of the monolayer of intestinal epithelial cells to shear forces and other physical trauma from particles within the lumen and may also limit direct contact of the epithelium with microorganisms.

The cellular components of the intestinal barrier consist of the complete array of epithelial cell types present within the intestine. These cells are polarized with a luminal, or apical, surface membrane composition that is distinct from the basolateral membranes. For example, the nutrient transporters found on the apical membrane, many of which use cotransport of  $\text{Na}^+$  ions to provide the energy and directionality of transport, are typically absent on basolateral membrane. In contrast, the  $\text{Na}^+, \text{K}^+$ -ATPase, which establishes the  $\text{Na}^+$  electrochemical gradient, is present on basolateral, but not apical membranes. In addition, the lipid composition of the membrane domains differs; the apical membrane is enriched in sphingolipids and cholesterol relative to the basolateral membrane. One result of this polarization of proteins and lipids is that the apical membranes of intestinal epithelial cells are generally impermeable to hydrophilic solutes in the absence of specific transporters. Thus, the mere presence of epithelial cells, particularly the apical membranes, contributes significantly to the mucosal barrier. Their absence, as occurs in mucosal erosions, leads to marked barrier defects whose magnitude correlates directly with the surface area involved.

While the intestinal epithelial cell membranes are essential to mucosal barrier function, the paracellular space between adjacent cells must also be sealed. This function is served by the apical junctional complex that is composed of tight junctions, adherens junctions, and desmosomes. The tight junctions, which form the paracellular barrier, are located most apically, and define the boundary between apical and basolateral plasma membrane domains [2]. The subjacent adherens junctions and desmosomes serve important structural and signaling roles, but are not thought to contribute directly to paracellular barrier function.

## Tight Junction Barrier Properties

Tight junctions have been categorized as 'leaky' and 'tight' [3]. Tight junctions within urinary bladder epithelium are 'tight', meaning that they are highly impermeable to even small solutes, such as ions as well as macromolecules. In contrast, tight junctions established by intestinal epithelia are leaky and allow some amount of paracellular flux. In either case, the tight junctions are the rate-limiting determinants of paracellular transport. In addition, because of the marked difference in permeabilities of the apical plasma membrane and tight junction in leaky epithelia, the tight junction is the primary determinant of mucosal permeability in the presence of an intact epithelium.

Gastrointestinal tight junctions demonstrate both ion and size selectivity. For example, intestinal epithelial tight junctions are typically more permeable to  $\text{Na}^+$  than to  $\text{Cl}^-$ . However, this ion selectivity can be modified by altering tight junction protein expression patterns [4, 5]. Size selectivity of the tight junction barrier also varies along the villus-crypt axis. Studies of jejunal tight junction structure and paracellular flux suggest that pores within crypt tight junctions are permeable to molecules with radii as large as 50  $\text{\AA}$ , while pores within villus tight junctions allow only molecules with radii of  $<6 \text{\AA}$  [6, 7]. In addition, the number of these pores is subject to acute regulation, with  $\text{Na}^+$  nutrient cotransport triggering an increase in the number of small, but not large, pores [6, 8, 9]. While the functional significance of this regulation has been a topic of controversy [10–12], available data suggest that increased tight junction permeability allows for paracellular amplification of water and nutrient absorption when the transcellular pathway is saturated [13–16].

## Barrier Defects in Crohn's Disease

Increased paracellular permeability was reported in patients with Crohn's disease (CD) over 25 years ago [17, 18]. Although such barrier loss may be caused by erosions that occur in active disease, some approaches used to measure permeability partially excluded this possibility by normalizing absorption of the inert sugar lactulose to that of the smaller, and more easily absorbed, inert sugar mannitol [17, 18]. The implication that increased permeability in CD reflects abnormal tight junction permeability was supported by transmission and scanning electron microscopy studies showing a reduction in the number of tight junction contacts as well as disruption of the nor-

mal anastomosing strand pattern seen by freeze fracture electron microscopy [19, 20]. Subsequent work showed that, in addition to being present in CD patients, increased paracellular permeability was also present in a subset of their healthy first-degree relatives [21, 22]. This suggested that barrier defects could be a cause of CD [23, 24], and more recent work has linked barrier defects in healthy relatives to specific mutations in *NOD2/CARD15* [25]. Unfortunately, the contribution of barrier defects to risk of developing disease in healthy relatives of CD patients has not been assessed. One case report has described a subject who had increased intestinal permeability at age 13, but no evidence of CD, and went on to develop disease 8 years later [26]. However, this patient had 2 first-degree relatives with CD and, therefore, was at increased risk of developing disease regardless of intestinal permeability. Nonetheless, this case does demonstrate a barrier defect prior to onset of CD and, therefore, supports the hypothesis that increased permeability may be an early step in the pathogenesis of this disorder.

Additional circumstantial evidence supporting a role for barrier defects in CD pathogenesis comes from a series of studies examining patients during remission. These analyses showed that patients with increased intestinal permeability were at greater risk of relapse over the subsequent year [27–29]. It should, however, be noted that one more recent study failed to reproduce these data [30]. In that study, reduced psychosocial stress correlated with decreased rates of relapse. The implications of this observation are of interest in terms of treatment and pathogenesis and may relate to barrier function, as stress can induce increases in small intestinal permeability [31]. Notably, the more recent work also identified the presence of colonic disease as an independent predictor of disease relapse [30]. This is particularly significant, since the probes used to assess permeability in that study, lactulose and mannitol, are degraded by colonic bacteria. Thus, an alternative explanation for the absence of a correlation between disease relapse and permeability in that study is that subclinical disease was present in the colon. The fact that lactulose and mannitol, as well as other commonly used paracellular probes, are degraded by colonic bacteria is the principle reason why the data above apply primarily to CD. However, it should be noted that some of the observations described above, included abnormal tight junction ultrastructure [32], have been demonstrated in ulcerative colitis. Further analysis of these issues in ulcerative colitis patients may now be possible, as newer probes able to assess colonic paracellular permeability have become available [33].

## **Tight Junction Alterations in Inflammatory Bowel Disease**

As mentioned above, tight junction structure and function are abnormal in CD and ulcerative colitis. Recent advances in our understanding of tight junction proteins components have made it possible to directly assess these events in human specimens. Remarkably, both epithelial cells from CD and ulcerative colitis patients demonstrate increased expression of claudin-2 [34, 35]. Claudin-2 expression in cell culture models increases  $\text{Na}^+$  conductance across the tight junction and also increases the number of small tight junction pores [4, 5, 36]. However, this may not be sufficient to explain the increased permeability to lactulose seen in CD patients. One potential explanation for observed increases in permeability of lactulose and other large molecules may be the removal of claudin-5, claudin-8, and occludin from the tight junction as well as degradation of these proteins in CD patients [37]. However, it may also be that acute cytokine-dependent tight junction regulation plays a critical role. For example, the tumor necrosis factor (TNF)-neutralizing antibody infliximab, which is remarkably effective in CD and some ulcerative colitis patients [38–40], also restores the intestinal barrier in CD patients [41]. While these effects may be secondary effects of reduced inflammatory activity after infliximab treatment, a direct role of tumor necrosis in regulating barrier function must be considered.

## **Tumor Necrosis Factor-Dependent Tight Junction Regulation**

TNF causes barrier loss in cultured intestinal epithelial monolayers [42, 43]. Given the ability of infliximab to restore barrier function in human patients, the means by which TNF causes increased paracellular permeability is of considerable interest. A major advance in understanding this process came from the observation that myosin light chain kinase inhibition was able to acutely restore barrier function in TNF-treated intestinal epithelial monolayers [44]. In addition to providing insight into TNF-dependent tight junction regulation, this result suggests that the underlying mechanism may overlap with those responsible for physiological barrier regulation by  $\text{Na}^+$ -nutrient cotransport, which also requires myosin light chain kinase activity [9]. Further studies showed that acute TNF treatment resulted in increased myosin light chain kinase expression [43, 45], as a result of transcriptional activation [46], in vitro and in vivo. Consis-

tent with a role in barrier loss in human disease, further study revealed increases in myosin light chain kinase expression and activity in intestinal epithelia from ulcerative colitis and CD patients [47]. Thus, it was hypothesized that TNF-dependent myosin light chain kinase activation might be a critical mechanism of barrier dysfunction in inflammatory bowel disease.

### **Myosin Light Chain Kinase Activity Is Required for TNF-Induced Acute Diarrhea**

An *in vivo* model was required to better define the role of TNF in barrier loss and diarrheal disease. This model was established by treating mice with either an anti-CD3 monoclonal antibody, to activate T cells, or by injecting purified recombinant TNF [48–50]. These treatments cause an acute, self-limited diarrhea that can be prevented by infliximab [48, 49]. Moreover, treatment of human subjects with anti-CD3 monoclonal antibody induces an acute, self-limited diarrhea that, like inflammatory bowel disease, can be treated with corticosteroids [51–53]. Thus, while the mouse model does not cause chronic disease, it has pathophysiological relevance to human inflammatory bowel disease.

T-cell activation induced increases in jejunal paracellular permeability within hours [49]. This was accompanied by jejunal epithelial myosin II regulatory light chain phosphorylation, consistent with myosin light chain kinase activation [49]. Moreover, myosin II regulatory light chain phosphorylation waxed and waned in parallel with the development and resolution of diarrhea and both phosphorylation and increased paracellular permeability were prevented by infliximab [49]. This suggested that TNF-dependent myosin light chain kinase activation might be required for *in vivo* barrier loss and diarrhea. To test this hypothesis, the response of mice lacking the long myosin light chain kinase [54], the only form expressed in intestinal epithelia [55], to T-cell activation was assessed. Mucosal cytokine production induced by T cell activation was similar in wild-type and long myosin light chain kinase knockout mice. However, intestinal epithelial myosin II regulatory light chain phosphorylation did not increase, there was no increase in paracellular permeability, and diarrhea was prevented [49]. In addition, the most prominent morphological correlate of tight junction dysregulation, endocytosis of the tight junction protein occludin, did not occur in long myosin light chain kinase knockout mice. Similarly, treatment of mice with a specific peptide inhibitor of myosin light

chain kinase prevented myosin II regulatory light chain phosphorylation, occludin endocytosis, barrier loss, and diarrhea. Thus, the TNF-dependent diarrhea that occurs after T-cell activation requires long myosin light chain kinase-mediated myosin II regulatory light chain phosphorylation [56].

### **Constitutive Myosin Light Chain Kinase Activation Accelerates Onset and Increases Severity of Experimental Inflammatory Bowel Disease**

The data presented thus far demonstrate that (1) myosin light chain kinase activation is necessary for acute TNF-induced barrier loss and diarrhea, (2) myosin light chain kinase expression and activity are increased in inflammatory bowel disease patients, (3) barrier loss is present in some first-degree relatives of CD patients, and (4) barrier loss precedes and may be a marker of impending disease reactivation. In addition, *in vitro* work has shown that expression of a constitutively active truncated myosin light chain kinase in cultured intestinal epithelial monolayers is sufficient to increase tight junction permeability [57]. Thus, to test the hypothesis that increases in tight junction permeability, similar to those detected in CD patients and a subset of their relatives, a transgenic mouse expressing the constitutively active truncated myosin light chain kinase within the intestinal epithelium was developed [58]. As expected, this mouse displayed increased myosin II regulatory light chain phosphorylation within intestinal epithelia and increased paracellular permeability within the small intestine and colon [58]. However, the mice developed, gained weight, and reproduced normally under standard specific pathogen-free housing. While some might consider this to indicate that the model was poorly executed, the absence of spontaneous disease in these mice actually recapitulates human data. Healthy first-degree relatives of CD patients with increased permeability are notable because they are healthy; with no evidence of disease. Many of these relatives will never develop disease. Thus, the transgenic mice expressing constitutively active myosin light chain kinase represent a unique opportunity to probe the mechanisms that maintain mucosal homeostasis despite the presence of barrier defects and to ask if such defects predispose an individual to inflammatory bowel disease.

Detailed analysis of the constitutively active myosin light chain kinase transgenic mice showed that TNF, interferon- $\gamma$ , and interleukin-10 expression were in-

creased within the colonic mucosa [58]. Interestingly, these increases were not present prior to weaning, raising the possibility that the rapid changes in luminal microbiota composition that accompany weaning may contribute to the observed mucosal immune activation. The transgenic mice also demonstrated a marked increase in the number of lamina propria T cells and a repositioning of CD11c+ dendritic cells to a subepithelial location. It will be a great interest in the future to determine what immune mechanisms prevent disease in these mice.

To determine if increased intestinal paracellular permeability could contribute to disease progression, the transgenic mice were studied using the CD4+CD45Rb<sup>hi</sup> adoptive transfer model of colitis, which shares many biochemical, immune, and morphological characteristics of human inflammatory bowel disease [59–62]. When constitutively active myosin light chain kinase transgenic mice were challenged with CD4+CD45Rb<sup>hi</sup> T cells, they developed colitis more rapidly than their non-transgenic littermates. Weight loss occurred earlier, mucosal cytokine expression was higher, and histologic damage more advanced in the transgenic mice. As disease progressed, weight loss in transgenic mice and non-transgenic littermates became similar, but mucosal cytokine expression remained higher and histologic damage more severe in the transgenic mice. In addition, survival of transgenic mice was reduced relative to non-transgenic littermates. Thus, pathophysiologically relevant regulation of intestinal epithelial tight junctions induces mucosal immune activation and, while insufficient to cause disease, can contribute to development and progression of colitis.

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## Conclusion

The tight junction is a critical determinant of mucosal barrier function. In the absence of mucin deficiency or gross epithelial damage, the tight junction is the primary determinant of paracellular permeability. Increases in intestinal permeability are present in healthy individuals and have been reported at increased frequency in first-degree relatives of CD patients. A role of barrier loss in CD pathogenesis is further suggested by the observation that increased intestinal permeability correlates with increased risk of relapse from remission. Finally, studies using pre-clinical animal models have demonstrated that TNF induces cytoskeletally mediated tight junction dysregulation. Conversely, cytoskeletally mediated tight junction dysregulation is able to increase mucosal TNF production, suggesting the presence of a cycle linking immune activation and epithelial barrier dysfunction. These data suggest that restoration of the intestinal epithelial barrier is an appropriate therapeutic target that may be an effective, non-immunosuppressive means of achieving or maintaining disease remission.

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## Disclosure Statement

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