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The tight junction in inflammatory disease: communication breakdown

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The intestinal epithelium restricts free passage of toxic and infectious molecules from the gut lumen while allowing selective paracellular absorption across the tight junction. Inflammatory bowel disease (IBD) patients demonstrate a loss of tight junction barrier function, increased pro-inflammatory cytokine production, and immune dysregulation; however, the relationship between these events is incompletely understood. Although tight junction barrier defects are insufficient to cause experimental IBD, mucosal immune activation is altered in response to increased epithelial permeability. Thus, an evolving model suggests that barrier dysfunction may predispose or enhance disease progression and therapies targeted to specifically restore the barrier function may provide an alternative or supplement to immunology-based therapies.

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Introduction

Crohn's disease and ulcerative colitis, collectively inflammatory bowel disease (IBD), affect 1.4 million Americans. Although the exact cause of IBD remains unknown, genetic susceptibility, environmental factors, and immune dysregulation all contribute to disease pathogenesis. In addition, IBD patients demonstrate increased intestinal paracellular permeability, which reflects decreased epithelial barrier function [1,2]. While it remains unclear whether barrier dysfunction precedes disease or results from active inflammation, increased intestinal permeability is also observed in unaffected first-degree relatives suggesting that a barrier defect may lead to disease progression [3,4].

An intact monolayer of intestinal epithelial cells protects the body from pathogens and other toxic luminal substances. Epithelial tight junctions maintain the intestinal barrier while regulating permeability of ions, nutrients, and water [5]. The tight junction is a multi-protein complex that forms a selectively permeable seal between adjacent epithelial cells and demarcates the boundary between apical and basolateral membrane domains (Figure 1).

Involvement of the tight junction in IBD

The tight junction is composed of multiple proteins including transmembrane proteins such as occludin, tricellulin, claudins and junctional adhesion molecule (JAM). The intracellular portions of these transmembrane proteins interact with cytoplasmic peripheral membrane proteins, including zona occludens (ZO)-1,-2,-3 and cingulin [6]. These tight junction and cytoplasmic proteins interact with F-actin and myosin II, thereby anchoring the tight junction complex to the cytoskeleton. Once thought to be static, the association of these proteins with the tight junction is highly dynamic [7••] and may play a role in epithelial barrier regulation.

Occludin was the first tight junction-associated integral membrane protein identified [8]. Although occludin knockout mice exhibit intact intestinal epithelial tight junctions and display no observable barrier defect [9,10], they have a complex disease phenotype that includes severe growth retardation, male sterility, chronic gastritis, and osteomalacia [10]. While these data have been interpreted by some to suggest the lack of an important role for occludin in tight junction integrity, *in vitro* studies demonstrate crucial roles in tight junction assembly and maintenance [11–13]. This suggests that further analysis of occludin knockout mice under stressed condition may reveal *in vivo* functions of occludin and provide new insight into mechanisms of tight regulation [5].

Tricellulin is related to occludin but is preferentially localized to the tricellular junction region where three cells meet [14]. Although tricellulin is crucial to maintenance of ion gradients within the inner ear [15,16], tricellulin expression in the intestine has not been described. Given the phylogenetic and structural similarities between occludin and tricellulin [14], it may be that the tricellulin accounts for normal intestinal barrier function in occludin knockout mice. This hypothesis could also be applied to inflammatory bowel disease, where intestinal epithelial occludin expression is reduced [17]. While untested the notion that tricellulin can

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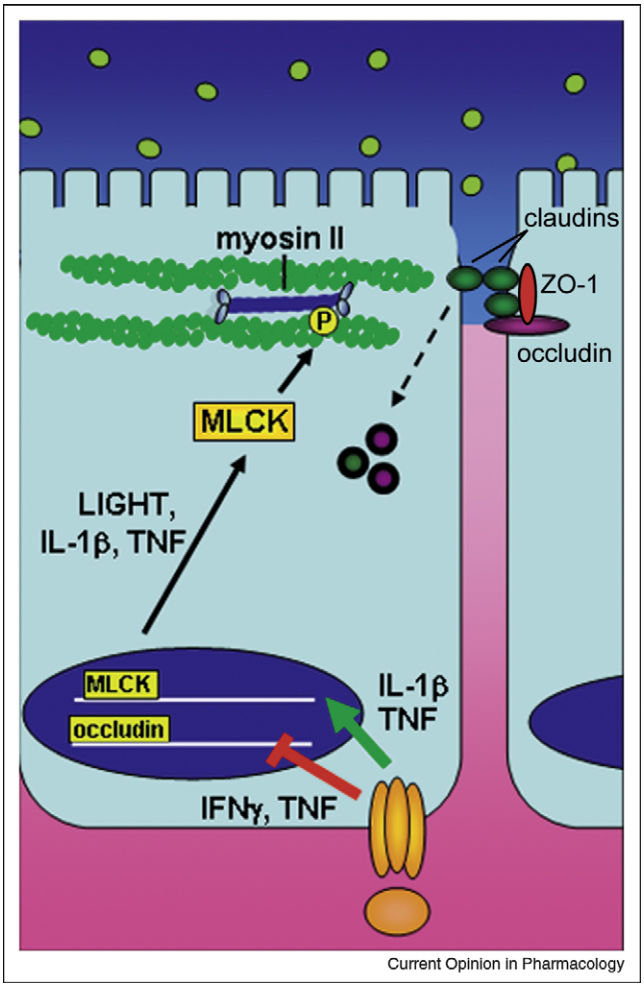
Figure 1



Cytokine regulation of epithelial barrier function. Pro-inflammatory cytokines such as TNF, IL-1β, and LIGHT promote barrier dysfunction by inhibiting transcription of junction proteins and inducing cytoskeleton-mediated redistribution of tight junction proteins. These cytokines promote transcription of MLCK, which when activated phosphorylates myosin II, resulting in reorganization of tight junction proteins, including endocytic removal from the apical junctional complex.

compensate for loss of occludin expression is consistent with the observation that occludin knockdown in cultured monolayers causes tricellulin to redistribute from tricellular to bicellular junctions [18•]. Thus, it will be important for future studies to assess the expression, trafficking, and function of tricellulin in IBD.

The observation that barriers can develop in the absence of occludin prompted a continued search essential barrier-forming components of the tight junction [19]. This led to the identification of claudin-1 and claudin-2 [20]. At least 24 different claudin proteins are present in mammals [21], and these proteins are the primary component of tight junction strands [22]. While the molecular anatomy of the tight junction is not yet clear, it is certain that claudins are able to form aqueous pores that permit ions and uncharged molecules to pass in a charge-selective and size-selective manner [23,24,25••]. This appears to be relevant to IBD, as claudin-2, which increases paracellular conductance of sodium ions and small uncharged molecules [25••,26], is increased at the tight junction in IBD [17,27]. By contrast, claudin-3, claudin-4, claudin-5, and claudin-8 are removed from the tight junction in IBD patients [27,28]. The mechanisms by which claudin expression is regulated are not fully understood, although, as discussed below, cytokine signaling is one important factor. Altered transcriptional regulation and vesicular trafficking of claudins, and other tight junction proteins, in disease may be important therapeutic targets in the future.

Causes of barrier dysfunction in IBD

Barrier dysfunction includes increased paracellular permeability resulting from enhanced flux across the tight junction, but may also be caused by epithelial damage, including apoptosis, erosion, and ulceration [17,28,29]. While some data suggest that the barrier is maintained despite epithelial apoptosis [30–32], there is not uniform agreement on differing results that probably reflect the extent of apoptosis and varying experimental systems. Nonetheless, there is consensus that damage to a single cell or small group of cells, such as that induced by cytokines or inflammatory cell transmigration, barrier integrity is rapidly repaired by an actomyosin-dependent purse string mechanism [33,34]. It also seems clear that extensive epithelial damage must compromise the mucosal barrier. Improved understanding of the processes that regulate mucosal healing and development of means to accelerate epithelial repair are, therefore, important goals for treatment of inflammatory bowel disease.

In contrast to the gross barrier loss that occurs with epithelial damage, barrier dysfunction due to tight junction regulation is more selective. Therefore, it is important to discriminate between increased intestinal permeability due to epithelial loss and that which reflects tight junction-dependent changes in paracellular permeability. The latter has been carefully studied as a function of cytokine production. These studies have primarily been performed *in vitro*, as *in vivo* models may be complicated by cytokine-dependent immune cell recruitment and activation within the mucosa. The most well-studied cytokine that causes barrier dysfunction due to epithelial tight junction regulation is tumor necrosis factor (TNF) [5]. This is likely relevant to disease, as TNF is a current target of current biologic therapies for IBD [35,36], and anti-TNF therapy restores the gut barrier in Crohn's disease [37]. Therefore, our laboratory and others have focused on understanding the mechanisms by which inflammatory cytokines regulate tight junction permeability (Figure 1). Freeze-fracture electron microscopy studies showed that TNF treatment of

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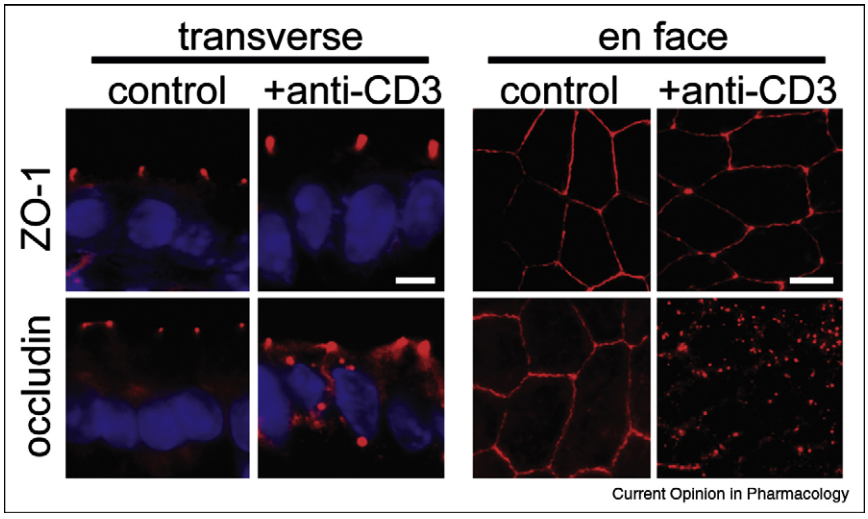
Figure 2



Tight junction morphology is altered in immune-mediated diarrhea. Mice were injected intraperitoneally with anti-CD3 antibody to induce acute immune-mediated diarrhea, which is accompanied by increased TNF and IFNγ production. Immunofluorescence localization of ZO-1 and occludin in small intestinal epithelium was assessed before or 3 h after anti-CD3 treatment. While ZO-1 distribution appears unchanged in transverse sections, when viewed en face, ZO-1 staining at the junction appears thinner and concentrated more at tricellular junctions with anti-CD3 treatment. Similarly, occludin internalization into vesicles is seen in both transverse and en face sections following treatment with anti-CD3 (from [44] with permission).

HT29/B6 cells resulted in decreased tight junction strand number and complexity and increased frequency of strand breaks [38]. TNF also inhibits occludin promoter activity [39] and causes redistribution of occludin, ZO-1, and claudin-1 [40] (Figure 2). *In vitro*, the major effector responsible for TNF-induced tight junction modulation is myosin light chain kinase (MLCK), and transcription and translation of epithelial MLCK are increased by TNF *in vitro* and *in vivo* [40–42]. Moreover, MLCK inhibition corrects TNF-induced barrier defects *in vitro* and *in vivo* [43,44]. MLCK expression and activity are also enhanced in experimental models of IBD [45] and in intestinal epithelium of human IBD patients [46]. While only correlative, the further observation that the degree of MLCK upregulation in human patients parallels disease activity is consistent with the hypothesis that increased mucosal cytokine production contributes to MLCK-mediated barrier loss [46].

Other pro-inflammatory cytokines may mediate barrier function through modulation of MLCK activity. For example, LIGHT, another TNF family member, also promotes MLCK-induced tight junction disruption [47]. Although TNF and LIGHT signal through different epithelial receptors [45,47], they both likely contribute to IBD [48]. While the details are less well defined, it also appears that IL-1β enhances paracellular permeability via MLCK [49]. Thus, MLCK represents a common effector used by multiple cytokines to modulate paracellular permeability and is an important target for future therapies to restore barrier function during active disease.

Roles of barrier regulation in colitis

First-degree relatives of Crohn's disease patients are at increased risk of developing IBD. The presence of barrier defects in some healthy relatives suggests that barrier loss may contribute to disease progression [1,2]. This hypothesis is supported by the demonstration in multiple studies that increased intestinal paracellular permeability during remission is a marker of impending disease reactivation [50]. Despite this, no mutations in tight junction proteins or defined regulators of barrier function have been reported in IBD [51]. However, the genetic linkage of certain *NOD2/CARD15* mutations to barrier defects [4] suggests that immune activation may be responsible for the early barrier defects observed in healthy relatives and in patients before disease reactivation. Given the essential role of MLCK in immune-mediated tight junction regulation, our laboratory generated a transgenic mouse expressing constitutively active MLCK (CA-MLCK) exclusively within an intestinal epithelium [52••]. Although CA-MLCK transgenic mice display chronic increased epithelial permeability, these mice did not develop disease [52••]. However, subclinical mucosal inflammation was present in these mice, as demonstrated by increased $CD4^+$ lamina propria mononuclear cells (LPMC) and enhanced migration of $CD11c^+$ positive dendritic cells to the superficial lamina propria. Mucosal expression of TNF and IFNγ was increased at six weeks of age, and these increases were not strictly dependent on mature lymphocytes, as findings were similar in $Rag1^{-/-}$/CA-MLCK mice. Moreover, adoptive transfer of $CD4^+CD45Rb^{hi}$ cells into $Rag1^{-/-}$/CA-MLCK mice resulted in accelerated disease development as well as

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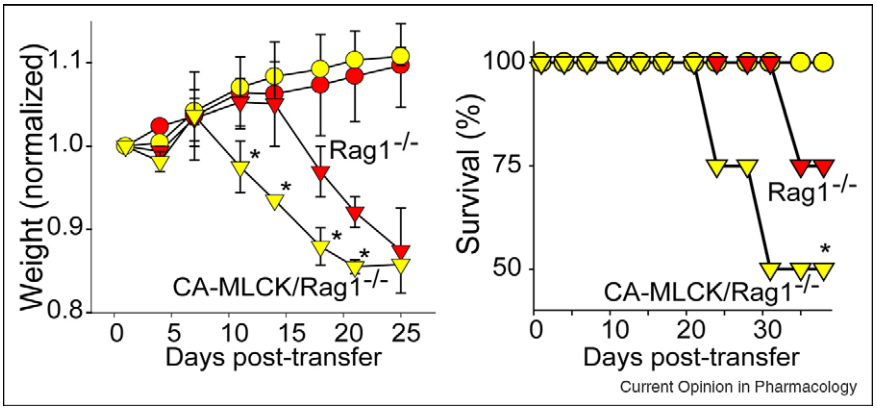
Figure 3



Increased paracellular permeability accelerates immune-mediated colitis. $CD4^+CD45Rb^{hi}$ (triangles) or $CD4^+CD25Rb^{lo}$ (circles) T cells were adoptively transferred into $RAG1^{-/-}$ mice (red symbols) or $RAG1^{-/-}$ mice expressing constitutively active MLCK (yellow symbols). $RAG1^{-/-}$/CA-MLCK recipients transferred with $CD4^+CD45Rb^{hi}$ T cells exhibited increased weight loss and mortality post-transfer than $RAG1^{-/-}$ recipients (from [52••] with permission).

more severe colitis than $Rag1^{-/-}$ mice (Figure 3). Thus, barrier dysfunction may predispose or contribute to progression of immune-mediated intestinal damage. Consistent with this, one recent study has shown that an antagonist against zonulin, which regulates intracellular tight junction disassembly, may limit mucosal immune activation in $IL\text{-}10^{-/-}$ mice [53••]. Development of therapeutic approaches to correct the molecular defects that give rise to barrier dysfunction, therefore, has great potential as a non-immunologic therapy for inflammatory bowel disease.

The absence of 'spontaneous' disease in CA-MLCK transgenic mice might prompt one to conclude that the barrier defect induced by the transgene was insufficient. However, the magnitude of barrier loss observed was similar to that in healthy relatives of Crohn's disease patients and, as noted above, the transgenic mice had increased susceptibility to adoptive transfer colitis [52••]. Interestingly, increased colonic mucosal IL-10 expression was present the CA-MLCK transgenic mice [52••]. This suggests that, in immunologically 'normal' individuals, barrier defects may trigger immunoregulatory responses that prevent inappropriate immune activation. It is important to recognize that these barrier defects are quantitatively and qualitatively different from the massive barrier loss induced by severe, widespread epithelial destruction and mucosal ulceration, as occurs in some enteric infections as well as the DSS model of colitis.

Consistent with the hypothesis that limited barrier defects may activate immunoregulatory processes, one recent study has shown that transient barrier defects can protect mice from future insults [54••]. In this study, barrier function was disrupted by local epithelial damage after intrarectal ethanol administration. In addition to increased intestinal permeability, these treatments enhanced IFNγ and IL-10 production by LPMC and caused expansion of a regulatory $CD4^+$ T cell population expressing latency-associated peptide (LAP). While expansion of $CD4^+LAP^+$ LPMCs was required for protection, adoptive transfer of these cells was insufficient to ameliorate colitis, suggesting that other factors contribute to the observed prevention of disease [54••].

Taken together, both of these studies show that barrier dysfunction alone is not sufficient to promote disease, but can alter susceptibility to colitis through regulation of mucosal immunity. While much work is needed to define the mechanisms of this immunoregulation, the increased exposure of the mucosa to luminal microbiota or their products by Toll-like receptors, including TLR2, may be involved [54••]. These studies highlight the importance of the subtle interplay between epithelial barrier function and mucosal immune regulation that may represent a future target for disease prevention.

Conclusions

Epithelial barrier dysfunction and inflammation are major contributors to the pathogenesis intestinal disease; however, much remains unknown about how these two processes contribute independently to disease initiation. Cytokine-induced barrier dysfunction is known to exacerbate colitis, perhaps due to increased translocation of microbial products. While barrier dysfunction alone is insufficient to cause disease, it can lead to subclinical activation of immune responses that may affect disease development at later times. Careful study of tight junction regulation and its contribution to disease initiation will probably provide new targets for the development of IBD therapeutics.

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- •• of outstanding interest

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