

Intestinal barrier function: Molecular regulation and disease pathogenesis

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Activity Objectives

1. To become familiar with the current literature regarding intestinal epithelial barrier function and disease pathogenesis.

2. To become familiar with the molecular components of the tight junction (TJ) complex and their role in the maintenance of intestinal epithelial barrier function.

3. To understand immune-mediated pathways involved in the regulation of intestinal epithelial barrier dysfunction.

4. To understand endogenous and exogenous factors that influence intestinal epithelial barrier function.

5. To understand the role that altered intestinal barrier function can play in the predisposition to food allergies, inflammatory bowel disease (IBD), celiac disease, and type 1 diabetes.

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The intestinal epithelium is a single-cell layer that constitutes the largest and most important barrier against the external environment. It acts as a selectively permeable barrier, permitting the absorption of nutrients, electrolytes, and water while maintaining an effective defense against intraluminal toxins, antigens, and enteric flora. The epithelium maintains its selective barrier function through the formation of complex protein-protein networks that mechanically link adjacent cells and seal the intercellular space. The protein networks connecting epithelial cells form 3 adhesive complexes: desmosomes, adherens junctions, and tight junctions. These complexes consist of

transmembrane proteins that interact extracellularly with adjacent cells and intracellularly with adaptor proteins that link to the cytoskeleton. Over the past decade, there has been increasing recognition of an association between disrupted intestinal barrier function and the development of autoimmune and inflammatory diseases. In this review we summarize the evolving understanding of the molecular composition and regulation of intestinal barrier function. We discuss the interactions between innate and adaptive immunity and intestinal epithelial barrier function, as well as the effect of exogenous factors on intestinal barrier function. Finally, we summarize clinical and experimental evidence demonstrating intestinal epithelial barrier dysfunction as a major factor contributing to the predisposition to inflammatory diseases, including food allergy, inflammatory bowel diseases, and celiac disease. (*J Allergy Clin Immunol* 2009;124:3-20.)

Key word: *Intestinal epithelium*

The intestinal epithelium is a single layer of cells lining the gut lumen and has 2 critical functions. First, it acts as a barrier to prevent the passage of harmful intraluminal entities, including foreign antigens, microorganisms, and their toxins.^{1,2} Its second

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Abbreviations used

AJ:	Adherens junction
BBDP:	BioBreeding diabetes-prone rat
CD:	Crohn's disease
EPEC:	Enteropathogenic <i>Escherichia coli</i>
HA/P:	Hemagglutinin protease
HRP:	Horseradish peroxidase
IBD:	Inflammatory bowel disease
iIEL:	Intestinal intraepithelial lymphocyte
iIL-9Tg:	Intestinal IL-9 transgenic mice
JAM:	Junctional adhesion molecule
MAPK:	Mitogen-activated protein kinase
MLC:	Myosin light chain
MLCK:	Myosin light chain kinase
MUPP1:	Multi-PDZ domain protein-1
NSAID:	Nonsteroidal anti-inflammatory drug
NO:	Nitric oxide
PALS1:	Protein associated with Lin Seven-1
PATJ:	PALS1-associated protein
PDZ:	Postsynaptic density-95/ <i>Drosophila</i> disc large/ <i>Zonula occludens-1</i> protein
PKC:	Protein kinase C
STAT:	Signal transducer and activator of transcription
TER:	Transepithelial resistance
TJ:	Tight junction
ZO:	<i>Zonula occludens</i>
Zot:	<i>Zonula occludens</i> toxin

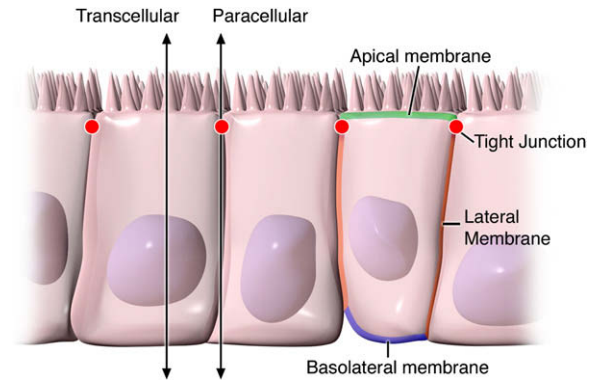


FIG 1. Pathways of epithelial permeability. Transcellular permeability is associated with solute or water movement through intestinal epithelial cells. Paracellular permeability is associated with movement in the intercellular space between epithelial cells and is regulated by TJs localized at the junction of the apical-lateral membranes.

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

STRUCTURAL COMPONENTS OF JUNCTIONAL COMPLEXES

AJs

AJs (also known as zonula adherens) are protein complexes on the lateral membrane that occur at points of cell-cell contact (Fig 2). They are formed by interactions between transmembrane proteins, intracellular adaptor proteins, and the cytoskeleton. The

major AJs are formed by cadherin-catenin interactions. E-cadherins (calcium-dependent adhesion molecules) are type I single-transmembrane-spanning glycoproteins that possess an intracellular C-terminus and extracellular N-terminus. The extracellular domain forms homotypical interactions with cadherins of neighboring cells to promote cell-cell adhesion. The intracellular domain contains a catenin-binding domain that interacts with members of the armadillo repeat superfamily, β -, γ -, and p120-catenin.^{11,17-21} The catenins link the AJ to the cytoskeletal network through direct binding to the C-terminal domain of F-actin or indirectly through interactions with other adaptor proteins, such as afadin.²²⁻²⁶ Cadherin-catenin complexes are important not only for linking adjacent cells but also for maintaining cell polarity, regulating epithelial migration and proliferation, and forming other adhesive complexes, such as desmosomes.^{19,21,27} In support of this, downregulation of E-cadherin in the intestinal epithelium weakens cell-cell adhesion and has been linked with perturbed intestinal epithelial proliferation and migration.^{28,29}

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

TJs

TJs are the apical-most adhesive junctional complexes in mammalian epithelial cells and form a continuous belt-like ring around epithelial cells at the border between the apical and lateral membrane regions (Fig 2).⁸ TJs are dynamic, multiprotein complexes that function as a selective/semipermeable paracellular barrier, which facilitates the passage of ions and solutes through the intercellular space while preventing the translocation of luminal antigens, microorganisms, and their toxins. The evolution of TJ biology emerged in the 1960s with the development of electron microscopy. Analysis of epithelial cells revealed a series of apparent fusions where the space between adjacent epithelial cells was eliminated.^{6,38,39} These so-called "kissing points" are morphologically different from AJs and desmosomes, where adjacent

cell membranes remain 15 to 20 nm apart.⁶ Since these initial observations, TJs have been found to consist of 4 unique families of transmembrane proteins: occludin, claudins, junctional adhesion molecules (JAMs), and tricellulin.

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

TJ FORMATION IN THE GASTROINTESTINAL (GI) TRACT

The intestinal epithelium forms the largest and most important barrier between our internal and external environments. The barrier is maintained by the expression of AJs and TJs, including cadherins, claudins, occludin, and JAM proteins, which seal together adjacent cells and provide cytoskeletal anchorage (Fig 3).⁴¹ Expression of junctional proteins in the intestine is highly regulated and dependent on the intestinal compartment (small or large intestine), villus/crypt localization, and cell membrane specificity (apical, lateral, or basolateral). The complex pattern of TJ expression in the intestine is related to the specific functions of a particular intestinal region and localization. Expression of AJ and TJ proteins is also regulated by means of phosphorylation.⁴²⁻⁵³ Phosphorylation can either promote TJ formation and barrier function or alternatively promote TJ protein redistribution and complex destabilization.^{54,55}

Occludin

The first TJ-specific integral membrane protein identified was occludin.⁵⁶ Occludin is expressed predominately at TJs in epithelial and endothelial cells but also by astrocytes, neurons, and dendritic cells.⁵⁶⁻⁵⁹ Occludin (60-82 kd) is a tetraspanning integral membrane protein with 2 extracellular loops, a short cytoplasmic N-terminus, and a long cytoplasmic C-terminus.^{11,13} Functional analysis indicates that the extracellular loops and transmembrane domains of occludin regulate selective paracellular permeability. Intracellularly, the C-terminus interacts with the PDZ domain-containing protein zonula occludens (ZO-1), which is required to link occludin to the actin cytoskeleton (Fig 3).^{60,61}

Several occludin isoforms have been identified and are thought to be a result of alternative mRNA splicing.^{62,63} Notably, several splice variants demonstrate altered subcellular distribution and interaction with other TJ molecules.^{62,63}

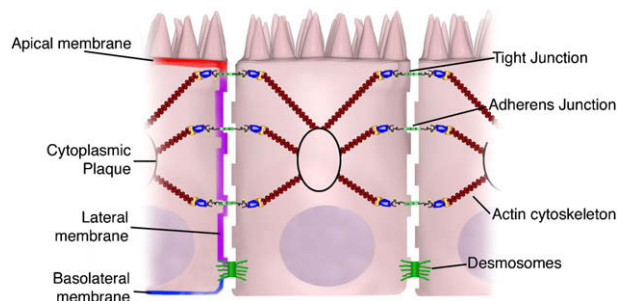


FIG 2. Overview of intestinal epithelial junctional complexes. The intestinal epithelium consists of a single layer of polarized epithelial cells. Adjacent cells are connected by 3 main junctional complexes: desmosomes, AJs, and TJs. Desmosomes are localized dense plaques that are connected to keratin filaments. AJs and TJs both consist of transcellular proteins connected intracellularly through adaptor proteins to the actin cytoskeleton. The collection of proteins in the junctional complexes forms cytoplasmic plaques.

Analysis of the splice variants revealed that the cytoplasmic C-terminal domain is essential for the intracellular trafficking of occludin to the lateral cell membrane and that the fourth transmembrane domain is critical for targeting occludin to the TJ and for ZO-1 interactions.⁶³

The function of occludin is not fully delineated; however, *in vitro* and *in vivo* data suggest a role for occludin in the regulation of paracellular permeability.^{64,65} Notably, the major allergen of the house dust mite Der p 1 has been found to proteolytically cleave occludin, leading to the disruption of the TJ complex and increased paracellular permeability.⁶⁶ Furthermore, hydrocortisone treatment of bovine retinal endothelial cells increased occludin expression 2-fold and enhanced monolayer barrier properties.⁶⁷ Although occludin is an important constituent of TJs, TJ formation and paracellular permeability barrier function are not dependent on occludin. Experimental analyses of occludin^{-/-} mice demonstrated equivalent numbers and organization of TJs and similar paracellular ion conductance as seen in wild-type mice.⁴² Furthermore, epithelial transport and barrier function were normal in occludin^{-/-} mice.⁴³ In addition to regulating paracellular permeability, there is evidence suggesting that occludin is involved in cellular adhesion.⁶⁸ Expression of occludin in occludin^{-/-} rat fibroblasts conferred cell-cell adhesion that was abrogated by synthetic peptides corresponding to the first extracellular loop of occludin, underscoring the importance of this region of occludin in cell adhesion.⁶⁹

In vitro analysis suggests that occludin localization to the TJ complex is regulated by phosphorylation. Several potential phosphorylation sites at tyrosine, serine, and threonine residues of occludin have been identified, and regulation of occludin phosphorylation is proposed to occur by kinases, including the nonreceptor tyrosine kinase c-Yes and protein kinase C (PKC), and phosphatases, including the serine/threonine protein phosphatase 2A.^{70,71} PKC η , a novel protein kinase predominantly expressed in the intestinal epithelium, has been shown to directly phosphorylate occludin at threonine residues (T403 and T404). Blockade of PKC η -mediated occludin phosphorylation disrupted junctional distribution of occludin and ZO-1 and compromised epithelial barrier function.⁷² These data suggest that occludin phosphorylation regulates occludin-ZO-1 interactions and the maintenance of intact TJ complexes and paracellular barrier function.

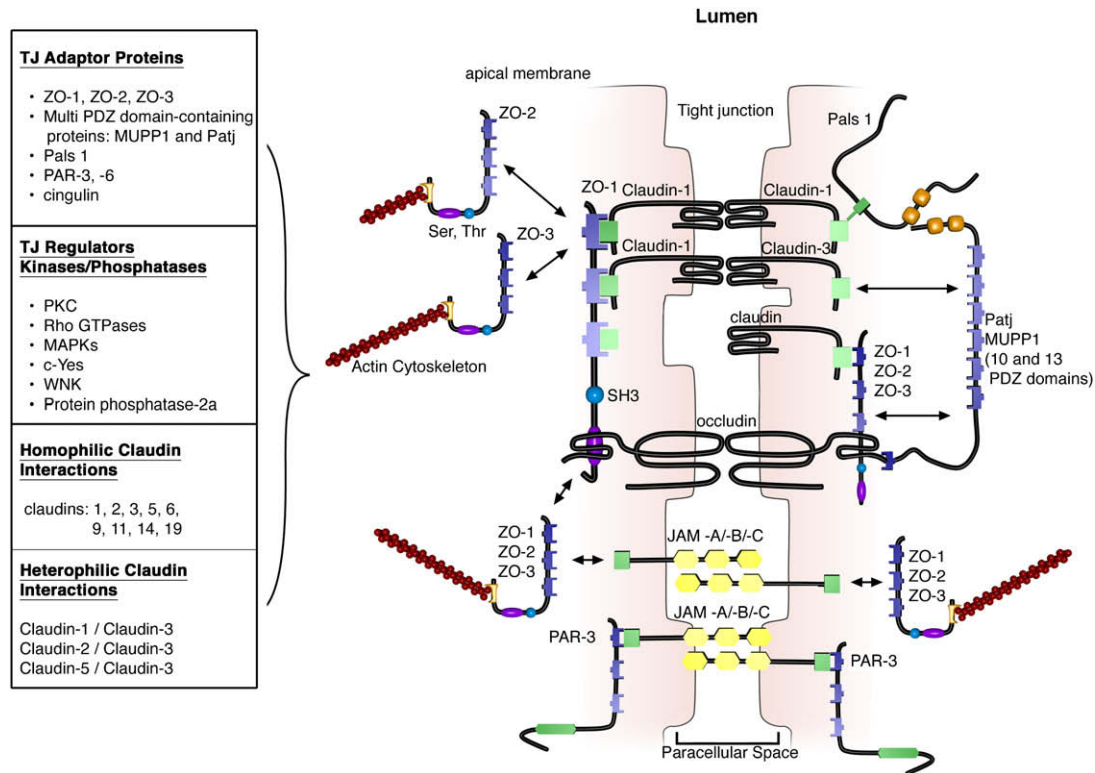


FIG 3. TJs are localized to the apical-lateral membrane junction. They consist of integral transmembrane proteins (occludin, claudins, and JAMs) that interact in the paracellular space with proteins on adjacent cells. Interactions can be homophilic (eg, claudin-1/claudin-1) or heterophilic (eg, claudin-1/claudin-3).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

Claudins

Claudins are 20- to 27-kd integral membrane proteins with 4 hydrophobic transmembrane domains, 2 extracellular loops, and N- and C-terminal cytoplasmic domains.^{7,73-75} The extracellular loops are critical for homophilic and/or heterophilic, TJ protein-protein interactions and the formation of ion-selective channels.⁷ The intracellular C-terminal domain is involved in anchoring claudin to the cytoskeleton through interactions with PDZ-binding domain proteins, including ZO-1, ZO-2, and ZO-3.⁷⁶⁻⁷⁸ Currently, 24 distinct claudin family gene members have been identified in human subjects, with a number of orthologues expressed in other species.^{6,79} They exhibit distinct tissue-, cell-, and developmental stage-specific expression patterns.⁸⁰⁻⁸⁵

Claudin-claudin interactions between adjacent cells can be either homophilic or heterophilic.^{86,87} Homophilic interactions have been demonstrated for claudin-1, claudin-2, claudin-3, claudin-5, claudin-6, claudin-9, claudin-11, claudin-14, and claudin-19. On the other hand, heterophilic interactions are more restricted and primarily have been observed with claudin-3, which can interact with claudin-1, claudin-2, and claudin-5.⁸⁶ Notably, there is specificity in heterophilic transinteractions. For example, transfection of fibroblasts with claudin-1, claudin-2, and claudin-3 led to claudin-3 interactions with both claudin-1 and claudin-2; however, no interactions between claudin-1 and claudin-2 were observed.⁸⁷ These selective interactions are thought to explain the diversity in TJ formations and provide a molecular basis for tissue-specific heterogeneity of barrier function.⁷⁵

Recent studies with claudin-deficient mice also provide corroborative data supporting a role for claudins in the regulation of barrier function (Table I). Claudin 1^{-/-} mice die within 1 day of birth because of significant transepidermal water loss.⁴⁴ In addition, transgenic overexpression of claudin-6 in the epidermis disrupted TJ formation and increased epithelial permeability.⁴⁵ Notably, experimental data suggests that claudins can have differential effects on paracellular permeability. For example, introduction of claudin-2 into MDCK I cells that express claudin-1 and claudin-4 induces a decrease in transepithelial resistance (TER), whereas transfection of claudin-3 had no effect, suggesting that claudin-2 markedly decreased claudin-1/claudin-4-based TJ strand tightness.⁸⁸ In support of these data, recent experimental evidence suggests that claudins can form size- and charge-specific paracellular channels. Transfection of claudin-8 into MDCK II cells that lack endogenous claudin-8 significantly reduced paracellular movement of monovalent and divalent cations without affecting anion and uncharged solute movement.⁸⁹ Experimental analyses suggest that the first extracellular loop of claudins play an important role in determining charge selectivity. Interchanging of the first or both extracellular domains of claudin-4 on claudin-2 profoundly decreased the ion conductance of Na⁺ relative to Cl⁻.⁹⁰ Furthermore, substitution of a negatively charged lysine to a positively charged aspartic acid (K65D) within the loop of claudin-15 caused an increase in Na⁺ permeability, whereas mutation in the same region of 3 positively charged amino acids to negatively charged aspartic acid, arginine, and aspartic acid

(E46K, D55R, and E64K) switched the ion selectivity of claudin-15 from the Na⁺ channel to the Cl⁻ channel.⁹¹ Pore density and size can also influence paracellular movement of charged and non-charged solutes.⁹²

Claudins also play a role in epithelial cell invasion and motility. Overexpression of claudin-3 and claudin-4 in human ovarian epithelial cells, which lack endogenous expression of these proteins, was associated with increased epithelial cell survival and enhanced invasion and motility.⁹³ Consistent with this observation, small interfering RNA-mediated knockdown of claudin-3 and 4 in ovarian cancer cell lines reduced invasion.⁹³ The effects of claudin-3 appear to be linked to altered matrix metalloproteinase-2 activity, which suggests that claudin-induced invasion might be mediated by metalloproteinase proteins.

As with occludin, claudin localization to the TJ complex and its function are regulated by post-translational phosphorylation and through interactions with PDZ-binding domains. The intracellular C-terminal domain of claudin possesses multiple regulatory sites, including potential serine and threonine phosphorylation sites and PDZ-binding domains.⁷ Phosphorylation of claudin-3 and 4 in ovarian cancer cells is linked to the regulation of paracellular permeability.^{94,95} For example, patients with pseudohypoaldosteronism type II (chloride shunt syndrome) present with hyperkalemic metabolic acidosis, hypertension, and dysregulated paracellular ion transport.⁹⁶ The molecular basis is linked to a loss-of-function mutation in the serine-threonine kinases WNK1 and WNK4, which regulate epithelial chloride cotransporters. This leads to an increase in the phosphorylation of claudin-1, claudin-2, claudin-3, and claudin-4 and an increase in paracellular permeability.⁹⁶ A number of signaling pathways have been implicated in the phosphorylation of claudins, including PKC, Rho guanosine triphosphatases, mitogen-activated protein kinases (MAPKs), and phosphatases.⁹⁷ For example, MAPK phosphorylation of claudin-1 is required for claudin-1-mediated barrier function.⁹⁸ Furthermore, claudin-1, claudin-2, claudin-7, claudin-8, claudin-16, and claudin-17 possess putative PKC phosphorylation sites.⁹⁷

All claudins, except claudin-12, end in the dipeptide sequence YV, which has been shown to interact with PDZ-binding domain proteins, including ZO-1, ZO-2, ZO-3, multi-PDZ domain protein 1 (MUPP1), and protein associated with lin seven (PALS1)-associated TJ protein (PATJ) (Fig 3).^{73,75,77,99} Many of these scaffolding proteins contain multiple PDZ domains, which facilitate the formation of dense localized protein complexes, also known as "cytoplasmic plaques." Furthermore, the scaffolding proteins can interact with signaling molecules, including heterodimeric guanosine triphosphate-binding proteins (Rab13 and Gα12), transcriptional factors, and RNA-processing factors, to link TJ complexes to the actin cytoskeleton and regulate aspects of epithelial polarization, differentiation, and function.^{6,21,38,100,101}

JAMs

JAMs are integral membrane proteins that belong to the immunoglobulin superfamily and have 2 immunoglobulin folds (VH and C2 type) in the extracellular domain.^{80,81} JAMs are expressed by multiple cell types, including epithelial, endothelial, and immune cells.^{82,83} They are subdivided based on the expression of type I or II PDZ-binding motifs in the intracellular C-terminus, which suggests that the 2 types interact with unique scaffolding and cytoplasmic proteins.⁸⁰ JAM-A, JAM-B, and JAM-C (or JAM-1, JAM-2, and JAM-3) have Type II binding motifs, whereas the atypical

JAMs, including JAM-4, Coxsackie and adenovirus receptor, and endothelial selective adhesion molecule contain Type I PDZ-binding domains.^{21,80} Similar to other TJ proteins, these JAM-PDZ interactions provide anchorage to the actin cytoskeleton (Fig 3).

The extracellular region of JAMs binds to multiple ligands through homophilic and heterophilic interactions, which are proposed to regulate the cellular functions and paracellular permeability of JAMs.^{80,81} Homophilic JAM-A or JAM-B interactions regulate the formation of functional TJs and cell-cell border formation,^{84,85} whereas heterophilic JAM interactions play a role in leukocyte-endothelial cell adhesion.⁸¹

Recent studies demonstrate the importance of JAM-A in the formation and assembly of TJs in intestinal epithelial cells. Small interfering RNA downregulation of JAM-A in SK-CO15 epithelial cells induced an increase in permeability.⁴⁶ Consistent with this, JAM-A^{-/-} mice had increased mucosal permeability, as indicated by enhanced dextran flux and decreased TER.⁴⁶ However, these mice also had an increase in claudin-10 and claudin-15 expression, which are thought to form selective pores in the TJ complex, enhancing paracellular permeability.^{91,102} Interestingly, JAM-A^{-/-} mice have increased susceptibility to chemical-induced colitis. Dextran sodium sulfate administration to JAM-A^{-/-} mice induced more severe colonic injury compared with that seen in wild-type control animals.⁴⁶ These studies suggest altered intestinal permeability as a susceptibility factor to intestinal disease.

DYSREGULATION OF TJ FORMATION AND INTESTINAL BARRIER FUNCTION

Cytokine mediated

In vitro and *in vivo* animal studies have demonstrated that intestinal permeability is regulated by multiple factors, including exogenous factors, epithelial apoptosis, cytokines, and immune cells (Fig 4). Immune-induced intestinal barrier dysfunction is thought to be critical in the predisposition to and exacerbation of numerous autoimmune and inflammatory conditions, including inflammatory bowel disease (IBD), food allergy, celiac disease, and diabetes.¹⁰³ For example, IFN-γ and TNF-α, which are central mediators of intestinal inflammatory diseases, including IBD, induce intestinal epithelial barrier function.¹⁰⁴⁻¹⁰⁶ Incubation of intestinal epithelial cell monolayers (Caco2 and T84) with IFN-γ and TNF-α promoted the reorganization of several TJ proteins (ZO-1, JAM-A, occludin, claudin-1, and claudin-4) and decreased epithelial barrier function.¹⁰⁷ The mechanism of action of these cytokines appears to be primarily mediated through myosin light chain kinase (MLCK)-mediated phosphorylation of the myosin light chain (MLC), which promotes TJ disruption.¹⁰⁷ In support of this, inhibition of TNF-α- and IFN-γ-induced MLC phosphorylation restored barrier function.¹⁰⁷ Alternatively, TNF-α and IFN-γ can disrupt TJs and increase intestinal permeability through dysregulation of claudin and occludin expression.¹⁰⁸

Experimental and clinical data support a role for T_H2 cytokines in the regulation of intestinal barrier function (Fig 4). Stimulation of colonic epithelial cells (T84 and HT-29/B6) with IL-4 or IL-13 induced an increase in intestinal permeability.^{109,111} Notably, altered barrier function was associated with the induction of epithelial apoptosis and expression of the pore-forming TJ claudin-2. *In vitro* data suggest that the effects of IL-4 and IL-13 on barrier function are primarily mediated by phosphoinositide 3-kinases.^{110,112} Blockade of phosphoinositide 3-kinase, but not

TABLE I. Transgenic or knockout mice and effects on intestinal barrier function

Protein	Transgenic or knockout	Function	Phenotype	References
Occludin	Gene deletion	TJ protein	No change in TJs or permeability	42, 43
Claudin-1	Gene deletion	TJ protein	Die within 1 d of birth	44
Claudin-6	Epidermis transgenic	TJ protein	Disrupted TJ formation and increased epithelial permeability	45
JAM-A	Gene deletion	TJ protein	Increased intestinal permeability Increased claudin-10 and claudin-15 expression Increased susceptibility to DSS colitis	46
IL-9	Intestinal transgenic	Cytokine	Increased intestinal mast cell Increased intestinal permeability Increased susceptibility to oral antigen sensitization and anaphylaxis	52
IL-10	Gene deletion	Cytokine	Increased permeability; spontaneously have chronic colitis similar to CD	48-50
STAT-6	Gene deletion	Signaling molecule	Protected against IL-4- and IL-13-induced intestinal epithelial barrier dysfunction	47
Mcpt1	Gene deletion	Mast cell protease	Protected against <i>T spiralis</i> infestation-induced intestinal epithelial barrier dysfunction	51
MLCK	Transgenic	Signaling molecule	Increased intestinal permeability Increased onset and severity of immune-related colitis	53

DSS, Dextran sulphate sodium; *mMCP-1*, murine mast cell protease 1.

signal transducer and activator of transcription (STAT)-6 activation, blocked IL-4/IL-13-induced barrier dysfunction.¹¹⁰ However, studies in STAT-6^{-/-} mice identified a role for STAT-6 signaling in IL-4- and IL-13-mediated intestinal epithelial barrier dysfunction.⁴⁷ Moreover, IL-4- and IL-13-induced altered permeability, glucose absorption, and chloride secretion were attenuated in STAT-6^{-/-} mice compared with values seen in wild-type mice.⁴⁷

The anti-inflammatory cytokine IL-10 has also been shown to regulate intestinal barrier function.^{48,49} Stimulation of ileal segments from Sprague-Dawley rats with IL-10 enhanced intestinal electroneutral sodium and chloride absorption and inhibited stimulated chloride secretion.¹¹³ In addition, treatment of T84 epithelial cell monolayers with IL-10 blocked IFN- γ -induced epithelial permeability.¹¹⁴ These results suggest that IL-10 plays a protective role in intestinal barrier function. In support of this, mice deficient in IL-10 have increased intestinal permeability.⁴⁸ Notably, IL-10^{-/-} mice spontaneously develop chronic intestinal inflammation, which is manifested by symptoms commonly associated with Crohn's disease (CD), including weight loss, mucosal hyperplasia, and chronic enterocolitis.⁵⁰ These data suggest that increased permeability might predispose IL-10^{-/-} mice to intestinal inflammation and colitis. Consistent with this hypothesis, increased permeability in IL-10^{-/-} mice was observed before disease onset.⁴⁸

Mechanistic studies to delineate IL-10-mediated intestinal permeability have implicated the zonulin pathway and TNF- α . Remarkably, inhibition of the zonulin receptor in IL-10^{-/-} mice led to decreased intestinal permeability and reduced colonic TNF- α secretion *ex vivo* and abrogated the spontaneous development of colitis.⁴⁹ These findings further support a role for

increased intestinal permeability in the development of intestinal inflammation and disease and a possible role for zonulin. The zonulin/zonulin receptor pathway is thought to regulate TJ formation through PKC-dependent actin reorganization.¹¹⁵ Whether decreased intestinal barrier function in IL-10^{-/-} mice is primarily due to an inherent defect in the zonulin/zonulin receptor pathway or, alternatively, a consequence of increased expression of cytokines, such as IFN- γ and TNF- α , remains to be delineated.

Immune cells

T cells. Anti-CD3-induced CD4⁺ T-cell activation in mice promotes an increase in transcellular and paracellular intestinal permeability and the release of proinflammatory cytokines, such as IFN- γ and TNF- α (Fig 4).^{116,117} Furthermore, injection of mice with TNF- α provokes a breakdown in intestinal barrier function, diarrhea, and PKC α -dependent inhibition of Na⁺/H⁺ exchange.¹¹⁸ T cells regulate transcellular permeability through the downregulation of Na⁺/K⁺ATPase and disruption of Na⁺ absorption, Na⁺-glucose cotransport, and inducible Cl⁻ secretion,^{116,117} whereas dysregulation of the paracellular permeability pathway is mediated through MLCK-dependent TJ disruption.¹¹⁷

γ/δ -positive intraepithelial lymphocytes (iIEL $\gamma\delta^+$), which are closely associated with the basolateral side of intestinal epithelial cells, have also been implicated in intestinal barrier maintenance.¹¹⁹ In response to enteric parasitic infestation, mice deficient in iIEL $\gamma\delta^+$ T cells have abnormal claudin-3, occludin, and ZO-1 localization; decreased occludin phosphorylation; and abnormal epithelial TJ formation.¹¹⁹ Notably, the alterations in intestinal barrier function could be attributed to a single subset

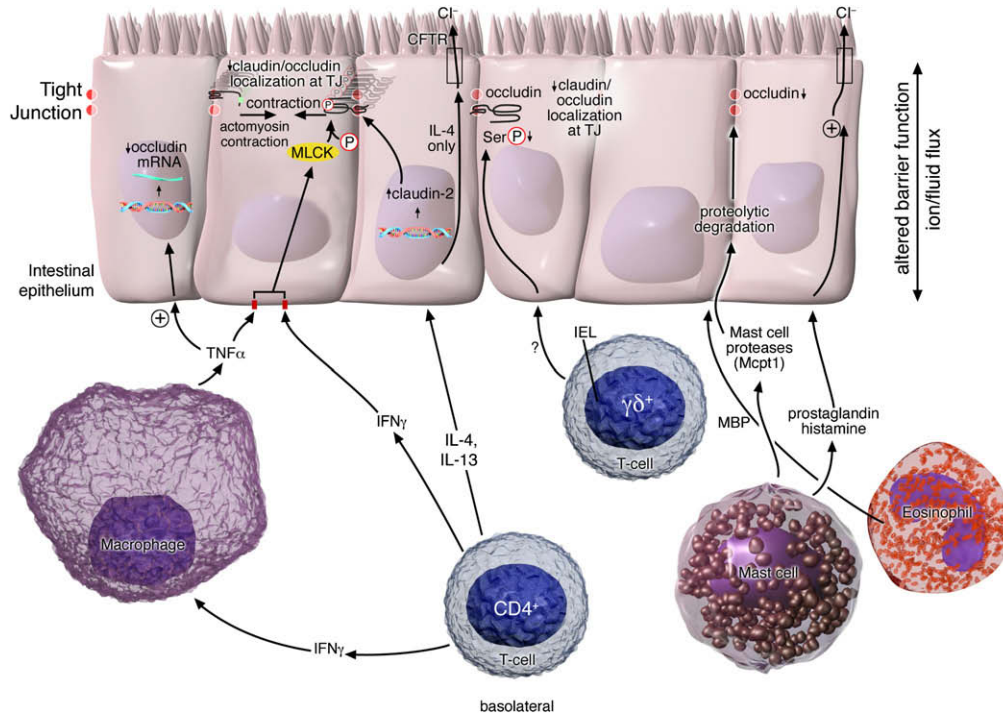


FIG 4. Immune regulation of intestinal barrier function. T cell-derived IFN- γ and TNF- α inhibit MLCK-mediated phosphorylation of MLC, leading to TJ junction disruption and intestinal barrier dysfunction. IFN- γ can also promote the redistribution of TJ proteins, JAM-A, occludin, claudin-1, and claudin-4 from the apical TJ border by means of a micropinocytosis process. TNF- α and IFN- γ can alternatively disrupt TJ stability and increase intestinal permeability through dysregulation of occludin expression. IL-4- and IL-13-induced increase in intestinal permeability is mediated through induction of epithelial apoptosis and expression of the pore-forming TJ protein claudin-2. IL-4, but not IL-13, regulates ion conductance through downregulation of epithelial cystic fibrosis transmembrane conductance regulator Cl⁻ channel expression. Intraepithelial iIEL $\gamma\delta^+$ lymphocytes (T-cell receptor V $\gamma 7^+$) iIEL cells are important in serine phosphorylation of occludin and TJ stabilization. Mast cell mediators, including the cytokine TNF- α , mast cell protease 1 (*mcpt-1*), and lipid mediators, including histamine, platelet-aggregating factor, and prostaglandins, promote increased Cl⁻ conductance and increase intestinal permeability. Mcpt1 degrades the TJ protein occludin, altering barrier function. Eosinophil-derived major basic protein downregulates TJ protein occludin-1 expression in colonic epithelial cells. *MBP*, Major basic protein.

of iIEL $\gamma\delta^+$ lymphocytes: T cells expressing V $\gamma 7^+$ encoded T cell receptors. Reconstitution of mice deficient in iIEL $\gamma\delta^+$ T cells with V $\gamma 7^+$ iIELs restored epithelial barrier function.¹¹⁹

Mast cells. Mast cells are present in all compartments of the GI tract.¹²⁰ On activation, they release a powerful array of inflammatory mediators, including histamine, 5-hydroxytryptamine, neutral proteases (tryptases, chymases, and carboxypeptidase A), prostaglandins, leukotrienes, platelet-activating factor, and multiple cytokines, including TNF- α , IL-3, IL-4, IL-5, IL-6, and GM-CSF.¹²¹⁻¹²³ Employing models of food allergy or helminthic infestation (*Nippostrongylus brasiliensis* or *Trichinella spiralis*), investigators have demonstrated mast cell involvement in intestinal barrier function.¹²⁴ Intraluminal challenge of egg albumin-sensitized rats induced a 15-fold increase in uptake of ⁵¹Cr-labeled EDTA compared with that seen in rats treated with unrelated protein.¹²⁵ The antigen-induced decreased barrier function was associated with mast cell degranulation and an increase in the short-circuit current, a measure of net ion transport.¹²⁵ The importance of mast cells was demonstrated by the absence of changes in barrier function in mast cell-deficient mice sensitized and challenged with egg albumin, which was restored by bone marrow reconstitution.^{126,127} Furthermore, several mast cell

mediators have been shown to modulate intestinal epithelial ion transport. Pretreatment of egg albumin-sensitized rats with histamine H₁ or 5-hydroxytryptamine 2 receptor antagonists significantly reduced oral antigen-induced short-circuit current alterations.^{126,128}

Experimental analyses employing models of parasitic infestation have identified a role for mast cell-derived proteases in intestinal barrier function.⁵¹ Murine infestation with the enteric nematode *T. spiralis* induced intestinal mastocytosis, occludin degradation, and increased intestinal permeability.⁵¹ The alterations in barrier function were demonstrated to be mast cell dependent as depletion of mast cells with a neutralizing anti-c-kit antibody ablated intestinal epithelial barrier dysfunction.⁵¹ Similarly, mice deficient in the murine mast cell protease-1 (Mcpt1) were also resistant to *T. spiralis* infestation-induced intestinal epithelial barrier dysfunction. Mcpt1 mediated increase in intestinal permeability during *T. spiralis* infection was linked to occludin degradation.⁵¹

Eosinophils. Increased eosinophils and eosinophil granular proteins, including major basic protein, eosinophil peroxidase, and eosinophilic cationic protein, are often associated with IBD and altered barrier function.¹²⁹⁻¹³² *In vitro* coculture of T84

intestinal epithelial cells with eosinophils or eosinophil-derived major basic protein decreased TER and increased permeability. Altered intestinal barrier function was associated with the down-regulation of occludin.¹³³

EXOGENOUS REGULATION OF INTESTINAL BARRIER FUNCTION

Alcohol

Chronic alcohol consumption has been shown to be associated with increased intestinal permeability, inhibition of vitamin and nutrient transport, and a reduction in sodium and water absorption.^{134,135} Experimental analyses suggest involvement of the byproduct of ethanol metabolism, acetaldehyde, and nitric oxide (NO) in alcohol-mediated barrier dysfunction. High levels of acetaldehyde have been detected in the intestines of rats after ethanol administration and elevated levels were associated with increased intestinal permeability and endotoxin translocation.¹³⁶ Furthermore, incubation of Caco2 cells with acetaldehyde increased monolayer permeability, and this increase was associated with increased tyrosine phosphorylation of ZO-1, E-cadherin, and β -catenin.⁵⁴ Exposure of Caco2 monolayers to ethanol also promotes inducible nitric oxide synthase expression, stimulating increased NO production and increased monolayer permeability.¹³⁷ NO-induced changes were associated with an increase in unstable non-polymerized tubulin and extensive damage to the microtubule cytoskeleton.

Experimental studies in rodents have also demonstrated that acute administration of alcohol induces mucosal damage in the upper small intestine, including villus ulceration, submucosal blebbing, and hemorrhagic erosions and intestinal barrier dysfunction.^{135,138,139} It is postulated that alcohol-induced intestinal permeability facilitates enhanced translocation of endotoxin to distant organs, leading to inflammation and tissue damage.^{138,140,141} Intragastric administration of endotoxin in the presence of alcohol to rodents led to significantly higher plasma endotoxin levels than animals fed endotoxin alone.^{138,141} Similar lesions have been found in healthy volunteers and active alcoholics following acute alcohol consumption,^{141,142} and plasma endotoxin levels in alcoholics were found to be 5-fold greater than in healthy control subjects.¹⁴³ Although not fully understood, evidence suggests the mechanism underlying alcohol-induced barrier dysfunction is related to the influx of inflammatory cells and release of various mediators, including cytokines, reactive oxygen species, leukotrienes, and histamine.^{144,145}

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drug (NSAID) use is associated with a high incidence of gastrointestinal side effects, and there is substantial evidence indicating that chronic use can alter intestinal barrier function and cause significant gastrointestinal damage, including ulcers, perforation, hemorrhage, and an exacerbation of IBD.¹⁴⁶⁻¹⁵³ Both acute and chronic ingestion of NSAIDs by healthy volunteers and patients promotes altered intestinal barrier dysfunction and hypermotility.^{148,154,155} *In vitro* studies with MKN28, a gastric epithelial cell line, have demonstrated that an aspirin-induced increase in permeability was accompanied by a significant decrease in the expression of claudin-7, but not claudin-3, claudin-4, ZO-1, or occludin.¹⁵⁶

NSAID-induced gastrointestinal injury was initially found to be a consequence of cyclooxygenase inhibition and decreased prostaglandin synthesis; however, it has become evident that intestinal damage is a multistage process.^{147,154,157} Experimental analyses have identified a contribution from neutrophils, microcirculatory disturbances, oxygen free radicals, and bile acids in NSAID-induced gastrointestinal damage.^{147,158,159} NSAIDs increase intestinal nitric oxide synthase expression and activity, leading to increased levels of NO and promoting increased intestinal permeability.¹⁶⁰ NSAIDs can also uncouple mitochondrial oxidative phosphorylation, which impairs the mitochondrial energy production necessary for TJ complex integrity, leading to increased intestinal inflammation and permeability.¹⁶¹ Finally, a recent study demonstrated that aspirin induced an increase in gastric epithelial cell permeability that was mediated by activation of p38 MAPK and a decrease in claudin-7 levels, and treatment with a p38 MAPK inhibitor attenuated this response.¹⁵⁶

Pathogens

The intestine is home, both permanently and transiently, to an extraordinarily complex microflora that provides an abundant source of potentially pathogenic organisms, toxins, and antigens. The dynamic and complex interactions between enteric pathogens and the intestinal epithelium often leads to disturbances in the intestinal barrier, altered fluid and electrolyte transport, and the induction of an inflammatory response.¹⁶² Enteric pathogens can disrupt the intestinal barrier directly by binding to cell-surface molecules and inducing changes in TJ protein expression. Alternatively, pathogens generate toxins and proteases, which can promote cell damage and apoptosis, alter epithelial ion transport, and disrupt TJs and the cytoskeleton. Herein, we will provide examples and brief descriptions of several mechanisms by which pathogens disrupt barrier function.

Vibrio cholerae. *V. cholerae* is a major enteric pathogen that alters intestinal barrier function through the disruption of TJs, dysregulation of intestinal ion and fluid transport, and the initiation of inflammatory cascades. A major toxin produced by *V. cholerae* is the cytotoxin hemagglutinin protease (HA/P), a zinc-binding metalloprotease that degrades TJ proteins and decreases barrier function.^{163,164} Studies of mutant toxin-attenuated strains of *V. cholerae* have identified HA/P as the principle toxin responsible for alterations of TJs and decreased TER in cultured MDCK and T84 cells.^{163,164} *In vitro* studies demonstrate that HA/P cleaves the extracellular domain of occludin. This disrupts intracellular occludin-ZO-1 interactions and destabilizes the TJ complex and cytoskeletal anchorage, resulting in increased paracellular permeability.¹⁶⁵

Another toxin elaborated by *V. cholerae* is zonula occludens toxin (Zot), an enterotoxin that reversibly increases intestinal epithelial permeability, disrupts the actin cytoskeleton, and induces fragmentation of ZO-1 and occludin.¹⁶⁶⁻¹⁶⁸ Zot binds to the zonulin receptor on the apical side of intestinal epithelial cells and activates phospholipase C, leading to PKC α -dependent polymerization of the actin cytoskeleton.^{167,169} Actin polymerization is thought to promote cytoskeletal reorganization and the destabilization of TJ complexes. Consistent with this hypothesis, pretreating intestinal epithelial monolayers with PKC α inhibitors prevented Zot-induced changes in actin polymerization and permeability.¹⁶⁷ A human homologue for Zot, zonulin, has been identified and found to bind to the same receptor and regulate intestinal permeability.¹⁷⁰ Zonulin is believed to regulate TJ function, and its dysregulation

has been implicated in several inflammatory diseases associated with intestinal barrier dysfunction, including IBD, type I diabetes, and celiac disease (see the “Clinical Review” section).

Enteropathogenic *Escherichia coli*. Enteropathogenic *Escherichia coli* (EPEC) is a diarrhea-causing bacteria that disrupts TJ proteins by adhering directly to the surface of epithelial cells. They form attaching and effacing lesions characterized by the localized destruction of the adjacent epithelial microvilli and the formation of a pedestal-like structure from the accumulation of cytoskeletal proteins, such as actin, beneath the site of attachment.¹⁷¹ EPEC uses a syringe-like type III secretion system to trigger TJ disruption and alterations in intestinal epithelial ion secretion.¹⁷²⁻¹⁷⁴ Infection of intestinal T84 monolayers with EPEC increased epithelial permeability, and this was associated with destabilization and dissociation of the ZO-1, occludin, and claudin-1 TJ complex from the lateral membrane.¹⁷³⁻¹⁷⁵ The molecular mechanisms associated with EPEC-mediated TJ alterations are still unclear; however, studies with pharmacologic agents that inhibit MLCK have implicated involvement of the MLCK pathway in the process.¹⁷⁶

Clostridium perfringens. While many bacterial products have been demonstrated to alter TJs, the enterotoxin of *Clostridium perfringens* (CPE) which is a common cause of food poisoning, directly interacts with and uses TJs as receptors.^{177,178} CPE binds to the extracellular loop of claudin-3 and 4 on the cell surface of enterocytes, forming small protein complexes in the plasma membrane.¹⁷⁸ These complexes promote oligomerization and the formation of larger plasma membrane complexes, which have been associated with increased plasma membrane permeability.^{179,180} CPE also interacts with occludin to promote its removal from the TJ and redistribution into the cytoplasm.¹⁷⁹ The redistribution of claudins and occludin induces destabilization of the TJ complex, leading to altered intestinal paracellular permeability. For example, exposure of MDCK monolayers to CPE induced a reversible decrease in TER and increase in permeability.¹⁸¹ Additionally, the large CPE- and TJ-containing complexes are believed to insert into the plasma membrane to form a functional pore that induces Ca²⁺ influx, which triggers host epithelial cell death by means of apoptosis or oncosis.^{182,183}

CLINICAL REVIEW

Introduction

A breakdown or impairment of the epithelial barrier has been implicated as a critical determinant in the predisposition to intestinal inflammation and a number of gastrointestinal diseases, including IBD and food allergy (Table II).^{*} Although altered intestinal barrier function (increased intestinal epithelial permeability) can be a consequence of disease exacerbation, clinical evidence suggests that it might be a primary causative factor predisposing to disease development. For example, healthy first-degree relatives of patients with IBD and celiac disease have increased intestinal permeability.²²⁷ Furthermore, altered intestinal permeability persisted in patients with asymptomatic celiac disease treated with a gluten-free diet^{202,203} and is predictive of clinical relapse in patients with clinically inactive IBD.^{204,205} In this section we summarize the current clinical data relevant to intestinal epithelial barrier function in chronic disease

susceptibility and describe the potential implications of these studies in disease pathogenesis.

Food allergy

Food allergies are adverse, immune-mediated reactions to ingested food proteins/antigens. It is hypothesized that intestinal barrier dysfunction might contribute to both antigen sensitization and also the IgE/mast cell-mediated anaphylactic effector phase of disease. The development of food allergies is dependent on the exposure of the food antigen to the mucosal immune system, which leads to antigen sensitization and the production of dietary antigen-specific CD4⁺ T_H2 cells and IgE. It is hypothesized that altered intestinal barrier function permits increased dietary antigen transport across the intestinal barrier and exposure of dietary antigens to the mucosal immune system, leading to the development of the dietary antigen-specific response. Consistent with this hypothesis, intestinal permeability in infants with food allergy, as assessed based on the lactulose/mannitol ratio in urine, was significantly increased compared with that seen in healthy young children.^{206,207} To determine whether the altered intestinal barrier function was a consequence of a recent adverse reaction to dietary antigen, the lactulose/mannitol ratio was examined in patients with food allergy who had been on an allergen-free diet for at least 6 months. Intestinal permeability remained increased in these subjects, indicating that increased intestinal permeability persisted in the absence of food antigen stimulation.²⁰⁶

Additional data supporting a role for increased intestinal permeability in the development of food antigen sensitization and food allergies are provided by recent clinical studies that demonstrate an association between increased intestinal permeability and the development of new-onset food allergies in patients after liver and heart transplantation. Patients treated with the immunosuppressant tacrolimus (FK506) have been shown to have increased intestinal permeability and increased levels of food antigen-specific IgE.²⁰⁸⁻²¹⁰ Notably, some of these patients had new-onset food allergies.^{211,212} The development of food allergies in immunosuppressed patients after transplantation was originally thought to be a consequence of the passive transfer of food antigen-specific IgE or lymphocytes from donors with food allergy to previously nonallergic recipients.^{213,214} However, studies have reported the development of food allergies in patients where the donor had no history of food allergy.^{215,216} Interestingly, *in vitro* and *in vivo* experiments with rats have demonstrated that tacrolimus induces a dose-dependent increase in intestinal permeability,²¹⁷ suggesting that tacrolimus-induced altered intestinal barrier function may be a possible explanation for the new-onset food allergies in immunosuppressed post-transplant patients.

Notably, tacrolimus has been shown to uncouple mitochondrial oxidative phosphorylation, leading to impaired mitochondrial energy production and a significant decrease in cellular ATP levels.^{217,218} Importantly, the formation of the intestinal barrier and maintenance of intercellular junctional complexes are energy-dependent processes, and decreased cellular ATP levels are responsible for inducing a breakdown in TJ complexes and barrier dysfunction.⁵⁶ Consistent with this, rats treated with tacrolimus were shown to have a dose-dependent increase in intestinal permeability that correlated with decreased intracellular ATP levels and CO₂ release.²¹⁷ Similarly, liver transplant patients treated with tacrolimus were found to have reduced mitochondrial

*See references 49, 52, 53, 104, 105, 162, 184-201.

TABLE II. Diseases associated with altered TJ protein expression and intestinal epithelial barrier function

Disease state	TJ proteins	Inflammatory cell/cytokine	Proposed mechanism	References
Food allergy	Not defined	Mast cells	Mast cell-mediated degradation of TJ proteins	52, 190
IBD	↓ Claudin-3, claudin-4, claudin-5, and claudin-8 ↑ Claudin-2	CD4 ⁺ T cells IL-10, IFN- γ , and TNF- α and MLCK	IFN- γ - and TNF- α -mediated activation of MLCK leading to TJ disruption and dysregulation of claudin and occludin expression	49, 53, 104, 105, 191, 192
Celiac disease	Occludin and ZO-1	Zonulin	Gliadin-induced zonulin secretion by intestinal epithelial cells Zonulin-induced downregulation of occludin/ZO-1	193, 194
Diabetes	Not defined	Zonulin	Increased zonulin secretion	195, 196
Stress	Occludin and ZO-1	Mast cells and corticotrophin-releasing hormone	Destabilization and redistribution of occludin/ZO-1	197-201

energy production associated with increased intestinal permeability and an increase in serum endotoxin levels.²¹²

The immunosuppressive activity of tacrolimus is through the inhibition of calcineurin, which is critical for IL-2-induced T-cell activation.²¹⁹ Inhibition of IL-2 has been shown to promote the T_H2 immune response.²²⁰ T_H2 cells secrete IL-4, IL-5, and -13, which promote IgE-mediated allergic inflammation and set the stage for food antigen sensitization and the induction of food allergies. There are most likely several mechanisms involved in the pathogenesis of food allergies in tacrolimus-immunosuppressed patients and increased intestinal permeability appears to be an important mediator to facilitate presentation of food antigens to the immune system and oral antigen sensitization.

We have recently provided experimental evidence supporting a role for altered intestinal permeability in oral antigen sensitization and the development of food allergies in mice.⁵² We generated a transgenic mouse that overexpresses the cytokine IL-9 specifically in the enterocytes of the small intestine (iIL-9Tg mice). A consequence of transgenic overexpression of IL-9 was a pronounced intestinal mastocytosis and altered intestinal permeability.⁵² Repeated oral administration of ovalbumin to iIL-9Tg BALB/c mice and not wild-type mice promoted the development of antigen-specific IgE, CD4⁺IL-4⁺ T cells, and symptoms of a food-induced allergic response in the absence of prior systemic sensitization or the use of adjuvant. Pharmacologic mast cell depletion in iIL-9Tg mice was found to restore intestinal permeability to levels comparable with those seen in wild-type mice. Remarkably, reconstitution of barrier function and decreased intestinal permeability in iIL-9Tg mice prevented orally induced antigen sensitization.⁵² These findings suggest that increased intestinal permeability facilitates enhanced antigen uptake and the oral induction of food antigen sensitization.

Intestinal barrier dysfunction is also thought to contribute to the severity of food allergen-induced clinical symptoms. Oral challenge of subjects with food allergy with food allergen induced an increase in the lactulose/mannitol ratio in urine.^{190,206} The level of intestinal barrier dysfunction positively correlated with the severity of clinical symptoms.²⁰⁶ Notably, treatment of the group

with food allergy with sodium cromoglycate, a mast cell stabilizer, before ingestion of food allergen significantly reduced lactulose permeability compared with that seen in subjects challenged with food allergen not receiving sodium cromoglycate, indicating a role for mast cells in dietary antigen-induced intestinal epithelial barrier dysfunction.¹⁹⁰

Consistent with clinical observations, animal models of GI anaphylaxis and food allergy have also demonstrated increased intestinal permeability after oral antigen challenge.^{52,221,222} Intraluminal challenge of egg-sensitized rats with egg albumin induced a 15-fold increase in uptake of ⁵¹C-labeled EDTA compared with that seen in rats treated with unrelated protein.¹²⁵ Studies using mast cell-deficient animals or pharmacologic agents to deplete mast cells have provided corroborative evidence demonstrating that mast cells are critical for altered intestinal barrier function during food-induced allergic reactions.^{52,126,127,222} Increased permeability after antigen challenge has been shown to initially be the result of increased antigen uptake and translocation through the transcellular route, as evidenced by an increase in horseradish peroxidase (HRP)-containing endosomes within minutes of HRP challenge in rats that had been sensitized.²²³ The second phase, which occurs after sensitization and is mast cell dependent, was associated with a disruption in the TJs and an increase in paracellular permeability.²²³ Collectively, these studies suggest a role for altered intestinal barrier function in patients with food allergy. Furthermore, these studies suggest a role for mast cells in the regulation of intestinal barrier dysfunction in patients with food allergy.

IBD

The IBDs CD and ulcerative colitis are chronic, relapsing-remitting inflammatory diseases. An emerging model of the pathogenesis of IBD suggests there are 3 essential factors: (1) a breakdown in intestinal barrier function; (2) exposure of luminal contents to immune cells in the lamina propria; and (3) an exaggerated immune response.²²⁴ However, it is currently unclear which factor is responsible for initiating this self-perpetuating

cycle leading to disease exacerbation. There is a growing body of data to suggest that increased intestinal permeability is a primary causative factor contributing to IBD pathogenesis. Patients with CD with clinically active disease have increased intestinal permeability, and in patients with inactive disease, increased intestinal permeability is predictive of clinical relapse.^{204,205} In addition to patients with IBD, increased intestinal permeability occurs in 10-25% of their healthy first-degree relatives, indicating that increased intestinal permeability likely preceded the onset of clinical disease.²²⁵⁻²²⁷ Furthermore, studies have found that a subset of patients who are at high risk for CD have either increased intestinal permeability at baseline or an exaggerated increase in permeability in response to stimulation.^{185,228} Notably, a case study on the long-term follow-up of a healthy 13-year-old girl who had increased intestinal permeability and a parent with CD reported that she subsequently developed CD 8 years later.²²⁹

There are extensive experimental studies employing models of experimental colitis demonstrating a link between altered intestinal barrier function and IBD. IL-10^{-/-}, mdr1a^{-/-}, and SAMP/Yit mice, which spontaneously develop IBD-like symptoms have increased intestinal permeability preceding disease development.^{48,230,231} For example, IL-10^{-/-} mice spontaneously develop CD-like colitis by 12 weeks of age.⁵⁰ These mice also had increased intestinal permeability, which was present before the onset of disease.⁴⁸ Notably, when IL-10^{-/-} mice were treated with a zonulin peptide inhibitor, intestinal permeability was reduced, and development of colitis was significantly attenuated.⁴⁹ Although intestinal permeability is a key player in the development of IBD, recent data suggest that increased intestinal permeability alone is not sufficient to predispose to IBD. Transgenic mice that constitutively express active MLCK in the intestinal epithelia had significant intestinal barrier dysfunction and increased intestinal permeability.⁵³ It was found that the decrease in barrier function did not predispose the mice to spontaneous development of colitis; however, it did accelerate the onset and severity of immune-mediated colitis in the MLCK transgenic mice.⁵³

A significant advance in IBD research came with the discovery of the nucleotide-binding oligomerization domain 2 (NOD2)/caspase-recruitment domain 15 (CARD15) gene as a CD genetic susceptibility locus.^{232,233} Mutations in the CARD15 gene have been identified in patients with CD and their healthy first-degree relatives.¹⁸⁵ Notably, 40% of relatives with 1 mutation and 75% of relatives with 2 mutations had increased intestinal permeability compared with that seen in control subjects.

Multiple molecular mechanisms for increased intestinal permeability in patients with IBD have been reported, including altered TJ protein expression for distribution, and increased epithelial apoptosis.²³⁴ Initial studies reported a downregulation in occludin expression in patients with IBD, with no change in claudin-1 expression²³⁵; however, the role occludin may play in barrier dysfunction has been questioned since occludin-deficient mice have normal intestinal barrier function.²³⁶ There are a number of studies demonstrating that significant barrier dysfunction is associated with the disruption of occludin. This seeming discrepancy is most likely a consequence of the dynamic nature of TJs, whereby TJ complexes can form normally in the absence of occludin, as in occludin-deficient mice; however, once occludin is intimately associated with the complex, its disruption can alter the barrier function of the TJ complex. Clinical studies have also reported an upregulation of the

barrier-reducing TJ protein claudin-2, in particular in the crypt epithelium, as well as decreased expression and redistribution of the sealing TJ proteins claudin-3, claudin-4, claudin-5, and claudin-8 in patients with IBD (Table II).^{112,236} However, these TJ modifications might be a consequence of disease pathogenesis, rather than a cause, as they were not altered in patients with inactive CD.²³⁶ Additionally, the breakdown in the protective barrier in patients with IBD leads to an inflammatory infiltrate and enhanced production of cytokines and other mediators that can further contribute to altered barrier function. Increased levels of IFN- γ and TNF- α have been demonstrated in the intestinal mucosa of patients with IBD, and both cytokines have been shown to alter intestinal epithelial barrier function *in vitro*.^{104,105,237,238} Notably, treatment of these patients with anti-TNF mAbs downregulated the inflammatory response and restored intestinal barrier function, leading to a decrease in intestinal permeability.^{191,192}

Celiac disease

Celiac disease is an immune-mediated enteropathy triggered by an inappropriate T cell-mediated response to ingested gluten and its component gliadin. Clinical and experimental studies suggest that altered intestinal barrier function might play an inciting role in the development of celiac disease by allowing gliadin to cross the intestinal barrier and activate the immune system. Patients with celiac disease have enhanced intestinal permeability and altered TJ morphology, and these disruptions persisted in asymptomatic patients who were on a gluten-free diet.^{202,203,239,240} There are data demonstrating that the increased intestinal permeability exists prior to disease onset, and this suggests that permeability might play an inciting role in the development of celiac disease. For example, a significant proportion of healthy first-degree relatives of patients with celiac disease also have increased intestinal permeability.²⁴¹ Consistent with these observations, inbred Irish Setter dogs, which spontaneously have a gluten-sensitive enteropathy similar to human celiac disease, have increased intestinal permeability.²⁴² Notably, the increase in permeability was present prior to gluten exposure and disease onset, and when animals reared on a gluten-free diet were first exposed to gluten, they immediately developed disease. These findings suggest that altered barrier function is a predetermining factor in celiac disease susceptibility.²²⁰

The environmental triggers for celiac disease, gluten and its toxic component gliadin, have been well studied, and both have been shown to directly stimulate zonulin production and induce an increase in intestinal permeability.^{202,203,239,240} Under physiologic circumstances, the intestinal epithelium is, for the most part, impermeable to gluten and gliadin; however, patients with celiac disease have been found to have compromised TJ integrity and enhanced paracellular permeability, which could allow gliadin to cross the intestinal barrier and activate the immune system. Gliadin regulates intestinal barrier function in part through the upregulation of zonulin, and gliadin has been found to bind to the CXCR3 receptor on intestinal epithelial and initiate a MyD88-dependent release of zonulin.²⁴³ Incubating human intestinal epithelial monolayers or biopsy specimens from patients with celiac disease with gliadin stimulated zonulin secretion and an increase in epithelial permeability.¹⁹³ Clinical studies have also demonstrated a positive correlation between increased intestinal permeability and intestinal zonulin levels

in patients with active celiac disease.¹⁹⁴ Furthermore, antagonism of zonulin prevented gliadin-induced permeability changes in intestinal biopsy specimens from patients with celiac disease.¹⁹³

Mechanistically, zonulin binds to the zonulin receptor on intestinal epithelial cells and induces a PKC-mediated rearrangement of the cytoskeleton, downregulation of ZO-1 and occludin, and disruption of TJ complex integrity, increasing epithelial permeability.^{193,244} In line with the *in vitro* and *ex vivo* studies demonstrating that zonulin alters ZO-1 expression, additional studies have demonstrated decreased expression of ZO-1 and redistribution of F-actin in patients with celiac disease.²⁴⁵ Remarkably, when patients eliminated gluten from their diet, the celiac disease went into remission, normal intestinal permeability was reinstated, and the abnormalities in ZO-1 and F-actin expression were reversed.²⁴⁶ In addition, celiac disease also clearly has a genetic component, as evidenced by 95% of patients with celiac disease being HLA-DQ2 positive.²⁴⁷ However, a role for this in barrier function is yet to be defined.

Type I diabetes

It is hypothesized that a combination of predisposing genetics, dysregulated intestinal barrier function, and aberrant immune responses play an inciting role in type I diabetes. Increased intestinal permeability has been reported in patients with type I diabetes at disease onset and is believed to facilitate increased exposure to antigens that can trigger autoimmune destruction of the insulin-producing pancreatic β cells.²⁴⁸⁻²⁵⁰ Additionally, ultrastructural examination of duodenum from diabetic patients revealed altered TJ structure and an increase in the paracellular space between epithelial cells and cells from healthy control subjects (Table II).²⁵¹ Studies with the BioBreeding diabetes-prone rat (BBDP), an inbred line in which autoimmune diabetes spontaneously develops when weaned onto a normal diet, supports a role for altered intestinal barrier function in the pathogenesis of type I diabetes. BBDP rats have been found to have increased intestinal permeability associated with decreased expression of the TJ protein claudin-1 before the onset of insulinitis and clinical diabetes.^{103,252} In support of this, a recent study examined barrier function in diabetic patients at various stages of disease progression. Intestinal permeability was increased in all diabetic groups; however, prediabetic subjects had the greatest increase, suggesting that increased intestinal permeability precedes the onset of clinical diabetes.^{103,252}

Similar to celiac disease and IBD, increased intestinal zonulin production is a potential mechanism leading to enhanced intestinal permeability in patients with type I diabetes. Experimental studies with prediabetic BBDP rats demonstrated increased intestinal zonulin secretion, which coincided with altered barrier function and preceded the development of autoantibodies.¹⁹⁵ Notably, treatment of the rats with a zonulin receptor antagonist reconstituted normal barrier function and abrogated disease development.¹⁹⁵ A recent clinical study examining patients with type I diabetes and their first-degree relatives found significantly higher serum zonulin levels in diabetic patients, which correlated with the degree of intestinal barrier dysfunction.¹⁹⁶ Increased serum zonulin levels were also found in 70% of prediabetic relatives, who were classified based on the presence of positive autoantibodies in the absence of clinical disease.¹⁹⁶ Taken together, these studies suggest that

an abnormal upregulation of zonulin can induce an increase in paracellular intestinal permeability that could facilitate the development of autoimmune diabetes.

Stress-induced barrier dysfunction and disease exacerbation

Psychologic and physical stress can induce a variety of changes in normal gastrointestinal function, including changes in gut motility and permeability, as well as alterations in ion, fluid, and mucus secretion and absorption.²⁵³ Animal models of acute and chronic stress demonstrate that stress induces changes in intestinal barrier function. Administration of acute, cold-restraint stress induced an increase in transcellular and paracellular intestinal permeability in rats.²⁵⁴ Electron microscopic examination revealed an increase in the number and size of HRP-containing endosomes in enterocytes from stressed rats compared with those from control animals; additionally, HRP was found within the paracellular spaces of epithelial cells in intestines from stressed rats but not in those from control rats.²⁵⁴ The increased intestinal permeability induced by immobilization stress has also been associated with a temporary redistribution of TJ proteins, including occludin and ZO-1 (Table II).¹⁹⁷ Experimental analyses have demonstrated the stress-induced permeability changes are mediated by mast cells, cholinergic and adrenergic nerves, and corticotropin-releasing hormone.¹⁹⁸ Studies have demonstrated that stress-induced permeability and ion secretion changes are attenuated in mast cell-deficient animals or after mast cell depletion or stabilization.¹⁹⁹⁻²⁰¹

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

SUMMARY

Dysregulation of the intestinal barrier has been associated with chronic immune diseases, including food allergy, IBD, and celiac disease. Whether intestinal epithelial barrier function is a primary causative factor in the predisposition to disease development remains unclear; however, clinical and experimental evidence supports a role for intestinal epithelial barrier dysfunction in disease pathogenesis. Recent experimental studies have identified a role for a number of exogenous factors, including bacterial pathogens and components of innate and adaptive immunity, in the regulation of intestinal barrier function. Understanding the interactions between innate and adaptive immunity and intestinal barrier function will provide important insight into the pathogenesis of inflammatory and autoimmune diseases. Furthermore, delineation of the molecular pathways involved in the regulation of intestinal barrier function will have important clinical implications both for the treatment and prevention of chronic

inflammatory disease, as well as the development of therapeutic agents targeted at modulating intestinal barrier function that could be useful for immunotherapy and drug and vaccine absorption.

What do we know?

- The intestinal epithelial barrier is maintained by complex protein-protein networks that form desmosomes, AJs, and TJs.
- Alterations of TJ protein formation and distribution, destabilization, or both of the TJ complexes lead to intestinal epithelial barrier dysfunction.

Intestinal barrier function is modulated by

- the immune system, including the T_H1 -cytokine IFN- γ , the T_H2 cytokines IL-4 and IL-13, TNF- α , T cells, mast cells, and eosinophils;
- ingestion of alcohol or NSAIDs; and
- enteric pathogens directly and through the elaboration of toxins and proteases.

Altered intestinal barrier function and increased intestinal permeability is associated with

- food allergies;
- IBD;
- celiac disease; and
- type I diabetes.

What is still unknown?

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

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