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Mechanisms of intestinal tight junctional disruption during infection

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1. ABSTRACT

Tight junctions are dynamic structures that may undergo structural and functional changes in response to both physiological and pathological circumstances. Several microbial pathogens impair intestinal barrier function by exploiting tight junctions. These pathogens have developed a broad and complex range of strategies to subvert host tight junction barrier function. The purpose of this review is to give an overview of the mechanisms whereby select enteric viruses, bacterial pathogens and parasites modulate intestinal tight junctional structure and function and how these effects may contribute to the development of chronic intestinal disorders.

2. INTRODUCTION

The intestinal epithelium has the difficult task of forming a selective barrier between the luminal environment and the subepithelial tissues. This barrier is essential to intestinal homeostasis. The intestinal epithelium must restrict the passage of potentially harmful luminal products while allowing for the transport of nutrients, water and electrolytes (1; 2). A single sheet of polarized epithelial cells is the primary component of the intestinal barrier and most hydrophobic substances are effectively blocked by the epithelial cell plasma membrane. Moreover, tight junctional complexes in the paracellular space between adjacent epithelial cells help control intestinal barrier function (2; 3). This intercellular seal, and thus paracellular permeability, is regulated primarily by tight junctional proteins (3; 4). This review discusses mechanisms whereby enteric pathogens disrupt these tight junctional complexes and how, in turn, these abnormalities may be implicated in gastrointestinal malfunction.

3. TIGHT JUNCTION STRUCTURE AND FUNCTION

Tight junctional complexes can be visualized by electron microscopy as a series of discreet membrane contacts between the plasma membranes of adjacent epithelial cells (4; 5). Freeze-fracture electron microscopy revealed that these contacts correspond to continuous,

branching intramembranous strands or fibrils that encircle the apical aspect of the lateral surface of each cell (6; 7). Fibrils from one cell interact with corresponding fibrils of an adjacent cell to close the paracellular space and, as a result, define the permeability characteristics of the epithelium (7). These fibrils are formed by transmembrane proteins, which are one component of a network of proteins that make up the tight junction. For a more detailed discussion on the structure and function of the tight junction-associated proteins, interested readers are directed to relevant reviews on this topic (5; 8-10). Tight junctions and adherens junctions consist of a complex interaction between several protein families:

1) The transmembrane proteins, occludin, claudins, junctional adhesion molecule 1 (JAM-1) and coxsackie and adenovirus receptor (CAR);
2) Cytoplasmic plaque proteins, an ever expanding list of proteins including, zonula-occludens (ZO)-1, -2 and-3; and
3) Several 'signaling proteins' consisting of a mixed group of cytosolic, membrane-bound or nuclear-associated proteins that are proposed to be involved in junction assembly, barrier regulation and gene transcription (5; 11).

Transmembrane proteins physically interact with corresponding proteins on membranes of adjacent cells to form the primary seal, and are thought to directly mediate paracellular permeability. The dense network of cytoplasmic plaque proteins are connected with the transmembrane proteins and act as adaptors by recruiting other cytosolic components to the tight junction complex, including protein kinases and GTPases (7; 12). In addition, the cytosolic proteins function as cytoskeletal linkers anchoring the transmembrane proteins to the perijunctional actomyosin ring (13). For instance, claudins, occludin and JAMs are attached to actin filaments and myosin light chain (MLC) of the perijunctional actomyosin ring by linker proteins of the ZO family (11; 12). The physical interactions with both actin filaments and MLC are thought to stabilize the tight junction, as well as being a key mechanism involved in the regulation and remodeling of tight junctions (3; 6).

The tight junction protein complex described above functions to form the primary component of a semipermeable gate that regulates passive movement of fluid and solutes through the paracellular space, as well as forming a 'fence' that segregates apically located membrane proteins from those at the basolateral membrane, thereby contributing to the generation of cell polarity (12). Therefore, it is not surprising that an intact tight junction barrier is essential to gut homeostasis. Disruption of this barrier is a common pathophysiological component of a number of infectious and inflammatory intestinal diseases (3; 12; 14). It is also becoming increasingly clear that tight junctions are dynamic structures that can be regulated in response to both physiological and pathological stimuli and, as a result, remodeling of the tight junction complex is emerging as a key mechanism of altered barrier function. Importantly, a number of intestinal pathogens are able to impair intestinal barrier function by targeting tight junction structure and/or function (15; 16). The following paragraphs discuss the mechanisms through which enteric microbes may directly disrupt tight junctional complexes.

4. TIGHT JUNCTIONAL DISRUPTION BY ENTERIC PATHOGENS

Several bacterial pathogens, parasites and viruses exploit tight junctions as a part of their pathogenic strategy (12; 15; 16). In general, key mechanisms identified to date include direct reorganization or degradation of specific tight junction proteins, reorganization of the cell cytoskeleton, and activation of host cell signaling events (14-16). More specifically, disruption of specific tight junction elements can result from degradation by pathogen derived proteases, changes in the phosphorylation state of the proteins, and altered protein synthesis. Furthermore, some enteric pathogens appear to subvert tight junction function by utilizing tight junction-associated proteins as receptors. Pathogen-induced alterations to the actin cytoskeleton often involve modification of host cell pathways, including those that involve the activation of myosin light chain kinase (MLCK) (17-18). This pathway is emerging as a common mechanism by which tight junction function is disrupted and involves contraction of the perijunctional actomyosin via phosphorylation of MLC by MLCK. The contraction, in turn, regulates paracellular permeability by placing tension on the tight junction complex (13). Several pathogens also appear to exploit tight junction function by altering the activity of the Rho family of GTPase binding proteins, which are involved in the assembly and/or organization of the actin cytoskeleton (14; 19-22). Thus, enteric pathogens have developed a broad and complex range of mechanisms to subvert host tight junction barrier function. The effect of select pathogens on tight junction structure and function are discussed below and summarized in Tables 1-3.

4.1 Viruses

4.1.1. Rotavirus

The best-studied viral pathogen in terms of its interaction with intestinal tight junctions is the rotavirus. This virus causes severe diarrhea in small children and infants in the absence of any consistent histological damage to the intestinal epithelium (23). However, a frequently observed feature following rotavirus infection is an increase in paracellular permeability (24-26). More research is needed to determine whether dysregulation of the paracellular pathway is an important factor in the development of rotaviral diarrhea. Rotavirus infection induces a drop in transepithelial resistance (TER) and increases paracellular flux of labeled probes across Caco-2 and MDCK cell monolayers (25; 27-29). The disrupted barrier function is accompanied by structural alterations to several tight junction-associated proteins, including ZO-1, occludin and claudin-1 (24; 25). Altered tight junction function induced by rotavirus does not appear to correlate with cell damage or death. Furthermore, pretreatment of infected cell monolayers with a MLCK inhibitor does not prevent the change in transepithelial resistance, indicating that phosphorylation of MLC is an unlikely mechanism in this infection model (25).

Table 1. Examples of viral pathogens that modify tight junctional barrier structure and function

Viral Pathogen	Mechanism	Tight junction dysfunction	Reference
Rotavirus	Reduced occludin gene synthesis PKA activation	Reduced nonphosphorylated form of occludin	(24-26)
Coxsackie virus and Adenovirus	Binding of virus fiber protein to CAR receptor	Redistribution and loss of ZO-1	(36; 37)
Reovirus	Binds JAM-A NFkappaB-induced apoptosis	Unknown	(38)

Protein kinase A, PKA; coxsackie virus and adenovirus receptor, CAR; zonula-occluden-1, ZO-1; junctional adhesion molecule-1, JAM-1

Table 2. Examples of bacterial pathogens that modify tight junctional barrier structure and function

Bacterial Pathogen	Mechanism	Tight junction dysfunction	Reference
Clostridium difficile	Inactivation of the Rho family of GTPases Activation of PKC	Modification of the actin cytoskeleton Redistribution of ZO-1 and occludin	(44-46)
Clostridium perfringens	*C. perfringens* enterotoxin binding to claudin-3 and 4	Disruption and degradation of claudin-4	(52)
Salmonella typhimurium	Altred Rho GTPase activity via T3SS effector proteins	Altered organization of F-actin, ZO-1 and occludin	(21; 22)
Vibrio cholerae	Zot activation of PKC HA/P	Redistribution of ZO-1 Degradation of occludin	(62; 63; 69)
Enteropathogenic *E.coli* (EPEC)	MLCK activation Dephosphorylation of occludin	Perijunctional actomyosin ring contraction Redistribution of occludin, claudin and ZO-1 Loss of protein-protein interactions	(18; 32 73)
Campylobacter jejuni	Unknown	Dephosphorylation and redistribution of occludin, reduced JAM-1 and claudin-4, and increased claudin-1	(33; 88)

Protein kinase C, PKC; zonula-occluden-1, ZO-1; type three secretion system, T3SS; zonula occludens toxin, Zot; hemagglutinin/protease, HA/P; myosin light chain kinase, MLCK; junctional adhesion molecule-1, JAM-1

Table 3. Examples of intestinal parasites that modulate tight junctional barrier structure and function

Parasite	Mechanism	Tight junction dysfunction	Reference
Giardia	MLCK Caspase-3 dependent apoptosis	Contraction of the actin cytoskeleton ZO-1, alpha-actinin, and claudin-1 disruptions	(17; 93; 95; 96)
Entamoeba histolytica	Cysteine proteinases	Degradation and dephosphorylation of ZO-1 and ZO-2	(99; 103)

Myosin light chain kinase, MLCK; zonula-occludens-1 and-2, ZO-1 and -2

Findings from a recent study indicate that infection with rotavirus causes a loss of occludin protein from the tight junction via a reduction in the nonphosphorylated form of occludin (26). This observation is consistent with the central role played by the phosphorylated state of occludin in tight junctional distribution and function (30; 31). The dephosphorylation of occludin may be associated with tight junctional reorganization and dysfunction in other models of infection (32; 33). Therefore, it appears that the mechanism responsible for rotavirus-induced barrier dysfunction may differ as the nonphosphorylated form of occludin is reduced, while there is no change in the phosphorylated form. In addition, the loss of the nonphosphorylated protein correlates with a significant decrease in occludin mRNA in rotavirus-infected Caco-2 cells. These changes occur in the absence of alterations in ZO-1 and ZO-3 expression (26). Thus, rotavirus infection seems to affect tight junction function, at least in part, by inducing the modification of occludin protein synthesis. The mechanism by which rotavirus specifically reduces the level of occludin mRNA remains unknown, but the changes to occludin mRNA and protein levels are sensitive to PKA inhibitors (26). Activation and translocation of PKA to the nucleus phosphorylates transcription factors that activate or inhibit responsive sites within the promoter region (26). Further studies are needed to determine how rotavirus may regulate occludin gene transcription, and whether it similarly affects other components of tight junctional complexes.

4.1.2. Adenovirus and Coxsackievirus

Coxsackie B virus and adenovirus attach and infect epithelial cells by binding to the coxsackie virus and adenovirus receptor, CAR (34). CAR is an integral membrane protein that is a member of the immunoglobulin receptor superfamily (35). CAR is now recognized as a tight junction associated protein that colocalizes with ZO-1 in a number of epithelial cell lines as determined by immunofluorescence and electron microscopy. Immunoprecipitation studies further demonstrated that there is a physical association between ZO-1 and CAR (36). Whether this is a direct association or an indirect link via additional junctional proteins remains to be established. In Chinese hamster ovary cells stably transfected with human CAR, the receptor is highly concentrated at sites of cell-cell contacts and mediates homotypic cell adhesion (36). The presence of CAR-CAR interactions also increases TER and reduces paracellular passage of labeled probes, in further support of the hypothesis that CAR contributes to the barrier function of tight junctions.

In human airway epithelia, binding of adenovirus viral fibers to the CAR receptor also blocks CAR-mediated cell adhesion, reduces TER, and increases paracellular permeability to both ions and viral particles (37). Furthermore, observations with electron microscopy revealed discontinuous cell-cell junctions close to, or directly above, infected cells, as well as small gaps between cells with adenovirus particles extending toward the apical surface. The functional permeability changes induced by viral fiber-CAR interactions are accompanied by a redistribution of beta-catenin and disruption of ZO-1 staining (37).

It is interesting that a virus would utilize a receptor that is normally hidden below or within the tight junction complex and it is still unclear how the virus initially gains access to such a receptor. However, coxsackie virus and adenovirus are not the only viral

particles to exploit tight junction proteins as receptors. The junctional adhesion molecule, JAM, which is also an integral membrane protein and a member of the immunoglobulin receptor superfamily, has been identified as a receptor for mammalian reovirus (38). Reovirus interaction with JAM is required for virus attachment and infection in several epithelial cell lines, as well as for NFκB-induced apoptosis during reovirus infection (38). Moreover, JAM-A deficiency results in enhanced mucosal permeability in association with altered expression of claudin-10 and -15 (39).

The functional consequence of reovirus infection on tight junction structure and function remain unclear. In support of a possible reovirus-induced alteration in barrier function, knockdown of JAM-A *in vitro* leads to decreased TER and enhanced permeability to dextran (40). Furthermore, reovirus-induced NFκB- dependent apoptosis may indirectly contribute to the loss of epithelial barrier function. Taken together, these observations indicate that, by exploiting a tight junction associated receptor, viral enteropathogens may induce tight junction dysfunction and open the paracellular pathway, possibly contributing to the development of diarrhea and promoting viral shedding and transmission.

4.2 Bacteria

4.2.1. Clostridium sp.

C. difficile is the primary cause of pseudomembranous colitis and antibiotic-associated diarrhea (20). Disease etiology is attributed to the production and release of two exotoxins, toxin A and toxin B. Both toxins are internalized into the host cell cytosol where they disrupt the function of the Rho family of GTP binding proteins, including Rho, Rac and Cdc-42 (14; 20). They do so by catalyzing the transfer of a glucosyl residue from UDP-glucose to Rho, Rac and Cdc-42, which renders the proteins inactive. The Rho family of proteins is important in the assembly and organization of the actin cytoskeleton; therefore, inactivation of these proteins leads to disassembly of actin stress fibers, disruption of actin-associated adhesion plaque proteins, as well as cell detachment and rounding (20; 41; 42). Because the actin cytoskeleton is linked to the tight junction complex, alterations to the actin cytoskeleton via inactivation of Rho proteins could then be expected to affect epithelial permeability (43). Indeed, *C. difficile* derived toxin A and toxin B are reported to induce F-actin condensation in conjunction with a reduction in TER and increased flux of labeled probes, implying that the increased epithelial permeability reflected disruptions of the tight junction (44; 45).

Further support for the importance of Rho proteins in regulating tight junction structure and epithelial permeability was provided by a study utilizing the *C. botulinum* toxin, C3 transferase (19). This toxin specifically inactivates the Rho protein without affecting Rac or Cdc-42. An experiment with C3 transferase demonstrated that inactivation of Rho leads to altered apical F-actin organization and, as a result, redistributes ZO-1 and disrupts epithelial barrier function (19). The same group later reported that *C. difficile* toxin exposure induced the displacement of ZO-1, ZO-2 and occludin from the lateral membrane. These changes occurred in parallel with the disorganization of F-actin and the drop in TER (46). The total cellular concentration of the tight junction proteins is not affected, indicating that the proteins are not degraded. However, the physical association of ZO-1 with actin is significantly impaired upon exposure to C3 transferase. Furthermore, toxin exposure reduces the amount of ZO-1 and high molecular weight occludin found within lipid raft-like membrane microdomains. Tight junction structural components are normally enriched within the raft-like membrane microdomains, and the organization of the proteins into these membrane microdomains is known to be essential to tight junction homeostasis (47). These observations demonstrate that the Rho family of proteins helps maintain ZO-1 and actin interactions, and preserves the affiliation of tight junctional proteins with membrane microdomains. Overall, *C. difficile* toxins appear to have developed strategies to exploit the Rho family of proteins to subvert tight junction structure and epithelial barrier function, and this likely contributes to the development of intestinal inflammation and diarrhea during the infection.

C. perfringens is another cause of food borne gastrointestinal illness and diarrhea and pathophysiology is associated with the *C. perfringens* enterotoxin, CPE (48). In contrast to *C. difficile*- derived toxin A and toxin B, CPE appears to modify epithelial permeability by directly altering specific tight junction proteins. The transmembrane proteins, claudin-3 and claudin-4 have been identified as receptors for CPE (49; 50). The *C. perfringens* toxin consists of a C-terminal receptor binding region and an N-terminal toxicity domain (51). Treatment of epithelial cell monolayers with the non-cytotoxic carboxy terminal end of CPE results in the removal of claudin-4 from the tight junction complex, and its eventual degradation (52). CPE-induced structural changes to claudin-4 correlate with a reduction in the number of tight junction strands, and an increase in paracellular permeability (52). Therefore, CPE binding to specific claudins may disrupt tight junction function via a non-cytotoxic pathway. The exact role this disruption plays in the development of symptoms associated with *C. perfringens* infection has yet to be determined.

4.2.2. Salmonella typhimurium

Salmonella enterica serovar *typhimurium* (*S. typhimurium*) is one of the most common food-borne pathogens worldwide, and is responsible for self-limiting gastroenteritis and diarrheal disease. Disease etiology is primarily a result of the intra-epithelial translocation of virulence factors via a type 3 secretion system (T3SS) encoded by the *Salmonella* pathogenicity island-1 (SP-1) (53; 54). Infection of polarized epithelial monolayers by *Salmonella* elicits a rapid and progressive drop in TER (55) that is accompanied by an increase in the paracellular flux of labeled markers across infected cell monolayers (21; 56; 57). Coinciding with the increased paracellular permeability, occludin is dephosphorylated and ZO-1 is degraded (57). In addition, the distribution of F-actin, ZO-1 and occludin is altered, and the perijunctional actomyosin

ring contracts (21; 56). However, contraction of the actomyosin ring does not appear to be a prerequisite for *Salmonella*-induced tight junctional dysfunction since pretreatment with staurosporine, a protein kinase inhibitor that prevents perijunctional contraction, failed to attenuate *Salmonella* invasion and subsequent tight junction dysfunction (58). Interestingly, infection with *S. typhimurium* is reported to be associated with a significant increase in the translocation of both pathogenic and non-pathogenic bacteria across epithelial cell monolayers, suggesting an important functional consequence of *Salmonella*-induced disruption to the tight junction barrier (57).

The type 3 secretion system injects numerous bacterially derived effector proteins into the host cell, some of which are reported to regulate actin dynamics by modulating the Rho family of GTPases (59; 60). Therefore, *S. typhimurium* effector proteins may modulate the activity of Rho proteins, thereby altering tight junction function. Experiments utilizing a mutant strain lacking the SP1 T3SS indicate that the *Salmonella*-induced drop in TER and reorganization of ZO-1 and occludin are dependent on SP1 effector proteins (21). In particular, the effector proteins, SopB, SopE, SopE2 and SipA are collectively required for *Salmonella*-induced barrier dysfunction and disruption of ZO-1 and occludin localization (22). Furthermore, pretreatment of epithelial monolayers with an inhibitor of geranylgeranyltransferase-1, which is required for activation of the Rho GTPases, significantly reduces the drop in TER observed following *Salmonella* infection (21; 22). Together, these data indicate that *S. typhimurium*-induced tight junction disruption is due, in part, to the activation of Rho GTPases via specific SP1 effector proteins.

4.2.3. Vibrio cholera

V. cholera is acquired through the ingestion of contaminated food or water, and causes life-threatening acute diarrhea. The enterotoxin cholera toxin (CT) was initially identified as the means by which *V. cholera* induces the profuse and severe diarrhea characteristic of cholera. Indeed, the A subunit of CT activates adenylate cyclase, which increases cyclic AMP synthesis, hence opening chloride ion channels and ultimately increasing the net secretion of fluid and electrolytes (61). *V. cholera* strains lacking the A subunit of CT are significantly reduced in their ability to cause diarrhea. However, these strains are still able to induce mild to moderate diarrhea in a significant proportion of infected individuals (62). Therefore, it was proposed that *V. cholera* may produce another toxin that contributes to the development of diarrhea (63). It is now well established that *V. cholera* produces a toxin other than CT, which increases intestinal permeability by altering the structure of tight junctions. In this instance, the increased permeability is accompanied by alterations in ZO-1 morphology, and the *Vibrio* toxin responsible was identified as zonula occludens toxin, or Zot (63). Upon exposure to Zot, epithelial F-actin is redistributed and actin polymerization is increased in epithelial monolayers exposed to Zot-containing supernatant (64). Inhibition of PKC activity abolishes Zot-induced actin reorganization, and significantly attenuates the increased intestinal permeability caused by Zot (64). Toxin activity also appears to be region-specific, since altered barrier function in response to Zot is only observed in the small intestine. The region-restricted activity of Zot is consistent with specific binding of the toxin to mature villus enterocytes predominately in the jejunum and small intestine (65). Together these results suggest that Zot binds to a specific intestinal epithelial cell surface receptor to initiate a cascade of intracellular events that involves activation PKC and polymerization of actin filaments. These events may ultimately lead to redistribution of ZO-1 and increased paracellular permeability.

Interestingly, an intestinal mammalian analogue of the Vibrio Zot protein has been identified (66). This protein, named zonulin, reversibly opens tight junctions and appears to bind to the same receptor and act through the same intracellular signaling mechanism as the bacterially derived Zot (66). The physiological function of zonulin remains unclear; however, it was reported that both pathogenic and non-pathogenic bacteria elicit luminal zonulin secretion from the mammalian small intestine and this was accompanied by increased tissue permeability (67). Thus, it appears that the exposure of the relatively sterile proximal gut to enteric bacteria can lead to a reduction in tight junction competency and increase paracellular permeability. This may initially represent a host defense response to induce diarrhea and help 'flush' the bacteria out of the small intestine. On the downside, the development of a 'leaky' intestine could also contribute to the development of a number of chronic pathophysiological conditions that are associated with increased intestinal epithelial permeability (3).

Another toxin elicited by *Vibrio cholera* that is of interest when discussing tight junction structure and function is the hemagglutinin/protease (HA/P). HA/P is a metalloproteinase capable of cleaving numerous substrates as well as activating the A subunit of CT (68). Culture supernatants from *V. cholera* strains that lack several toxins associated with virulence, including CT and Zot, are still able to cause a drop in TER across epithelial monolayers, and the cytotoxic factor responsible was identified as the HA/P (69). The barrier disruption induced by HA/P was due, in part, to ZO-1 and F-actin reorganization. Furthermore, a specific bacterial metalloprotease inhibitor significantly reduces the morphological changes induced by HA/P, suggesting that proteolytic activity may be required (69). It was subsequently demonstrated that HA/P degrades the transmembrane protein occludin (70). A metalloprotease inhibitor significantly attenuates the HA/P-induced degradation of occludin, whereas another protease, trypsin, fails to induce the same effect, indicating that the degradation of occludin may be specific to the proteolytic activity of HA/P. Moreover, exposure to HA/P leads to the rearrangement of ZO-1, but does not degrade ZO-1. These observations indicate that HA/P disrupts tight junction barrier function by the specific proteolysis of occludin, and the reorganization of ZO-1 and F-actin.

Interestingly, the related species *Vibrio parahaemolyticus* also significantly decreases TER and increases paracellular permeability across human colonic adenocarcinoma cells, in association with disruptions of the actin cytoskeleton, ZO-1, and occludin (71). However, these changes appear to occur independently of the major virulence factors produced by *V. parahaemolyticus* suggesting that additional virulence factors may disrupt tight junctional function during this infection.

4.2.4. Escherichia coli

Several pathogenic strains of *Escherichia coli* cause intestinal disease in humans and animals. Among these, enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) are two of the most well recognized causes of infectious diarrhea worldwide. EPEC causes severe infantile diarrhea, particularly in developing countries, while EHEC causes hemorrhagic colitis and hemolytic uremic syndrome. Both strains belong to a family of non-invasive, attaching and effacing pathogens and despite the unique characteristic of being minimally invasive, both pathogens have been reported to alter intestinal epithelial barrier function (18; 72; 73). EPEC, in particular, has a profound effect on intestinal epithelial permeability. *In vitro* studies demonstrated that EPEC induces a time and dose dependent drop in TER across intestinal epithelial cell monolayers, in association with increased paracellular permeability (72; 73). One mechanism by which EPEC affects paracellular permeability is via the phosphorylation of MLC. Inhibition of MLCK prevents EPEC-induced changes to tight junction barrier function, while inhibition of PKC or tyrosine kinases has no effect (18). Therefore, it appears that EPEC increases the phosphorylation of MLC by MLCK. This, in turn, induces contraction of the perijunctional actoymyosin ring, thereby placing tension on the tight junctional complexes and opening the paracellular pathway (13; 18).

EPEC infection disrupts protein-protein interactions such that the amount of occludin associated with both ZO-1 and claudin-1 is significantly reduced (74). This loss of protein-protein interactions is accompanied by a translocation of apically localized tight junctional proteins to the lateral membrane. These morphological changes also correlate with a decrease in the phosphorylated form of occludin (74). This is in support of other findings that reported a rapid and progressive dephosphorylation of occludin following EPEC infection of intestinal epithelial monolayers (32). Phosphorylation of occludin is important for its association with the membrane at the level of the tight junction, consistent with the observed relocation of occludin to the intracellular compartment following EPEC-induced dephosphorylation (32). These events coincide with a drop in TER, providing further evidence that the dephosphorylation of occludin represents another likely mechanism by which EPEC alters tight junction function. Whether the contraction of the perijunctional actomyosin ring via phosphorylation of MLC, and the dephosphorylation and redistribution of occludin are causally related events remains unknown; however, it is plausible given the intimate physical link between the actin cytoskeleton and the tight junctional complex.

EPEC utilizes a type III secretion system to inject several effector proteins into the host cell (75). *In vitro* studies demonstrate that the effector protein, EspF, is key to the tight junction barrier dysfunction induced by EPEC and *in vivo* studies using mouse models of EPEC infection corroborate these findings (74, 76-78). Indeed, C57BL/6 mice become successfully colonized by EPEC, and barrier function in both the colon and ileum are significantly diminished one day after infection. These functional abnormalities correlate with a redistribution of occludin, while ZO-1 seems not to be affected. In contrast, EPEC strains lacking the EspF effector protein have no effect on barrier function or occludin distribution, despite colonizing the mouse gut at similar levels to the wild type strain. Interestingly, the effect of the wild type EPEC strain on barrier function and occludin localization persist at five days post infection, and the EspF mutant strain fails to attenuate the barrier dysfunction at this time point. Thus, it appears that the EPEC-induced tight junction barrier disruption may be EspF dependent at earlier time points of infection, while other factors likely contribute to barrier dysfunction later in infection. Altered barrier function at the later time point was shown to coincide with increased production of the pro-inflammatory cytokine TNF-alpha, indicating that host factors likely play a role (78). This observation is consistent with the documented effects of TNF-alpha and IFN-gamma on tight junctional structure and function (4; 79-82).

Intestinal epithelial cells infected with the closely related pathogen EHEC also display a decrease in TER and an increase in the transepithelial flux of radiolabelled probes, although the barrier disruption inflicted by EHEC is delayed and less pronounced than that caused by EPEC (83; 84). However, as with EPEC, altered barrier function occurs in association with the redistribution of both ZO-1 and occludin (83; 84). Inhibition of MLCK, PKC or calmodulin partially inhibits the changes induced by EHEC infection (83). Interestingly, the effector protein, EspF, shown to be important to the barrier dysfunction induced by EPEC, does not appear to play a role during EHEC infection (84).

Cytotoxic necrotizing factor (CNF)-1, a toxin produced by necrotizing *E. coli*, constitutively activates the Rho family of GTPases (85; 86). *E. coli* CNF-1 has been used as a model system to examine the role of Rho GTPases on tight junctional structure and function. Treatment of intestinal epithelial cell monolayers with CNF-1 reduces TER and increases paracellular permeability across intestinal epithelial cell monolayers (85). The functional alterations in barrier function are accompanied by displacement of ZO-1 and occludin, and reorganization of JAM-1 (86). The CNF-1-induced activation of Rho GTPases and barrier disruption is in contrast to the mechanism utilized by pathogens such as *C. difficile*, which inactivate Rho GTPases (14; 20). Thus, both the activation and inactivation of the Rho GTPases may result in tight junctional disruptions.

4.2.5. Campylobacter jejuni

C. jejuni is a leading cause of human enterocolitis (87; 88). Infection of human intestinal epithelial cells with

C.jejuni triggers a significant decrease in TER and a corresponding redistribution of occludin (33; 89). These changes are associated with a reduced level of hyperphosphorylated occludin, while the amount and distribution of ZO-1 remain unchanged. In addition, in infected cells, the levels of lipid-raft associated claudin-1 and JAM-1 are increased and decreased respectively, while the perijunctional actomyosin ring remains intact (33). The mechanisms involved in *C. jejuni*-induced barrier dysfunction remain unclear.

More recently, *C.jejuni*-induced barrier dysfunction was associated with altered claudin-4 distribution and content (90). Importantly, infection by *C.jejuni* seems to facilitate the translocation of non-pathogenic, non-invasive *E. coli* across the epithelium, indicating that *C.jejuni* may promote the translocation of otherwise non-invasive microbial flora across the intestinal epithelial barrier (90; 91). This observation is supported by the recent report that another enteric pathogen, *S. typhimurium* facilitates the translocation of both pathogenic and non-pathogenic bacteria across the intestinal epithelial barrier (57).

4.2.6. Shigella flexneri

S. flexneri is responsible for bacillary dysentery in humans and is a serious cause of morbidity and mortality, particularly in children from developing countries. The bacterium was initially reported to invade intestinal epithelial cells almost exclusively from the basolateral membrane by utilizing specialized M cells to cross the epithelial barrier (92). A more recent study reported that both apical and basolateral infection with wild type and non-invasive strains of *S. flexneri* elicits a drop in TER across intestinal epithelial cell monolayers (93). Confocal microscopy demonstrated that both strains of *S. flexneri* are able to translocate through the tight junctional seal into the paracellular space, suggesting that *Shigella* can penetrate the epithelial barrier independently of the M cell route (93).

Treatment of epithelial cell monolayers with wild type or non-invasive *S. flexneri* results in the dephosphorylation of occludin (93). In addition, *S. flexneri* specifically targeted claudin-1, leading to a reduction in the expression of this protein following infection. The altered expression of both claudin-1 and occludin is accompanied by a redistribution of these proteins away from the tight junctional complex. Similarly, both wild type and non-invasive strains of *S. flexneri* are capable of removing ZO-1 from the tight junctional complex. In contrast, ZO-2 levels are initially decreased, followed by a recovery, and subsequent increase in ZO-2 expression. The increase in ZO-2 expression is accompanied by the recruitment of ZO-2 from the cytosol into the tight junctional complexes, perhaps compensating for the temporal loss of ZO-1 and ZO-2. Interestingly, the changes to ZO-2 expression and distribution closely resembled the regulation of E-cadherin. It is speculated that the regulation of ZO-2 and E-cadherin is a protective response elicited by the host epithelial cell in an attempt to compensate for the *S. flexneri*- induced alterations to tight junction structure and function. Alternatively, the response may be induced by the bacteria itself in order to restore tight junction function and limit the host immune response following infection (93). Further studies are required to determine mechanisms by which *S. flexneri* alters the expression and distribution of tight junction- associated proteins.

4.3 Parasites

Although much interest has been placed on tight junction dysfunction due to bacterial infection, more complex organisms like intestinal parasites have also been shown to affect tight junctional structure and function. Two of the better-studied protozoan parasites, in terms of their effects on epithelial barrier function, are discussed below (Table 3).

4.3.1. Giardia sp.

Giardia lamblia (syn. *G. duodenalis, G. intestinalis*) is an intestinal protozoan parasite that is a common cause of waterborne disease characterized by acute or chronic diarrhea, dehydration, abdominal cramping and weight loss (94; 95). In some cases infected individuals can remain asymptomatic, or can develop chronic infections. Interestingly, symptoms can be present in the absence of any significant morphological damage to the mucosa. The mechanism(s) responsible for the development of diarrhea remain incompletely understood. Nevertheless, several reports indicate that epithelial barrier dysfunction may represent a key player in the pathophysiology of *Giardia* infection (17; 96-99).

G. lamblia infection of human intestinal monolayers and non-transformed duodenal epithelial cells induces a significant reduction in TER and increased paracellular permeability to macromolecules (96; 100). These functional alterations are accompanied by disruptions of the actin cytoskeleton and altered ZO-1 localization (17; 96). Treatment with a MLCK inhibitor attenuates the effects of *Giardia* on paracellular permeability, F-actin and ZO-1, indicating that the tight junctional abnormalities are due, at least in part, to a *Giardia*-induced phosphorylation of MLC (17). In addition, it appears that there is a link between the altered epithelial permeability and increased epithelial cell apoptosis upon exposure to this parasite. *G. lamblia* induces enterocyte apoptosis in epithelial monolayers and this effect can be blocked by a caspase-3 inhibitor, which also prevents the disruption of ZO-1 and increased permeability following infection (98). Thus, *Giardia*-induced tight junctional dysfunction seems to be regulated by both the MLCK and pro-apoptotic caspase-3 pathways.

In vivo studies in mice infected with *Giardia* support the findings from *in vitro* studies, demonstrating that *Giardia* infection increases intestinal permeability (17). More recently, another report established that chronic giardiasis in humans disrupts intestinal barrier function. Loss of barrier function in infected patients correlated with reduced expression of claudin-1, and increased epithelial apoptosis (99). Thus, tight junction barrier disruption was identified as one mechanism by which *Giardia* could contribute to diarrheal disease in infected humans.

4.3.2. Entamoeba histolytica

E. histolytica is the cause of human intestinal and extraintestinal amebiasis. Infection is initiated by the adhesion and invasion of trophozoites into the enteric mucosa, followed by disruption of the epithelial microvilli and tight junctions and, ultimately, cell lysis (101). Clinical features range from mild diarrhea to severe dysentery, which seem to be associated with the loss of intestinal barrier function. A rapid drop in TER and increased flux of labeled paracellular markers across epithelial monolayers provide evidence that the loss of barrier function occurs at the level of the tight junction (101). Moreover, selective Entamoeba-induced changes in tight junction associated proteins have been reported, including the loss of protein-protein interactions, the degradation of ZO-1 and the dephosphorylation of ZO-2 (102). Although *E. histolytica* is known to induce cell lysis and tissue destruction, this does not appear to contribute to the initial barrier dysfunction given that the drop in TER occurs as early as 15-30 minutes after infection, whereas cell death and the subsequent epithelial cell exfoliations are not observed until 3-6 hours post-infection. Moreover, low numbers of trophozoites that fail to induce cell death are still able to reduce transepithelial barrier function (102).

Several virulence factors are produced by *E. histolytica* including the Gal/GalNAc-specific amebic lectin and amebic cysteine proteinases (103; 104). The Gal/GalNAc specfic lectin is associated with trophozoite adherence to the enteric mucosa. Agents that bind to this lectin, thereby preventing adhesion of trophozoites, have been shown to prevent *E. histolytica*- induced tight junctional dysfunction (102; 105). The involvement of several signaling molecules, including PKA, PKC, MLCK and GTPases that are known to regulate tight junction function, have also been examined. However, inhibition of these key signaling molecules fails to restore tight junctional function following *E. histolytica* infection (106). In contrast, the *E. histolytica* induced drop in TER and degradation of ZO-1 were prevented by inhibition of the *E. hystolytica* cysteine proteinases (106). Thus, it appears that adhesion and cysteine proteinases are required for *E. hystolytica*-induced tight junctional barrier dysfunction.

5. RELEVANCE OF TIGHT JUNCTIONAL DISRUPTION BY ENTERIC PATHOGENS TO CHRONIC INTESTINAL DISORDERS

An intact tight junctional complex is essential to intestinal homeostasis; therefore, disruption of tight junction structure by enteric pathogens may have significant detrimental effects on intestinal function. Disruption of the tight junction results in the opening of the paracellular pathway, allowing water and electrolytes to leak into the lumen under hydrostatic pressure. This may contribute to the diarrhea that is commonly associated with acute viral, bacterial and parasitic infections discussed in this review. In addition, loss of intestinal barrier function may allow luminal antigens to access the underlying lamina propia and activate the host's immune compartment. Increasing evidence suggests that this may contribute to the development of chronic intestinal disorders like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), as well as autoimmune disorders like coeliac disease and diabetes.

IBD is a chronic, relapsing and remitting inflammation of the gastrointestinal tract that is composed of two major diseases, Crohn's disease and ulcerative colitis. Increased epithelial permeability has been proposed as a key pathophysiological mechanism in IBD (3; 12). The integrity of the intestinal barrier is reported to be impaired in patients with Crohn's disease and ulcerative colitis (107-109). A recent study reported a structural correlate to impaired barrier function in Crohn's disease patients, including the reduced expression of occludin, claudin-3, claudin-5 and claudin-8, as well as the appearance of discontinous tight junction strands (110). Similarly, colonic mucosa from patients with ulcerative colitis revealed a reduced expression of occludin, ZO-1, claudin-1 and JAM (111). Whether such changes are a cause or a consequence for the severe inflammatory response of the mucosa remains controversial.

Interestingly, in Crohn's disease patients in clinical remission, increased intestinal epithelial permeability is often reported to precede a relapse by as much as one year (112-114). Furthermore, a subset of healthy first degree relatives of Crohn's disease patients is reported to have increased intestinal permeability (115-119). Further research is needed to determine whether altered intestinal barrier function, triggered by environmental factors like enteric pathogens, may precede the development of disease in genetically susceptible individuals.

The development of IBD has not yet been linked to a specific environmental trigger; however, given the detrimental effects of enteric pathogens on intestinal homeostasis, it is not surprising that the incidence of active IBD in patients with a prior episode of infectious gastroenteritis is more than double that of the rate see in individuals with no prior gastroenteritis. Interestingly, *Campylobacter jejuni* was the most commonly detected bacteria in active IBD patients with a prior episode of gastroenteritis (120). In addition, bacterial infections have also been associated with triggering relapses in IBD patients (120; 121).

Post-infectious IBS has been associated with bacterial pathogens including, *Campylobacter, Salmonella and Shigella* (122; 123). Infection with the parasite *Giardia*, can also lead to the development of IBS-like symptoms (124). Furthermore, gut permeability is increased in post-infectious IBS and diarrhea-predominant IBS (125). The link between bacterial infection, increased intestinal permeability and the development of IBS is further supported by the "Walkerton outbreak" in which increased permeability was reported in individuals who developed post infectious IBS two years after gastroenteritis due to *E. coli* O157:H7 or *Campylobacter jejuni* (123; 126).

Dysregulated intestinal barrier function has also been implicated in several autoimmune disorders, including

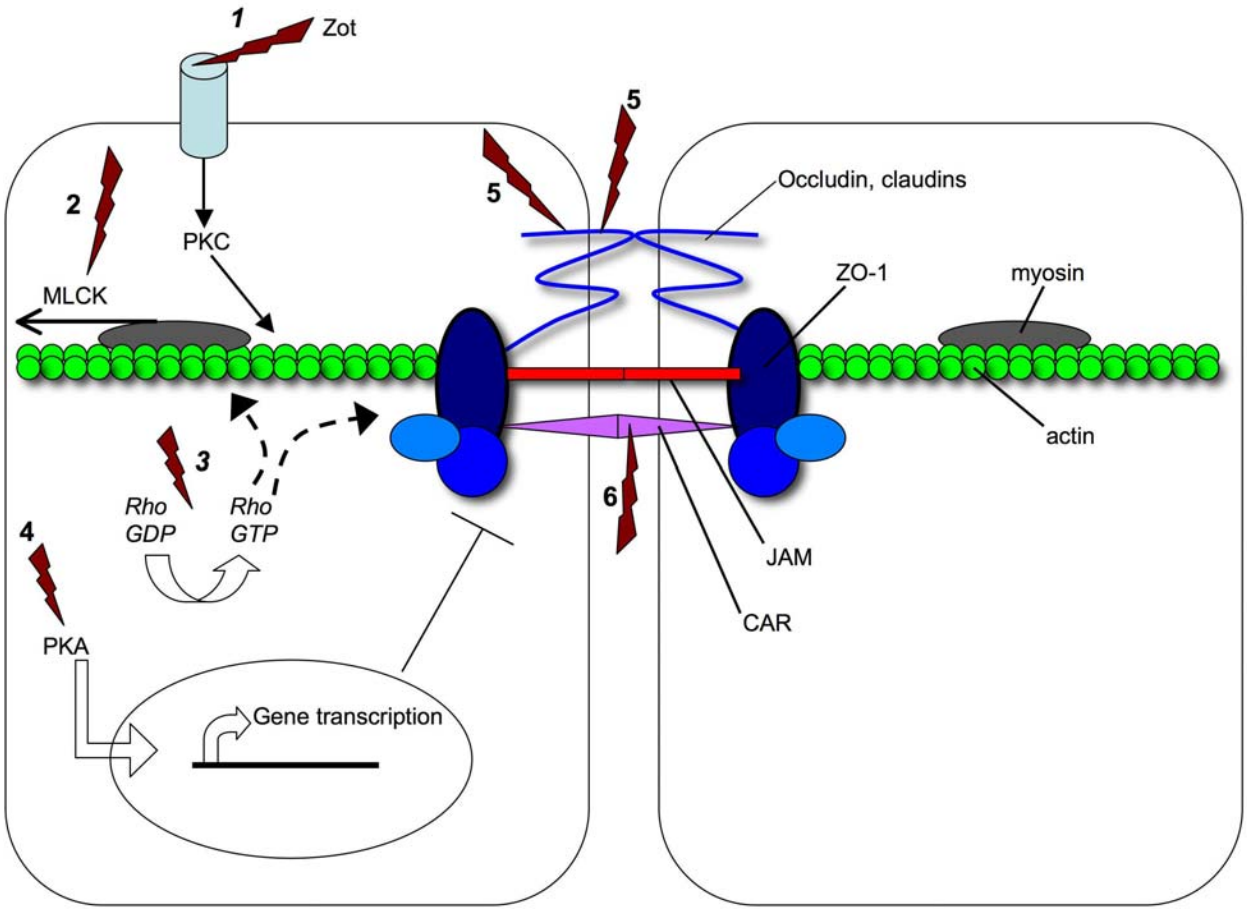

Figure 1. Mechanisms utilized by select enteric pathogens to disrupt tight junctional structure and function. 1. Bacterially-derived proteins or toxins may interact with a cell surface receptor to initiate a cascade of events, leading to the activation of PKC and subsequent actin rearrangement (ex. *Vibrio cholera*-derived zonula occludin toxin). 2. Contraction of the perijunctional actomyosin ring (arrow) via activation of myosin light chain kinase (ex. enteropathogenic *Escherichia coli* (EPEC), *Giardia*). 3. Modulation of the actin cytoskeleton and/or tight junctional complexes (hatched arrows) by altering the activity of the Rho family of GTPase binding proteins (ex. *Clostridium difficile*, *Salmonella typhimurium, E.coli* CNF-1). 4. Altered synthesis of tight junctional associated proteins via the activation of PKA (ex. *Rotavirus*). 5. Direct modification of tight junctional proteins extra- or intra-cellularly, including the phosphorylated state of occludin (ex. EPEC, *Vibrio cholera*-derived hemagglutinin/protease, *Campylobacter jejuni*). 6. Utilization of tight junctional proteins as receptors for enteric pathogens (ex. *Clostridium perfringens*-enterotoxin, *reovirus*, *adenovirus* and *coxsackie* virus).

coeliac disease (127). Although the underlying mechanisms remain unclear, risk factors contributing to the development of coeliac disease include infection by enteric pathogens, specifically rotavirus and adenovirus (128; 129). Interestingly, the tight junctional regulator zonulin is significantly increased in the intestine of individuals with active coeliac disease, suggesting a causal role for zonulin in disease pathogenesis (130). As discussed in this review, the *V. cholera*- derived toxin, Zot, mimics the effect of zonulin (66). Moreover, the presence of enteric bacteria in the mouse small intestine activates the zonulin pathway, and impairs barrier function (67). Thus, it is possible that enteric infections, followed by tight junctional disruptions, may contribute to the pathophysiology of coeliac disease.

Another autoimmune disorder associated with increased intestinal permeability is type-1 diabetes (131-133). Using an *in vitro* rat model of type-1 diabetes, intraluminal zonulin levels are significantly elevated in diabetic-prone rats compared to diabetic-resistant rats (134). The increased zonulin levels occur in parallel with a decrease in small intestinal TER, and this response is followed by the production of autoantibodies to pancreatic beta cells. Importantly, blockade of the zonulin receptor decreased the incident of type-1 diabetes in the diabetic-prone rats by 70% (134). Human studies confirm the *in vitro* rat study, demonstrating elevated zonulin levels in 42% of patients with type-1 diabetes compared to age matched control subjects (135). The increased zonulin levels are accompanied by enhanced intestinal permeability and altered claudin-1 and claudin-2 gene expression. Furthermore, elevated zonulin levels appear to precede the onset of disease in patients who went on to develop type-1 diabetes (135).

6. CONCLUSIONS

A broad range of enteric pathogens are known to disrupt epithelial tight junctions and alter intestinal permeability. It is becoming increasingly clear that intestinal microbes utilize a diverse array of strategies to overcome the tight junction barrier. Some of these strategies are summarized in Figure 1. Defining the mechanisms by which these pathogens subvert tight junction function will facilitate our understanding of the pathophysiological processes of enteric infections. This, in turn, may lead to the development of new therapeutic approaches to counteract the altered epithelial permeability associated with acute enteric infections, and may help control a variety of chronic disorders of the intestine.

7. REFERENCES

1. Hollande, F., A. Shulkes, and G.S. Baldwin: Signaling the junctions in gut epithelium. *Sci STKE* 2005, e13 (2005)
2. Turner, JR.: Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. *Am J Pathol* 169, 1901-1909 (2006)
3. Clayburgh, D.R., L. Shen, and J.R. Turner: A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 84,282-291 (2004)
4. Nusrat, A., J.R., Turner, and J.L. Madara: Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. *Am J Physiol Gastrointest Liver Physiol* 279, G851-G857, (2000)
5. Anderson, J.M.: Molecular structure of tight junctions and their role in epithelial transport. *News Physiol Sci* 16, 126-130 (2001)
6. Ivanov, A.I., A. Nusrat, and C.A. Parkos: Endocytosis of the apical junctional complex: mechanisms and possible roles in regulation of epithelial barriers. *Bioessays* 27, 356-365 (2005)
7. Mitic, L.L., C.M. Van Itallie, and J.M. Anderson: Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. *Am J Physiol Gastrointest Liver Physiol* 279, G250-G254, (2000)
8. Cereijido, M., L. Shoshani, and R.G. Contreras: Molecular physiology and pathophysiology of tight junctions. I. Biogenesis of tight junctions and epithelial polarity. *Am J Physiol Gastrointest Liver Physiol* 279, G477-G482 (2000)
9. Matter, K. and M.S. Balda: Signalling to and from tight junctions. *Nat Rev Mol Cell Biol* 4, 225-236 (2003)
10. Schneeberger, E.E. and R.D. Lynch: The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol* 286, C1213-C1228 (2004)
11. Buret, A.G., J.P. Fedwick, and A.N. Flynn: Host epithelial interactions with Helicobacter pylori: a role for disrupted gastric barrier function in the clinical outcome of infection? *Can J Gastroenterol* 19, 543-552 (2005)
12. Laukoetter, M.G., M. Bruewe,r and A. Nusrat: Regulation of the intestinal epithelial barrier by the apical junctional complex. *Curr Opin Gastroenterol* 22, 85-89 (2006)
13. Turner, J.R.: 'Putting the squeeze' on the tight junction: understanding cytoskeletal regulation. *Semin Cell Dev Biol* 11, 301-308 (2000)
14. Fasano, A. and J.P. Nataro: Intestinal epithelial tight junctions as targets for enteric bacteria-derived toxins. *Adv Drug Deliv Rev* 56, 795-807 (2004)
15. Sears, C.L.: Molecular physiology and pathophysiology of tight junctions V. assault of the tight junction by enteric pathogens. *Am J Physiol Gastrointest Liver Physiol* 279, G1129-G1134 (2000)
16. Berkes, J., V.K. Viswanathan, S.D. Savkovic, and G. Hecht: Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut* 52, 439-451 (2003)
17. Scott, K.G., J.B. Meddings, D.R. Kirk, S.P. Lees-Miller, and A.G. Buret: Intestinal infection with Giardia spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology* 123, 1179-1190 (2002)
18. Yuhan, R., A. Koutsouris, S.D. Savkovic, and G. Hecht: Enteropathogenic Escherichia coli-induced myosin light chain phosphorylation alters intestinal epithelial permeability. *Gastroenterology* 113, 1873-1882 (1997)
19. Nusrat, A., M. Giry, J.R. Turner, S.P. Colgan, C.A. Parkos, D. Carnes, E. Lemichez, P. Boquet, and J.L. Madara: Rho protein regulates tight junctions and perijunctional actin organization in polarized epithelia. *Proc Natl Acad Sci U S A* 92,10629-10633 (1995)
20. Pothoulakis, C.: Effects of Clostridium difficile toxins on epithelial cell barrier. *Ann N Y Acad Sci* 915, 347-356 (2000)
21. Tafazoli, F., K.E. Magnusson, and L. Zheng: Disruption of epithelial barrier integrity by Salmonella enterica serovar typhimurium requires geranylgeranylated proteins. *Infect Immun* 71, 872-881 (2003)
22. Boyle, E.C., N.F. Brown, and B.B. Finlay: Salmonella enterica serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function. *Cell Microbiol* 8, 1946-1957 (2006)
23. Ciarlet, M. and M.K. Estes: Interactions between rotavirus and gastrointestinal cells. *Curr Opin Microbiol* 4, 435-441 (2001)
24. Obert, G., I. Peiffer, and A.L. Servin: Rotavirus-induced structural and functional alterations in tight junctions of polarized intestinal Caco-2 cell monolayers. *J Virol* 74, 4645-4651 (2000)
25. Dickman, K.G., S.J. Hempson, J. Anderson, S. Lippe, L. Zhao, R. Burakoff, and R.D. Shaw: Rotavirus alters paracellular permeability and energy metabolism in Caco-2 cells. *Am J Physiol Gastrointest Liver Physiol* 279, G757-G766 (2000)
26. Beau, I., J. Cotte-Laffitte, R. Amsellem, and A.L Servin: A protein kinase A-dependent mechanism by which rotavirus affects the distribution and mRNA level of the functional tight junction-associated protein, occludin, in human differentiated intestinal Caco-2 cells. *J Virol* 81, 8579-8586 (2007)
27. Svensson, L., B.B. Finlay, D. Bass, C.H. von Bonsdorff, and H.B Greenberg: Symmetric infection of rotavirus on polarized human intestinal epithelial (Caco-2) cells. *J Virol* 65, 4190-4197 (1991)

28. Tafazoli, F., C.Q. Zeng, M.K. Estes, K.E. Magnusson, and L. Svensson: NSP4 enterotoxin of rotavirus induces paracellular leakage in polarized epithelial cells. *J Virol* 75, 1540-1546 (2001)
29. Nava, P., S. Lopez, C.F. Arias, S. Islas, and L. Gonzalez-Mariscal: The rotavirus surface protein VP8 modulates the gate and fence function of tight junctions in epithelial cells. *J Cell Sci* 117, 5509-5519 (2004)
30. Sakakibara, A., M. Furuse, M. Saitou, Y. ndo-Akatsuka, and S. Tsukita: Possible involvement of phosphorylation of occludin in tight junction formation. *J Cell Biol* 137, 1393-1401 (1997)
31. Wong, V.: Phosphorylation of occludin correlates with occludin localization and function at the tight junction. *Am J Physiol* 273, C1859-C1867 (1997)
32. Simonovic, I., J. Rosenberg, A. Koutsouris, and G. Hecht: Enteropathogenic Escherichia coli dephosphorylates and dissociates occludin from intestinal epithelial tight junctions. *Cell Microbiol* 2,305-315 (2000)
33. Chen, M.L., Z. Ge, J.G. Fox, and D.B. Schauer: Disruption of tight junctions and induction of proinflammatory cytokine responses in colonic epithelial cells by Campylobacter jejuni. *Infect Immun* 74, 6581-6589 (2006)
34. Bergelson, J.M., J.A. Cunningham, G. Droguett, E.A. Kurt-Jones, A. Krithivas, J.S. Hong, M.S. Horwitz, R.L. Crowell, and R.W. Finberg: Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* 275, 1320-1323 (1997)
35. Goosney, D.L. and G.R. Nemerow: Adenovirus infection: taking the back roads to viral entry. *Curr Biol* 13, R99-R100 (2003)
36. Cohen, C.J., J.T. Shieh, R.J. Pickles, T. Okegawa, J.T. Hsieh, and J.M. Bergelson: The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci U S A* 98, 15191-15196 (2001)
37. Walters, R.W., P. Freimuth, T.O. Moninger, I. Ganske, J. Zabner, and M.J. Welsh: Adenovirus fiber disrupts CAR-mediated intercellular adhesion allowing virus escape. *Cell* 110, 789-799 (2002)
38. Barton, E.S., J.C. Forrest, J.L. Connolly, J.D. Chappell, Y. Liu, F.J. Schnell, A. Nusrat, C.A. Parkos, and T.S. Dermody: Junction adhesion molecule is a receptor for reovirus. *Cell* 104, 441-451 (2001)
39. Laukoetter, M.G., P. Nava, W.Y. Lee, E.A. Severson, C.T. Capaldo, B.A. Babbin, I.R. Williams, M. Koval, E. Peatman, J.A. Campbell, T.S. Dermody,TS, A. Nusrat, and C.A. Parkos: JAM-A regulates permeability and inflammation in the intestine in vivo. *J Exp Med* 204, 3067-3076 (2007)
40. Mandell, K.J., B.A. Babbin, A. Nusrat, and C.A. Parkos: Junctional adhesion molecule 1 regulates epithelial cell morphology through effects on beta1 integrins and Rap1 activity. *J Biol Chem* 280, 11665-11674 (2005)
41. Ridley, A.J. and A. Hall: The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70, 389-399, (1992)
42. Hall, A.: Rho GTPases and the actin cytoskeleton. *Science* 279, 509-514 (1998)
43. Madara, J.L., R. Moore, and S. Carlson: Alteration of intestinal tight junction structure and permeability by cytoskeletal contraction. *Am J Physiol* 253, C854-C861 (1987)
44. Hecht, G., C. Pothoulakis, J.T. LaMont, and J.L. Madara: Clostridium difficile toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. *J Clin Invest* 82, 1516-1524 (1988)
45. Hecht, G., A. Koutsouris, C. Pothoulakis, J.T. LaMont, and J.L. Madara: Clostridium difficile toxin B disrupts the barrier function of T84 monolayers. *Gastroenterology* 102, 416-423 (1992)
46. Nusrat, A., C. von Eichel-Streiber, J.R. Turner, P. Verkade, J.L. Madara, and C.A. Parkos: Clostridium difficile toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect Immun* 69, 1329-1336 (2001)
47. Nusrat, A., C.A. Parkos, P. Verkade, C.S. Foley, T.W. Liang, W. Innis-Whitehouse, K.K. Eastburn, and J.L. Madara: Tight junctions are membrane microdomains. *J Cell Sci* 113 (Pt 10), 1771-1781 (2000)
48. McClane, B.A., P.C. Hanna, and A.P. Wnek: Clostridium perfringens enterotoxin. *Microb Pathog* 4, 317-323 (1988)
49. Katahira, J., H. Sugiyama, N. Inoue, Y. Horiguchi, M. Matsuda, and N. Sugimoto: Clostridium perfringens enterotoxin utilizes two structurally related membrane proteins as functional receptors in vivo. *J Biol Chem* 272, 26652-26658 (1997)
50. Katahira, J., N. Inoue, Y. Horiguchi, M. Matsuda, and N. Sugimoto: Molecular cloning and functional characterization of the receptor for Clostridium perfringens enterotoxin. *J Cell Biol* 136, 1239-1247 (1997)
51. McClane, B.A.: The complex interactions between Clostridium perfringens enterotoxin and epithelial tight junctions. *Toxicon* 39, 1781-1791 (2001)
52. Sonoda, N., M. Furuse, H. Sasaki, S. Yonemura, J. Katahira, Y. Horiguchi, and S. Tsukita: Clostridium perfringens enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. *J Cell Biol* 147, 195-204 (1999)
53. Galan, J.E.: Interaction of Salmonella with host cells through the centisome 63 type III secretion system. *Curr Opin Microbiol* 2, 46-50 (1999)
54. Galan, J.E. and A. Collmer: Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 284, 1322-1328 (1999)
55. Finlay, B.B. and S. Falkow: Salmonella interactions with polarized human intestinal Caco-2 epithelial cells. *J Infect Dis* 162, 1096-1106 (1990)
56. Jepson, M.A., C.B. Collares-Buzato, M.A. Clark, B.H. Hirst, and N.L. Simmons: Rapid disruption of epithelial barrier function by Salmonella typhimurium is associated with structural modification of intercellular junctions. *Infect Immun* 63, 356-359 (1995)
57. Kohler, H., T. Sakaguchi, B.P. Hurley, B.A. Kase, H.C. Reinecker, and B.A. McCormick: Salmonella enterica serovar Typhimurium regulates intercellular junction proteins and facilitates transepithelial neutrophil and

bacterial passage. *Am J Physiol Gastrointest Liver Physiol* 293. G178-G187 (2007)
58. Jepson, M.A., H.B. Schlecht, and C.B. Collares-Buzato: Localization of dysfunctional tight junctions in Salmonella enterica serovar typhimurium-infected epithelial layers. *Infect Immun* 68, 7202-7208 (2000)
59. Fu, Y. and J.E. Galan: A salmonella protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* 401, 293-297 (1999)
60. Hardt, W.D., L.M. Chen, K.E. Schuebel, X.R. Bustelo, and J.E. Galan: S. typhimurium encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* 93, 815-826 (1998)
61. Sears, C.L. and J.B. Kaper: Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. *Microbiol Rev* 60, 167-215 (1996)
62. Levine, M.M., J.B. Kaper, D. Herrington, G. Losonsky, J.G. Morris, M.L. Clements, R.E. Black, B. Tall, and R. Hall: Volunteer studies of deletion mutants of Vibrio cholerae O1 prepared by recombinant techniques. *Infect Immun* 56, 161-167 (1988)
63. Fasano, A., B. Baudry, D.W. Pumplin, S.S. Wasserman, B.D Tall, J.M. Ketley, and J.B. Kaper: Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions. *Proc Natl Acad Sci U S A* 88, 5242-5246 (1991)
64. Fasano, A., C. Fiorentini, G. Donelli, S. Uzzau, J.B. Kaper, K. Margaretten, X. Ding, S. Guandalini, L. Comstock, and S.E. Goldblum: Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, in vitro. *J Clin Invest* 96, 710-720 (1995)
65. Fasano, A., S. Uzzau, C. Fiore, and K. Margaretten: The enterotoxic effect of zonula occludens toxin on rabbit small intestine involves the paracellular pathway. *Gastroenterology* 112, 839-846 (1997)
66. Wang, W., S. Uzzau, S.E. Goldblum, and A. Fasano: Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci* 113 Pt 24, 4435-4440 (2000)
67. El Asmar, R., P. Panigrahi, P. Bamford, I. Berti, T. Not, G.V. Coppa, C. Catassi, and A. Fasano: Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. *Gastroenterology* 123, 1607-1615 (2002)
68. Booth, B.A., M. Boesman-Finkelstein, and R.A. Finkelstein: Vibrio cholerae hemagglutinin/protease nicks cholera enterotoxin. *Infect Immun* 45, 558-560 (1984)
69. Wu, Z., D. Milton, P. Nybom, A. Sjo, and K.E. Magnusson: Vibrio cholerae hemagglutinin/protease (HA/protease) causes morphological changes in cultured epithelial cells and perturbs their paracellular barrier function. *Microb Pathog* 21, 111-123 (1996)
70. Wu, Z., P. Nybom, and K.E. Magnusson: Distinct effects of Vibrio cholerae haemagglutinin/protease on the structure and localization of the tight junction-associated proteins occludin and ZO-1. *Cell Microbiol* 2, 11-17 (2000)
71. Lynch, T., S. Livingstone, E. Buenaventura, E. Lutter, J. Fedwick, A.G. Buret, D. Graham, and R. DeVinney: Vibrio parahaemolyticus disruption of epithelial cell tight junctions occurs independently of toxin production. *Infect Immun* 73, 1275-1283 (2005)
72. Spitz, J., R. Yuhan, A. Koutsouris, C. Blatt, J. Alverdy, and G. Hecht: Enteropathogenic Escherichia coli adherence to intestinal epithelial monolayers diminishes barrier function. *Am J Physiol* 268, G374-G379 (1995)
73. Philpott, D.J., D.M. McKay, P.M. Sherman, and M.H. Perdue: Infection of T84 cells with enteropathogenic Escherichia coli alters barrier and transport functions. *Am J Physiol* 270, G634-G645 (1996)
74. Muza-Moons, M.M., E.E. Schneeberger, and G.A. Hecht: Enteropathogenic Escherichia coli infection leads to appearance of aberrant tight junctions strands in the lateral membrane of intestinal epithelial cells. *Cell Microbiol* 6, 783-793 (2004)
75. Hecht, G.: Microbes and microbial toxins: paradigms for microbial-mucosal interactions. VII. Enteropathogenic Escherichia coli: physiological alterations from an extracellular position. *Am J Physiol Gastrointest Liver Physiol* 281, G1-G7 (2001)
76. McNamara, B.P., A. Koutsouris, C.B. O'Connell, J.P. Nougayrede, M.S. Donnenberg, and G. Hecht: Translocated EspF protein from enteropathogenic Escherichia coli disrupts host intestinal barrier function. *J Clin Invest* 107, 621-629 (2001)
77. Elliott, S.J., C.B. O'Connell, A. Koutsouris, C. Brinkley, M.S. Donnenberg, G. Hecht, and J.B. Kaper: A gene from the locus of enterocyte effacement that is required for enteropathogenic Escherichia coli to increase tight-junction permeability encodes a chaperone for EspF. *Infect Immun* 70, 2271-2277 (2002)
78. Shifflett, D.E., D.R. Clayburgh, A. Koutsouris, J.R. Turner, and G.A. Hecht: Enteropathogenic E. coli disrupts tight junction barrier function and structure in vivo. *Lab Invest* 85, 1308-1324 (2005)
79. Wang, F., W.V. Graham, Y. Wang, E.D. Witkowski, B.T Schwarz, and J.R. Turner: Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. *Am J Pathol* 166, 409-419 (2005)
80. Ma, T.Y., M.A. Boivin, D. Ye, A. Pedram, and H.M. Said: Mechanism of TNF-{alpha} modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am J Physiol Gastrointest Liver Physiol* 288, G422-G430 (2005)
81. Madara, J.L. and J. Stafford: Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 83, 724-727 (1989)
82. Ye, D., I. Ma, and T.Y. Ma: Molecular mechanism of tumor necrosis factor-alpha modulation of intestinal epithelial tight junction barrier. *Am J Physiol Gastrointest Liver Physiol* 290, G496-G504 (2006)
83. Philpott, D.J., D.M. McKay, W. Mak, M.H. Perdue, and P.M. Sherman Signal transduction pathways involved in enterohemorrhagic Escherichia coli-induced alterations in T84 epithelial permeability. *Infect Immun* 66, 1680-1687 (1998)
84. Viswanathan, V.K., A. Koutsouris, S. Lukic, M. Pilkinton, I. Simonovic, M. Simonovic, and G. Hecht: Comparative analysis of EspF from enteropathogenic and enterohemorrhagic Escherichia coli in alteration of epithelial barrier function. *Infect Immun* 72, 3218-3227 (2004)

85. Gerhard, R., G. Schmidt, F. Hofmann, and K. Aktories: Activation of Rho GTPases by Escherichia coli cytotoxic necrotizing factor 1 increases intestinal permeability in Caco-2 cells. *Infect Immun* 66, 5125-5131 (1998)
86. Hopkins, A.M., S.V. Walsh, P. Verkade, P. Boquet, and A. Nusrat: Constitutive activation of Rho proteins by CNF-1 influences tight junction structure and epithelial barrier function. *J Cell Sci* 116, 725-742 (2003)
87. Health Canada. http://www.hc-sc.gc.ca/fn-an/res-rech/res-prog/microbio/campylobacter_e.html. (2008)
88. Centers for Disease Control and Prevention. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/campylobacter_g.htm (2008)
89. MacCallum, A., S.P. Hardy, and P.H. Everest: Campylobacter jejuni inhibits the absorptive transport functions of Caco-2 cells and disrupts cellular tight junctions. *Microbiology* 151, 2451-2458 (2005)
90. Lamb-Rosteski, J.M., L.D. Kalischuck, G.D. Inglis, and A.G. Buret: Epidermal growth factor inhibits Campylobacter jejuni-induced loss of epithelial barrier function, claudin-4 disruption, and Escherichia coli translocation. *Infect Immun* In press, (2008)
91. Kalischuk, L.D., G.D. Inglis, and A.G. Buret: Campylobacter jejuni induces translocation of non-invasive intestinal bacteria in vivo and in vitro (Abstract). *Can.J.Gastroenterol.* In press, (2008)
92. Mounier, J., T. Vasselon, R. Hellio, M. Lesourd, and P.J. Sansonetti: Shigella flexneri enters human colonic Caco-2 epithelial cells through the basolateral pole. *Infect Immun* 60, 237-248 (1992)
93. Sakaguchi, T., H. Kohler, X. Gu, B.A. McCormick, H.C. Reinecker: Shigella flexneri regulates tight junction-associated proteins in human intestinal epithelial cells. *Cell Microbiol* 4, 367-381 (2002)
94. Farthing, M.J.: Giardiasis. *Gastroenterol Clin North Am* 25, 493-515 (1996)
95. Marshall, M.M., D. Naumovitz, Y. Ortega, and C.R. Sterling: Waterborne protozoan pathogens. *Clin Microbiol Rev* 10, 67-85 (1997)
96. Teoh, D.A., D. Kamieniecki, G. Pang, and A.G. Buret: Giardia lamblia rearranges F-actin and alpha-actinin in human colonic and duodenal monolayers and reduces transepithelial electrical resistance. *J Parasitol* 86, 800-806 (2000)
97. Hardin, J.A., A.G. Buret, M.E. Olson, M.H. Kimm, and D.G. Gall: Mast cell hyperplasia and increased macromolecular uptake in an animal model of giardiasis. *J Parasitol* 83, 908-912 (1997)
98. Chin, A.C., D.A. Teoh, K.G. Scott, J.B. Meddings, W.K. MacNaughton, and A.G. Buret: Strain-dependent induction of enterocyte apoptosis by Giardia lamblia disrupts epithelial barrier function in a caspase-3-dependent manner. *Infect Immun* 70, 3673-3680 (2002)
99. Troeger, H., H.J. Epple, T. Schneider, U. Wahnschaffe, R. Ullrich, G.D. Burchard, T. Jelinek, M. Zeitz, M. Fromm, and J.D. Schulzke: Effect of chronic Giardia lamblia infection on epithelial transport and barrier function in human duodenum. *Gut* 56, 328-335 (2007)
100. Buret, A.G., K. Mitchell, D.G. Muench, and K.G. Scott: Giardia lamblia disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitology* 125, 11-19 (2002)
101. Li, E., W.F. Stenson, C. Kunz-Jenkins, P.E. Swanson, R. Duncan, and S.L. Stanley, Jr.: Entamoeba histolytica interactions with polarized human intestinal Caco-2 epithelial cells. *Infect Immun* 62, 5112-5119 (1994)
102. Leroy, A., T. Lauwaet, B.G. De, M. Cornelissen, and M. Mareel: Entamoeba histolytica disturbs the tight junction complex in human enteric T84 cell layers. *FASEB J* 14,1139-1146 (2000)
103. Nasirudeen, A.M.: Cell death and human intestinal protozoa: a brief overview. *Curr Issues Intest Microbiol* 6, 77-82 (2005)
104. Huston, C.D., E.R. Houpt, B.J. Mann, C.S. Hahn, and W.A. Petri, Jr.: Caspase 3-dependent killing of host cells by the parasite Entamoeba histolytica. *Cell Microbiol* 2, 617-625 (2000)
105. Lopez-Vancell, R., I. Montfort, and R. Perez-Tamayo: Galactose-specific adhesin and cytotoxicity of Entamoeba histolytica. *Parasitol Res* 86, 226-231 (2000)
106. Lauwaet, T., M.J. Oliveira, B. Callewaert, B.G. De, M. Mareel, and A. Leroy: Proteinase inhibitors TPCK and TLCK prevent Entamoeba histolytica induced disturbance of tight junctions and microvilli in enteric cell layers in vitro. *Int J Parasitol* 34,785-794 (2004)
107. Gibson, P.R.: Increased gut permeability in Crohn's disease: is TNF the link? *Gut* 53, 1724-1725 (2004)
108. Secondulfo, M., M.L. de, R. Fiandra, L. Caserta, M. Belletta, M.T. Tartaglione, G. Riegler, F. Biagi, G.R. Corazza, and R. Carratu: Intestinal permeability in Crohn's disease patients and their first degree relatives. *Dig Liver Dis* 33, 680-685 (2001)
109. Schmitz, H., C. Barmeyer, M. Fromm, N. Runkel, H.D. Foss, C.J. Bentzel, E.O. Riecken, and J.D. Schulzke: Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology* 116, 301-309 (1999)
110. Zeissig, S., N. Burgel, D. Gunzel, J. Richter, J. Mankertz, U. Wahnschaffe, A.J. Kroesen, M. Zeitz, M. Fromm, and J.D. Schulzke: Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 56, 61-72 (2007)
111. Kucharzik, T., S.V. Walsh, J. Chen, C.A. Parkos, and A. Nusrat: Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am J Pathol* 159, 2001-2009 (2001)
112. Wyatt, J., H. Vogelsang, W. Hubl, T. Waldhoer, and H. Lochs: Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 341, 1437-1439 (1993)
113. D'Inca, R., V. Di Leo, G. Corrao, D. Martines, A. D'Odorico, C. Mestriner, C. Venturi, G. Longo, and G.C. Sturniolo: Intestinal permeability test as a predictor of clinical course in Crohn's disease. *Am J Gastroenterol* 94, 2956-2960 (1999)
114. Arnott, I.D., K. Kingstone, and S. Ghosh: Abnormal intestinal permeability predicts relapse in inactive Crohn disease. *Scand J Gastroenterol* 35, 1163-1169 (2000)
115. Breslin, N.P., C. Nash, R.J. Hilsden, N.B. Hershfield, L.M. Price, J.B. Meddings, and L.R. Sutherland: Intestinal permeability is increased in a proportion of spouses of patients with Crohn's disease. *Am J Gastroenterol* 96, 2934-2938 (2001)

116. Hollander, D.: Permeability in Crohn's disease: altered barrier functions in healthy relatives? *Gastroenterology* 104, 1848-1851 (1993)
117. Peeters, M., B. Geypens, D. Claus, H. Nevens, Y. Ghoos, G. Verbeke, F. Baert, S. Vermeire, R. Vlietinck, and P. Rutgeerts: Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterology* 113, 802-807 (1997)
118. Katz, K.D., D. Hollander, C.M. Vadheim, C. McElree, T. Delahunty, V.D. Dadufalza, P. Krugliak, and J.I. Rotter: Intestinal permeability in patients with Crohn's disease and their healthy relatives. *Gastroenterology* 97, 927-931 (1989)
119. Munkholm, P., E. Langholz, D. Hollander, K. Thornberg, M. Orholm, K.D. Katz, and V. Binder: Intestinal permeability in patients with Crohn's disease and ulcerative colitis and their first degree relatives. *Gut* 35, 68-72 (1994)
120. Garcia Rodriguez, L.A., A. Ruigomez, and J. Panes: Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. *Gastroenterology* 130, 1588-1594 (2006)
121. Weber, P., M. Koch, W.R. Heizmann, M. Scheurlen, H. Jenss, and F. Hartmann: Microbic superinfection in relapse of inflammatory bowel disease. *J Clin Gastroenterol* 14, 302-308 (1992)
122. Spiller, R.C.: Role of infection in irritable bowel syndrome. *J Gastroenterol* 42 Suppl 17, 41-47 (2007)
123. Marshall, J.K., M. Thabane, A.X. Garg, W.F. Clark, M. Salvadori, and S.M. Collins: Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. *Gastroenterology* 131, 445-450 (2006)
124. D'Anchino, M., D. Orlando, and F.L. De: Giardia lamblia infections become clinically evident by eliciting symptoms of irritable bowel syndrome. *J Infect* 45, 169-172 (2002)
125. Dunlop, S.P., J. Hebden, E. Campbell, J. Naesdal, L. Olbe, A.C. Perkins, and R.C. Spiller: Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 101, 1288-1294 (2006)
126. Marshall, J.K., M. Thabane, A.X. Garg, W. Clark, J. Meddings, and S.M. Collins: Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Aliment Pharmacol Ther* 20, 1317-1322 (2004)
127. Schulzke, J.D., C.J. Bentzel, I. Schulzke, E.O. Riecken, and M. Fromm: Epithelial tight junction structure in the jejunum of children with acute and treated celiac sprue. *Pediatr Res* 43, 435-441 (1998)
128. Stene, L.C., M.C. Honeyman, E.J. Hoffenberg, J.E. Haas, R.J. Sokol, L. Emery, I. Taki, J.M. Norris, H.A. Erlich, G.S. Eisenbarth, and M. Rewers: Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. *Am J Gastroenterol* 101, 2333-2340 (2006)
129. Kagnoff, M.F., Y.J. Paterson, P.J. Kumar, D.D. Kasarda, F.R. Carbone, D.J. Unsworth, and R.K. Austin: Evidence for the role of a human intestinal adenovirus in the pathogenesis of coeliac disease. *Gut* 28, 995-1001 (1987)
130. Fasano, A., T. Not, W. Wang, S. Uzzau, I. Berti, A. Tommasini, and S.E. Goldblum: Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet* 355, 1518-1519 (2000)
131. Meddings, J.B., J. Jarand, S.J. Urbanski, J. Hardin, and D.G. Gall: Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat. *Am J Physiol* 276, G951-G957 (1999)
132. Damci, T., I. Nuhoglu, G. Devranoglu, Z. Osar, M. Demir, and H. Ilkova: Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients. *Eur J Clin Invest* 33, 397-401 (2003)
133. Secondulfo, M., D. Iafusco, R. Carratu, L. Demagistris, A. Sapone, M. Generoso, A. Mezzogiomo, F.C. Sasso, M. Carteni, R. De Rosa, F. Prisco, and V. Esposito: Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients. *Dig Liver Dis* 36, 35-45 (2004)
134. Watts, T., I. Berti, A. Sapone, T. Gerarduzzi, T. Not, R. Zielke, and A. Fasano: Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. *Proc Natl Acad Sci U S A* 102, 2916-2921 (2005)
135. Sapone, A., L. de Magistris, M. Pietzak, M.G. Clemente, A. Tripathi, F. Cucca, R. Lampis, D. Kryszak, M. Carteni, M. Generoso, D. Iafusco, F. Prisco, F. Laghi, G. Riegler, R. Carratu, D. Counts, and A. Fasano: Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 55, 1443-1449 (2006)

Abbreviations: JAM: junctional adhesion molecule, CAR: coxsackie virus and adenovirus receptor, ZO: zonula occludens, MLC: myosin light chain, MLCK: myosin light chain kinase, TER: transepithelial resistance, PKA: protein kinase A, CPE: clostridium perfringens enterotoxin, T3SS: type three secretion system, CT: cholera toxin, PKC: protein kinase C, Zot: zonula occludens toxin, HA/P: hemagglutinin/protease, EPEC: enteropathogenic Escherichia coli, EHEC: enterohaemorrhagic Escherichia coli, IBD: inflammatory bowel disease, IBS: irritable bowel syndrome

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