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REVIEW

Animal models for studying epithelial barriers in neonatal necrotizing enterocolitis, inflammatory bowel disease and colorectal cancer

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ABSTRACT

The intestinal epithelial cells line the luminal surface of the entire gastrointestinal tract which is crucial for the absorption of nutrients and prevention of pathogens entering from the external environment. The epithelial barrier plays an important role in organ development, disease pathogenesis, and aging. The major component of an epithelial barrier is the single columnar epithelium and tight junctions. Tight junctions are located at the most apical region of the junctional complex and contain many integral membrane proteins, such as occludin, the claudin family, and junctional adhesion molecules (JAMs). The disruption of intestinal epithelial barriers may lead to several pathophysiological conditions causing malabsorption of nutrition and chronic inflammation. In this review, we provide an update on the alterations of epithelial barriers associated with gut diseases using experimental animal models; we appraise the role of tight junctions in neonatal necrotizing enterocolitis (NEC), inflammatory bowel disease (IBD), and colorectal cancer; we also compare some common features as well as differences and similarities in the pathophysiology of intestinal inflammation in neonatal (NEC) and adult (IBD) gut.

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Introduction

The intestinal defense mechanisms can be classified as: (1) physical barriers including tight junctions (TJs) and adherens junctions (AJs) between intestinal epithelial cells; (2) proteins such as mucin and IgA; (3) immune cells/mediators including leukocytes, lymphocytes, macrophages, and eosinophils; and (4) other factors (e.g., gastric acidity, intestinal peristalsis, normal microbiome).[1] The intestinal epithelial cells provide the primary defensive line that protects the human body from massive pathogen invasion. TJs are dynamic structures that connect neighboring epithelial cells and form continuous intercellular contacts between epithelial cells which create a dynamic barrier to the paracellular movement of water, ions, and molecules. TJs also support paracellular flux (a passive process driven by concentration gradients and limited by molecule size) and prevent pathogen invasion.[2]

Immediately after birth, the gastrointestinal tract is essential for life. Defective TJs are an important pathogenic factor that contributes to various intestinal diseases, such as necrotizing enterocolitis (NEC), inflammatory bowel disease (IBD), colorectal cancer (CRC) and others.[1-4] NEC is the most common gastrointestinal emergency in the neonatal ICU, while IBD and CRC are the common adult gastrointestinal diseases without a cure so far. To date, the exact biologic role of TJs remains unclear and this necessitates the precise identification of defective TJ factors that may contribute to the above-mentioned intestinal diseases. However, evidence from human tissues and animal models indicates that the primary event involves TJ disruption and therefore TJs become a possible therapeutic target for the treatment of intestinal diseases. This review summarizes recent TJ findings as related to major intestinal diseases and their animal models. The major differences and similarities in the pathophysiology of neonatal NEC and adult IBD are also discussed.

Animal models with altered expression of different TJ proteins

TJs, also known as occluding junctions or zonulae occludens, seal adjacent epithelial cells in a narrow

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belt-like band at the apical ends of their lateral membranes. TJs consist of the transmembrane protein occludin, claudins, tricellulin, junctional adhesion molecule (JAM), the peripheral protein zonula occludens 1–3 (ZO-1, ZO-2, ZO-3), and cingulin.[5] The formation of functional TJs plays a critical role in the maintenance of gut permeability and intestinal barrier function. Occludin is a transmembrane protein that provides structural support for the TJ.[6] Claudins are a large family of 27 isoforms that regulate paracellular permeability.[1] Claudins within the gastrointestinal tract show selective expression whereby only claudin-6, −16, −19, −22 and −24 are not expressed in rodent intestinal epithelium as indicated by RT-PCR.[7] Claudins also show selective expression in human intestinal epithelium. For example, claudin-13 is not expressed in human intestinal epithelium but is present in rodent intestinal epithelium.[7] Although several claudin genetically-engineered mice (e.g., claudin-1, −2, −5, −7, −11, −14, −15, and −16) currently exist, only claudin-1, −2, −7, and −15 genetically-engineered mice demonstrate intestinal mucosa abnormalities.[8-12] The epithelial cell adhesion molecule (EpCAM) is a TJ-associated protein which contributes to the formation of functional TJs and recruitment of claudins.[13] The genetically-engineered TJ mouse models that show a disruption of the epithelial barrier in intestines are listed in Table 1 and below.

Occludin knockout mouse

Occludin is a tetra-spanning membrane protein and is the first TJ integral membrane protein discovered.[6] The long cytoplasmic domain of occludin associates with ZO proteins which provide direct linkage to the actin cytoskeleton.[14] This linkage participates in

Table 1. Genetically-engineered mice models with altered TJ protein expression.

Animal	Gene alteration	GI phenotypes	Other phenotypes	References
Occludin$^{-/-}$	Global occludin deletion	Chronic gastric inflammation, no significant anomaly in intestinal epithelium	Growth retardation, brain calcification, testicular atrophy, salivary gland dysfunction, and compact bone weakening	[15, 16]
JAM-1$^{-/-}$	Global JAM-1 deletion	Normal epithelial architecture but increased leukocyte infiltration, increased mucosal permeability		[28]
Tie-2-Cre-JAM-1$^{-/-}$	Endothelial/haematopoietic -specific JAM deletion	Similar phenotypes as JAM-1$^{-/-}$		[29]
Cldn1$^{-/-}$	Global claudin-1 deletion		Dehydration, die on postnatal d 1	[33]
Cl^{-}1Tg	Villin-claudin-1 transgenic mice (Intestinal claudin-1 overexpression)	Increased intestinal epithelial proliferation, inhibited goblet cell differentiation		[9]
APC-Cldn1	APC^{min} mice crossed with Villin-claudin-1 transgenic mice	Enlarged colon tumor size, reduced mucosal defense related genes	Decreased mouse survival rate	[34]
Cldn2$^{-/-}$	Global claudin-2 deletion	Reduced intestinal permeability for Na^+ and K^+, enhanced experimental colorectal inflammation		[10, 44]
Cl^{-}2TG	Villin-claudin-2 transgenic mice (Intestinal claudin-2 overexpression)	Protected against experimental colitis, increased colonocyte proliferation		[46]
Cldn7$^{-/-}$	Global claudin-7 deletion	Intestinal inflammation, mucosal ulcerations	Salt wasting, dehydration, and growth retardation, die within 12 d	[11]
cCldn7$^{-/-}$	Intestinal claudin-7 deletion	Colon inflammation	die within 28 d	[48]
Cldn15$^{-/-}$	Global claudin-15 deletion	Mega-intestine, reduced intestinal permeability for Na^+ and K^+		[12, 44]
EpCAM$^{-/-}$	Global EpCAM deletion (LoxP sites are inserted into exon 2 and 3)	Intestinal hemorrhage, obstruction and perforation. Down-regulation of claudins 2, 3, 7, and 15 and impaired TJ barrier function	Most die within 10 d	[13]
EpCAM$^{\beta geo/\beta geo}$	Global EpCAM deletion (LoxP sites are inserted into intron 1 and 3)	Same as EpCAM$^{-/-}$	Same as EpCAM$^{-/-}$	[13]
EpCAM$^{\Delta 4/\Delta 4}$	Universal EpCAM exon 4 deletion (LoxP site at exon 4)	Congenital tufting enteropathy, blunting of the intestinal villi, intestinal permeability defects	Neonatal lethality and growth retardation. Die within 7 d	[54]
mTrop1/EpCAM	Global mTrop1/EpCAM deletion (Gene-trapping method)	Intestinal tufts, villous atrophy, colon crypt hyperplasia	Neonatal lethality and growth retardation, die within 4 d	[55]

apoptosis as the Rho signaling pathway connects the extracellular apoptotic signal from occludin and then elicits the reorganization of the actin cytoskeleton.[15] A number of animal model studies indicate that occludin does not contribute to the structural assembly of the TJ proteins.[16-18] Global occludin knockout mice (occludin$^{-/-}$) demonstrate no significant anomaly in either TJ morphology or TJ barrier function in intestinal epithelium. However, global occludin knockout mice (occludin$^{-/-}$) show growth retardation, chronic gastric inflammation and epithelial hyperplasia, brain calcification, testicular atrophy, salivary gland dysfunction, and compact bone weakening.[16] These findings were confirmed by another occludin knockout mouse model developed years later.[15] Furthermore, an in depth study showed that occludin deletion promotes the differentiation of mucus cells but reduces the production of parietal and chief cells, which suggests that occludin acts in gastric epithelial differentiation and in the homeostasis of the gastric environment.[15] Although occludin$^{-/-}$ mice show no detectable TJ barrier dysfunction, occludin deficiency significantly reduces the resistance to pathological conditions.[18] Occludin$^{-/-}$ mice fed with increasing amounts of ethanol for one month demonstrated not only elevated paracellular permeability of intestinal epithelium, but also the redistribution of other junctional proteins (e.g., ZO-1, E-cadherin, β-catenin). In addition, colon morphology was severely disrupted in occludin$^{-/-}$ mice on an ethanol diet versus wild type mice on an ethanol diet.[18] Moreover, occludin gene ablation in embryonic stem cells does not prevent these cells from forming a polarized epithelium with functional TJs. Taken together, these studies suggest that occludin is not required for TJ formation. But instead, occludin acts as a gate for the intestinal barrier and provides the structural support to prevent pathogen invasion. In this regard, a decreased expression of occludin usually occurs in Crohn's disease, ulcerative colitis, and celiac disease.[3,19]

JAM-1 knockout mouse

Junctional adhesion molecule (JAM) is a single transmembrane protein first found in the immunoglobulin superfamily.[20] The JAM family consists of JAM-1 (JAM-A/F11R), JAM-2 (JAM-B, VE-JAM, hJAM-2 or mJAM-3), JAM-3 (JAM-C, hJAM-3 or mJAMM-2), JAM-4, and JAM-L.[21] JAM-1 and JAM-2 are predominantly expressed in lymphatic cells, endothelial and epithelial cells.[20,22] JAM-3 is predominantly expressed in leukocytes.[23] JAM-4 is predominantly expressed in kidney glomerulus and small intestinal epithelium.[24] The various functions of JAMs include TJ assembly, leukocyte transmigration, angiogenesis, and platelet aggregation.[24-26] Knockdown of JAM-1 in SK-CO15 colonic epithelial cells increases paracellular permeability, decreases cell-matrix adhesion, and decreases β-integrin on the cell surface, all of which indicates that JAM-1 critically regulates the function of the epithelial barrier.[27] Many studies using JAM-1 knockout mice have identified JAM-1 as one of the contributors in intestinal inflammatory diseases.[28-31] Global JAM-1 deletion mice (JAM-1$^{-/-}$) do not display any abnormality in epithelial morphology, but do display a disruption of epithelial barrier function. In this regard, the trans-epithelial electrical resistance in JAM-1$^{-/-}$ mice decreases whereas the intestinal epithelial permeability increases, both of which suggest JAM-1 involvement in gating the intestinal epithelial barrier.

JAM-1$^{-/-}$ mice demonstrate intestinal inflammation with mucosal leukocyte infiltration.[28] Surprisingly, the intestinal mucosa of JAM-1$^{-/-}$ mice shows reduced injury compared with wild type mice when challenged with dextran sodium sulfate (DSS). Moreover, the intestinal mucosa of DSS-challenged JAM-1$^{-/-}$ mice shows an increase in epithelial proliferation.[28] However, whether the above-mentioned observations are due to a direct effect of JAM-1 deletion in intestinal epithelium or other cell types remains unclear since JAM-1 is also highly expressed in endothelial cells and leukocytes.[20] To elucidate the role of JAM-1 in intestinal epithelial cells specifically, the endothelial and haematopoietic-specific JAM inactivation mice (Tie-2-Cre-JAM-1$^{-/-}$) were created and challenged with DSS along with JAM-1$^{-/-}$ mice and wild type mice.[29] In DSS induced experimental colitis, JAM-1$^{-/-}$ mice develop massive leukocyte infiltration, accelerated colitis, a high level of inflammatory cytokines, and an increased mortality rate compared with Tie-2-Cre-JAM-1$^{-/-}$ mice and wild type mice. However, Tie-2-Cre-JAM-1$^{-/-}$ mice do not show any significant difference in DSS challenging compared with wild-type mice upon DSS challenge.[29] These data suggest that JAM-1 regulates the integrity and permeability of the intestinal epithelial barrier, which relies on JAM-1 expression exclusively in intestinal

epithelium, not in endothelium or haematopoietic cells. This indicates that JAM-1 plays an essential role in the maintenance of intestinal homeostasis.

Claudin-1 knockout/ knockin mouse

Claudin-1 and −2 were first discovered in the claudin family by Furuse et al.[32] A global knockout of claudin-1 mouse (gCldn1$^{-/-}$) model was created by Furuse et al. and their studies collectively showed that claudin-1 is a key regulator of epidermal barrier function. However, gCldn1$^{-/-}$ mice die on postnatal d 1 due to dehydration probably caused by a severely compromised epidermis.[33] A villin-claudin-1 transgenic mouse (Cl−1Tg) model was created by Pope et al. that specifically overexpresses claudin-1 in intestinal epithelium.[9] Claudin-1 overexpression not only promotes intestinal epithelial proliferation, but also inhibits goblet cell differentiation via modulation of the Notch signaling pathway.[9] An APC-Cldn1 mouse model was also created by Pope et al by crossing villin-claudin-1 transgenic mice with APC^{min} mice (a common intestinal tumorigenesis mouse model).[34] The claudin-1 overexpression in APC-Cldn1 mice results in enlarged colon tumor size, reduced mucosal defense related genes (*atoh1, muc2, muc3, muc4, tff3*), and a decreased mouse survival rate compared with APC^{min} mice. In addition, the expression of Notch- and Wnt-signaling pathways related genes (i.e., *Tcf4, Lef1, Fzd10, Axin2, Wnt6, Wnt10, Mmp9*) significantly increases.[34] This study indicates that claudin-1 promotes intestinal tumorigenesis by modulating the Notch- and Wnt-signaling pathways. However, the role of claudin-1 in intestinal cancer remains controversial. For example, a recent clinical study showed low claudin-1 serum levels in colorectal cancer patients.[35] Colorectal cancer patients with high claudin-1 expression levels have a relatively better prognosis.[36] Nevertheless, numerous studies still support the notion that claudin-1 overexpression leads to intestinal tumorigenesis.[37-39]

Claudin-2 knockout/ knockin mouse

Unlike other claudin proteins, claudin-2 forms a leaky paracellular junction in the intestinal epithelium allowing the trans-epithelial movement of sodium, calcium and fluid.[40-42] Claudin-2 localizes to both crypt cells and villus cells in the small intestine, but localizes only to undifferentiated crypt cells in the colon.[43] A global knockout of claudin-2 mouse model showed that claudin-2 deletion decreases intestinal permeability for Na^+, but claudin-2 is not the indispensable determiner since claudin-15 deletion further decreases luminal Na^+ in the adult small intestine.[44] Claudin-2 upregulation (which associates with a leak flux) occurs in inflammatory bowel disease (IBD), intestinal infections, and patients with diarrhea.[45] Interestingly, claudin-2 downregulation induces intestinal inflammation. The decreased expression of claudin-2 enhances the expressions of IL-6, IL-1β and myosin light chain kinase (MLCK).[10] In cldn2$^{-/-}$ mice, TNFα-induced colorectal inflammation via NF-κB signaling activation is enhanced and MLCK expression level is increased compared with that of control mice.[10] On the other hand, claudin-2 overexpression in villin-induced claudin-2 transgenic mice (Cl−2TG) induces a resistance to intestinal injury as Cl−2TG mice are protected from DSS-induced colitis. Claudin-2 protects the intestinal mucosa from injury by suppressing immune cells and promoting colonocyte proliferation.[46] These studies suggest that claudin-2 contributes to intestinal disease formation, but claudin-2 may not be the determining factor.

Claudin-7 knockout mouse

Claudin-7 holds the position as the most dominant claudin protein in the entire intestine from duodenum to colon.[47] Claudin-7 localizes to both the apical and basolateral regions of the intestinal epithelium.[11] Our studies have shown that claudin-7 deletion in mice disrupts the integrity of the intestinal epithelium and causes a severe intestinal defect. The role of claudin-7 is best illustrated in global claudin-7 ablation mice (gCldn7$^{-/-}$) which demonstrate inflammation, mucosal ulcerations, and epithelial cell sloughing along the entire intestine although the TJ structure remains due to the presence of other TJ components.[11] gCldn7$^{-/-}$ mice also show significantly increased levels of matrix metalloproteinase-3 (MMP-3), an enzyme that degrades the extracellular matrix.[11] However, intestinal-specific deletion of claudin-7 mice (cCldn7$^{-/-}$) only develop inflammation in the mouse colon and demonstrate a small intestine phenotype that resembles wild type mice. A bacterial product called N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP; 438 Da) initiates colonic inflammation.[48] gCldn7$^{-/-}$ mice die within 10 d whereas cCldn7$^{-/-}$ mice die

within 28 d which indicates that claudin-7 is crucial in maintaining the intestinal epithelial integrity and disruption of claudin-7 is life threatening. These findings taken together highlight the position that a global claudin-7 deletion (gCldn7$^{-/-}$) and an intestinal-specific claudin-7 deletion (cCldn7$^{-/-}$) cause differences in the pathogenesis and molecular mechanisms associated with intestinal abnormalities.

Claudin-15 knockout mouse

Claudin-15 is found along the entire length of the mouse intestine whereby claudin-15 is strongly expressed in the duodenum and jejunum and weakly expressed in the ileum and colon.[47] The claudin-15 expression pattern shows a developmental dependency from infancy to adulthood in the mouse intestine. In the infant mouse intestine, claudin-15 is only detected in the TJs of the crypts, while in the adult mouse intestine, claudin-15 is detected in the TJs of both villi and crypts. Claudin-15 deletion promotes proliferation of intestinal cryptic cells, which leads to mega-intestine but without presentation of severe intestinal disruption.[12] Global claudin-15 ablation mice (gCldn15$^{-/-}$) are born, grow normal, and show no alteration of any other claudin proteins. However, gCldn15$^{-/-}$ mice show aberrantly low luminal Na^+ concentrations and glucose malabsorption in the adult small intestine using paracellular flux studies.[44] Interestingly, an increased claudin-15 expression in the intestines of lactating rats accompanies an increased absorption of Na^+, Ca^{2+}, K^+, and nutrients.[49] The above-mentioned findings suggest that claudin-15 plays a prominent role in the paracellular permeability of Na^+ and glucose absorption and also in the regulation of intestinal epithelium proliferation. In addition, claudin-2 and −15 double knockout mice also display defects in paracellular Na^+ flow and reduced absorption of glucose, amino acids, and fats in the gut and die by postnatal d 25 from malnutrition.[50]

EpCAM knockout mouse

Epithelial cell adhesion molecule (EpCAM; also known as CD326, TACSTD1, or TROP-1) is a 40 kDa transmembrane glycoprotein originally identified as a tumor marker since high EpCAM expression levels associate with cancers of epithelial origin while low EpCAM expression levels associate with normal simple epithelia.[51-53] EpCAM localizes at cell–cell junctions, including TJs. EpCAM mutations were found to cause congenital tufting enteropathy (CTE) using single nucleotide polymorphism (SNP) homozygosity mapping of CTE patients.[54] CTE is a severe, life threatening inherited disorder of the small intestine that presents with watery diarrhea, dehydration, electrolyte imbalance, and metabolic decompensation in the early few d of life. EpCAM knockout mouse models have been created using Cre-LoxP recombination technology. In this regard, the EpCAM$^{\beta geo/\beta geo}$ mouse model possesses 2 loxP sites inserted into intron 1 and 3 while the EpCAM$^{-/-}$ mouse model possesses 2 deletions at exon 2 and 3.[13] In addition, the EpCAM$^{\Delta 4/\Delta 4}$ mouse model was generated from a construct lacking exon 4 in EpCAM and the mTrop1/EpCAM–null knockout mouse model was generated using mTrop1 gene trapping technology.[55,56] All of the above-mentioned knockout mice do not survive for more than 10 d. EpCAM$^{-/-}$ mice demonstrate a downregulation of claudins −2, −3, and −15, with claudin-7 undetectable.[13] As a whole, the above-mentioned findings suggest that EpCAM plays a role in formation of functional TJs and recruitment of claudins.

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal ICU and the second most common cause of morbidity in premature infants.[51] The incidence of NEC correlates to low gestational age and low birth weight with a >20% incident increase in <500 g birth weight infants. The incidence of NEC also correlates with enteral feeding since 90–95% of NEC premature infants were previously enteral fed. In this regard, accumulating evidence points to breast feeding and human breast milk as NEC protective. Unfortunately, a high NEC mortality rate (up to 40%) persists despite recent advances in neonatal medicine.[19,52-55]

NEC characteristically presents with apnea, bradycardia, feeding intolerance, increased gastric residuals, abdominal distension, and bloody stools. These symptoms may progress rapidly to erythema, ecchymosis of the abdominal wall with intestinal perforation, peritonitis, and systemic hypotension which require intensive medical support.[57] NEC pathology results primarily from intestinal coagulation necrosis with major histological findings that include mixed acute

and chronic inflammation, ulceration, hemorrhage, and transmural bland necrosis. Other findings include mucosal edema, bacteria overgrowth and pneumatosis intestinalis. The specific findings vary and depend upon the progression of the disease and the presence of underlying pathogenic factors.[58]

Although the exact etiology is still unclear, multiple risk factors leading to NEC have been identified, such as prematurity, enteral feeding, ischemic insult and bacterial colonization. These risk factors all lead to a common outcome called bowel coagulation necrosis. How these risk factors interact and initiate NEC remains under study.[58,59] However, current studies suggest that an intrinsic imbalance exists between anti-inflammatory and pro-inflammatory mediators in the premature infant. In this regard, ischemic mucosal injury, enteral feeding, or bacteria would favor uncontrolled inflammation, mucosal permeability, and diffuse apoptosis in the premature infant because the defense and repair mechanisms of premature infants are still underdeveloped and dramatically weaker compared with older children and adults.[58] Based on the risk factors and pathophysiology of NEC, animal models were created by inducing prematurity, enteral feeding, ischemic insult, or bacterial colonization. However, the creation of NEC animal models that closely mimic *in vivo* infant NEC remains challenging since NEC is a multifactorial and complex disease. Presently, the most popular NEC animal models have been established in the rat, mouse, and piglet.[60]

Rodent necrotizing enterocolitis models

A rat NEC model was established in newborn rats fed with formula and exposed to hypoxia and hypothermia (i.e., breathing 100% nitrogen gas for 50 s followed by cold stress at 4°C for 10 min) for 7 d after delivery.[61] This newborn rat model was based on the concept that hypoxia and hypothermia produce vasculature contraction and selective circulatory ischemia, especially the mesentery circulation. Decreasing mesenteric blood flow may initiate the changes leading to necrotizing enterocolitis.[61] Another rat NEC model was established in 1-d old preterm rats fed with formula along with Escherichia coli (E. coli) and exposed to hypoxia for 3 d.[62] This 1-d old preterm rat model includes the widely accepted risk factors and shows a 70–90% NEC-inducing success rate which makes it the most reliable and widely used rat NEC model. However, feeding rat pups demand a challenging work load and therefore the protocol has been modified to simplify the experimental procedure. Hypoxic rats with or without breast-feeding show no differences in intestinal pathology/morphology which indicates that hypoxia is the most important NEC risk factor and therefore a rat NEC model can be established under a breast-feeding condition.[61,63] The rat has several advantages over the mouse in NEC studies which include a larger size that allows sophisticated surgical manipulations and collection of an ample tissue volume along with a physiology that more closely mimics the human than the mouse.

Mouse NEC models are also widely used based on the benefits of a uniform genetic background, low cost, and relative ease in genetic modification to evaluate the role of a specific gene involvement in NEC. The first mouse NEC model (a modification of the preterm rat model) was established in preterm mice fed with formula without E. coli and exposed to hypoxia and hypothermia.[64] In the preterm mouse model, the mice (66%) develop abdominal distention, cyanosis, and respiratory distress, along with gross and microscopic evidence of intestinal necrosis that resemble NEC. A second mouse NEC model was established in neonatal mice fed with formula without E. coli and exposed to hypoxia and hypothermia.[65] In the neonatal mouse model, only 43% of mice develop symptoms that resemble NEC. A third mouse NEC model was established in 10-d old mice fed with formula and exposed to hypoxia.[66] In the 10-d old mouse model, mice develop NEC symptoms after 4 d of treatment. It is important to point out that other organs, such as kidneys, were also affected in a mouse NEC model reported recently by Garg et al.[67]

Piglet necrotizing enterocolitis models

The piglet has several advantages to choose as NEC models over the rodent which include an embryonic development pattern and anatomic features that closely mimic the human. In this regard, piglet gastrointestinal maturation occurs in postnatal week 1 while human gastrointestinal maturation occurs *in utero* which suggests that a near-term piglet may be used to establish a piglet NEC model that mimics the preterm human.[68] The first piglet NEC model was established in full-term piglets exposed to hypoxia (partial

pressure in arterial oxygen less than 30% for 1 h).[69] This model was later modified to include formula feeding, hypothermia, and ischemic/reperfusion to obtain a higher NEC-inducing success rate.[70] The most popular piglet NEC model was established in pre-term piglets fed with formula.[71] In the pre-term piglet model, the piglets (> 50%) develop fatal NEC symptoms after 3–4 d of treatment. This pre-term piglet model stands noteworthy as it demonstrates that the combination of a pre-term birth and formula feeding alone can cause NEC symptoms without the need for hypoxia or hypothermia exposure. Studies using the above-mentioned animal models to investigate the pathophysiology of NEC have been well summarized in previous reviews.[14,60,70,72]

The piglet possesses several features that advantage the use of the piglet in NEC studies over the rodent which include a larger size that allows sophisticated surgical manipulations, the use of preclinical drug studies, and the development of early non-invasive diagnostic methods and clinical care. However, the piglet possesses several features that dis-advantage the use of the piglet in NEC studies over the rodent which include the high cost, special care of a piglet litter, and the lack of transgenic models. The rodent (especially the mouse) has several advantages in NEC studies due to relatively large litters, a short gestation period, lower housing and maintenance cost, and a uniform genetic background that allows for genetic manipulation. Therefore, the selection of a particular NEC animal model should take into account the special features of the animal, conditions of care, along with the cost and limitations of the experimental environment that might complicate the results.[60,72]

Disruption of TJs in NEC

Functional TJs are critical for the maintenance of gut permeability and intestinal barrier function. Preterm infants have an under-developed intestinal barrier and TJs compared to term infants.[73] Disruption of TJs in NEC animals has been reported in many cases.[74-78] However, whether the disruption of the physical/anatomic barrier is the initial step or a consecutive effect in the genesis of NEC remains unknown. Until now, studies of TJs are mostly based on the rodent NEC models (Table 2).

The first report of TJ disruption in NEC animals using 1-d old preterm rats fed with formula (no bacteria) and exposed to hypoxia and hypothermia for 3 d found that the occludin expression level increases only at the site of NEC injury while the claudin-3 expression level increases in all crypt regions in 4-d old NEC rats.[77] Epidermal growth factor (EGF) suppresses the above-observed increased occludin and claudin-3 expression levels.[77] These findings suggest that both occludin and claudin-3 are involved in NEC pathophysiology. In addition, TJ disruption in NEC animals using preterm rats fed with formula and lipopolysaccharide (LPS) without hypoxia or hypothermia are found that occludin, claudin-3, claudin-1, and ZO-1 expression levels increase in 1-d old NEC rats but not 3-d old NEC rats, which may be due to different NEC-inducing protocols.[74] LPS stimulates the production of several pro-inflammatory cytokines in intestinal epithelium including tumor necrosis factor, IL-6 and IL-8, all of which independently promote the inflammatory cascade characteristic of NEC.[79] These studies demonstrate that different NEC risk factors may lead to different outcomes in TJ disruption. ZO-1 expression level also increases in 1-d old preterm rats fed with formula (no bacteria) and exposed to hypoxia and hypothermia for 3 d. Erythropoietin prevents the loss of ZO-1 at the TJ thereby protecting the intestinal barrier.[75] Contradictory results have also been reported. Occludin, claudin-3, and ZO-1 expression levels decrease in 4-d old NEC rats when LPS feeding was combined with prolonged hypoxia started at postnatal d 1 (see Table 2).[78] This study indicates that specific NEC-inducing factors contribute to different TJ protein expression levels. Interestingly, the downregulated TJ proteins can be increased by bifidobacterium, a probiotic organism in the intestinal flora of breast-fed infants.[78] Högberg et al demonstrated that the expression of genes associated with TJs and cell adhesion is altered in early experimental NEC using newborn rats exposed to hypoxia/re-oxygenation on d 1 after delivery and then analyzed for gene expression after sacrifice on d 2.[76] They found that claudin-1, −14, and −15 expression levels decrease and claudin-8 expression level increases, suggesting a TJ disruption in NEC that involves multiple claudin isoforms.

Mouse models have also been used to explore the role of TJs in NEC. Bergmann et al documented that intestinal permeability increases at only 12 h after stress using a mouse NEC model involving hypoxia-hypothermia plus commensal bacteria inoculation. They found that occludin and claudin-4

Table 2. Changes of TJ protein expression in animal models of NEC.

Animal	Factors induce NEC				Other factor	Treatment time	TJs changes compared with control animals	References
	Preterm	Formula feeding	Hypoxia	Cold stress				
Rat	1 d	Every 5 h	100% N_2 60 sec	4°C for 10 min		96 h	Occludin↑, Claudin-3↑	[77]
Rat	1 d				LPS	96 h	Claudin-1,-3↑	[74]
Rat	1 d	Every 4 h	100% N_2 3 min, followed by 100% O_2 3 min	4°C for 10 min	LPS	72 h	Occludin↓, Claudin-1,-3↓, ZO-1↓	[78]
Rat	1 d	Every 3 h	95% N_2 10 min			5 d	Claudin-3↑	[75]
Rat			100% CO_2 10 min, followed by 100% O_2 10 min			24 h	Occludin↑, Claudin-1,-14,-15↓, Claudin-8↑ *	[76]
Mouse		Every 3 h	100% N2 for 60 sec	4°C for 10 min	bacteria mixture (adult mice)	72 h	Claudin-2↑ at 6 h#, Claudin-2↑ at 48h, Claudin-7↑ after 6 h#	[80]

Notes. *gene expression measured by microarray;
#Gene expression measured by q-PCR; All others were measured for protein levels

internalization occurs at 12 h after stress, claudin-2 expression level increases at 6 h after stress, and claudin-4 and −7 expression levels decrease at 24 h after stress. In addition, none of the pups show any histological evidence of NEC at 12 h, 1/10 pups show severe NEC at 24 h, 1/9 pups show NEC at 36 h, and 3/8 pups show NEC at 48 h. Therefore, an increase in intestinal permeability precedes NEC and associates with occludin and claudin-4 internalization.[80] The above studies provide the insight that altered components of the physical/anatomic barrier (e.g., claudins, occludin) as well as contributory inflammatory and other factors occur early in the NEC disease process before any histological changes appear. Despite numerous studies it remains difficult to reach a clear conclusion that clarifies the cause vs. the consequence. For example, do changes in claudin expression levels cause NEC? Since inflammatory mediators modify claudin expression, one may speculate that stress (e.g., hypoxia-hypothermia, etc.) increases inflammatory mediators which in turn alters claudin expression levels.[81-85] It may also be possible that changes in inflammatory mediators and claudin expression levels happen at the same time and act synergistically as part of the process leading to NEC. Although a paucity of data points to the existence of a primary defect in the components of the physical/anatomic barrier as the initial pathogenesis of NEC, accumulating evidence indicates that changes in the physical/anatomic barrier closely correlates with the development of the deadly NEC disease.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing idiopathic inflammatory disorder of the gastrointestinal tract that compromises the integrity of the intestinal epithelium. Crohn's disease (CD) and ulcerative colitis (UC) are the principal types of IBD. CD may appear anywhere from the mouth to anus with full thickness inflammation of the gastrointestinal wall, while UC occurs only in the colon with mucosal and submucosal inflammation and ulcers. Patients usually show symptoms such as severe diarrhea, pain, fatigue, and weight loss which may be debilitating and lead to life-threatening complications.[86] IBD is known as an expensive disease without a cure because of its unclear etiology and pathogenesis. However, a combination of genetic and environmental factors has been recognized as a possible cause that leads to immunological responses and inflammation in the intestine. A common disorder found in IBD is a disruption of the intestinal epithelial barrier which usually associates with decreased expression of TJ proteins in the inflamed mucosa and increased intestinal permeability. The disruption of the intestinal epithelial barrier allows the luminal contents and pathogens to be subjected to the mucosal immune system thereby triggering a more severe inflammatory reaction. Below we summarize the studies that address the significance of TJs in human and animal IBD.

Disruption of TJs in IBD patient samples

TJ disruption within the intestines has been reported in IBD patients. Occludin localizes in the apical region of an epithelial cell in normal intestine.[14] However, occludin redistributes to the basolateral region of intestinal epithelial cells and shows a decreased expression level in CD patients.[87,88] Occludin protein and mRNA expression levels decrease in UC patients vs. healthy controls. Furthermore, the occludin mRNA expression level increases following recovery and decreases in UC remission, which suggests that redistribution of occludin and decreased occludin expression level in intestinal epithelium are characteristic of IBD and that occludin is a suitable marker for IBD prognosis.[89] Similarly, ZO-1 (a TJ scaffold protein) redistributes and shows a decreased expression level in UC and CD patients.[4,87] Claudin-2 forms a leaky paracellular junction.[40] Claudin-2 upregulation is reported many times in the inflammatory intestinal epithelium in UC and CD patients.[4,5,88,90-92] The expression levels of paracellular sealing claudins differ in UC patients vs. CD patients. For example, claudin-5 and −8 downregulation occurs in the sigmoid colon of CD patients whereas claudin-1, −4, and −5 remain unchanged.[87] However, another study reported that claudin-3 and −4 downregulation occurs in the colon of CD patients.[90]

The contradictory results may be due to a different sampling location of the colon used in the experiments and the location-specific expression features of claudins. Claudin-4 and −7 downregulation occurs in active UC patients.[88,93] Although claudin-1 and −3 remain unchanged in UC patients,[88] several studies have shown that claudin-1 expression level increases and that the increased claudin-1 expression positively correlates with inflammatory activity.[91,93] Similarly,

claudin-18 upregulation occurs in UC patients (see Table 3).[94] Based on the above-mentioned studies, one may comfortably speculate that TJ disruption in intestinal epithelium is a hallmark of IBD. The changes in TJ components not only differ in UC vs. CD patients, but also display a location-specific feature. Animal models and colonic cell lines have been used to investigate the role of different molecules in IBD and to uncover the underlying IBD pathogenesis.

Disruption of TJs in IBD animal models

A large number of animal models have been created for IBD study. These models are generally divided into either genetically-modified models or chemically-induced models. The detailed description of these animal models has been summarized by Janelle et al.[95] In this review, we mainly focus on the animal models with TJ alterations.

The most common IBD animal models are chemically-induced IBD rodents. Chemicals such as dextran sulfate sodium (DSS) and Trinitrobenzene sulfonic acid (TNBS) are used as fast, economic and effective strategies to induce IBD-like intestinal injury. DSS is a water-soluble, negatively charged sulfated polysaccharide synthesized by certain bacteria from sucrose.[96] Mice fed with DSS show signs of diarrhea, gross rectal bleeding, and weight loss along with histological evidence of intestinal epithelial erosion initially and then followed by inflammation resembling UC symptoms.[97,98] The mechanism by which DSS induces intestinal inflammation is unclear but is likely the result of damage to the epithelial monolayer lining. Short-term treatment with a high dose of DSS causes acute colitis, whereas administering either a longer course of DSS at low dose, or cyclical re-administration of high doses, can induce a chronic colitis with fibrosis.[99] TJ disruptions similar to those found in human IBD intestine are found in these animal models. For example, ZO-1 downregulation and claudin-1 upregulation occur in the intestinal epithelia of DSS-induced IBD mice. More importantly, ZO-1 downregulation happens before intestinal inflammation which suggests that ZO-1 alterations are not a consequence of inflammatory disruption due to DSS-induced colitis, but instead ZO-1 alterations precede the pathogenesis of IBD.[100] Occludin and claudin-3 downregulation occurs in the intestinal epithelia of

Table 3. Alterations of TJ proteins in intestines from IBD patients, IBD animal models, and CRC patients.

	Occludin	ZO-1	Claudin-1	Claudin-2	Claudin-3	Claudin-4	Claudin-5	Claudin-7	Claudin-8	Claudin-18	Claudin-23	References
IBD patient intestines												
UC	—	↓		↑		↓						[4]
				↑	↓	↓						[90]
	—		↑	↑		↑						[91]
	—	—	↑	—	↑	↑						[92]
			↑	↑		↓		↓				[93]
										↑		[94]
CD	—	↓		↑		↓						[4]
	↓		—	↑		—	↓	—	↓			[86]
				↑	↓	↓						[90]
IBD animal intestines												
DSS-induced colitis		↓										[100]
	↓				↓							[101]
TNBS-induced colitis										↑		[94]
	↓			—								[104]
		↑										[105]
SAMP1/Yit mice	↓			↑								[109]
CRC patient intestines												
	↑	↑	↑	↑	↑	↑	↑					[112]
			↑									[116, 120, 122]
										↑		[114]
								↑				[113]
			↑			↑						[109]
						↓						[118]
			↑		↑	↑		—				[119]
								↓				[121, 124]
											↑	[123]

Note. "↑" increase; "↓" decrease; " - " no difference in the intestinal epithelium. Empty space: did not detect

DSS-treated mice and this downregulation can be elevated by somatostatin treatment (a neuropeptide in D cells).[101] Intestinal epithelial permeability significantly increases after somatostatin treatment, suggesting that somatostatin regulates TJs and may be used for therapeutic evaluation.

TNBS is a nitroaryl oxidizing acid that acts as a hapten. A single high dose of TNBS generates an acute inflammatory response, whereas cyclical re-administration leads to a delayed-type hypersensitivity reaction. Long-term administration of low dose TNBS results in chronic colitis that resembles human CD.[102] It has been reported that TNBS-induced mouse colitis share the similar pathogenesis involving NOD2, a key CD susceptibility gene.[103] Claudin-18 upregulation occurs in intestinal epithelia of both IBD patients and TNBS-treated mice[94] as does occludin[104] and ZO-1, −2, −3 downregulation.[105] In addition, claudin-1, −4, −7 expression levels remain unchanged in intestinal epithelia of TNBS-treated mice which comports with the findings in CD patients. Interestingly, pore-forming claudin-2 upregulation occurs in colonic epithelia of CD patients; however, claudin-2 levels remain unchanged after TNBS treatment which indicates that claudin-2 may function differently in TNBS-induced colitis.[104]

Genetically-modified IBD animal models that present with primary TJ disruption include the interleukin 10-deficient mouse (IL-$10^{-/-}$) and the SAMP1/Yit mouse. IL-10 is an anti-inflammatory cytokine. Mice administrated with total parenteral nutrition show increased intestinal paracellular permeability and decreased levels of occludin and ZO-2, which is reversed by IL-10 treatment.[106] IL-$10^{-/-}$ mice develop colitis spontaneously after 12 weeks of age and show an increase in ileal and colonic permeability at 2 weeks of age before exhibiting histologic signs of intestinal inflammation.[107] These observations suggest that IL-10 has a role in the protection of the intestinal barrier. Increased intestinal permeability occurs as a primary defect before the onset of mucosal inflammation suggesting that disruption of the intestinal permeability may be an important etiology in the development of colitis in IL-$10^{-/-}$ mice. However, the mechanism of how IL-10 affects the intestinal permeability is still unknown.

SAMP1/Yit mouse is another CD animal model. The SAMP1/Yit mouse is a model of IBD in which its histologic features closely resemble CD and the injury resides in the ileum.[108] This mouse model is ideal for the investigation of therapeutic approach for ileitis as the ileal phenotype occurs spontaneous without genetic, chemical or immunological manipulation and displays a time course of pathogenesis as the disease progress.[108] The SAMP1/Yit mouse model mimics CD and shows a primary defect in TJs. Occludin downregulation and claudin-2 upregulation together with increased paracellular permeability occur in intestinal epithelia of the SAMP1/Yit mouse. These changes happen before the onset of inflammation at the early stage of the disease which suggests that epithelial defects are the primary cause of SAMP1/Yit induced IBD.[109]

Regulation of TJs in IBD

The expression of TJ proteins is disrupted in both human and animal IBD models and can be regulated to improve intestinal epithelial paracellular permeability. In this context, vitamin D reduces claudin-1 and −2 expression levels and elevates claudin-4 and −7 expression levels in UC patients, which is accompanied with a decrease in inflammatory cytokines IL-6 and IL-13.[94] IL-6 causes claudin-2 upregulation through the PI3K signaling pathway in mice and Caco-2 cells (colonic adenocarcinoma epithelial cells).[5] Similarly, IL-13 also causes claudin-2 upregulation in T84 (colonic adenocarcinoma epithelial cells) which can be abolished by blocking the PI3K signaling pathway using LY294002.[88] These findings demonstrate that inflammatory cytokines regulate claudin-2 in a PI3K signaling pathway-dependent manner. The idea that the regulation of TJ protein expression may provide a therapeutic target to treat IBD inspires further investigation into the role of TJs in the disease process. Accumulated evidence reveals that signaling pathway molecules regulate the integrity of TJs. In this regard, IL-9 reduces claudin-1 expression level but elevates occludin, claudin-4 and −7 expression levels.[104] STAT5b modulates the zonula occluden family and NF-κB activation to maintain the integrity of the colonic epithelial barrier.[105] IFNc/TNFα reduces claudin-2 and −3 expression levels in T84 cells.[88] TNFα elevates the claudin-1 expression level in IEC-18 cells (rat ileum epithelial cells).[110] In conclusion, studies concerning TJ regulations remain limited in animal models

so that the proof of its potential clinical application requires further investigative studies.

Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer worldwide. The classic cancer type is colorectal adenocarcinoma - a malignant epithelial tumor that originates from superficial glandular epithelial cells lining the colon and rectum.[111] Altered expression of various TJ proteins in intestinal epithelium during CRC tumorigenesis has been reported.[112-118] Accumulated data revealed that the protein levels of claudin-1, −3, −4, −18 and −23 increase whereas the protein level of claudin-7 decreases in colorectal tumor tissues vs. normal control tissues.[39,114-117,119-123] In addition, occludin, ZO-1, ZO-2, claudin-1, −2, −3 and −5 gene expression levels increase, whereas JAM-2, claudin-7, −8, −15 gene expression levels decrease in human CRC vs. normal control tissues (see Table 3).[112,115,119,124]

TJ disruption in CRC patient samples

Claudin-1 plays a major role in CRC tumorigenesis based on numerous studies that show elevated claudin-1 protein and mRNA levels.[39,112,115,122] Claudin-1 is the most extensive studied target of TJs in CRC. Claudin-1 expression level not only changes in CRC, but claudin-1 also acts in multiple intracellular signals. For example, claudin-1 expression level particularly increases in metastatic colorectal cancer. Smad4 (a tumor suppressor protein in human CRC) down-regulates claudin-1 in human colorectal carcinoma samples.[125] In addition, claudin-1 down-regulates E-cadherin expression by up-regulating ZEB-1 expression in SW480 and SW620 colonic adenocarcinoma cell lines. ZEB-1 is a transcriptional repressor of the E-cadherin gene. The loss of E-cadherin expression indicates an epithelial to mesenchymal transition (EMT) and association with cell invasion and metastasis. Claudin-1 promotes cell growth and proliferation through Wnt and phosphotidylinositol-3-kinase/Akt signaling pathways and allows a cell to avoid anoikis through ZEB-1 and in a Src-Akt-Bcl^{-}2-dependent manner.[39,126] TNF-α (pro-inflammatory cytokine) regulates claudin-1 to promote an EMT in HT-29 cells. TNF-α treated HT-29 cells exhibit elevated claudin-1, activation of ERK1/2 and Src signaling, and increased tumorigenic tendency. Interestingly, knockdown of claudin-1 in HT-29 cells abolishes this phenomenon.[38] Based on these studies, claudin-1 may serve not only as a CRC marker but also as a therapeutic target to regulate tumorigenesis. Although many studies have indicated that elevated claudin-1 promotes CRC, the matter remains somewhat controversial. In contrast to studies that indicate claudin-1 acts as a tumor promoter through EMT, other studies suggest that low claudin-1 expression level associates with a poor CRC prognosis and metastasis. Nakagawa et al. compared the prognosis of 119 patients with high and low claudin-1 expression levels that underwent CRC surgery. They reported that patients with low claudin-1 expression level show poorer overall survival and disease-free survival vs. patients with high claudin-1 expression level. Moreover, claudin-1 knockdown significantly increases cell invasiveness in 3 human CRC cell lines (DLD-1, HCT116 and LoVo).[36] Similarly, a low claudin-1 expression level associates with CRC recurrence after surgery and high grade CRCs.[127] Based on the concept that claudin-1 is altered in CRC, claudin-1 may serve as a tumor marker for CRC diagnosis. Serum claudin-1 levels were significantly lower in non-metastatic and metastatic CRC patients vs. healthy controls in a study of 140 CRC patients. The low serum claudin-1 levels were accompanied with high CEA levels, a known CRC tumor marker.[35] These controversial data indicate that the correlation of claudin-1 with the prognosis of human CRC and the molecular mechanisms that regulate CRC are still poorly understood.

Common features of NEC and IBD – a pathophysiological comparison

Although IBD is an adult chronic gastrointestinal disorder and NEC is a neonatal acute gastrointestinal condition, the two diseases are similar in that their pathophysiology involves altered mucosal defenses, disruption of commensal bacteria, and an inappropriate immune response.[128]

First, in NEC, intestinal defenses are compromised as the neonatal gastrointestinal tract is not fully developed, especially in immature neonates. Neonatal gut is vulnerable to pathogens because of the decreased immunoglobulin A (IgA), the immaturity of the intestinal barrier and mucus layer. Although the gastrointestinal tract in adult is fully developed, TJ proteins are disrupted in the intestinal epithelium of IBD patients as previously discussed. Mucus layer is

thinner and discontinuous in UC yet thicker in CD comparing to healthy subjects.[129,130] Hence, the intestinal defenses are also compromised in IBD. Low level of IgA increases the risk of bacteria and virus attachment to mucous membrane. Bacteria and bacteria products normally restricted to the lumen in the healthy intestine may translocate across the mucosa into the systemic circulation in the compromised intestine. Bacterial components, such as lipopolysaccharides (LPSs), peptidoglycans and flagellins, can bind to immune cells, leading to over-activation of neutrophil, macrophages and monocytes et al, thus stimulating the secretion of inflammatory cytokines and causing tissue damage.

Second, gut microbiota is essential for maturation of normal immune function and host defense. Immediately after birth, bacteria start to colonize in the gastrointestinal tract, which promotes the intestinal maturation. Breast milk contains probiotics that suppress pathogenic bacterial colonization, modulate the immune system through increased IgA or cytokine regulation, and regulate the intestinal barrier. Studies have shown that gut microbiota in breast-fed infants are dominated by the probiotics bifidobacteria and bacteroides, while formula-fed infants are dominated by streptococci, staphylococci and lactobacilli.[131] Bifidobacteria is able to stabilize claudins and maintain the TJ barrier in a mouse NEC model.[80] In addition, dysbacteriosis is commonly observed in NEC. For example, colonization of Clostridium butyricum, C. neonatale, C. proteolyticum, and uropathogenic Escherichia coli strains are detected in the feces of NEC patients.[132,133] Based on the fact that formula feeding is one of the risk factors for NEC, formula feeding is commonly used to create experimental animal models.[75,77,78,80,134] In the induced piglet NEC, ileal mucosa is characterized by the increased number of Streptomyces spp., Leptolyngbya spp, Clostridium butyricum, C. neonatale, and C. proteolyticum.[134] These studies suggest that dysbacteriosis is a highly significant risk factor for development of NEC. Dysbacteriosis is also a major contribution factor to IBD. It presents as a lack of probiotics and a reduced diversity of microbiota, which allows the overgrowth of pathogenic bacteria to induce an excessive inflammatory response. A 30–50% less diversity of microbiota has been associated with active CD and UC, respectively.[135] A lower number of gram positive and a higher number of gram negative bacteria than in healthy subjects have been reported in IBD patients. Depletion of Faecalibacterium and Bifidobacterium and over-growth of Escherichia coli are reported in several studies.[135,136] These studies demonstrate that dysbacteriosis is a common phenomenon in both NEC and IBD. Although the bacterial species are different in neonatal and adult guts, they share common probiotics such as bifidobacterial and over-grown bacteria such as E. Coli et al.

Third, both NEC and IBD are associated with an inappropriate immune response. LPS and other bacterial toxins can activate the host immune response in both NEC and IBD. Immune cells respond to LPS in the lamina propria and stimulate a pro-inflammatory reaction by secretion of cytokines and inflammatory mediators. IL-6, IL-8, IL-12, IL-18, tumor necrosis factor α (TNF-a), interferon gamma (IFNγ), platelet-activating factor (PAF), reactive oxygen species (ROS) and nitric oxide synthase (NOS) are elevated in NEC patients.[137] IL-10 and IL-11 are thought to be the anti-inflammatory cytokines. IL-10 knockout mice exacerbate the severity of NEC and intraperitoneal administration of IL-10 has been shown to decrease the severity of intestinal injury.[138,139] A high level of IL-11 in 21 neonatal NEC patients presents a lower rate of developing pan necrosis.[140] In IBD, studies have shown that IL-1b, IL-6, IL-8, IL-12, IL-18, IL-23, TNF-a, IFNγ, ROS, nitric oxide, and leukotrienes trigger inflammation and lead to an aberrant immune and inflammatory response in the intestine.[5,83,136,141] Among these cytokines, IL-6 increases TJ permeability by stimulating the expression of channel-forming claudin-2.[5] IFNγ decreases the protein level of occludin and ZO-1 in T84 cells.[141] Similar as in NEC, IL-10 is generally considered to be an anti-inflammatory cytokine in IBD. IL-10-deficient mice spontaneously develop intestinal inflammation and are used as one of the experimental animal models for colitis.[142] Recombinant IL-10 treatment of IBD animals has been shown to significantly inhibit both antigen presentation and subsequent pro-inflammatory cytokine (IL-1b, TNF-α, IL-6) release, therefore, reducing the intestinal inflammation.[143]

A systematic comparison of the gene expression in the ileum of neonatal NEC with adult active CD is analyzed using RNA-Sequence. The data show that 60% of the significant altered signaling pathways identified in NEC are also identified in CD. At the individual gene level, 175 (21.8% of the total) of the genes

altered in NEC also appear to be significantly altered in adult CD.[144] These genes are most involved with immune functions, such as altered T and B cell signaling, B cell development, and the role of pattern recognition receptors for bacteria and viruses. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) mutation is the first genetic risk factor that is involved in the development of CD.[145] NOD2 activates the NF-κB pathway and regulates the secretion of pro-inflammatory and protective molecules that maintain the homeostasis of intestinal epithelium. A study assessing the risk of NOD2 variant prevalence in NEC was performed in 10 NEC patients. No mutations of NOD2 was found in these NEC patients. It is concluded that NOD2 does not play a major role in genetic susceptibility to NEC.[146] However, in another study, NOD2 mutation is genotyped on 9082 very low body weight infants for a prospective, population-based cohort study. It is found that very low body weight infants carrying ≥ 2 NOD2 variant alleles have an increased risk for NEC.[147] The difference here may be due to the size of the research subjects and experimental design.

Until now, there is no further evidence indicating that the genetic risk factor significantly associated with NEC has been found. NEC is often very aggressive and the mortality rate is up to 40%. To prevent or treat NEC, we need to develop an early diagnostic tool allowing for the identification of risk factors for neonates. Given the fact that NEC and IBD share many similarities in pathophysiology and genetic expression, studies targeting IBD may offer clues for better understanding of NEC, and vice versa.

Conclusion and future work

The TJ is a crucial determinant of intestinal mucosal barrier function. The functional importance and alteration of TJs are well established in human NEC, IBD and CRC. However, the mechanism of TJ dysregulation is still largely unknown. Currently, most of the studies of TJs in IBD and CRC are established in human intestinal tissues and cell lines. Human IBD and CRC colonic tissues are easily accessed because of the high disease occurring rate. Since many IBD and CRC mouse models have been well established,[95,128] further studies targeting the mechanism of distinct effects of TJs and modulation of TJs for therapeutic purposes will be applied to the animal models with NEC, IBD and CRC.

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