Epithelial Barriers in Homeostasis and Disease

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tight junction, claudin, occludin, myosin light chain kinase, tumor necrosis factor, inflammatory bowel disease

Abstract

Epithelia form barriers that are essential to life. This is particularly true in the intestine, where the epithelial barrier supports nutrient and water transport while preventing microbial contamination of the interstitial tissues. Along with plasma membranes, the intercellular tight junction is the primary cellular determinant of epithelial barrier function. Disruption of tight junction structure, as a result of specific protein mutations or aberrant regulatory signals, can be both a cause and an effect of disease. Recent advances have provided new insights into the extracellular signals and intracellular mediators of tight junction regulation in disease states as well as into the interactions of intestinal barrier function with mucosal immune cells and luminal microbiota. In this review, we discuss the critical roles of the tight junction in health and explore the contributions of barrier dysfunction to disease pathogenesis.

TISSUES AS COMPARTMENTS

Tight junction: the most apical intercellular junction and rate-limiting step in paracellular flux

Zonula occludens 1 (ZO-1): a tight junction–associated peripheral membrane protein with multiple sites for protein-protein interactions, often referred to as a scaffold protein

Multicellular organisms, from sea sponges to mammals, require tissue compartmentalization to interface with the external environment while supporting specialized functions internally. Although sea sponges have only a single germ layer, the superficial cells differentiate to form a skin that defines the surface of the organism. This is slightly more complicated in diploblastic animals, such as jellyfish and sea anemones, that develop two distinct germ layers during embryogenesis. Both layers are covered by epithelial cells. The internal layer, or gastroderm, contains a glandular epithelium that secretes digestive enzymes, whereas the outer exoderm differentiates into several cell types, including epitheliomuscular and nerve cells. Although the development of more advanced animals, triploblasts, is more complex and includes formation of three cell layers during embryogenesis, the developmental themes present in diploblasts are maintained. The endoderm forms the gastrointestinal tract; the nervous system and external skin develop from the ectoderm; and the mesoderm gives rise to musculoskeletal, genitourinary, and circulatory systems. Each of these layers includes cells specialized to establish distinct compartments, including gastrointestinal and respiratory epithelia derived from endoderm, epidermal squamous epithelium and accessory glands derived from ectoderm, and renal epithelium and vascular endothelium derived from mesoderm (**Figure 1**). Thus, cells that define the interface between the organism and the external world are a characteristic of all multicellular eukaryotes.

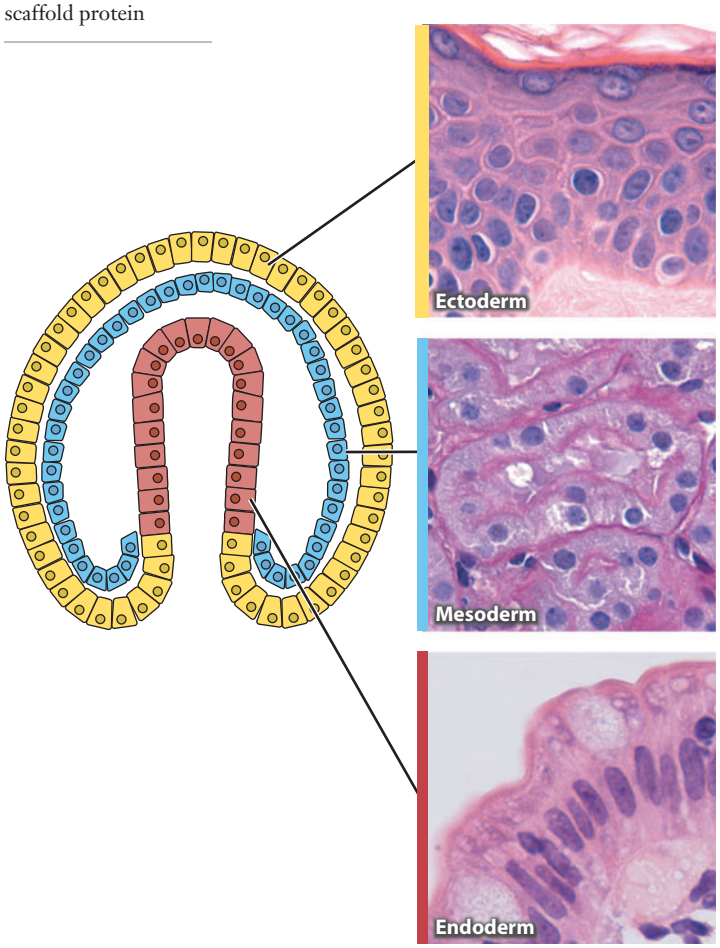

Figure 1

Barrier-forming epithelia are derived from each of the three primary germ layers. Triploblasts have three germ layers: ectoderm (*yellow*), mesoderm (*blue*), and endoderm (*red*). Cells derived from each layer establish distinct compartments, including squamous epithelium of the skin, renal tubular epithelium, and small intestinal epithelium.

To establish defined boundaries, cells that cover the external surface and line internal compartments must form barriers to prevent unrestricted exchange of materials. The critical nature of this barrier, and consequences of barrier loss, can be demonstrated in burn patients, in whom prognosis is directly related to the surface area damaged. Burns represent a gross form of barrier loss, where there is direct damage to the epithelial cells and exposure of underlying tissues. This damage results in a markedly increased risk of infection, as the cutaneous epithelial barrier is absent, as well as significant water and protein loss. Thus, cells that define the boundary are essential to barrier maintenance. The plasma membranes of these cells effectively prevent most hydrophilic solutes from crossing the boundary, but the paracellular, or shunt, pathway between cells must also be sealed. This function is the responsibility of the apical junctional complex (**Figure 2**), which is composed of the tight junction, or zonula occludens (ZO) and the

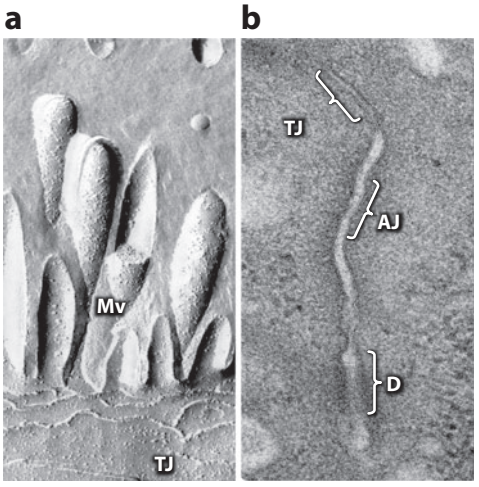

Figure 2

The apical junctional complex. (*a*) Freeze-fracture electron micrograph showing apical microvilli (Mv) and tight junction strands (TJ) in a cultured intestinal epithelial cell (courtesy of Dr. Eveline Schneeberger, Harvard Medical School). (*b*) Transmission electron micrograph showing junctional complexes between two villous enterocytes. The tight junction (TJ) is the most apical, followed by the adherens junction (AJ). The desmosomes (D) are located basolaterally.

subjacent adherens junction, or zonula adherens. The desmosomes, or macula adherens (1), are located along the lateral membranes beneath the adherens junction. Whereas tight junctions seal the paracellular pathway, the adherens junctions and desmosomes provide the strong bonds necessary to maintain cellular proximity and allow tight junction assembly. Adherens junctions are also critical for epithelial polarization and differentiation, mucosal morphogenesis, and tumor suppression, processes that occur through a variety of interactions with other proteins, including actin and beta-catenin.

THE PROBLEM WITH ABSOLUTE BARRIERS

The above discussion implies that the best evolutionary approach may be to create intercellular junctions that are perfectly impermeant. The initial description of the tight junction suggested that this might be the case. Farquhar & Palade (1, p. 375) described the tight junction as "characterized by fusion of the adjacent cell membranes resulting in obliteration of the intercellular space." However, this model was rapidly discarded when it became apparent that, despite the presence of tight junctions in all epithelia, the electrical resistance of the paracellular pathway varied by as much as 1000-fold between different epithelia. This variation was also noted when paracellular permeability was assessed through use of electron-dense tracers, such as ionic lanthanum, that were excluded from the tight junctions of a high-resistance epithelium but penetrated tight junctions of a low-resistance epithelium (2). It therefore became clear that tight junctions in various tissues can have very different barrier properties.

Barrier function: the ability of epithelial- and endothelial-lined surfaces to restrict free passage of water, ions, and larger solutes

When considered in the abstract, the spectrum of tight junction barrier function makes teleological sense. An absolute barrier would prevent any communication, at least via the paracellular pathway, with the external environment. In the case of sea sponges, impermeant paracellular junctions would prevent the exchange of oxygen and nutrients for waste products and rapidly lead to the death of the organism. Similarly, impermeant tight junctions between mammalian small intestinal epithelial cells could impede the absorption of water and nutrients that are necessary for life. Thus, it may not be surprising that mammalian small intestinal epithelia have low-resistance tight junctions. In contrast, gallbladder epithelium, which must prevent concentrated bile acids from entering the circulation, forms tight junctions with high resistance. However, as discussed in detail below, the sieving properties of the tight junction are not fixed. Thus, in addition to the wide variation in basal tight junction barrier function between different tissues, barrier properties can be acutely regulated (3).

MOLECULAR ANATOMY OF THE TIGHT JUNCTION

Since the discovery of the first tight junction–associated protein in 1986 (4), more than 50 additional tight junction–associated proteins have

Claudins: a large family of tight junction–associated transmembrane proteins that are critical for barrier function and paracellular transport

PDZ: a protein domain, named for PSD95, DlgA, and ZO-1, that supports protein interactions

Occludin: a tight junction–associated transmembrane protein with controversial function and no homology to claudin proteins

been described. These can be thought of in broad classes, although specific consideration of certain proteins is also necessary. At present, the most well-understood tight junction proteins are the claudins, a large family that includes at least 24 members (5–7). These proteins have four transmembrane helices with a very short intracellular amino-terminal sequence and a somewhat longer carboxy-terminal tail. The first extracellular loop is approximately 50 residues long, although there is some variation among claudin family members. More importantly, sequence variation within the first extracellular loop determines tight junction charge selectivity (8), consistent with the view that the array of claudin proteins expressed in a given cell type defines the paracellular pore (9) through which ions and perhaps nonionic solutes travel. The second extracellular loop of claudins is smaller than the first, ranges from 16 to 33 amino acids in length, and is poorly characterized. The cytoplasmic carboxy-terminal tail varies in length from 21 to 63 residues and is the least conserved region of claudin proteins, suggesting that the tail may also be a site of functional regulation. Importantly, almost all claudin proteins terminate with a three-amino-acid motif that binds to PDZ domains, as explained below (10).

The cytoplasmic plaque, or peripheral membrane, proteins of the tight junction include ZO-1 (**Figure 3**) as well as the related proteins ZO-2 and ZO-3 (4, 11, 12). Each of these proteins contains three PDZ domains within the amino-terminal portion of the protein. PDZ domains, which were initially identified in PSD95, DlgA, and ZO-1, are specialized for protein interactions. In the case of ZO-1, ZO-2, and ZO-3, the most amino-terminal PDZ domain binds to the carboxy terminal of claudins (10). This is functionally critical because deletion of the PDZ-binding motif prevents efficient claudin targeting to the tight junction (13). Moreover, ZO-1 and ZO-2 are each able to direct claudins to developing tight junctions; simultaneous elimination of ZO-1 and ZO-2 expression prevents claudin recruitment, tight junction formation,

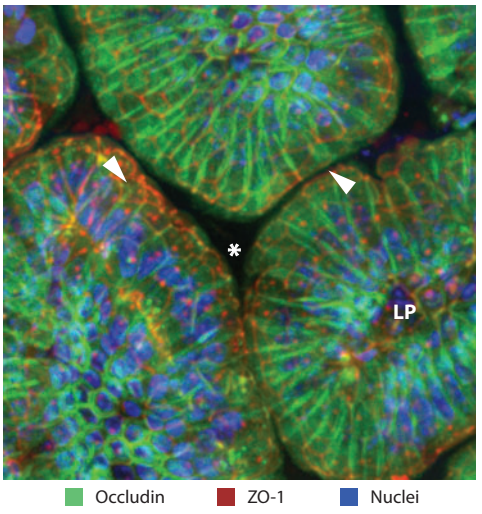

Figure 3

Tight junction proteins in villus enterocytes. Three-dimensional projection of jejunal villi from a transgenic mouse expressing enhanced green fluorescent protein–occludin and monomeric red fluorescent protein 1–zonula occludens 1 (ZO-1; *arrowheads*) under control of the villin promoter. Nuclei were stained blue. The gut lumen (*asterisk*) can be seen between the villi, and the lamina propria (LP) can be seen beneath the single layer of villus epithelial cells.

and development of barrier function (14). Although the mechanisms that direct ZO-1 to the nascent tight junction require further investigation, it is clear that poorly characterized carboxy-terminal regions of ZO-1 are required (14, 15) and that ZO-1 initially interacts with alpha-catenin at sites of cell-cell contact before separating from the developing adherens junction to establish the tight junction (16, 17). A variety of other peripheral membrane proteins have been associated with the tight junction, but their functional roles have yet to be defined (18, 19).

Several other groups of proteins, including transmembrane, cytoskeletal, and signaling proteins (20), are present at the tight junction. As discussed in detail below, actin and myosin are essential structural elements of the tight junction. In addition, occludin, the first transmembrane protein discovered at the tight junction (21), continues to be enigmatic. An

abundance of data making use of cultured cell lines and *Xenopus* embryos suggests that this protein is very important to barrier development and regulation (22–28). However, occludin knockout embryonic stem cells can differentiate into polarized epithelial cells with functional tight junctions (29), and although occludin knockout mice have severe disease with a complex phenotype (30), they do not appear to have defects in intestinal transport or barrier function (31). Thus, although the knockout mice may have partially compensated for the absence of occludin, the specific in vivo role of this protein remains controversial. Notably, human mutations in the occludin-related tight junction protein tricellulin have been linked to autosomal recessive hearing loss (32–34).

PROTEIN INTERACTIONS AND TIGHT JUNCTION STRUCTURE

Although a large number of proteins associated with the tight junction have been identified, the manner in which they work together to form the selectively permeable barrier is only beginning to be defined. For example, the pore created by claudins is not understood, although some data suggest that hexamers within single cells (35) may dimerize via the second extracellular loop (36, 37) and can form close associations with occludin (38). Other data demonstrate that occludin can self-associate, primarily through domains within the long cytoplasmic carboxy-terminal tail (38), and that other sites within the tail bind to PDZ domains of ZO-1, ZO-2, and ZO-3 (10, 39, 40). Additional multi–PDZ domain–containing proteins, some with as many as 13 such domains (41), are present at the tight junction and are thought to form a scaffold that supports the tight junction. Finally, interactions between tight junction proteins and a variety of kinases and cytoskeletal proteins have also been described (42–46). As a result of this plethora of potential binding interactions, the steady-state tight junction has been modeled as a heavily cross-linked, stable structure (47). This is supported by studies in cultured epithelial monolayers and fibroblasts that show the majority of claudin-1 to be stably anchored at the tight junction (13, 36). However, this model is being revised as a result of recent analyses of the dynamic behavior of tight junction proteins in cultured epithelial monolayers (13). Studies that use well-validated fluorescent-tagged fusion proteins have shown that the majority of tight junction–associated ZO-1 and occludin are present in a form that allows them to rapidly move in and out of specific regions within the tight junction. Although this finding could indicate that ZO-1 and occludin exchange as a complex, further analyses have shown that ZO-1 exchanges with a cytoplasmic pool by means of an energy-dependent process (13). In contrast, occludin exchange occurs by energy-independent diffusion within the plasma membrane (13). Thus, although ZO-1 and occludin may interact at the tight junction (39), this interaction is likely to be transient and highly dynamic. It is tempting to speculate that the observed behavior may reflect dynamic regulation of the pore complex. Tight junction protein dynamics may also explain how flux of charged and noncharged solutes is differentially regulated as well as the recent observation that, for noncharged solutes, at least two size classes of pores exist (48). Interestingly, overexpression of claudin-2 increases conductance of small uncharged molecules and cations, but it has no effect on paracellular flux of larger molecules (48), suggesting that claudins can regulate size, as well as charge, selectivity. Nevertheless, the basic structural biology of the tight junction remains an important subject for future studies.

TIGHT JUNCTION PROTEIN MUTATIONS AND DISEASE

Claudin function was first demonstrated in vivo when the genetic cause of a rare autosomal recessive disease, characterized by hypomagnesemia with hypercalciuria and nephrocalcinosis, was identified to be mutation of the claudin-16 gene, *CLDN16* (49). Expression of claudin-16 allows development of cation-selective tight

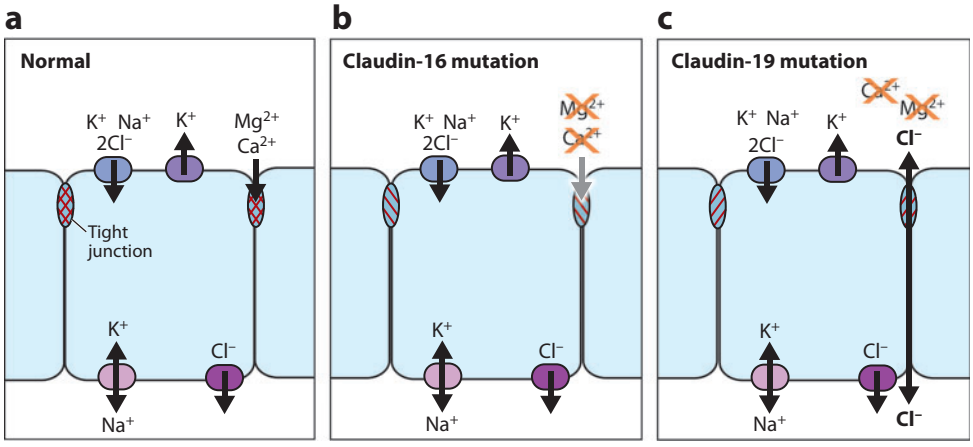

Figure 4

Effects of claudin mutations on renal ion transport. Interactions between claudin-16 and claudin-19 support paracellular divalent cation absorption within the renal tubule. (*a*) In functioning epithelia, transcellular transporters (NKCC2, ROMK, Na^+/K^+-ATPase, and Clc family members) generate a positive charge within the tubular lumen. This drives paracellular reabsorption of Mg^{2+} and Ca^{2+} across the tight junction through claudin-16-dependent pores. (*b*) When claudin-16 mutations are present, the tight junction is impermeant to Mg^{2+} and Ca^{2+}, resulting in urinary wasting of these cations. (*c*) In contrast, claudin-19 mutations disrupt the tight junction barrier to Cl^-, thereby allowing paracellular anion flux and neutralizing the positive potential within the lumen. This removes the driving force for Mg^{2+} and Ca^{2+} reabsorption, again resulting in urinary wasting of these ions.

junctions, namely those that preferentially allow cations to cross (50). Renal tubular Mg^{2+} and Ca^{2+} reabsorption occurs in the thick ascending limb of Henle, the same site at which claudin-16 is expressed, is predominantly paracellular, and is driven by a positive ionic potential within the tubular lumen. In the absence of functional claudin-16, Mg^{2+} and Ca^{2+} cannot be reabsorbed, so they remain in the tubule and either form stones or are wasted in the urine (**Figure 4**). Analysis of a large number of affected families showed that many of the claudin-16 mutants are incorrectly trafficked and fail to localize to the tight junction; these mutations include one that interferes with claudin-16 binding to ZO-1 (51, 52). However, several mutations, primarily those within the extracellular loops, traffic correctly, supporting a model in which the residues within these domains determine the biophysical characteristics of the tight junction pore (7, 8). Further analysis of patients with hypomagnesemia and nephrocalcinosis identified several families in which no claudin-16 mutation could be identified. Moreover, in addition to renal disease, these patients also had severe visual impairment. Linkage analyses identified mutation of claudin-19, which is highly expressed in the kidney and eye, in each of these families (53). In vitro studies suggest that there is a direct physical interaction between claudin-16 and claudin-19, that the claudin-19 mutations associated with disease interfere with this interaction, and that claudin-19 expression prevents paracellular Cl^- ion flux (54). Thus, mutant claudin-19 allows paracellular flux of Cl^- ions, which neutralizes the positive potential of the tubular lumen and eliminates the driving force for paracellular cation reabsorption (**Figure 4**). In addition to demonstrating cooperativity between two different claudins, these data also suggest that, whereas some claudin proteins form pores that allow preferential passage of specific ions, other claudins may contribute to barriers that prevent passage of specific ions.

Mutations in other claudin proteins have also been linked to disease (**Table 1**), and ZO-2 mutations have been linked to familial hypercholanemia (55). Interestingly, the mutation identified within ZO-2 weakens the interaction with carboxy-terminal claudin tails, suggesting that the disease is the result of inefficient claudin trafficking. Overall, the phenotypes of these patients, as well as animal models, have established the critical roles of specific claudins, their tissue-specific patterns of expression, and precise intracellular trafficking in barrier function. However, the interactions among the myriad of proteins present at the tight junction have been incompletely defined. The molecular mechanisms by which claudin defects alter tight junction function and modify the physical structure of tight junction pores require further investigation if we are to fully understand these diseases.

BARRIER DEFECTS ARE ASSOCIATED WITH INTESTINAL DISEASE

The capacity for acute modulation of tight junction barrier properties and its potential role in disease was first appreciated nearly 30 years ago, when separate groups showed (*a*) that the actin-binding drugs cytochalasin B and phalloidin disrupted both function and morphology of tight junctions within *Necturus* gallbladder epithelium, (*b*) that the tight junction was physically associated with actin and myosin filaments (46), and (*c*) that administration of phalloidin to rats disrupted the structure of hepatocyte tight junctions, increased their permeability, and induced cholestasis, presumably as a result of reflux of secreted bile acids from the canalicular space (56). Shortly thereafter, it was discovered that small intestinal permeability was increased in patients with active Crohn's disease as well as in patients with untreated celiac disease (57). Although the inert permeability probes used are markers of paracellular, but not transcellular, flow, this observation could potentially be secondary to mucosal ulceration in Crohn's disease. This explanation does not apply to celiac disease, as ulceration is not typically present. Subsequent work has confirmed epithelial tight junction defects in celiac disease patients (58), and, remarkably, genetic polymorphisms of the tight junction–associated scaffold protein *MAGI2* have been linked to celiac disease, ulcerative colitis, and Crohn's disease (59, 60). However, unlike tight junction protein mutations described in other diseases, *MAGI2* polymorphisms are only present in a fraction of patients and, thus far, have only been detected in noncoding regions. Thus, if tight junction protein polymorphisms are a cause of these diseases, the pathogenesis is far more complicated than that of monogenic diseases such as hypomagnesemia with hypercalciuria and nephrocalcinosis. Similarly, acute, reversible changes in barrier function are associated with infectious enteritis due to viral or bacterial infections (61–63).

Further study of Crohn's disease patients suggested that increased paracellular permeability could be present in patients with inactive disease, even in macroscopically normal mucosa (64). Moreover, analysis of unaffected first-degree relatives of Crohn's disease patients, who have an increased risk of developing Crohn's disease, showed that up to 10% of these healthy subjects have increased small intestinal permeability (65, 66). Thus, it has been hypothesized that defective small intestinal barrier function may be a risk factor for the development of Crohn's disease. Consistent with this suggestion, one healthy relative with increased small intestinal permeability was reported to have developed Crohn's disease eight years later (67). However, note that this single case report describes a subject with elevated familial risk of developing Crohn's disease, regardless of barrier status, and that what is truly required for assessment of the risk conferred by increased small intestinal permeability is a prospective study comparing development of disease in relatives with and without barrier defects. Such an assessment has not been made, but several studies have reported that, in patients with inactive Crohn's disease, barrier defects are strong prognostic indicators of disease reactivation (68, 69).

Table 1 Tight junction protein mutations and disease[a]

Protein	Gene	Human mutation(s)	Protein defect	Human disease	Mouse model	Phenotype
Claudin-1	*CLDN1*	Frameshift/truncation	Not expressed	Ichthyosis, sclerosing cholangitis	*CLDN1* KO	Skin barrier defect, water loss, perinatal death
Claudin-5	*CLDN5*	–	–	None reported	*CLDN5* KO	Blood-brain barrier defect, perinatal death
Claudin-6	*CLDN6*	–	–	None reported	*CLDN6* TG	Skin barrier defect, perinatal death
Claudin-11	*CLDN11*	–	–	None reported	*CLDN11* KO	CNS myelin and Sertoli cell defects, deafness
Claudin-14	*CLDN14*	Frameshift/truncation, substitution	Truncation, misfolding	Nonsyndromic deafness	*CLDN14* KO	Phenocopy of human disease
Claudin-15	*CLDN15*	–	–	None reported	*CLDN15* KO	Megaintestine
Claudin-16	*CLDN16*	Truncation, splice site, missense	Trafficking defects	Hypomagnesemia with hypercalciuria and nephrocalcinosis	*CLDN16* KD	Phenocopy of human disease
Claudin-19	*CLDN19*	Missense	Trafficking defects	Hypomagnesemia with hypercalciuria, visual impairment	*CLDN19* KO	Schwann cell barrier defect
Occludin	*OCLN*	–	–	None reported	*OCLN* KO	Complex phenotype
Tricellulin	*MARVELD2*	Splice site/truncation	Truncation	Autosomal recessive nonsyndromic hearing loss	–	–
ZO-1	*TJP1*	–	–	None reported	*TJP1* KO	Embryonic lethal
ZO-2	*TJP2*	Substitution	Reduced claudin-1 affinity	Familial hypercholanemia	*TJP2* KO	Embryonic lethal
ZO-3	*ZO-3/TJP3*	–	–	None reported	*TJP3* KO	No phenotype
Peripheral myelin protein 22	*PMP22*	Deletion, duplication, mutations	Various	Peripheral polyneuropathy	Multiple	Myelination defects

[a] Abbreviations: CNS, central nervous system; KD, knockdown; KO; knockout; ZO, zonula occludens.

PHYSIOLOGICAL MECHANISMS OF TIGHT JUNCTION REGULATION

Although data from human patients do suggest an association between tight junction barrier defects and intestinal disease (**Table 2**), the processes responsible for this regulation are only partially understood. On the basis of the hypothesis that tight junction dysregulation in disease may be due to disruption of normal mechanisms, physiological tight junction regulation was investigated. The best-characterized example of tight junction regulation in response to physiological stimuli remains the reversible increase in intestinal paracellular permeability induced by apical Na^+-nutrient cotransport (70–72). This increase in paracellular permeability is thought to allow passive paracellular flux of nutrients, along with water, in situations where transcellular transport pathways have been saturated (73, 74).

Ultrastructural analyses of tight junctions showed that activation of Na^+-nutrient cotransport induced condensation of perijunctional microfilaments (75). This was interpreted as an indicator of actomyosin contraction, but at the time the tools needed to study these biochemical events in intact tissue did not exist. To circumvent this problem, an in vitro model that recapitulated Na^+-glucose cotransport–induced tight junction regulation was developed through use of cultured intestinal epithelial cell monolayers (71). Unlike intact tissue, these monolayers were amenable to biochemical analysis. Based on the ultrastructural data, phosphorylation of myosin II regulatory light chain (MLC), a trigger for actomyosin contraction, was examined and shown to

Transcellular transport: active or passive movement of water and solutes through cells by means of specific transmembrane transport proteins

Myosin II regulatory light chain (MLC): one of two types of light chains within conventional, or type II, myosin; phosphorylation activates a conformational change that stimulates actomyosin contraction

Table 2 Intestinal diseases and disease models associated with barrier defects[a]

Disease/model	Species	Cause of barrier defect
Crohn's disease	Human	Idiopathic, TNF, microbiota?, MLC hyperphosphorylation?
Ulcerative colitis	Human	Idiopathic, immune dysregulation?, MLC hyperphosphorylation
CD4+CD45Rb^{hi} transfer	Mouse	TNF, microbiota, MLC hyperphosphorylation
Constitutively active MLCK transgene expression	Mouse	MLC hyperphosphorylation, barrier dysregulation
IL-10 knockout	Mouse	Immune dysregulation, microbiota, TNF?
Systematic T cell activation	Human/mouse	Cytokine release (TNF, LIGHT)
Dominant negative N-cadherin transgene expression	Mouse	Defective epithelial maturation, migration, and adherens junctions
SAMP1/YitFc	Mouse	Unknown
Graft versus host disease	Human/mouse	TNF signaling?
Autoimmune enteropathy	Human/mouse	*FOXP3* mutation, excessive T cell activation
Celiac disease	Human	Abnormal mucosal immune response stimulated by gliadin peptides
Clostridium difficile infection	Human/mouse	Toxin glucosylates rho and triggers actin disruption
Irritable bowel syndrome	Human	Enteric nervous system defects?
Enteric glial cell destruction	Mouse	Enteric nervous system defects
Enteropathogenic *Escherichia coli* infection	Human/mouse	Proteins injected by type III secretion apparatus
Shigella	Human	Toxin causes direct epithelial cell damage
Giardia lamblia	Human/mouse	Microvillous damage, apoptosis, MLC hyperphosphorylation
Rotavirus	Human	Enterocyte damage

[a]Abbreviations: LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry mediator on T cells; MLC, myosin II regulatory light chain; MLCK, MLC kinase; TNF, tumor necrosis factor.

Smooth muscle/nonmuscle myosin II regulatory light chain kinase (MLCK): the Ca^{2+}-calmodulin-dependent, serine-threonine kinase that phosphorylates MLC; two primary forms, long and short, are expressed

TNF: tumor necrosis factor

IFN-γ: interferon gamma

increase following activation of Na^+-glucose cotransport (71). Moreover, pharmacological MLC kinase (MLCK) inhibition prevented both MLC phosphorylation and barrier regulation induced by Na^+-glucose cotransport in cultured monolayers and also blocked barrier regulation in intact intestinal mucosa (71). These data suggest that MLCK is a critical physiological regulator of tight junction permeability. Within months, similar data showing an association of MLCK-dependent MLC phosphorylation with increased tight junction permeability induced by enteropathogenic *Escherichia coli* infection were published (62), suggesting that MLCK-dependent MLC phosphorylation may be both a physiological and a pathophysiological regulator of barrier function. Furthermore, in vitro studies of epithelial cells with inducible expression of constitutively active MLCK demonstrate that enzymatic MLCK activation is sufficient to trigger downstream events necessary for barrier regulation (76).

MLCK ACTIVATION IS REQUIRED FOR TNF-INDUCED BARRIER LOSS

Tumor necrosis factor (TNF) is a critical mediator of Crohn's disease, as demonstrated by the remarkable efficacy of anti-TNF antibody therapy (77, 78). This intervention also corrects barrier defects in Crohn's disease patients (79), suggesting that TNF signaling to epithelial cells may contribute to disease-associated increases in permeability. This hypothesis is supported by the observation that TNF induces barrier loss in cultured epithelial monolayers (80). Moreover, TNF is a central mediator in ischemic intestinal damage (81) and has been associated with intestinal injury occurring in graft versus host disease after allogeneic bone marrow transplantation (82). Thus, the mechanisms by which TNF induces epithelial barrier defects are of considerable pathophysiological and clinical importance.

Early studies suggested that TNF-induced epithelial apoptosis could be one mechanism of barrier dysregulation (83). However, in vitro studies make it clear that barrier function can be maintained in the face of significant apoptosis (84) and that apoptosis is not required for TNF-induced barrier loss (85). Alternatively, suppression of the occludin promoter by TNF and interferon gamma (IFN-γ) could be involved (86), but reduced occludin protein expression does not coincide, temporally, with TNF-induced barrier loss. The recognition that TNF-induced loss of barrier function is associated with increased MLC phosphorylation was a major advance in our understanding of the mechanisms of cytokine-dependent tight junction regulation (85). In addition, MLCK inhibition can reduce MLC phosphorylation and restore barrier function in TNF-treated intestinal epithelial cell monolayers (85). This finding suggests that activation of MLCK enzymatic activity is central to TNF-induced barrier loss. Subsequent studies showed that in vitro TNF-induced barrier loss is associated with increased transcription and translation of MLCK (87, 88) and that intestinal epithelial MLCK transcription is induced by TNF in vivo (89). Moreover, both MLCK protein expression and MLC phosphorylation, the latter an indicator of MLCK enzymatic activity, are increased and correlate with the degree of inflammatory activity in patients with Crohn's disease and ulcerative colitis (90). Thus, MLCK-dependent MLC phosphorylation is induced by TNF in vitro and in vivo and is also present in human inflammatory bowel disease.

LONG MLCK MEDIATES EPITHELIAL TIGHT JUNCTION REGULATION

MLCK is a Ca^{2+}-calmodulin regulated serine-threonine protein kinase that phosphorylates MLC (**Figure 5**). There are three MLCK genes in vertebrates, including humans: skeletal *MYLK2* on human chromosome 20, cardiac *MYLK3* on human chromosome 16, and smooth muscle/nonmuscle *MYLK* on human chromosome 3. The smooth muscle/nonmuscle gene produces three separate

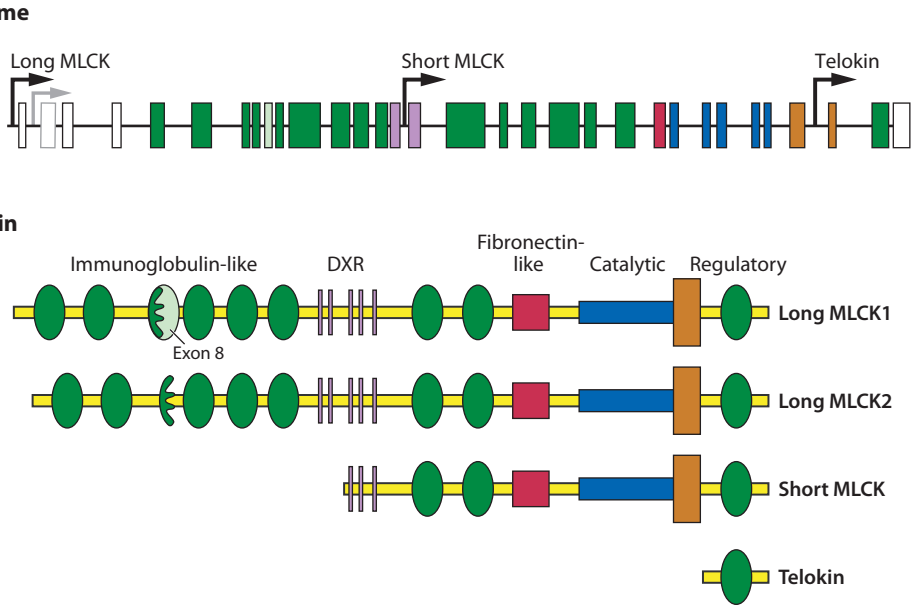

Figure 5

Genomic and protein structure of *MYLK* and myosin II regulatory light chain kinase (MLCK). The smooth muscle/nonmuscle *MYLK* gene produces three transcripts: long MLCK, short MLCK, and telokin. Exons within the genomic structure are indicated by boxes that are open for untranslated exons and filled for coding exons. Promoters are indicated with arrows. The second long MLCK promoter and the alternative first exon are shown in gray. The MLCK protein contains multiple conserved domains, which are colored to correspond to the exon structure. MLCK1 and MLCK2 isoforms differ by the inclusion of exon 8 (*light green*) in MLCK1, which completes an immunoglobulin-like domain in the protein.

transcripts (91). The first (known as the smooth muscle or short) MLCK is 110–130 kDa in size. Knockout of this gene is lethal in the early postnatal period (92). Inducible, tissue-specific short MLCK knockout mice have recently been described (93). Deletion of short MLCK in smooth muscle results in hypotension, urinary bladder dysfunction, and severe gastrointestinal dysmotility (93). All of these defects are probably secondary to reduced MLC phosphorylation and impaired smooth muscle contraction (93). A less well-studied product of the smooth muscle/nonmuscle MLCK gene is telokin, a ~21-kDa protein corresponding to the carboxy terminal of the larger proteins that lacks enzymatic activity. Some studies suggest that telokin may mediate desensitization of smooth muscle to repeated stimulation.

The second enzymatically active product of the smooth muscle/nonmuscle *MYLK* gene is long MLCK. This ~225-kDa isoform contains all of the sequence present in short MLCK as well as an amino-terminal extension and is expressed in endothelial and epithelial cells (94, 95). Variable mRNA splicing within the extension creates several different isoforms, of which only (*a*) the full-length form, long MLCK1, and (*b*) a spliced form lacking a single exon, long MLCK2, are expressed in intestinal epithelia (95). Short MLCK is not expressed in intestinal epithelial cells (95). Although the relative roles of MLCK1 and MLCK2 are incompletely understood, in vitro work indicates that phosphorylation of MLCK1, but not MLCK2, by Src increases enzymatic activity (96). In human intestinal epithelium, MLCK1 expression is restricted to villus enterocytes and surface colonocytes, where it is specifically targeted to the perijunctional actomyosin ring (90, 95). Together with in vitro data showing that

CD3: component of the T cell receptor

knockdown of MLCK1 expression enhances barrier function (95), these data suggest that MLCK1 may be the isoform required for TNF-induced tight junction regulation.

The factors that regulate differential splicing of long MLCK have not been defined, but rapid amplification of 5′ complementary DNA ends was used to detect two distinct MLCK 5′ untranslated regions in human intestinal epithelial cell mRNA. This indicates that there are two distinct promoters for long MLCK (89). Each of these promoters is upstream of the short MLCK promoter, which is located within a long MLCK intron. The long MLCK promoters use alternative exons encoding 5′ untranslated regions, and therefore the translational start site and transcribed coding sequences are identical (89). One of the long MLCK promoters has been characterized and found to contain elements responsive to activator protein 1 (AP-1) as well as to nuclear factor kappa B (NF-κB), and it appears that either of these can promote transcription (89). This finding explains conflicting results of in vitro studies examining the mechanisms by which TNF induces long MLCK expression (87, 88). Further data suggest that NF-κB may be the primary mediator in undifferentiated crypt-like cells and that AP-1 predominates in well-differentiated absorptive enterocytes (88, 89). Long MLCK expression is also increased in patients with inflammatory bowel disease (90), where it is associated with the perijunctional actomyosin ring (90). However, analyses of the transcription factors responsible for long MLCK expression in vivo have not been reported.

LONG MLCK IS REQUIRED FOR T CELL–INDUCED ACUTE DIARRHEA

T cell activation induced by systemic administration of anti-CD3 antibodies causes acute, self-limited diarrhea in humans and experimental animals (97, 98). This diarrhea is TNF dependent and is associated with intestinal barrier dysfunction (99, 100). Anti-CD3 antibody treatment also causes perijunctional cytoskeletal condensation similar to that observed after activation of Na^+-nutrient cotransport (75, 101). This suggested that in vivo barrier loss after T cell activation may be MLCK dependent and that, consistent with this hypothesis, anti-CD3 treatment causes TNF-dependent intestinal epithelial MLC phosphorylation (101). The magnitude of MLC phosphorylation correlates with the extent of intestinal fluid accumulation, suggesting the possibility of a direct relationship. To study this interaction, the effect of anti-CD3 antibody treatment on long MLCK knockout mice was examined. These mice lack an exon within the unique long MLCK region of *MYLK* and therefore express short MLCK and telokin normally (102). Long MLCK knockout mice are phenotypically normal in the absence of exogenous stressors, suggesting that, unlike short MLCK, long MLCK is not essential for life. Nevertheless, intestinal epithelial MLC phosphorylation is not induced by anti-CD3 treatment in long MLCK knockout mice (101). Moreover, long MLCK knockout mice are entirely protected from the barrier dysfunction and diarrhea that are typically induced by anti-CD3 treatment (101). These data demonstrate that long MLCK is required for acute, self-limited diarrhea induced after systemic T cell activation. However, because these mice lack long MLCK in all tissues, and because long MLCK has been reported to be involved in neutrophil function (103), deletion of long MLCK in cells other than intestinal epithelia may have been responsible for the observed protection from diarrhea. To address this hypothesis, the intestines of wild-type mice were directly perfused with a highly specific peptide inhibitor of MLCK. This MLCK inhibitor entered, but did not cross, the epithelium and was not detected in subepithelial tissues (101). The MLCK inhibitor blocked increased intestinal epithelial MLC phosphorylation induced by T cell activation (101). Moreover, barrier function loss and diarrhea were prevented by the MLCK inhibitor in a dose-dependent fashion, suggesting that the degree of MLCK activation and MLCK phosphorylation can be titrated

(101). Notably, neither genetic nor enzymatic MLCK inhibition reduced MLC phosphorylation in the absence of T cell activation, suggesting that other kinases are responsible for basal MLC phosphorylation (101). Overall, the data demonstrate that the barrier dysfunction and diarrhea occurring after T cell activation require intestinal epithelial long MLCK.

Although the requirement for long MLCK in chronic disease has not yet been tested, circumstantial data suggest that it may be critical. One example comes from genetically modified mice that overexpress TNF as a result of deletion of adenine and uridine (AU)-rich elements within the 3′ untranslated region (104). These mice develop joint and intestinal diseases that are similar to rheumatoid arthritis and Crohn's disease, respectively (104). However, only joint disease occurs when TNF is overexpressed in TNF receptor 2 (TNFR2) knockout mice (104). Although there are many potential roles for TNFR2, it is tempting to speculate that these include long MLCK regulation within intestinal epithelium, as in vitro studies have shown that TNFR2, but not TNFR1, is essential for TNF-induced activation of long MLCK (105). This finding could also be related to the observation that TNFR2 knockout mice are resistant to graft versus host disease after allogeneic bone marrow transplantation (106).

BARRIER LOSS ALONE IS NOT SUFFICIENT TO CAUSE DIARRHEA

Although the data described above imply that the water secretion induced by T cell activation results from water flow through the tight junction, there are some inconsistencies. For example, although MLCK inhibition, either genetic or enzymatic, completely restored barrier function to levels seen in control animals and also prevented water secretion after anti-CD3 treatment, water absorption was not completely restored to that of control animals. Conversely, TNF-neutralizing antibodies completely restored water absorption but only partially prevented barrier dysfunction after T cell activation. These data suggest that molecules other than TNF contribute to the observed MLCK-dependent barrier dysfunction. Moreover, the data indicate that, whereas intestinal epithelial MLCK activation is required for water secretion to occur, other mechanisms that contribute to water absorption may also be impaired after T cell activation. To identify these events, investigators assessed the spectrum of cytokines expressed in intestinal mucosa after anti-CD3-induced T cell activation. Transcription of IFN-γ, TNF, and a core TNF family member, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry mediator on T cells (LIGHT), is markedly increased after anti-CD3 treatment (107).

LIGHT: lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry mediator on T cells

Given that in vitro studies demonstrated that LIGHT was able to cause MLCK-dependent barrier dysfunction in a manner similar to TNF (108), but that IFN-γ did not induce barrier defects or diarrhea in vivo (107), LIGHT was considered as the potential TNF-independent mediator of barrier dysfunction. Consistent with this hypothesis, systemic administration of recombinant LIGHT induces barrier dysfunction via MLCK activation that is similar to that induced by recombinant TNF (107). The effects of LIGHT and TNF are additive, and mice treated with both cytokines develop barrier dysfunction that is quantitatively identical to that induced by T cell activation (107). However, whereas TNF alone causes only half the barrier loss of T cell activation, the magnitude of water secretion induced by these treatments is similar (107). In contrast, LIGHT does not induce water secretion, but rather slightly enhances absorption (107).

The divergent effects of TNF and LIGHT on water transport are explained by the observation that TNF, but not LIGHT, markedly inhibits Na^+ absorption (107). The loss of Na^+ absorptive activity in TNF-treated animals is due to activation of protein kinase C alpha (PKCα) within intestinal epithelial cells. In turn, PKCα triggers endocytosis of the apical Na^+-H^+ antiporter NHE3, a major mechanism of intestinal Na^+ absorption, thereby disrupting the transepithelial Na^+

Paracellular transport: passive movement of water and solutes through the space between adjacent cells (paracellular space)

gradient that provides the driving force for intestinal water absorption (107, 109). Although absorption and maintenance of the transepithelial Na^+ gradient in LIGHT-treated animals explain the absence of diarrhea, they do not fully explain the slight increase in water absorption observed after LIGHT treatment. This can be explained by enhanced paracellular water absorption across tight junctions with increased permeability (**Figure 6**). Thus, in addition to defining cooperativity between transcellular and paracellular transport, these data demonstrate that significant paracellular water transport does occur in the intestine. Moreover, as expected for paracellular transport, water is either absorbed or secreted depending on the direction of the driving force. Thus, given that activation of Na^+-glucose cotransport leads to increased paracellular permeability, it is not surprising that transcellular transport of Na^+ and glucose enhances water absorption (72). Together with the increase in NHE3 activity induced by Na^+-glucose cotransport (110), this coupling of transcellular ion transport with paracellular water absorption may also explain why oral rehydration therapy with water containing carbohydrates and Na^+ is more effective than water alone as treatment for high-volume diarrheal diseases such as cholera (111).

TIGHT JUNCTION PROTEIN ENDOCYTOSIS IS TRIGGERED BY LONG MLCK ACTIVATION

Other than condensation of the perijunctional actomyosin ring, the predominant alteration in tight junction structure induced by T cell activation or administration of recombinant TNF is endocytosis of the transmembrane protein occludin (101, 107). No changes in distribution of claudin proteins have been detected. Occludin endocytosis requires MLCK activity, as it does not occur in long MLCK knockout mice or in mice treated with an MLCK inhibitor (**Figure 7**). This is difficult to understand in a mechanistic sense, given that occludin knockout mice are reported to have normal intestinal barrier function (31). However, the responses of occludin knockout mice to stress

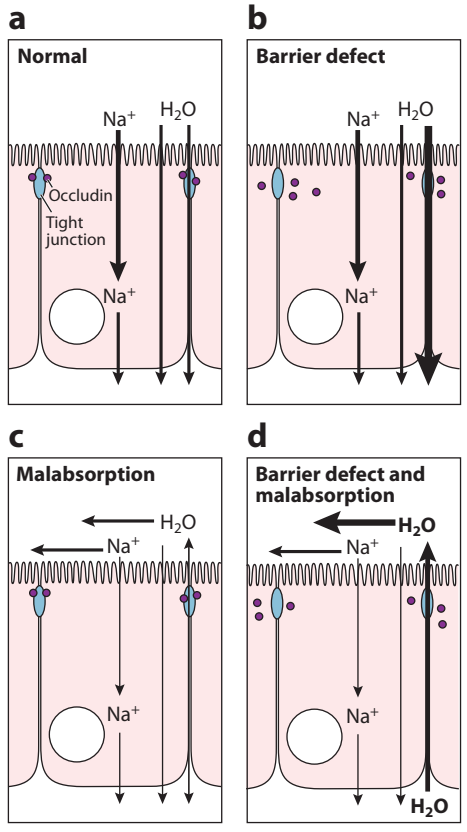

Figure 6

Tumor necrosis factor (TNF)-mediated intestinal epithelial barrier defects. (*a*) In normal small bowel, Na^+ is absorbed transcellularly, resulting in an osmotic gradient that drives paracellular water absorption. (*b*) An isolated tight junction barrier defect—such as that following LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry mediator on T cells)-induced MLCK (myosin II regulatory light chain kinase) activation and occludin internalization—does not affect Na^+ absorption and allows mild enhancement of water absorption. (*c*) Na^+ malabsorption disrupts the osmotic gradient and allows some water to cross the tight junction into the lumen. (*d*) In contrast, a barrier defect combined with Na^+ malabsorption allows massive amounts of water to cross the tight junction, resulting in diarrhea.

have not been reported, and it is possible that, as with long MLCK, genetic knockout does not perturb basal function but rather alters regulatory responses. Thus, although the specific

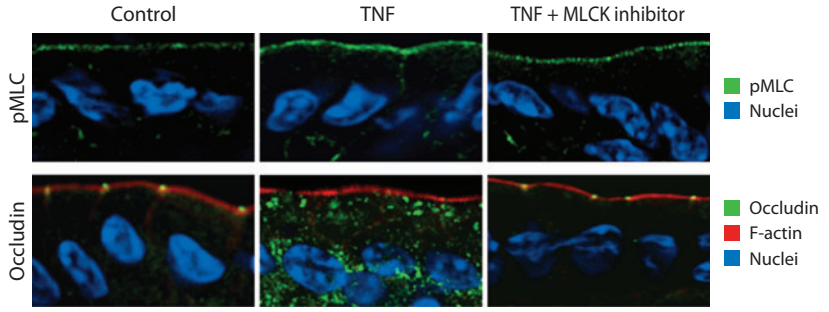

Figure 7

Effects of tumor necrosis factor (TNF) on myosin II regulatory light chain (MLC) phosphorylation and occludin localization. In vivo TNF treatment increases phosphorylation of MLC at the perijunctional actomyosin ring of jejunal villus enterocytes. (*Top row*) MLC phosphorylation can be blocked by MLC kinase (MLCK) inhibition. (*Bottom row*) Similarly, TNF-induced occludin internalization can be blocked by MLCK inhibition. Abbreviation: pMLC, phosphorylated MLC.

function of occludin is not clear, endocytosis of occludin is a sensitive morphologic correlate of TNF-induced loss of barrier function. Identification of the mechanisms of occludin endocytosis may therefore provide important clues to the molecular reorganization of the tight junction that results in barrier dysfunction. Stimulus-induced occludin internalization in vitro has been reported to occur via three primary routes: macropinocytosis, clathrin-coated endocytosis, and caveolar endocytosis (3, 108). Detailed analysis performed through in vivo imaging of transgenic animals expressing an enhanced green fluorescent protein–occludin fusion protein, as well as pharmacological and genetic inhibitors of each endocytic pathway, has recently shown that this TNF-induced process occurs via caveolae (A.M. Marchiando, L. Shen, W.V. Graham, B.T. Schwarz, Y. Guan, A.J.M. Watson, M.H. Montrose & J.R. Turner, unpublished observations). Moreover, inhibition of caveolar endocytosis prevents TNF-induced barrier loss as well as water secretion. Importantly, caveolar endocytosis is not required for MLCK activation or MLC phosphorylation, thereby demonstrating that occludin endocytosis is a downstream mediator in MLCK-dependent tight junction regulation. In addition, along with MLCK inhibition, the data suggest that reversible blockade of caveolar endocytosis within intestinal epithelia may provide a therapeutic approach to preventing barrier loss and diarrhea. This method may be particularly effective because the intestinal epithelium is readily accessed by orally delivered agents, and therefore nonabsorbable caveolar endocytosis inhibitors might be used without systemic toxicity.

BARRIER DYSFUNCTION IN CHRONIC DISEASE

The observation that T cell activation or cytokine administration causes acute, MLCK-dependent diarrhea in vivo is mechanistically consistent with the reversible intestinal epithelial barrier regulation that occurs as a consequence of Na^+-nutrient cotransport. Both the rapidity of onset and ready reversibility suggest that modification of existing tight junction components, rather than synthesis of new proteins, may be central to this form of regulation. Although this mechanism is active in human disease (90), the examples provided by diseases associated with inherited claudin mutations indicate that modulation of tight junction claudin protein expression may be a second mechanism of barrier dysfunction in chronic disease. This appears to be the case, as studies of intestinal epithelium from inflammatory bowel disease patients have demonstrated increased expression of claudin-2 as well as

reduced expression and endocytosis of claudin-5, claudin-8, and occludin (112–115). Thus, tight junction barrier properties can be altered by genomic and postgenomic mechanisms in inflammatory bowel disease (115).

Animal models provide important insights into the development of barrier dysfunction in inflammatory bowel disease. One example is the interleukin (IL)-10 knockout mouse, which develops colitis of variable severity, depending on the composition of luminal bacteria and genetic background of the mouse (116, 117). Such mice, in which the primary defect is immune, demonstrate barrier defects to small molecules prior to the development of overt disease (118). Thus, in this case it is clear that an immune defect is responsible for initiating processes leading to a barrier defect. Similarly, the outbred SAMP1/YitFc mouse, which develops spontaneous ileitis, also demonstrates increased claudin-2 expression and intestinal barrier dysfunction prior to onset of clinically evident disease (119). By extension, it is therefore possible that a subclinical immune-mediated process explains the barrier defects seen in healthy first-degree relatives of Crohn's disease patients who have increased small intestinal permeability (65, 66). The observation that increased intestinal permeability in these healthy individuals is associated with certain *NOD2* polymorphisms suggests that this may indeed be the case (120, 121). However, the increased permeability that portends disease reactivation in Crohn's disease patients may also reflect subclinical immune activation prior to the development of clinical symptoms (68, 69). The possibility that barrier dysfunction leads to immune activation is only beginning to be explored.

BARRIER DYSFUNCTION CAUSES IMMUNE ACTIVATION, BUT NOT OVERT DISEASE, IN VIVO

As noted above, barrier defects have been described prior to the onset of overt disease in several models of inflammatory bowel disease. However, the barrier dysfunction that develops in these models is secondary either to immune perturbation or to undefined defects. Experimental models show that primary barrier defects are induced by disruption of adherens junctions and epithelial differentiation or by wholesale epithelial damage can trigger disease (122), but they differ from the size-selective alterations described in Crohn's disease patients during remission and in their healthy first-degree relatives (65, 123). Recently a mouse model of primary barrier dysfunction triggered by MLCK activation was reported (124). The mice in this study express a constitutively active MLCK within the intestinal epithelium and, as a result, have increased intestinal epithelial MLC phosphorylation and increased intestinal permeability (124). Barrier function is restored upon MLCK inhibition, demonstrating that this is the cause of the observed defects. Importantly, the barrier defects present in constitutively active MLCK transgenic mice are similar in both size selectivity and extent to the barrier defects reported in Crohn's disease patients and their healthy first-degree relatives (65, 66, 123, 125, 126). Moreover, epithelial proliferation, polarization, and differentiation, as well as overall mucosal architecture, are not affected by constitutively active MLCK expression. Thus, unlike other models targeting epithelial function (122, 127–130), transgenic expression of constitutively active MLCK creates a targeted, pathophysiologically relevant model of tight junction dysregulation (124). Like the subset of healthy Crohn's disease relatives with barrier defects, constitutively active MLCK transgenic mice are healthy and able to grow normally. Thus, these mice model the physiological state of the healthy Crohn's disease relatives and are a unique tool with which to address the question of whether primary, pathophysiologically relevant increases in intestinal epithelial paracellular permeability can lead to mucosal immune activation and disease.

Despite the absence of clinical disease, detailed analysis of constitutively active MLCK transgenic mice demonstrated mild mucosal immune activation (124). Transcription of

IFN-γ and TNF is increased in the colonic mucosa, and the T-bet/GATA-3 ratio is elevated, consistent with Th1 polarization (124). The complement of mucosal CD4+ lymphocytes within the mucosa is also increased, and mucosal CD11c+ dendritic cells are repositioned into the superficial lamina propria, closest to the lumen. Although the functional significance of dendritic cell translocation is not clear, it is interesting to note that enrichment of dendritic cells within the superficial mucosa has been observed during the early stages of *Campylobacter* colitis in human patients (131) and has also been associated with experimental colitis in rats (132).

One might also ask why the constitutively active MLCK transgenic mice did not develop disease. One explanation may be the observed increase in mucosal IL-10 transcripts that accompanied increased IFN-γ and TNF transcription (124). Consistent with this hypothesis, both IFN-γ and IL-10 are induced by direct epithelial damage, which causes transient intestinal barrier defects that are not size selective (133). Thus, in a host with an intact immune system, epithelial barrier defects may trigger mucosal immunoregulatory responses that prevent development of chronic disease.

An area in need of further study is the interaction of luminal contents, most likely the microbiota, and barrier dysfunction. For example, constitutively active MLCK transgenic mice do not demonstrate mucosal immune activation prior to 6 weeks of age (124), suggesting that the microbial alteration associated with weaning may contribute to the observed changes. Conversely, barrier dysfunction does not develop in IL-10 knockout mice raised in germ-free environments or in mice treated with antibiotics or probiotic organisms (117, 118, 134). These interventions also limit the development of inflammation in IL-10 knockout mice (117, 118, 134), suggesting integration of luminal microbiota, epithelial barrier function, and mucosal immune regulation.

PRIMARY BARRIER DYSFUNCTION ENHANCES IMMUNE-MEDIATED INTESTINAL DISEASE

No longitudinal studies correlating risk of developing disease with intestinal permeability in healthy relatives of Crohn's disease patients have been reported. Thus, the hypothesis that increased intestinal permeability is a risk factor for the development of disease has not been tested directly. Even if one assumes that barrier defects are a primary event contributing to Crohn's disease development, it is clear that increased permeability may be present many years before the onset of Crohn's disease in human subjects (67). Thus, one or more additional predisposing factors or stimuli that can contribute to disease are probably present. Nonetheless, because of their similarity to healthy Crohn's disease relatives with asymptomatic barrier loss, the constitutively active MLCK transgenic mice are an ideal experimental model with which to examine the contribution of tight junction barrier loss to the development of colitis. These mice were challenged through use of the established $CD4CD45Rb^{hi}$ adoptive transfer model of immune-mediated colitis (135). This model targets a pathway distinct from epithelial barrier regulation and has many similarities to human inflammatory bowel disease, including critical roles for IFN-γ, TNF, and IL-23 (135–138). Following adoptive transfer, constitutively active MLCK transgenic mice lose weight earlier than did their littermate controls. Although weight loss of littermates catches up to transgenic mice at more advanced stages of disease, mucosal cytokine expression remains higher, histopathology more severe, and survival poorer in constitutively active MLCK transgenic mice (124). Thus, although it is insufficient to initiate disease, targeted, pathophysiologically relevant intestinal epithelial tight junction dysregulation can accelerate the development and enhance the severity of immune-mediated colitis.

INTERACTIONS AMONG THE EPITHELIAL BARRIER, MICROBIOTA, AND IMMUNE SYSTEM THAT PREVENT OR PROMOTE DISEASE

The data described above support a model of inflammatory bowel disease, and perhaps other inflammation-associated diseases of the intestines such as celiac disease, that incorporates roles for luminal microbiota, epithelium, and the innate and adaptive arms of the mucosal immune system (139). This model proposes a cycle by which small defects in any of these components are amplified in a genetically susceptible host (**Figure 8**). For example, an episode of infectious enteritis can damage the epithelial barrier and allow luminal microbiota to enter the lamina propria in any individual. This probably generates a dominant immunoregulatory signal, similar to that seen in mice as a result of constitutively active MLCK expression (124) or after transient epithelial damage (133), that prevents development of chronic disease in most subjects. However, defects in IL-10 production, such as in IL-10 knockout mice (116) or in patients with polymorphisms adjacent to the *IL-10* gene (140), may limit this anti-inflammatory response (141, 142). As a result, mucosal immune activation may proceed unchecked and lead to release of cytokines, such as TNF, that increase barrier dysfunction, which in turn would cause further leakage of luminal bacteria and perpetuate the proinflammatory cycle. Alternatively, the defect could be a primary deficiency of epithelial barrier function. In this case, aberrant mucosal immune regulation or altered composition of the luminal microbiota could allow initiation of the proinflammatory cycle. Finally, defects in innate immunity, such as those associated with abnormalities in Toll-like receptors (143–146), or defects in adaptive immunity, documented by the presence of antibodies to bacterial components (147, 148), may trigger this self-amplifying cycle of inflammation. Whatever the causes of such cycles, analyses of these interactions could help to unify observations based on genetic and functional analyses of inflammatory bowel disease patients with the results of studies of in vitro and in vivo models of disease. This model may also explain the therapeutic effects of immunosuppressants, immunomodulators (77), and probiotics (149), as well as the potential benefits of barrier preservation (150). Thus, improved understanding of the role of the epithelial barrier, which

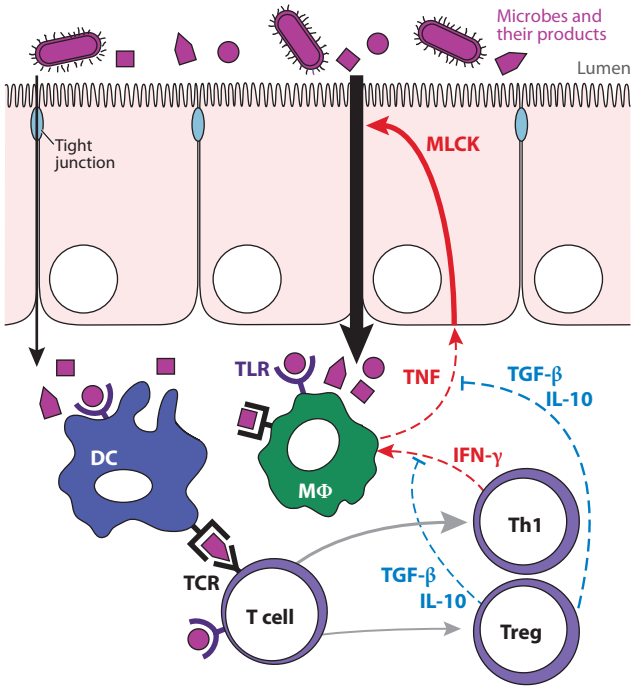

Figure 8

Model of interactions among the epithelial barrier, luminal microbiota, and immune system. The epithelial barrier normally restricts passage of luminal contents, including microbes and their products, but a small fraction of these materials do cross the tight junction. This simplified diagram shows how dendritic cells (DC), macrophages (MΦ), and T cells react to these materials via Toll-like receptors (TLRs) and antigen-specific T cell receptors (TCRs). The naïve T lymphocyte (T cell) responds to antigenic and other stimuli within the lamina propria, becoming a Th1-polarized cell (Th1), a T regulatory cell (Treg), or other differentiated T cell types (not shown). These innate and adaptive immune cells release cytokines that exert proinflammatory (TNF and IFN-γ) and anti-inflammatory (IL-10, TGF-β) effects. If proinflammatory signals dominate and signal to the epithelium, MLCK can be activated to cause barrier dysfunction, which would allow an increase in the amount of luminal material presented to immune cells. In the absence of appropriate immune regulation, this activation may cause further immune activation, cytokine release, and barrier loss, resulting in a self-amplifying cycle that can result in disease. Abbreviations: IL, interleukin; MLCK, myosin II regulatory light chain kinase; TGF, transforming growth factor; TNF, tumor necrosis factor.

importantly mediates the interactions between luminal microbiota and immune cells, may lead to novel therapies for inflammatory diseases of the gastrointestinal tract.

SUMMARY POINTS

1. Epithelia form barriers that are required for life but must be selectively permeable, and sometimes regulated, to ensure the correct composition of, and transport between, distinct compartments.
2. When the epithelium is intact, the tight junction defines the transepithelial permeability of leaky epithelia; altered barrier properties, due to mutations of tight junction proteins, have been linked to specific diseases.
3. Barrier defects associated with intestinal diseases are often due to disruption of normal regulatory mechanisms that control gene expression, tight junction structure, and cytoskeletal signaling.
4. Long MLCK is a principal mediator of the tight junction response to physiological and pathophysiological stimuli.
5. Paracellular barrier dysfunction may synergize with defective transcellular transport to cause diarrhea and other manifestations of disease.
6. Membrane traffic, including endocytosis, may be a means by which cytoskeletal signals regulate barrier function.
7. Although insufficient to initiate disease, defective barrier function may be a primary or secondary event that contributes to disease progression.
8. Chronic intestinal diseases that include barrier dysfunction probably represent defects in multiple aspects of epithelial, microbial, and immune interactions.

FUTURE ISSUES

1. Although many tight junction proteins have been identified, the molecular anatomy of the paracellular pore and the means by which it is altered in response to regulatory stimuli have not been defined.
2. The signal transduction pathway by which cytokines, such as TNF, activate MLCK and regulate barrier function is only beginning to be understood.
3. The complex interplay that characterizes epithelial, microbial, and immune interactions and either prevents or promotes disease requires further study.
4. The potential of barrier-restoring agents, such as MLCK inhibitors, to prevent development of disease in at-risk individuals, reduce recurrence during clinical remission, or treat active disease remains to be defined.

DISCLOSURE STATEMENT

J.R.T. holds U.S. patent 7,585,844, related to PIK, the specific myosin light chain kinase inhibitor used in some of the studies described herein. This has been commercially licensed. The other

authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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