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Intestinal Barrier Function: Molecular Regulation and Disease Pathogenesis

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Abstract

The intestinal epithelium is a single-cell layer that constitutes the largest and most important barrier against the external environment. It acts as a selectively permeable barrier permitting the absorption of nutrients, electrolytes and water, while maintaining an effective defense against intraluminal toxins, antigens and enteric flora. The epithelium maintains its selective barrier function through the formation of complex protein-protein networks that mechanically link adjacent cells and seal the intercellular space. The protein networks connecting epithelial cells form three adhesive complexes: desmosomes, adherens junctions and tight junctions. These complexes consist of transmembrane proteins that interact extracellularly with adjacent cells and intracellularly with adaptor proteins that link to the cytoskeleton. Over the past decade, there has been increasing recognition of an association between disrupted intestinal barrier function and the development of autoimmune and inflammatory diseases. In this review, we summarize the evolving understanding of the molecular composition and regulation of intestinal barrier function. We discuss the interactions between innate and adaptive immunity and intestinal epithelial barrier function, as well as the impact of exogenous factors on intestinal barrier function. Finally, we summarize clinical and experimental evidence demonstrating intestinal epithelial barrier dysfunction as a major factor contributing to the predisposition to inflammatory diseases including food allergy, inflammatory bowel diseases and celiac disease.

Introduction

The intestinal epithelium is a single layer of cells lining the gut lumen and has two critical functions. Firstly, it acts as a barrier to prevent the passage of harmful intraluminal entities including foreign antigens, microorganisms and their toxins [1, 2]. Its second function is to act

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as a selective filter allowing the translocation of essential dietary nutrients, electrolytes and water from the intestinal lumen into the circulation [1, 3–5]. The intestinal epithelium mediates selective permeability via two major routes: transepithelial/transcellular and paracellular pathways [6] (Figure 1). Transcellular permeability is generally associated with solute transport through the epithelial cells and predominantly regulated by selective transporters for amino acids, electrolytes, short chain fatty acids and sugars [3–5]. Paracellular permeability is associated with transport in the space between epithelial cells, and is regulated by intercellular complexes localized at the apical-lateral membrane junction and along the lateral membrane [7]. Contact between intestinal epithelial cells includes three components that can be identified at the ultrastructural level: desmosomes, adherens junctions (AJs) and tight junctions (TJs) (Figure 2) [8]. The adhesive junctional complexes consist of transmembrane proteins that link adjacent cells to the actin cytoskeleton via cytoplasmic scaffolding proteins. The AJs and desmosomes are thought to be more important in the mechanical linkage of adjacent cells [9–11]. The TJs, on the other hand, are the apical-most junctional complex and responsible for sealing the intercellular space and regulating selective paracellular ionic solute transport [6, 12–14]. The AJ and TJ complexes are also important in the regulation of cellular proliferation, polarization and differentiation [6, 11–16].

Structural Components of Junctional Complexes

Adherens junctions (AJs)

AJs (also known as zonula adherens) are protein complexes on the lateral membrane that occur at points of cell-cell contact (Figure 2). They are formed by interactions between transmembrane proteins, intracellular adaptor proteins and the cytoskeleton. The major AJs are formed by cadherin-catenin interactions. Epithelial (E)-cadherins (calcium-dependent adhesion molecules) are Type-I single transmembrane spanning glycoproteins that possess an intracellular C-terminus and extracellular N-terminus. The extracellular domain forms homotypical interactions with cadherins of neighboring cells to promote cell-cell adhesion. The intracellular domain contains a catenin-binding domain that interacts with members of the armadillo repeat superfamily, β-, γ- and p^{120}-catenin [11, 17–21]. The catenins link the AJ to the cytoskeletal network via direct binding to the C-terminal domain of F-actin or indirectly through interactions with other adaptor proteins such as afadin [22–26]. Cadherin-catenin complexes are important not only for linking adjacent cells, but also for maintaining cell polarity, regulating epithelial migration and proliferation and the formation of other adhesive complexes such as desmosomes [21, 27, 19]. In support of this, downregulation of E-cadherin in the intestinal epithelium weakens cell-cell adhesion and has been linked with perturbed intestinal epithelial proliferation and migration [28, 29].

Nectin-afadin interactions form another important AJ complex [21, 30, 31]. Nectins (nectin-1-4) are immunoglobulin-like proteins that undergo homophilic and heterophilic interactions with nectins on adjacent cells [32]. Nectins can interact with the cytoskeleton via afadin, an F-actin binding protein, or alternatively via interactions with other F- or α-actin binding proteins including ponsin/SH3P12, vinculin and afadin dil domain-interacting protein [33–37].

Tight junctions (TJs)

TJs are the apical-most adhesive junctional complexes in mammalian epithelial cells that form a continuous belt-like ring around epithelial cells at the border between the apical and lateral membrane regions (Figure 2) [8]. TJs are dynamic, multi-protein complexes that function as a selective/semipermeable paracellular barrier, which facilitates the passage of ions and solutes through the intercellular space, while preventing the translocation of luminal antigens, microorganisms and their toxins. The evolution of TJ biology emerged in the 1960's with the development of electron microscopy. Analysis of epithelial cells revealed a series of apparent fusions, where the space between adjacent epithelial cells was eliminated [6, 38, 39]. These so-called "kissing points" are morphologically different from AJs and desmosomes, where adjacent cell membranes remain 15–20nm apart [6]. Since these initial observations, TJs have been found to consist of four unique families of transmembrane proteins: occludin, claudins, junctional adhesion molecules (JAMs) and tricellulin.

The extracellular domains of transmembrane TJ proteins in adjacent cells anastomose to form the TJ seal. These interactions include those involving proteins in the same membrane (in cis) and those involving proteins in adjacent cells (in trans). In addition, TJ proteins can form either homophilic (with the same protein) or heterophilic (between non-identical TJ proteins) interactions. Similar to the AJs, the intracellular domains interact with various scaffolding proteins, adaptor proteins and signaling complexes to regulate cytoskeletal attachment, cell polarity, cell signaling and vesicle trafficking (Figure 3). The intracellular regions of AJs possess PDZ-binding domains, which recruit and interact with PDZ domain containing proteins. The PDZ domain (**P**ost synaptic density-95/**D**rosophila disc large/ **Z**onula occludens-1 protein) is a common structural domain of 80–90 amino acids that functions to anchor transmembrane proteins to the cytoskeleton. The intracellular domains can also interact with non-PDZ-binding domain containing proteins such as cingulin, which can interact with junctional membrane proteins, the actin cytoskeleton and signaling proteins [40]. The complex network of network of intracellular protein interactions is also known as the "cytoplasmic plaque".

Tight junction formation in the gastrointestinal tract

The intestinal epithelium forms the largest and most important barrier between our internal and external environments. The barrier is maintained by the expression of AJs and TJs, including cadherins, claudins, occludin and JAM proteins, which seal together adjacent cells and provide cytoskeletal anchorage (Figure 3) [41]. Expression of junctional proteins in the intestine is highly regulated and dependent on the intestinal compartment (small or large intestine), villus/crypt localization and cell membrane specificity (apical, lateral or basolateral). The complex pattern of TJ expression in the intestine is related to the specific functions of a particular intestinal region and localization. Expression of AJ and TJ proteins are also regulated by phosphorylation (Table 1). Phosphorylation can either promote TJ formation and barrier function, or alternatively promote TJ protein redistribution and complex destabilization [42, 43].

i. Occludin—The first TJ-specific integral membrane protein identified was occludin [44]. Occludin is expressed predominately at TJs in epithelial and endothelial cells, but also by astrocytes, neurons and dendritic cells [44,45–47].Occludin (60–82 kDa) is a tetraspanning integral membrane protein with two extracellular loops, a short cytoplasmic N-terminus and a long cytoplasmic C-terminus [11, 13]. Functional analysis indicates that the extracellular loops and transmembrane domains of occludin regulate selective paracellular permeability. Intracellularly, the C-terminus interacts with the PDZ-domain containing protein ZO-1, which is required to link occludin to the actin cytoskeleton (Figure 3) [48, 49].

Several occludin isoforms have been identified and are thought to be a result of alternative mRNA splicing [50, 51]. Notably, several splice variants demonstrate altered subcellular distribution and interaction with other TJ molecules [50, 51]. Analysis of the splice variants revealed that the cytoplasmic C-terminal domain is essential for the intracellular trafficking of occludin to the lateral cell membrane, and that the fourth transmembrane domain is critical for targeting occludin to the TJ and for ZO-1 interactions [51].

The function of occludin is not fully delineated; however, *in vitro* and *in vivo* data suggest a role for occludin in the regulation of paracellular permeability [52, 53]. Notably, the major allergen of the house dust mite, Der p 1, has been found to proteolyticly cleave occludin leading to the disruption of the TJ complex and increased paracellular permeability [54]. Furthermore hydrocortisone treatment of bovine retinal endothelial cells increased occludin expression two-fold and enhanced monolayer barrier properties [55]. Although occludin is an important constituent of TJs, TJ formation and paracellular permeability barrier function are not dependent on occludin. Experimental analyses of occludin$^{-/-}$ mice demonstrated equivalent numbers and organization of TJs and similar paracellular ion conductance as wild-type mice [56]. Furthermore, epithelial transport and barrier function were normal in occludin$^{-/-}$ mice [57]. In addition to regulating paracellular permeability, there is evidence suggesting occludin is involved in cellular adhesion [58]. Expression of occludin in occludin$^{-/-}$ rat fibroblasts conferred cell–cell adhesion that was abrogated by synthetic peptides corresponding to the first extracellular loop of occludin, underscoring the importance of this region of occludin in cell adhesion [59].

In vitro analysis suggests that occludin localization to the TJ complex is regulated by phosphorylation. Several potential phosphorylation sites at tyrosine, serine, and threonine residues of occludin have been identified and regulation of occludin phosphorylation is proposed to occur by kinases, including the non-receptor tyrosine kinase c-Yes and protein kinase C (PKC), and phosphatases including the serine/threonine protein phosphatase 2A [60, 61] (Figure 3). PKCη, a novel protein kinase predominantly expressed in the intestinal epithelium, has been shown to directly phosphorylate occludin at threonine residues (T403 and T404). Blockade of PKCη-mediated occludin phosphorylation disrupted junctional distribution of occludin and ZO-1 and compromised epithelial barrier function[62]. These data suggest that occludin phosphorylation regulates occludin-ZO-1 interactions and the maintenance of intact TJ complexes and paracellular barrier function.

ii. Claudins—Claudins are 20–27kDa integral membrane proteins with four hydrophobic transmembrane domains, two extracellular loops and N- and C-terminal cytoplasmic

domains [7, 63–65]. The extracellular loops are critical for homophilic and/or heterophilic TJ protein-protein interactions and the formation of ion-selective channels [7]. The intracellular C-terminal domain is involved in anchoring claudin to the cytoskeleton via interactions with PDZ-binding domain proteins, including ZO-1, -2 and -3 [66–68] (Figure 3). Currently, 24 distinct claudin family gene members have been identified in humans with a number of orthologues expressed in other species [6, 69]. They exhibit distinct tissue-, cell- and developmental stage-specific expression patterns [88–93]

Claudin-claudin interactions between adjacent cells can be either homophilic or heterophilic [70, 71]. Homophilic interactions have been demonstrated for claudins 1, 2, 3, 5, 6, 9, 11, 14 and 19. On the other hand, heterophilic interactions are more restricted and primarily have been observed with claudin-3, which can interact with claudins-1, -2 and -5 [70]. Notably, there is specificity in heterophilic trans-interactions. For example, transfection of fibroblasts with claudins-1, -2 and -3 led to claudin-3 interactions with both claudin-1 and -2; however no interactions between claudin-1 and -2 were observed [71]. These selective interactions are thought to explain the diversity in TJ formations and provide a molecular basis for tissue-specific heterogeneity of barrier function [65].

Recent studies, with claudin-deficient mice also provide corroborative data supporting a role for claudins in the regulation of barrier function. Claudin-$1^{-/-}$ mice die within one day of birth due to significant transepidermal water loss [72]. In addition, transgenic overexpression of claudin-6 in the epidermis disrupted tight junction formation and increased epithelial permeability [73]. Notably, experimental data suggests that claudins can have differential effects on paracellular permeability. For example, introduction of claudin-2 into MDCK I cells that express claudin-1 and -4 induces a decrease in transepithelial resistance (TER); whereas transfection of claudin-3 had no effect suggesting that claudin-2 markedly decreased claudin-1/claudin-4 based TJ strand tightness[74]. In support of these data recent experimental evidence suggests that claudins can form size- and charge-specific paracellular channels. Transfection of claudin-8 into MDCK II cells which lacks endogenous claudin-8 significantly reduced paracellular movement of mono- and divalent cations without affecting anion and uncharged solute movement [75]. Experimental analyses suggest that the first extracellular loop of claudins play an important role in determining charge selectivity. Interchanging of the first or both extracellular domains of claudin-4 on claudin-2 profoundly decreased the ion conductance of Na^+ relative to Cl^- [76]. Furthermore, substitution of a negatively-charged lysine to a positively charged aspartic acid (K65D) within the loop of claudin-15 caused an increase in Na^+ permeability, while mutation in the same region of three positively charged amino acids to negatively charged aspartic acid, arginine and aspartic acid (E46K, D55R and E64K) switched the ion selectivity of claudin-15 from Na^+ to Cl^- channel [77]. Pore density and size may also influence paracellular movement of charged and non charged solutes [78].

Claudins also play a role in epithelial cell invasion and motility. Overexpression of claudins-3 and -4 in human ovarian epithelial cells, which lack endogenous expression of these proteins, was associated with increased epithelial cell survival and enhanced invasion and motility [79]. Consistent with this observation, siRNA-mediated knockdown of claudins-3 and -4 in ovarian cancer cell lines reduced invasion [79]. The effects of claudin-3 appear to be

linked to altered matrix metalloprotease-2 activity, which suggests that claudin-induced invasion may be mediated by metalloprotease proteins.

As with occludin, claudin localization to the TJ complex and its function are regulated by post-translational phosphorylation and via interactions with PDZ-binding domains. The intracellular C-terminal domain of claudin possesses multiple regulatory sites including potential serine and theronine phosphorylation sites and PDZ-binding domains [7]. Phosphorylation of claudins-3 and -4 in ovarian cancer cells is linked to the regulation of paracellular permeability [80, 81]. For example, patients with pseudohypoaldosteronism type II (PHA II; or chloride shunt syndrome) present with hyperkalemic metabolic acidosis, hypertension and dysregulated paracellular ion transport [82]. The molecular basis is linked to a loss-of-function mutation in the serine-threonine kinases, WNK1 and WNK4, which regulate epithelial chloride cotransporters. This leads to an increase in the phosphorylation of claudins-1-4 and an increase in paracellular permeability [82]. A number of signaling pathways have been implicated in the phosphorylation of claudins including PKC, Rho GTPases, mitogen-activated protein kinases (MAPKs) and phosphatases [83]. For example, MAPK phosphorylation of claudin-1 is required for claudin-1-mediated barrier function [84]. Furthermore, claudins-1, -2, -7, -8, -16 and -17 possess putative PKC phosphorylation sites [83]

All claudins, except claudin-12, end in the dipeptide sequence YV, which has been shown to interact with PDZ-binding domain proteins include ZO-1, -2 and -3, multi-PDZ domain protein 1 and PALS1-associated TJ protein [63, 65, 6785] (Figure 3). Many of these scaffolding proteins contain multiple PDZ domains, which facilitates the formation of dense localized protein complexes, also known as “cytoplasmic plaques”. Furthermore, the scaffolding proteins can interact with signaling molecules, including heterodimeric GTP binding proteins (Rab13 and Gα12), transcriptional factors and RNA-processing factors, to link TJ complexes to the actin-cytoskeleton and regulate aspects of epithelial polarization, differentiation and barrier function (For review see [6, 21, 38, 86, 87]).

iii. Junctional adhesion molecules (JAMs)—JAMs are integral membrane proteins that belong to the immunoglobulin superfamily and have two immunoglobulin folds (VH- and C2-type) in the extracellular domain [88, 89]. JAMs are expressed by multiple cell types, including epithelial, endothelial and immune cells [90, 91]. They are subdivided based on the expression of Type I or II PDZ-binding motifs in the intracellular C-terminus, which suggests that the two types interact with unique scaffolding and cytoplasmic proteins [88]. JAM-A, -B and -C (or JAM1-3) have Type II binding motifs, while the atypical JAMs, including JAM-4, coxsackie and adenovirus receptor (CAR) and endothelial selective adhesion molecule contain Type I PDZ-binding domains [21, 88]. Similar to other TJ proteins, these JAM-PDZ interactions provide anchorage to the actin cytoskeleton (Figure 3).

The extracellular region of JAMs bind to multiple ligands through homophilic and heterophilic interactions, which are proposed to regulate the cellular functions and paracellular permeability of JAMs [88, 89]. Homophilic JAM-A or -B interactions regulate the formation of functional TJs and cell-cell border formation [92, 93], while heterophilic JAM interactions play a role in leukocyte-endothelial cell adhesion [89].

Recent studies demonstrate the importance of JAM-A in the formation and assembly of TJs in intestinal epithelial cells. siRNA downregulation of JAM-A in SK-C015 epithelial cells induced an increase in permeability [94]. Consistent with this, JAM-A$^{-/-}$ mice had increased mucosal permeability as indicated by enhanced dextran flux and decreased TER [94]. However, these mice also had an increase in claudin-10 and -15 expression, which are thought to form selective pores in the TJ complex, enhancing paracellular permeability [77, 95]. Interestingly, JAM-A$^{-/-}$ mice have increased susceptibility to chemical-induced colitis. Dextran sodium sulfate administration to JAM-A$^{-/-}$ mice induced more severe colonic injury as compared to WT control animals [94]. These studies suggest altered intestinal permeability as a susceptibility factor to intestinal disease.

Dysregulation of TJ formation and intestinal barrier function

i. Cytokine-mediated

In vitro and *in vivo* animal studies have demonstrated that intestinal permeability is regulated by multiple factors including exogenous factors, epithelial apoptosis, cytokines and immune cells (Figure 4). Immune-induced intestinal barrier dysfunction is thought to be critical in the predisposition to and exacerbation of numerous autoimmune and inflammatory conditions, including IBD, food allergy, celiac disease and diabetes [96]. For example, interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα), which are central mediators of intestinal inflammatory diseases including IBD, induce intestinal epithelial barrier function [97–99]. Incubation of intestinal epithelial cell monolayers (Caco2 and T84) with IFNγ and TNFα promoted the reorganization of several TJ proteins: ZO-1, JAM-A, occludin, claudins-1 and -4 and decreased epithelial barrier function [100]. The mechanism of action of these cytokines appears to be primarily mediated via myosin light chain kinase (MLCK)-mediated phosphorylation of myosin light chain (MLC), which promotes TJ disruption [100]. In support of this, inhibition of TNFα- and IFNγ-induced MLC phosphorylation restored barrier function [100]. . Alternatively, TNFα and IFNγ can disrupt TJs and increase intestinal permeability via dysregulation of claudin and occludin expression [101].

Experimental and clinical data supports a role for Th_2 cytokines in the regulation of intestinal barrier function (Figure 4). Stimulation of colonic epithelial cells (T84 and HT-29/B6) with IL-4 or -13 induced an increase in intestinal permeability [102–104]. Notably, altered barrier function was associated with the induction of epithelial apoptosis and expression of the pore-forming TJ claudin-2. *In vitro* data suggests that the effects of IL-4 and -13 on barrier function is primarily mediated by phosphoinositide 3-kinases [103, 105]. Blockade of phosphoinositide 3-kinase, but not STAT-6 activation blocked IL-4/IL-13-induced barrier dysfunction [103]. However, studies in STAT-6$^{-/-}$ mice identified a role for STAT-6 signalling in IL-4 and -13-mediated intestinal epithelial barrier dysfunction [106]. Moreover, IL-4- and IL-13-induced altered permeability, glucose absorption and chloride secretion was attenuated in STAT-6 deficient mice compared to WT mice [106].

The anti-inflammatory cytokine IL-10 has also been shown to regulate intestinal barrier function [107, 108]. Stimulation of ileal segments from Sprague-Dawley rats with IL-10 enhanced intestinal electroneutral sodium and chloride absorption and inhibited stimulated

chloride secretion [109]. In addition, treatment of T84 epithelial cell monolayers with IL-10 blocked IFNγ-induced epithelial permeability [110]. These results suggest that IL-10 plays a protective role in intestinal barrier function. In support of this, mice deficient in IL-10 have increased small intestinal permeability [107]. Notably, IL-$10^{-/-}$ mice spontaneously develop chronic intestinal inflammation, which is manifested by symptoms commonly associated with Crohn's Disease (CD), including weight loss, mucosal hyperplasia and chronic enterocolitis [111]. These data suggest that increased permeability may predispose IL-$10^{-/-}$ mice to intestinal inflammation and colitis. Consistent with this hypothesis, increased permeability in IL-$10^{-/-}$ mice was observed prior to disease onset [107].

Mechanistic studies to delineate IL-10-mediated intestinal permeability have implicated the zonulin pathway and TNFα. Remarkably, inhibition of the zonulin receptor in IL-$10^{-/-}$ mice led to decreased intestinal permeability, reduced colonic TNFα secretion *ex vivo* and abrogated the spontaneous development of colitis [108]. These findings further support a role for increased intestinal permeability in the development of intestinal inflammation and disease and a possible role for zonulin. The zonulin/zonulin receptor pathway is thought to regulate TJ formation via PKC-dependent actin reorganization [112]. Whether decreased intestinal barrier function in IL-$10^{-/-}$ mice is primarily due to an inherent defect in the zonulin/zonulin receptor pathway or alternatively, a consequence of increased expression of cytokines such as IFNγ and TNFα remains to be delineated.

ii. Immune cells

T-cells—Anti-CD3-induced $CD4^+$ T-cell activation in mice promotes an increase in transcellular and paracellular intestinal permeability, and the release of proinflammatory cytokines such as IFNγ and TNFα [113, 114] (Figure 4). Furthermore, injection of mice with TNFα provokes a breakdown in intestinal barrier function, diarrhea and PKCα-dependent inhibition of Na^+/H^+ exchange [115]. T-cells regulate transcellular permeability through the downregulation of Na^+/K^+-ATPase, and disruption of Na^+ absorption, Na^+-glucose cotransport and inducible Cl^- secretion [113, 114]. Whereas, dysregulation of the paracellular permeability pathway, is mediated via MLCK-dependent TJ disruption [114].

Gamma/delta-positive intestinal intraepithelial lymphocytes (iIELγδ$^+$), which are closely associated with the basolateral side of intestinal epithelial cells, have also been implicated in intestinal barrier maintenance [116]. In response to enteric parasitic infestation, mice deficient in iIELγδ$^+$ T-cells have abnormal claudin-3, occludin and ZO-1 localization, decreased occludin phosphorylation and abnormal epithelial TJ formation [116]. Notably, the alterations in intestinal barrier function could be attributed to a single subset of iIELγδ$^+$ lymphocytes: T-cells expressing Vγ7^+ encoded T-cell receptors. Reconstitution of mice deficient in iIELγδ$^+$ T-cells with Vγ7^+ iIELs restored epithelial barrier function [116].

Mast cells—Mast cells are present in all compartments of the gastrointestinal (GI) tract [117]. Upon activation, they release a powerful array of inflammatory mediators including histamine, 5-hydroxytryptamine (5-HT), neutral proteases (tryptases, chymases and carboxypeptidase A), prostaglandins, leukotrienes, platelet activating factor and several cytokines including TNFα, IL-3, -4, -5, -6 and GM-CSF [118–120]. Employing models of food

allergy or helminthic infestation (*Nippostrongylus brasiliensis* or *Trichinalla spiralis*), investigators have demonstrated mast cell involvement in intestinal barrier function [121] (Figure 4). Intraluminal challenge of egg albumin-sensitized rats induced a 15-fold increase in uptake of 51Cr-labeled EDTA compared to rats treated with unrelated protein [122]. The antigen-induced decreased barrier function was associated with mast cell degranulation and an increase in the short-circuit current, a measure of net ion transport [122]. The importance of mast cells was demonstrated by the absence of changes in barrier function in mast cell-deficient mice sensitized and challenged with egg albumin, which, was restored by bone marrow reconstitution [123, 124]. Furthermore, several mast cell mediators have been shown to modulate intestinal epithelial ion transport. Pretreatment of egg albumin-sensitized rats with histamine-H1 or 5-HT2 receptor antagonists significantly reduced oral antigen-induced short-circuit current alterations [123, 125].

Experimental analyses employing models of parasitic infestation have identified a role for mast cell-derived proteases in intestinal barrier function [126]. Murine infestation with the enteric nematode, *T. spiralis*, induced intestinal mastocytosis, occludin degradation and increased intestinal permeability [126]. The alterations in barrier function were demonstrated to be mast cell-dependent as depletion of mast cells with a neutralizing anti-c-kit antibody ablated intestinal epithelial barrier dysfunction [126]. Similarly, mice deficient in the murine mast cell protease 1 (mMCP-1) were also resistant to T. spiralis infestation-induced intestinal epithelial barrier dysfunction. Mast cell/MCP-1 regulation of intestinal permeability during T. Spiralis infection was linked to occludin degradation [126].

Eosinophils—Increased eosinophils and eosinophil granular proteins, including major basic protein, eosinophil peroxidase and eosinophilic cationic protein are often associated with IBD and altered barrier function [127–130]. In vitro coculture of T84 intestinal epithelial cells with eosinophils or eosinophil-derived major basic protein decreased TER and increased permeability. Altered intestinal barrier function was associated with the downregulation of occludin [131].

Exogenous Regulation of Intestinal Barrier Function

Alcohol

Chronic alcohol consumption has been shown to be associated with increased intestinal permeability, inhibition of vitamin and nutrient transport and a reduction in sodium and water absorption [132, 133]. Experimental analyses suggest involvement of the byproduct of ethanol metabolism, acetaldehyde and nitric oxide (NO) in alcohol-mediated barrier dysfunction. High levels of acetaldehyde have been detected in the intestine of rats following ethanol administration. Increased levels of acetaldehyde was associated with increased intestinal permeability and endotoxin translocation [134]. Furthermore, incubation of Caco2 cells with acetaldehyde increased monolayer permeability and this increase was associated with elevated tyrosine phosphorylation of ZO-1, E-cadherin, and β-catenin [42]. Exposure of Caco2 monolayers to ethanol also promotes inducible nitric oxide synthase expression, stimulating increased NO production and increased monolayer permeability [135]. NO-induced changes were associated with an increase in unstable, non-polymerized tubulin and extensive damage to the microtubule cytoskeleton.

Experimental studies in rodents have also demonstrated that acute administration of alcohol induces mucosal damage in the upper small intestine including villus ulceration, submucosal blebbing and hemorrhagic erosions and intestinal barrier dysfunction [133, 136, 137]. It is postulated that alcohol-induced intestinal permeability facilitates enhanced translocation of endotoxin to distant organs leading to inflammation and tissue damage [136, 138, 139]. Intragastric administration of endotoxin in the presence of alcohol to rodents lead to significantly higher plasma endotoxin levels than animals fed endotoxin alone [139, 136]. Similar lesions have been found in healthy volunteers and active alcoholics following acute alcohol consumption [139, 140] and plasma endotoxin levels in alcoholics were found to be 5-fold greater than healthy controls [141]. While not fully understood, evidence suggests the mechanism underlying alcohol-induced barrier dysfunction is related to the influx of inflammatory cells and release of various mediators, including cytokines, reactive oxygen species, leukotrienes and histamine [142, 143].

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAID use is associated with a high incidence of GI side effects, and there is substantial evidence indicating that chronic use can alter intestinal barrier function and cause significant GI damage, including ulcers, perforation, hemorrhage and an exacerbation of IBD [144–151]. Both acute and chronic ingestion of NSAIDs by healthy volunteers and patients promotes altered intestinal barrier dysfunction and hypermotility [146, 152, 153]. *In vitro* studies with MKN28, a gastric epithelial cell line have demonstrated that aspirin-induced increase in permeability was accompanied by a significant decrease in the expression of claudin-7, but not claudins-3, -4, ZO-1 or occludin [154]

NSAID-induced GI injury was initially found to be a consequence of cyclooxygenase inhibition and decreased prostaglandin synthesis; however, it has become evident that intestinal damage is a multi-stage process [145, 152155]. Experimental analyses have identified a contribution from neutrophils, microcirculatory disturbances, oxygen free radicals and bile acids in NSAID-induced GI damage [145, 156, 157]. NSAIDs increase intestinal nitric oxide synthase expression and activity, leading to increased levels of NO, promoting increased intestinal permeability [158]. NSAIDs can also uncouple mitochondrial oxidative phosphorylation, which impairs the mitochondrial energy production necessary for TJ complex integrity leading to increased intestinal inflammation and permeability [159]. Finally, a recent study demonstrated that aspirin induced an increase in gastric epithelial cell permeability that was mediated by activation of p38 MAPK and a decrease in claudin-7, and treatment with a p38 MAPK inhibitor attenuated this response [154].

Pathogens

The intestine is home, both permanently and transiently, to an extraordinarily complex microflora that provides an abundant source of potentially pathogenic organisms, toxins and antigens. The dynamic and complex interactions between enteric pathogens and the intestinal epithelium often leads to disturbances in the intestinal barrier, altered fluid and electrolyte transport and the induction of an inflammatory response [160]. Enteric pathogens can disrupt the intestinal barrier either directly, by binding to cell surface molecules and inducing changes in TJ protein expression. Alternatively, pathogens generate toxins and

proteases, which can promote cell damage and apoptosis, alter epithelial ion transport and disrupt TJs and the cytoskeleton. Herein, we will provide examples and brief descriptions of several mechanisms by which pathogens disrupt barrier function.

Vibrio cholera—*V cholera* is a major enteric pathogen that alters intestinal barrier function through the disruption of TJs, dysregulation of intestinal ion and fluid transport and the initiation of inflammatory cascades. A major toxin produced by *V cholera* is the cytotoxin, hemagglutinin protease (HA/P), a zinc-binding metalloprotease that degrades TJ proteins and decreases barrier function [161, 162]. Studies of mutant toxin-attenuated strains of *V cholera* have identified HA/P as the principle toxin responsible for alterations of TJs and decreased TER in cultured MDCK and T84 cells [161, 162]. *In vitro* studies demonstrate that HA/P cleaves the extracellular domain of occludin. This disrupts intracellular occludin-ZO-1 interactions and destabilizes the TJ complex and cytoskeletal anchorage, resulting in increased paracellular permeability [163].

Another toxin elaborated by *V cholera* is zonula occludens toxin (Zot), an enterotoxin that reversibly increases intestinal epithelial permeability, disrupts the actin cytoskeleton and induces fragmentation of ZO-1 and occludin [164, 165, 166]. Zot binds to the zonulin receptor on the apical side of intestinal epithelial cells and activates phospholipase C leading to PKCα-dependent polymerization of the actin cytoskeleton [165167]. Actin polymerization is thought to promote cytoskeletal reorganization and the destabilization of TJ complexes. Consistent with this hypothesis, pretreating intestinal epithelial monolayers with PKCα inhibitors prevented Zot-induced changes in actin polymerization and permeability [165]. A human homologue for Zot, zonulin, has been identified and found to bind to the same receptor and regulate intestinal permeability [168]. Zonulin is believed to regulate TJ function and its dysregulation has been has been implicated in several inflammatory diseases associated with intestinal barrier dysfunction including IBD, Type I Diabetes and celiac disease (see clinical review).

Enteropathogenic E. coli (EPEC)—EPEC is a diarrhea-causing bacteria that disrupts TJ proteins by adhering directly to the surface of epithelial cells. They form attaching and effacing lesions, characterized by the localized destruction of the adjacent epithelial microvilli and the formation of a pedestal-like structure from the accumulation of cytoskeletal proteins, such as actin, beneath the site of attachment [169]. EPEC uses a syringe-like type III secretion system to trigger TJ disruption and alterations in intestinal epithelial ion secretion [170–172]. Infection of intestinal T84 monolayers with EPEC increased epithelial permeability and this was associated with destabilization and dissociation of ZO-1, occludin and claudin-1 TJ complex from the lateral membrane [171–173]. The molecular mechanisms associated with EPEC-mediated TJ alterations are still unclear; however, studies utilizing pharmacological agents that inhibit MLCK, have implicated involvement of the MLCK pathway in the process [174].

Clostridium perfringens—While many bacterial products have been demonstrated to alter TJs, the enterotoxin of *Clostridium perfringens* (CPE), which is a common cause of food poisoning, directly interacts with and utilizes TJs as receptors [175, 176]. CPE binds to the extracellular loop of claudins-3 and -4 on the cell surface of enterocytes forming small

protein complexes in the plasma membrane [176]. These complexes promote oligomerization and the formation of larger plasma membrane complexes, which have been associated with increased plasma membrane permeability [177, 178]. CPE also interacts with occludin to promote its removal from the TJ and redistribution into the cytoplasm [177]. The redistribution of claudins and occludin induce destabilization of the TJ complex leading to altered intestinal paracellular permeability. For example, exposure of MDCK monolayers to CPE induced a reversible decrease in TER and increase in permeability [179]. Finally, the large CPE and TJ-containing complexes are believed to insert into the plasma membrane to form a functional pore that induces Ca^{2+} influx that triggers host epithelial cell death by apoptosis or oncosis [180, 181].

Clinical Review

Introduction

A breakdown or impairment of the epithelial barrier has been implicated as a critical determinant in the predisposition to intestinal inflammation and a number of gastrointestinal (GI) diseases including inflammatory bowel disease (IBD) and food allergy [160, 182–187] (Table 2). While altered intestinal barrier function (increased intestinal epithelial permeability) can be a consequence of disease exacerbation, clinical evidence suggests that it may be a primary etiologic factor predisposing to disease development. For example, healthy first-degree relatives of IBD and celiac patients have increased intestinal permeability. Furthermore, altered intestinal permeability persisted in asymptomatic celiac disease patients treated with a gluten-free diet [188, 189] and is predictive of clinical relapse in patients with clinically inactive IBD [190, 191]. In this section, we summarize the current clinical data relevant to intestinal epithelial barrier function in chronic disease susceptibility and describe the potential implications of these studies in disease pathogenesis.

Food Allergy

Food allergies are adverse, immune-mediated reactions to ingested food proteins/antigens. It is hypothesized that intestinal barrier dysfunction may contribute to both antigen sensitization and also the IgE/mast cell-mediated anaphylactic effector phase of disease. The development of food allergies is dependent on the exposure of the food antigen to the mucosal immune system, which leads to antigen sensitization and the production of dietary antigen-specific CD4+ Th2 cells and IgE. It is hypothesized that altered intestinal barrier function permits increased dietary antigen transport across the intestinal barrier and exposure of dietary antigens to the mucosal immune system leading to the development of the dietary antigen-specific response. Consistent with this hypothesis, intestinal permeability in infants with food allergy as assessed by lactulose/mannitol ratio in the urine, was significantly increased compared to healthy young children [192, 193]. To determine whether the altered intestinal barrier function was a consequence of a recent adverse allergic reaction to dietary antigen, lactulose/mannitol ratio was examined in food allergic patients who had been on an allergen-free diet for at least six months. Intestinal permeability remained elevated in these individuals indicating that increased intestinal permeability persisted in the absence of food antigen stimulation [192].

Additional data supporting a role for increased intestinal permeability in the development of food antigen sensitization and food allergies is provided by recent clinical studies that demonstrate an association between increased intestinal permeability and the development of new-onset food allergies in patients following liver and heart transplantation. Patients treated with the immunosuppressant tacrolimus (FK506) have been shown to have increased intestinal permeability and elevated levels of food antigen-specific IgE [194–196]. Notably, some of these patients developed new-onset food allergies [197, 198]. The development of food allergies in immunosuppressed post-transplant patients was originally thought to be a consequence of the passive transfer of food antigen-specific IgE or lymphocytes from food-allergic donors to previously non-allergic recipients [199, 200]. However, studies have reported the development of food allergies in patients where the donor had no history of food allergy [201, 202]. Interestingly, *in vitro* and *in vivo* experiments with rats have demonstrated that tacrolimus induces a dose-dependent increase in intestinal permeability [203] suggesting that tacrolimus-induced altered intestinal barrier function may be a possible explanation for the new-onset food allergies in immunosuppressed post-transplant patients.

Notably, tacrolimus has been shown to uncouple mitochondrial oxidative phosphorylation, leading to impaired mitochondrial energy production and a significant decrease in cellular ATP [203, 204]. Importantly, formation of the intestinal barrier and the maintenance of intercellular junctional complexes are energy-dependent processes and decreased cellular ATP is responsible for inducing a breakdown in TJ complexes and barrier dysfunction [44]. Consistent with this rats treated with tacrolimus were shown to have a dose-dependent increase in intestinal permeability that correlated with decreased intracellular ATP levels and CO_2 release [203]. Similarly, liver transplant patients treated with tacrolimus were found to have reduced mitochondrial energy production associated with increased intestinal permeability and an increase in serum endotoxin levels [198].

The immunosuppressive activity of tacrolimus is through the inhibition of calcineurin, which is critical for IL-2 induced T-cell activation [205], Inhibition of IL-2, has been shown to promote T-helper 2 immune response [233]. Th2 cells secrete IL-4, IL-5 and IL-13, which promote IgE-mediated allergic inflammation and set the stage for food antigen sensitization and the induction of food allergies. There are most likely several mechanisms involved in the pathogenesis of food allergies in tacrolimus-immunosuppressed patients and increased intestinal permeability appears to be an important mediator to facilitate presentation of food antigens to the immune system and oral antigen sensitization.

We have recently provided experimental evidence supporting a role for altered intestinal permeability in oral antigen sensitization and the development of food allergies in mice [206]. We generated a transgenic mouse that overexpresses the cytokine interleukin-9 specifically in the enterocytes of the small intestine (iIL-9Tg). A consequence of transgenic overexpression of IL-9 was a pronounced intestinal mastocytosis and altered intestinal permeability [206]. Repeated oral administration of OVA to iIL-9Tg BALB/c mice and not WT mice promoted the development of antigen-specific IgE, $CD4^+$ $IL\text{-}4^+$ T-cells and symptoms of a food allergic response in the absence of prior systemic sensitization or the use of adjuvant. Pharmacological mast cell depletion in iIL-9Tg mice was found to restore intestinal permeability to levels comparable to WT mice. Remarkably, reconstitution of

barrier function and decreased intestinal permeability in iIL-9Tg mice prevented orally-induced antigen sensitization [206]. These findings suggest that increased intestinal permeability facilitates enhanced antigen uptake and the oral induction of food antigen sensitization.

Intestinal barrier dysfunction is also thought to contribute to the severity of food allergen-induced clinical symptoms. Oral challenge of food allergic individuals with food allergen induced an increase in lactulose/mannitol ratio in the urine [192, 207]. The level of intestinal barrier dysfunction positively correlated with the severity of clinical symptoms [192]. Notably, treatment of the food allergic group with sodium cromoglycate a mast cell stabilizer prior to ingestion of food allergen, significantly reduced lactulose permeability compared to food allergen-challenged individuals not receiving sodium cromoglycate indicating a role for mast cells in dietary antigen-induced intestinal epithelial barrier dysfunction [207].

Consistent with clinical observations animal models of GI anaphylaxis and food allergy have also demonstrated increased intestinal permeability following oral antigen challenge [206, 208, 209]. Intraluminal challenge of egg-sensitized rats with egg albumin induced a 15-fold increase in uptake of 51cr-labelled EDTA as compared to rats treated with unrelated protein [122]. Studies utilizing mast cell-deficient animals or pharmacological agents to deplete mast cells have provided corroborative evidence demonstrating that mast cells are critical for altered intestinal barrier function during food allergic reactions [206, 209, 123, 124]. Increased permeability following antigen challenge has been shown to initially be the result of increased antigen uptake and translocation by the transcellular route, as evidenced by an increase in HRP-containing endosomes within minutes of HRP challenge in rats that had been sensitized [210]. The second phase, which occurs after sensitization and is mast cell-dependent, was associated with a disruption in the TJs and an increase in paracellular permeability [210]. Collectively, these studies suggest a role for altered intestinal barrier function in food allergy. Furthermore, these studies suggest a role for mast cells in the regulation of intestinal barrier dysfunction in food allergy.

Inflammatory Bowel Disease (IBD)

The IBDs, Crohn's disease (CD) and ulcerative colitis, are chronic, relapsing-remitting inflammatory diseases. An emerging model of the pathogenesis of IBD suggests there are three essential factors: 1) a breakdown in intestinal barrier function; 2) exposure of luminal contents to immune cells in the lamina propria; and 3) an exaggerated immune response [211]. However, it is currently unclear which factor is responsible for initiating this self-perpetuating cycle, leading to disease exacerbation. There is a growing body of data to suggest that increased intestinal permeability is a primary etiologic factor contributing to IBD pathogenesis. CD patients with clinically active disease have increased intestinal permeability and in patients with inactive disease, increased intestinal permeability is predictive of clinical relapse [190, 191]. In addition to patients with IBD, increased intestinal permeability occurs in 10–25% of their healthy first-degree relatives, indicating that increased intestinal permeability likely preceded the onset of clinical disease [212–214]. Furthermore, studies have found that a subset of patients who are at high risk for CD have either increased intestinal permeability at baseline or an exaggerated increase in

permeability in response to stimulation [183, 215]. Notably, a case study on the long-term follow-up of a healthy 13-year-old girl who had elevated intestinal permeability and a parent with CD patient reported that she subsequently developed CD 8 years later [216].

There are extensive experimental studies employing models of experimental colitis that demonstrate a link between altered intestinal barrier function and IBD. Mouse models predisposed to developing IBD-like symptoms, including IL-10$^{-/-}$, mdr1a$^{-/-}$ and SAMP/Yit mice have established that increased intestinal permeability precedes disease development [107, 217, 218]. For example, IL-10$^{-/-}$ mice spontaneously develop a CD-like colitis by 12 weeks of age [111]. These mice also had increased intestinal permeability, which was present prior to the onset of disease [107]. Notably, when IL-10$^{-/-}$ mice were treated with a zonulin peptide inhibitor, intestinal permeability was reduced and development of colitis was significantly attenuated [108]. While intestinal permeability is a key player in the development of IBD, recent data suggests that increased intestinal permeability alone is not sufficient to predispose to the development of IBD. Transgenic mice that constitutively express active myosin light chain kinase MLCK in the intestinal epithelia had significant intestinal barrier dysfunction and increased intestinal permeability [219]. It was found that the decrease in barrier function did not predispose the mice to spontaneous development of colitis; however, it did accelerate the onset and severity of immune-mediated colitis in the MLCK transgenic mice [219].

A significant advance in IBD research came with the discovery of the nucleotide-binding oligomerization domain 2 (NOD2)/caspase-recruitment domain 15 (CARD15) gene as a CD genetic susceptibility loci [220, 221]. Mutations in the CARD15 gene have been identified in patients with CD and their healthy first-degree relatives [183]. Notably, 40% of relatives with one mutation and 75% of relatives with two mutations had increased intestinal permeability compared to controls.

Multiple molecular mechanisms for increased intestinal permeability in IBD have been reported, including altered TJ protein expression and/or distribution and increased epithelial apoptosis [222]. Initial studies reported a downregulation in occludin expression in IBD patients with no change in claudin-1 expression in IBD patients [223]; however, the role occludin may play in barrier dysfunction has been questioned since occludin-deficient mice have normal intestinal barrier function [224]. There are a number of studies demonstrating significant barrier dysfunction is associated with the disruption of occludin. This seeming discrepancy is most likely a consequence of the dynamic nature of TJs, whereby TJ complexes can form “normally” in the absence of occludin, as in occludin-deficient mice; however, once occludin is intimately associated with the complex, its disruption can alter the barrier function of the TJ complex. Clinical studies have also reported an upregulation of the barrier-reducing TJ protein claudin-2, in particular in the crypt epithelium, as well as decreased expression and redistribution of the sealing TJ proteins claudin-3, -4, -5 and -8 in IBD [105, 224] (Table 2). However, these TJ modifications might be a consequence of disease pathogenesis, rather than a cause, as they were not altered in patients with inactive CD [224]. Additionally, the breakdown in the protective barrier in IBD leads to an inflammatory infiltrate and enhanced production of cytokines and other mediators that can further contribute to altered barrier function. Increased levels of IFNγ and TNFα have been

demonstrated in the intestinal mucosa of IBD patients, and both cytokines have been shown to alter intestinal epithelial barrier function *in vitro* [97, 98, 225, 226]. Notably, treating CD patients with monoclonal anti-TNF antibodies downregulated the inflammatory response and restored intestinal barrier function leading to a decrease in intestinal permeability [227, 228].

Celiac Disease

Celiac disease is an immune-mediated enteropathy triggered by an inappropriate T cell-mediated response to ingested gluten and its component gliadin. Clinical and experimental studies suggest that altered intestinal barrier function may play an inciting role in the development of celiac disease by allowing gliadin to cross the intestinal barrier and activate the immune system. Patients with celiac disease have enhanced intestinal permeability and altered TJ morphology; and these disruptions persisted in asymptomatic patients who were on a gluten-free diet [188, 189, 229, 230]. There is data demonstrating that the increased intestinal permeability exists prior to disease onset and suggests that permeability may play an inciting role in the development of celiac disease. For example, a significant proportion of healthy first degree relatives of celiac patients also have increased intestinal permeability [231]. Consistent with these observations inbred Irish Setter dogs, which spontaneously develop a gluten-sensitive enteropathy similar to human celiac disease, have increased intestinal permeability [232]. Notably, the increase in permeability was present prior to gluten exposure and disease onset, and when animals reared on a gluten-free diet were first exposed to gluten, they immediately developed disease. These findings suggest that altered barrier function is a predetermining factor in celiac disease susceptibility [233].

The environmental trigger for celiac disease, gluten and its toxic component gliadin, have been well studied and both have been shown to directly stimulate zonulin production and induce an increase in intestinal permeability [188, 189, 229, 230]. Under physiological circumstances the intestinal epithelium is, for the most part, impermeable to gluten and gliadin; however, patients with celiac disease have been found to have compromised TJ integrity and enhanced paracellular permeability, which could allow for gliadin to cross the intestinal barrier and activate the immune system. Gliadin regulates intestinal barrier function in part by the upregulation of zonulin and gliadin has been found to bind to the CXCR3 receptor on intestinal epithelial and initiate a MyD88-dependent release of zonulin [234]. Incubating human intestinal epithelial monolayers or biopsies from celiac patients with gliadin stimulated zonulin secretion and an increase in epithelial permeability [235]. Clinical studies have also demonstrated a positive correlation between increased intestinal permeability and intestinal zonulin levels in patients with active celiac disease [236]. Furthermore, antagonism of zonulin prevented gliadin-induced permeability changes in intestinal biopsies from patients with celiac disease [235].

Mechanistically, zonulin binds to the zonulin receptor on intestinal epithelial cells and induces a PKC-mediated rearrangement of the cytoskeleton, downregulation of ZO-1 and occludin and disruption of TJ complex integrity increasing epithelial permeability [235, 237]. In line with the *in vitro* and *ex vivo* studies demonstrating that zonulin alters ZO-1 expression, additional studies have demonstrated decreased expression of ZO-1 and redistribution of F-

actin in patients with celiac disease [238]. Remarkably, when patients eliminated gluten from their diet, the celiac disease went into remission, normal intestinal permeability was reinstated and the abnormalities in ZO-1 and F-actin expression were reversed [239]. In addition, celiac disease also clearly has a genetic component as evidenced by 95% of celiac patients being HLA-DQ2 positive [240]. However, a role for this in barrier function is yet to be defined.

Type I Diabetes

It is hypothesized that a combination of predisposing genetics, dysregulated intestinal barrier function and aberrant immune responses play an inciting role in type I diabetes. Increased intestinal permeability has been reported in patients with type I diabetes at disease onset and is believed to facilitate increased exposure to antigens that can trigger autoimmune destruction of the insulin-producing, pancreatic beta cells [241–243]. Additionally, ultra-structural examination of duodenum from diabetic patients revealed altered TJ structure and an increase in the paracellular space between epithelial cells as compared to healthy controls [244] (Table 2). Studies using the diabetes-prone BioBreeding rat (BBDP), an inbred line that spontaneously develops autoimmune diabetes when weaned onto a normal diet, supports a role for altered intestinal barrier function in the pathogenesis of type I diabetes. BBDP rats have been found to have increased intestinal permeability associated with decreased expression of the TJ protein claudin-1 prior to the onset of insulitis and clinical diabetes [96, 245]. In support of this, a recent study examined barrier function in diabetics at various stages of disease progression. Intestinal permeability was increased in all diabetic groups; however, pre-diabetics had the greatest increase, suggesting that increased intestinal permeability precedes the onset of clinical diabetes [96, 245].

Similar to celiac disease and IBD, increased intestinal zonulin production is a potential mechanism leading to enhanced intestinal permeability in Type I diabetes. Experimental studies with pre-diabetic BBDP rats demonstrated increased intestinal zonulin secretion, which coincided with altered barrier function and preceded the development of autoantibodies [246]. Notably, treatment of the rats with a zonulin receptor antagonist reconstituted normal barrier function and abrogated disease development [246]. A recent clinical study examining type I diabetics and their first-degree relatives found significantly higher serum zonulin levels in diabetic patients, which correlated with the degree of intestinal barrier dysfunction [247]. Elevated serum zonulin was also found in 70% of pre-diabetic relatives, who were classified by the presence of positive autoantibodies in the absence of clinical disease [247]. Taken together, these studies suggest that an abnormal upregulation of zonulin can induce an increase in paracellular intestinal permeability that could facilitate the development of autoimmune diabetes.

Stress-Induced Barrier Dysfunction and Disease Exacerbation

Psychological and physical stress can induce a variety of changes in normal GI function, including changes in gut motility and permeability as well as alterations in ion, fluid and mucus secretion and absorption [248]. Animal models of acute and chronic stress demonstrate that stress induces changes in intestinal barrier function. Administration of acute, cold-restraint stress induced an increase in transcellular and paracellular intestinal permeability in

rats [249]. Electron microscopic examination revealed an increase in the number and size of HRP-containing endosomes in enterocytes from stressed rats as compared to controls; additionally, HRP was found within the paracellular spaces of epithelial cells in the intestine from stressed rats but not control rats [249]. The increased intestinal permeability induced by immobilization stress has also been associated with a temporary redistribution of TJ proteins, including occludin and ZO-1 (Table 2) [250]. Experimental analyses have demonstrated the stress-induced permeability changes are mediated by mast cells, cholinergic and adrenergic nerves and corticotropin-releasing hormone [251]. Studies have demonstrated that stress-induced permeability and ion secretion changes are attenuated in mast cell-deficient animals or following mast cell depletion or stabilization [252–254].

Psychological stress has been shown to influence the clinical course of chronic intestinal disorders including IBD and irritable bowel syndrome [255–257]. Long-term stress has been associated with an increased risk and number of relapses in patients with UC [255]. Additionally, studies using animal models of colitis have found that stress induces a worsening of colitis, enhances disease reactivation, reduces colonic mucus production and increases colonic permeability [258, 259]. Furthermore, stress has been linked to the onset and exacerbation of irritable bowel syndrome and functional gastrointestinal disorders [260]. The increased intestinal permeability induced by stress is believed to play an important role in disease progression and relapse. Blocking stress-induced barrier changes may represent a novel therapy to circumvent stress-induced IBD and irritable bowel syndrome relapse.

Summary

Dysregulation of the intestinal barrier has been associated with chronic immune diseases including food allergy, IBD and celiac disease. Whether or not intestinal epithelial barrier function is a primary etiologic factor in the predisposition to disease development remains unclear; however clinical and experimental evidence supports a role for intestinal epithelial barrier dysfunction in disease pathogenesis. Recent experimental studies have identified a role for a number of exogenous factors including bacterial pathogens and components of innate and adaptive immunity in the regulation of intestinal barrier function. Understanding the interactions between innate and adaptive immunity and intestinal barrier function will provide important insight into the pathogenesis of inflammatory and autoimmune diseases. Furthermore, delineation of the molecular pathways involved in the regulation of intestinal barrier function will have important clinical implications both to the treatment and prevention of chronic inflammatory disease as well as development of therapeutic agents targeted at modulating intestinal barrier function that could be useful for immunotherapy and as well as drug and vaccine absorption.

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Abbreviations

AJs	adherens junctions
TJs	tight junctions
JAMs	junctional adhesion molecules
PDZ	**P**ost synaptic density-95/**D**rosophila disc large/**Z**onula occludens-1 protein
PKC	protein kinase C
TER	transepithelial resistance
MAPK	mitogen-activated protein kinases
IFNγ	interferon-gamma
TNFα	tumor necrosis factor alpha
MLCK	myosin light chain kinase
IL	interleukin
IBD	inflammatory bowel disease
CD	Crohn's disease
iIEL	intestinal intraepithelial lymphocytes
NO	nitric oxide
iNOS	inducible nitric oxide synthase
GI	gastrointestinal
mMCP-1	murine mast cell protease-1
NSAIDs	non-steroidal anti-inflammatory drugs
HA/P	hemagglutinin protease
Zot	zonula occludens toxin
EPEC	enteropathogenic E. coli
CPE	Clostridium perfringens
HRP	horseradish peroxidase
iIL-9 Tg	intestinal interleukin-9 transgenic mice
mdr1a$^{-/-}$	multi-drug resistance gene deficient mice
NOD2/CARD15	nucleotide-binding oligomerization domain 2/caspase-recruitment domain 15
CXCR3	CXC chemokine receptor-3

BBDP	BioBreeding diabetes-prone rat

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What do we know?

- The intestinal epithelial barrier is maintained by complex protein-protein networks that form desmosomes, adherens junctions and tight junctions (TJs).
- Alterations of TJ protein formation and distribution and/or destabilization of the TJ complexes leads to intestinal epithelial barrier dysfunction.

Intestinal barrier function is modulated by:

- The immune system, including the Th1-cytokine IFNγ, Th2-cytokines IL-4 and IL-13, TNFα, T-cells, mast cells and eosinophils
- Ingestion of alcohol or NSAIDs
- Enteric pathogens directly and through the elaboration of toxins and proteases.

Altered intestinal barrier function and increased intestinal permeability is associated with:

- Food allergies
- Inflammatory bowel disease
- Celiac Disease
- Type I Diabetes

What is still unknown?

- Molecular pathways involved in the regulation of homeostatic intestinal barrier function.
- Molecular components of the tight junctional complex within the intestinal epithelium.
- The contribution of individual TJ proteins to barrier function and formation and stabilization of TJ complexes.
- Molecular mechanisms of inflammation-induced intestinal barrier dysfunction.
- Key inflammatory cells involved in the regulation of intestinal barrier dysfunction in chronic inflammatory diseases including food allergy, IBD and celiac disease.
- Whether intestinal barrier dysfunction is a primary etiologic factor predisposing to chronic inflammatory disease development.
- Molecular basis of mast cell-mediated intestinal barrier dysfunction in food allergy.

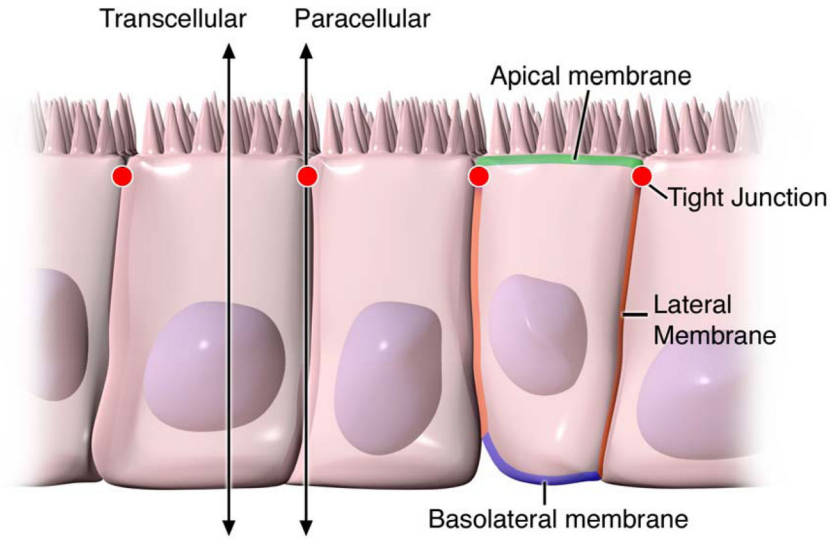

Figure 1. Pathways of epithelial permeability

Transcellular permeability is associated with solute or water movement through intestinal epithelial cells. Paracellular permeability is associated with movement in the intercellular space between epithelial cells and is regulated by tight junctions localized at the junction of the apical-lateral membranes.

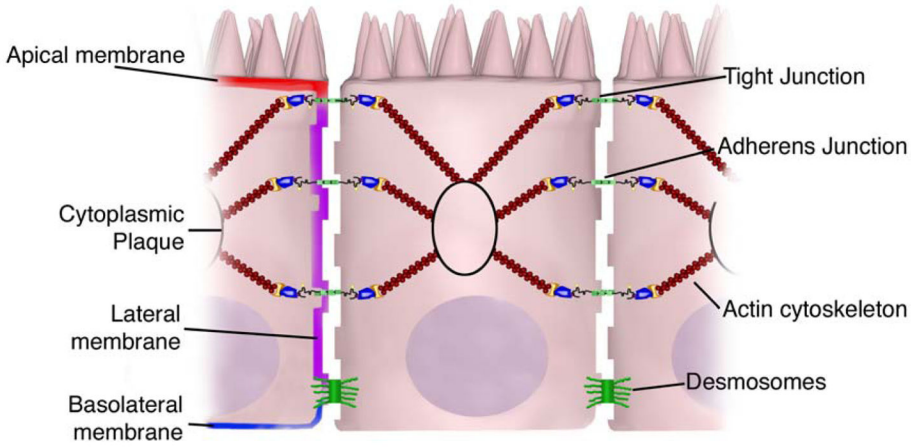

Figure 2. Overview of intestinal epithelial junctional complexes

The intestinal epithelium consists of a single layer of polarized epithelial cells. Adjacent cells are connected by 3 main junctional complexes: desmosomes, adherens junctions and tight junctions. Desmosomes are localized dense plaques that are connected to keratin filaments. Adherens and tight junctions both consist of transcellular proteins connected intracellularly via adaptor proteins to the actin cytoskeleton. The collection of proteins in the junctional complexes form “cytoplasmic plaques”.

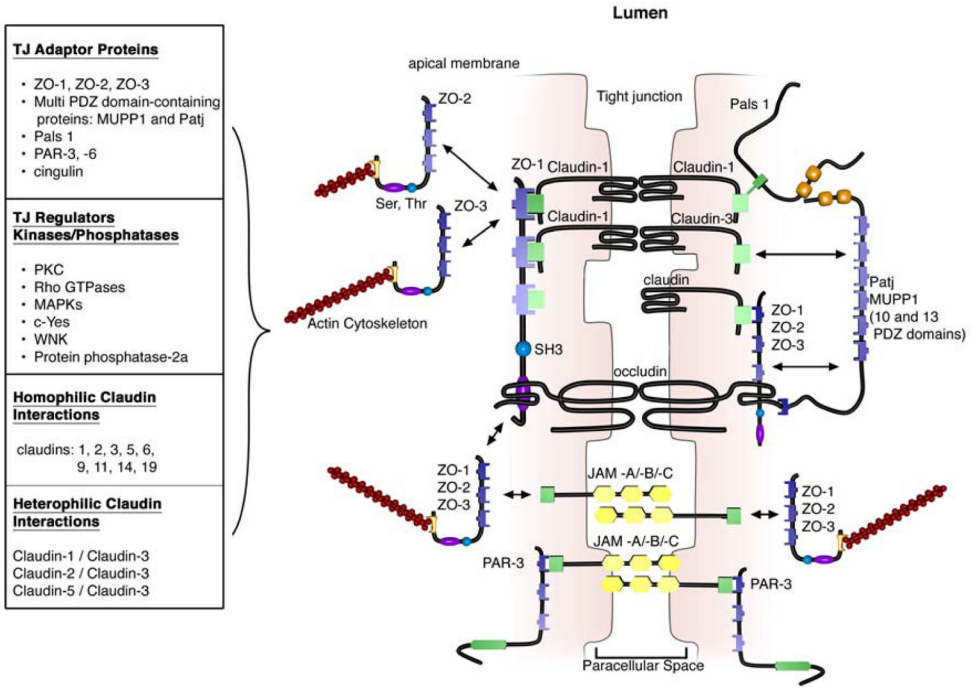

Figure 3. Tight Junctions

TJs are localized to the apical-lateral membrane junction. They consist of integral transmembrane proteins (occludin, claudins and junctional adhesion molecules (JAMs)) that interact in the paracellular space with proteins on adjacent cells. Interactions can be homophilic (eg claudin-1/claudin-1) or heterophilic (claudin-1/claudin-3). The intracellular domains of transmembrane proteins interact with PDZ-domain-containing adaptor proteins that mechanically link the TJ complex to the actin cytoskeleton. TJ proteins are regulated by phosphorylation by kinases, phosphatases and other signaling molecules.

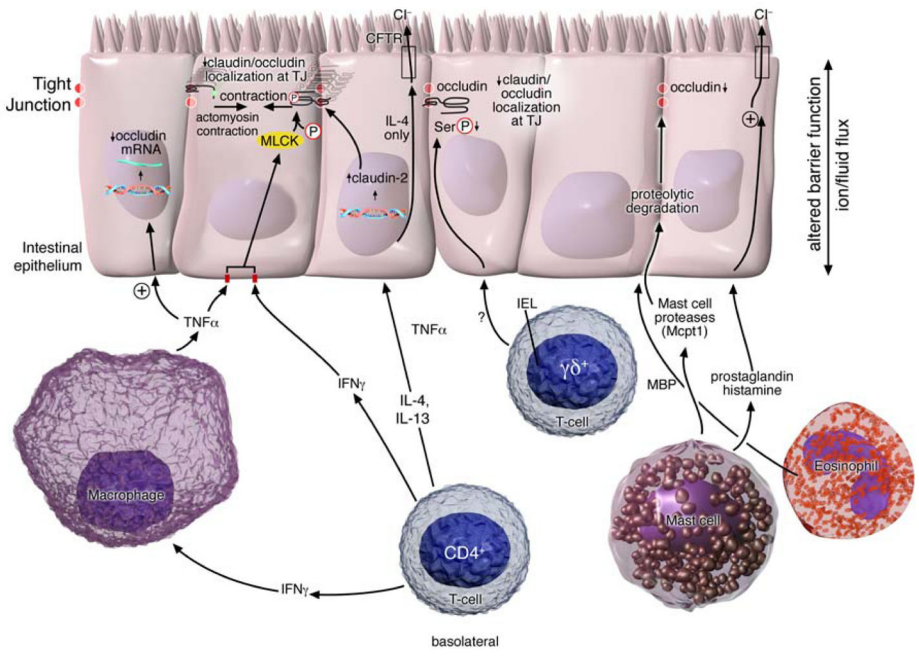

Figure 4. Immune regulation of intestinal barrier function

T-cell-derived IFNγ and TNFα inhibit MLCK-mediated phosphorylation of myosin light chain leading to TJ junction disruption and intestinal barrier dysfunction. IFNγ can also promote the redistribution of TJ proteins, JAM-A, occludin, claudin-1 and claudin-4 from the apical TJ border by a micropinocytosis process. TNFα and IFNγ may alternatively disrupt TJ stability and increase intestinal permeability via dysregulation of occludin expression. IL-4 and IL-13-induced increase in intestinal permeability is mediated via induction of epithelial apoptosis and expression of the pore-forming tight junction protein claudin-2. IL-4 and not IL-13 regulates ion conductance via downregulation of epithelial CFTR Cl^- channel expression. Intraepithelial (iIEL)$\gamma\delta^+$ lymphocytes (TCR-V$\gamma7^+$) iIEL cells are important in serine phosphorylation of occludin and TJ stabilization. Mast cell mediators including cytokine TNFα, mast cell protease 1 (mcpt-1) and lipid mediators including histamine, PAF and prostaglandins promote increased Cl^- conductance and increase intestinal permeability. Mcpt1 degrades the TJ protein occludin altering barrier function Eosinophil derived MBP down regulates tight junction protein occludin-1 expression in colonic epithelial cells.

Table 1

Transgenic or knockout mice and effects on intestinal barrier function.

Protein	Transgenic Or knockout	Function	Phenotype	reference
Occludin	Gene deletion	TJ protein	No change in TJs or permeability	56 and 57
Claudin-1	Gene deletion	TJ protein	Die within 1 day of birth	72
Claudin-6	Transgenic	TJ protein	Epidermis disrupted TJ formation and increased epithelial permeability	73
JAM-A	Gene deletion	TJ protein	Increased intestinal permeability Elevated claudin-10 and 15 expression Increased susceptibilty to DSS colitis	94
IL-9	Intestinal transgenic	cytokine	Elevated intestinal mast cell Increased intestinal permeability Increased susceptibility to oral antigen sensitization and anaphylaxis	206
IL-10	Gene deletion	cytokine	Increased permeability; Spontaneously develop chronic colitis similar to CD	107, 108 and 111
STAT-6	Gene deletion	Signaling molecule	Protected against IL-4- and IL-13-induced intestinal epithelial barrier dysfunction	106
mMCP-1	Gene deletion	Mast cell protease	Protected against T. spiralis infestation-induced intestinal epithelial barrier dysfunction	126
MLCK	Transgenic	Signaling molecule	Increased intestinal permeability Increased onset and severity of immune-related colitis.	219

Table 2

Diseases associated with altered TJ protein expression and Intestinal epithelial barrier function.

Disease State	TJ proteins	Inflammatory cell/Cytokine	Proposed Mechanism	references
Food Allergy	Not defined	Mast cells	Mast cell-mediated degradation of TJ proteins	206 and 207
IBD	↓ Claudin-3, -4, -5 and -8 ↑ Claudin-2	$CD4^+$ T-cells IL-10, IFNγ and TNFα and MLCK	IFNγ and TNFα-mediated activation of MLCK leading to TJ disruption and dysregulation of claudin and occludin expression.	97, 98, 108, 219, 227 and 228
Celiac Disease	Occludin and ZO-1	Zonulin	Gliadin-induced Zonulin secretion by intestinal epithelial cells. Zonulin-induced downregulation of occludin/ZO-1.	235 and 236
Diabetes	Not defined	Zonulin	Increased zonulin secretion	246 and 247
Stress	Occludin and ZO-1	Mast cells and corticotrophin-releasing hormone	Destabilization and redistribution of occludin/ZO-1	250–254