REVIEW ARTICLE

Intestinal Epithelial Barrier Function and Necrotizing Enterocolitis

Elizabeth Managlia[1], Xiaocai Yan[2], Isabelle G De Plaen[3]

Abstract

Necrotizing enterocolitis (NEC) is a major cause of morbidity and mortality in premature infants. NEC is characterized by intestinal tissue inflammation and necrosis. The intestinal barrier is altered in NEC, which potentially contributes to its pathogenesis by promoting intestinal bacterial translocation and stimulating the inflammatory response. In premature infants, many components of the intestinal barrier are immature. This article reviews the different components of the intestinal barrier and how their immaturity contributes to intestinal barrier dysfunction and NEC.

Keywords: Intestinal barrier, Necrotizing enterocolitis, Preterm neonate.

Newborn (2022): 10.5005/jp-journals-11002-0003

Introduction

Necrotizing enterocolitis (NEC) is a disease affecting the gastrointestinal (GI) tract of premature infants thought to result from an immature immune system, impaired microvasculature development, and an impaired mucosal barrier. In this review, the different components of the intestinal barrier and their functions are discussed (Fig. 1), with emphasis on how these are affected by the NEC disease process and by factors and interventions known to protect against NEC. The GI tract is a highly vascularized organ where the exchange of water and nutrients occurs via a single layer of epithelial cells. Precise regulation of the gut barrier function is essential to maintain the critical balance between its absorptive function and its role at preventing potentially harmful digestive enzymes, bile acids, and bacteria present in the lumen to get into the tissues. Breakdown of the intestinal barrier in neonates is thought to be a critical step in the development and the progression of NEC.

In human neonates, intestinal permeability to sugar decreases during the first week of life.[1] This is more pronounced in breastfed newborns, compared to those that are fed formula,[1] suggesting a beneficial role of breast milk on the intestinal barrier. The intestinal barrier has been shown to be impaired during NEC in humans[2] and animals.[3,4] We showed that the intestinal permeability is increased in response to NEC-inducing stresses prior to the development of intestinal injury in a mouse model of NEC,[5] which suggests that alterations of the intestinal barrier may play a role in NEC pathogenesis. In adults, an increase in intestinal permeability has been shown to precede Crohn's disease[6] and its relapse,[7] and is thought to contribute to the disease. In premature infants, several components of the intestinal barrier are immature and therefore may predispose them to NEC.

The Mucus Layer

The Mucus

The intestinal epithelium is protected against harmful luminal bacteria and toxins by a thick gelatinous mucus layer secreted by specialized epithelial cells called goblet cells. In the colon, while bacteria reside and thrive in the outer mucus layer,[8] the inner mucus layer is physically impenetrable to bacteria.[9] However, in the small intestine, the mucus layer is loose, unattached, and can easily be penetrated by microorganisms.[10] To decrease the risk of infection, the small intestinal mucus layer is regularly flushed by a process consisting of liquid secretion and motor activity. The migrating motor complex generated by the enteric nervous

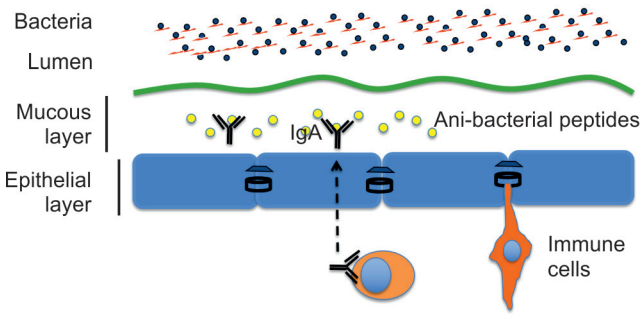

Fig. 1: Components of the intestinal barrier

[1–3]Division of Neonatology, Department of Pediatrics, Ann and Robert H Lurie Children's Hospital of Chicago, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, United States; Center for Intestinal and Liver Inflammation Research, Stanley Manne Children's Research Institute, Ann and Robert H Lurie Children's Hospital of Chicago, Northwestern University, Chicago, Illinois, United States

Corresponding Author: Isabelle G De Plaen, Division of Neonatology, Department of Pediatrics, Ann and Robert H Lurie Children's Hospital of Chicago, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, United States; Center for Intestinal and Liver Inflammation Research, Stanley Manne Children's Research Institute, Ann and Robert H Lurie Children's Hospital of Chicago, Northwestern University, Chicago, Illinois, United States, Phone: +312-503-7179, e-mail: isabelledp@northwestern.edu

How to cite this article: Managlia E, Yan X, De Plaen IG. Intestinal Epithelial Barrier Function and Necrotizing Enterocolitis. Newborn 2022;1(1):32–43.

Source of support: This article was supported by a NIH grant award DK116568 (IGDP).

Conflict of interest: None

system likely allows the loose mucus to migrate to the colon.[10] The mucus is composed of mucins, lipids, and water. Mucins are large glycoproteins made of a central protein backbone rich in proline, threonine, and serine which are *O*-glycosylated with large glycans.[11] These glycans cannot be digested by digestive enzymes thus protecting the central mucins from degradation by endogenous proteases. Mucins are produced, stored, and released by goblet cells. Once secreted, gel-forming mucins lubricate and protect the gastrointestinal tract. While Muc2 is the main secreted mucin in the small intestine,[12] others are MUC5AC, MUC5B, MUC6, and MUC7.[10] As opposed to secreted mucins, membane-bound mucins (MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, and MUC17) have a transmembrane domain that enables them to be anchored into the cell membrane. These play a role in protection, apical cell surface sensing, and signaling.[10]

In the fetus, Muc2 mRNA is expressed at 12 weeks of gestation in the jejunum, ileum, and colon.[12] Muc2 was found to be rapidly synthesized in the small intestine of preterm infants who have undergone an enterostomy for necrotizing enterocolitis.[13] While Muc2 developmental regulation is not fully known, a few studies suggest that its deficiency may play a role in NEC. In the intestinal tissue samples of patients with NEC, the number of mucus-containing small intestinal goblet cells is decreased compared to age-matched control samples.[14] In a neonatal rat NEC model, Muc2 mRNA and protein have been found to be decreased as well as the number of Muc2 positive cells when compared to dam fed animals.[15] In immature but not mature mice, TNF injection resulted in the loss of mucus-containing goblet cells but induced Muc2 and Muc3 mRNA upregulation in the mature ileum.[14] Muc2 deficient mice spontaneously develop colitis.[16] Several recent studies correlate increased Muc2 production with decreased NEC severity.[17–20] In addition, in premature neonates, the immaturity of the enteric nervous system and of the migrating motor complexes[21] may delay the normal migration of the bacteria-containing mucous layer from the small intestine to the colon. Both of these mechanisms may increase the risk of bacterial product translocation through the intestinal mucosa and contribute to NEC pathogenesis.

Antibacterial Products, Enzymes, and Soluble Factors

The mucus layer not only limits the diffusion of toxins but allows the generation of a gradient of antibacterial products secreted by Paneth cells and enterocytes.[10] Paneth cells secrete different antibacterial products such as alpha-defensins, cathelicidins, lysosyme, and secreted phospholipase A2 (sPLA2). The production of Paneth cell antimicrobial peptides is affected by the composition of the microbiota[22] and increases with age.[23–26] Paneth cells reside at the base of the crypt in close contact with the epithelial stem cell compartment. The potent cocktail of antimicrobial products produced by paneth cells is therefore thought to play a role in the protection of these vital cells from bacterial invasion. Mice lacking Paneth cells have increased bacterial translocation[27] possibly predisposing them to the development of NEC. Conflicting reports exist on the number of Paneth cells in NEC patients.[28–30] As antibacterial peptide detection is used to identify Paneth cells and immature Paneth cells do not yet produce antibacterial peptides, discrepencies in the number of these cells reported in the literature may ultimately be due to a variable degree of maturation of this cell population or to degranulation of the Paneth cells during the NEC process. NEC typically occurs in the neonatal period when these peptides are not being produced at high levels thus supporting the premise that decreased Paneth cell differentiation and maturation may be a predisposing factor for NEC. Interestingly, Paneth cell metaplasia and increased expression of the paneth cell product alpha-defensin upon recovery from NEC has been reported.[30] A recent study suggests that Paneth cells may play an important role in NEC as immature mice (P14-16) treated with the zinc chelator dithizone to ablate Paneth cells develop NEC-like disease when infected with *Klebsiella pneumoniae*.[31]

The alpha-defensins (called cryptdins in mice) are the most abundant antimicrobial peptides made by Paneth cells. These are produced in an inactive form and converted into an active peptide after cleavage by proteases such as matrilysin (also called MMP-7). Active defensins are able to permeabilize gram-positive and gram-negative bacterial cell membranes. Altered alpha-defensin expression has been shown in NEC.[29]

Cathelicidins and beta-defensin are antimicrobial peptides produced by different cell types including epithelial cells and several populations of immune cells such as neutrophils, NK cells, B cells, and monocytes. They differ in structure from the alpha-defensins but have similar cationic amphipathic properties and are also effective against gram-positive and gram-negative organisms. In addition, they present chemotactic activity for neutrophils, monocytes, and T cells. In mice, their expression is extremely high during the neonatal period and decreases with maturity concomitant to an increase in Paneth cell maturity and antimicrobial peptide production. In a rat model of NEC, treatment with human beta-defensin-3 improved NEC and promoted mucosal integrity by reducing inflammatory mediators and reduced autophagy-activated proteins.[32] Furthermore, rats treated with Bifidobacterium increased beta-defensin-2 which provided protection from NEC.[33] However, whether deficient production of these antimicrobial peptides contributes to NEC remains unknown.

Lysozyme is a highly cationic protein and enzyme which cleaves β-1-4 glycosidic bonds of gram-positive bacteria. This causes the destabilization of the bacterial peptidoglycans leading to cell lysis. Gram-negative bacteria are resistant to this mechanism due to their outer shell that encases their peptidoglycan layer. Lysozyme has been shown to be absent in the Paneth cells of newborn infants with NEC[28] and may play an important role in preventing bacterial invasion in the neonatal intestine.

Secreted phospholipase A2 (sPLA2) is another antimicrobial protein produced by Paneth cells. It acts on gram-positive bacteria through a mechanism that hydrolyzes phospholipids. Group II phospholipase A2 is developmentally regulated after birth and was found to increase from D15 to D21 in the neonatal rat ileum.[34] The expression of sPLA2 has been shown to be increased in a neonatal mouse NEC model.[35] Phospholipase A2 is an enzyme critical for the production of platelet-activating factor (PAF),[36] which has been shown to mediate intestinal injury[37] and experimental NEC.[38]

Intestinal alkaline phosphatase (IAP) is a brush border enzyme produced by intestinal epithelial cells. It is expressed in decreasing concentrations from the duodenum to the ileum. IAP has multiple functions including the regulation of lipid metabolism, the regulation of bicarbonate secretion, as well as the detoxification of bacterial lipopolysaccharide (LPS).[39] By dephosphorylating the Lipid-A moiety of LPS, IAP prevents its interaction with toll-like receptor 4 (TLR-4). The level of IAP expression has been found to be decreased in NEC.[40] Furthermore, IAP supplementation in experimental NEC models decreased the severity of the disease, attenuated the systemic inflammatory response,[41] and increased barrier function with upregulation of claudins-1, and -3, as well as

occludin and ZO-1 following treatment.[42] In the stool of premature infants with NEC, relative IAP content was increased but had biochemical dysfunction.[43]

Trefoil Factor 3 (TFF3), like Muc2, is an important protein secreted by goblet cells. TFF3 is thought to play a role in the maintenance of the mucus layer and surface integrity by facilitating mucin polymerization.[44] Furthermore, TFFs play a significant role in epithelial cell restitution following injury promoting enterocyte migration, proliferation, and survival.[45] Mice lacking TFF and subjected to experimental DSS colitis die of complications due to impaired restitution.[46] Large amounts of TFF3 are present in breastmilk[47] and TFF3 expression has been found to be decreased in NEC tissues.[48] Treatment of mice with recombinant human TFF3 during experimental NEC reduced inflammation which suggests a protective role of TFF3 in NEC.[49]

IgA molecules are transported from the basolateral to apical epithelial surface and secreted. These antibodies act in the mucus layer to inhibit attachment of microorganisms to the epithelial cells. In term neonates, the synthesis of secretory IgA is very low and takes 2 weeks or more after birth for normal production.[50] Colostrum is rich in IgA.[51] Breast milk from mothers of preterm infants was found to have higher IgA compared to those of term infants.[52] Breastmilk-derived IgA has been shown to shape the host-microbiota relationship of preterm neonates[53] and pups reared by IgA-deficient mothers are more susceptible to experimental NEC, suggesting that IgA is critical for preventing NEC in a mouse model.[53]

Lactoferrin is an iron-binding protein present in breast milk and in most exocrine fluids such as tears, saliva, bile, and pancreatic secretions.[54] Lactoferrin provides protection against bacterial translocation via several mechanisms: (1) by binding iron which is necessary for bacterial growth; (2) via the toxic effect of its metabolite, lactoferricin, which disrupts gram-negative bacteria cell membranes; (3) by binding microbe-associated molecular pattern (MAMP) such as endotoxin, CpG, flagellin, and secondary inflammation; (4) by promoting the growth of probiotics;[55] (5) by promoting intestinal epithelial cell proliferation.[56] Lactoferrin concentration is very high in human colostrum.[57] Oral lactoferrin prophylaxis has been found to reduce the incidence of late-onset sepsis in infants weighing less than 1500 g.[58] Recent work describes its effect on neonatal myeloid cells in their conversion to myeloid-derived-suppressor-cells, thus blocking intestinal inflammation and experimental NEC.[59] While large-scale randomized clinical trials are needed, current evidence does not support a protective effect against NEC of exogenous lactoferrin when given alone.[58–60]

The Epithelial Layer

Paracellular Permeability

Paracellular permeability is the passage of molecules across intercellular structures. Indeed, adjacent cells of the intestinal epithelial barrier are secured by several complex structures that are named, from luminal to basolateral side, the tight junction complex, the adherens junction complex, and the desmosome (Fig. 2). Besides a recent study showing that desmoglein-2, a component of desmosome, is increased with increased NEC severity in a pig model,[61] no current data exist on desmosomes in NEC. Detailed studies looking at tight junction and adherens junction complexes have been performed and are discussed below.

The Tight Junction Complexes

Intercellular tight junctions are protein structures formed on the apical surface between adjacent cells of the epithelial barrier which regulates the passage of water, ions, and large solutes across the epithelium via the paracellular pathway (Fig. 3).[62,63] Tight junctions are made of several structural and functional proteins which regulate their function, such as occludin and claudins.[64] Their extracellular domains interact with the proteins on the adjacent cell membrane while their cytoplasmic tails associate with scaffold proteins called zonula occludens (ZO-1, ZO-2, and ZO-3).[65] These protein complexes associate with a variety of kinases and cytoskeletal proteins such as actin and myosin to regulate barrier function. The apical surface of the epithelial cell is circled by a belt of actin and myosin. Upon phosphorylation of the light chain of myosin (MLC) by activated myosin light chain kinase (MLCK), the actin-myosin ring contracts leading to tight junction reorganization and increased paracellular permeability.[66] Several inflammatory mediators such as TNF and IL-6 have been found to increase paracellular permeability.[67,68] TNF for example has been shown to activate MLCK.[69] During human development, TJ complexes have been detected in the fetal intestine as early as 10 weeks of gestation.[70] We have shown that pups exposed to an experimental NEC protocol have TJ restructuring with increased apical-to-basal tight junction length prior to the development of intestinal injury.[5]

The claudin family of proteins has been shown to control charge selectivity, and ion and small molecule permeability[64,71] and mutations in claudins have been shown to disrupt paracellular transport.[72] Several members of the claudin family are expressed in the intestine. While the role of claudins 7, 12, and 15 is unclear, claudins 1, 3, 4, 5, and 8 have been shown to tighten the barrier or decrease permeability. In contrast, claudin-2 is pore-forming and its expression leads to increased permeability. In mice, the gene expression of the intestinal claudins has been shown to be developmentally regulated.[73] In neonatal rat pups, hypoxia/reoxygenation induced the downregulation of claudin 1, 14, and 15 and the upregulation of claudin 8 and of the gap junction protein, beta 3.[74] In a neonatal rat model of NEC, the expression of claudin 3 and occludin has been found to be increased at 96 hours in the intestine of stressed pups compared to controls.[3] This may be a compensatory mechanism of the intestine to re-establish its barrier function. Indeed, in neonatal mice, intestinal claudin 3 expression has been found to increase in the first 2–3 weeks after birth and this increase to be associated with a decreased in intestinal barrier permeability.[75] Claudin 3 increase was dependent upon bacterial colonization.[75] In humans, when measured in the urine, claudin 3 has been shown to be a useful diagnostic marker for the detection of NEC in infants.[76] In a neonatal mouse NEC model, we found that claudin 2 expression is increased at 6 hours while claudin 4 and 7 are decreased at 24 hours.[5] Claudin 2 protein is increased in the intestine of neonatal mice submitted to a NEC protocol for 48 hours and in human NEC tissues compared to controls.[5] Interestingly, claudin 2 is also increased in the intestinal tissues of patients with inflammatory bowel disease[77,78] and to be positively correlated with inflammatory activity.[78] IL-6 has been shown to increase the expression of claudin 2, thus increasing TJ permeability.[79] Whether the increase in claudin 2 is injurious or serves as a compensatory protective mechanism remains to be determined. Indeed, claudin 2 increases paracellular channel-like permeability to monovalent cations such as sodium.[80] The resulting absorption of nutrients such as glucose and amino acids may prevent malnutrition

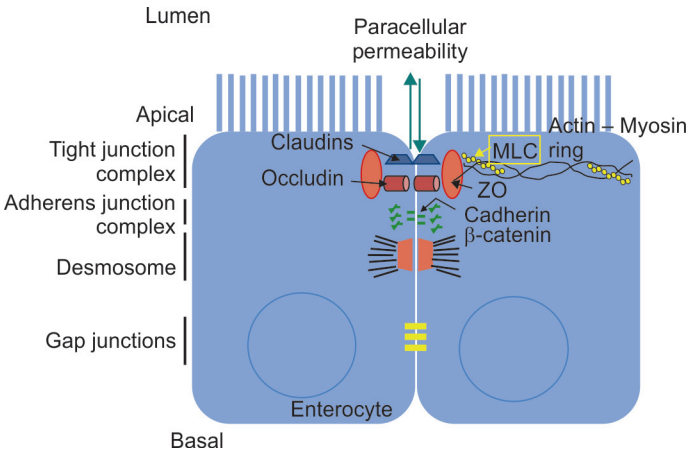

Fig. 2: Intercellular structures playing a role in paracellular permeability

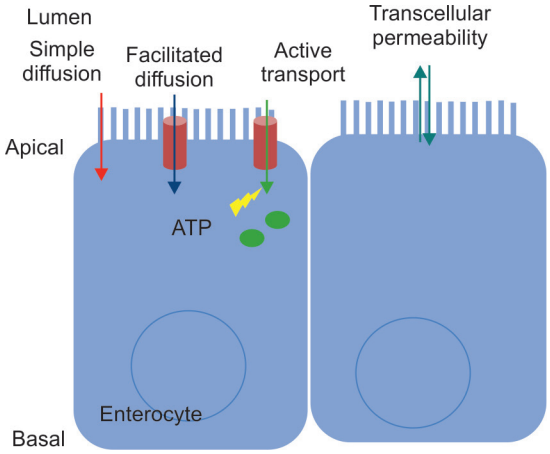

Fig. 3: Transcellular mechanisms responsible for transcellular permeability

during a time of stress, and therefore may be protective. Another potential protective mechanism may be, by increasing intestinal water loss, help flushing pathogens from the intestinal lumen. The distribution of TJ proteins is altered during several disease processes.[77,81] In patients with ulcerative colitis, claudin 4 association with TJs has been noted to be lost in the colonic epithelium.[77] In a neonatal rat model of NEC, the association of ZO-1 with TJ has been found to be lost at day 5.[82] In neonatal mice, we found that claudins 2, 4 and occludin are densely localized to TJ structures and claudin 7 is mainly associated with enterocyte lateral membranes.[5] When neonatal mice were exposed to a NEC protocol for 12 hours, which is a time when injury has typically not yet occured, the association of claudin 4 with TJ structures was markedly decreased, and occludin and claudins 4 were mainly found in the cytoplasm.[5] Furthermore, administration of *Bifidobacter* (*B.) infantis* preserved claudin 4 and occludin distribution at TJ, significantly attenuated stress-induced intestinal permeability and NEC in a mouse model.[5]

The Adherens Junction Complexes

The adherens junction complexes are made of transmembrane epithelial cadherin (E-cadherin) proteins and cytosolic proteins named alpha and beta-catenin. E-cadherin forms homophilic interactions with the neighboring cell thus conferring bonds that are necessary for epithelial strength and support. Additionally, adherens junctions are critical in directing cell polarity along the apical-basolateral axis.[83] While their role in NEC is not known, these proteins have been shown to be differently localized in the intestine of pups submitted to a NEC protocol compared to dam fed controls,[15] and this change was prevented by the inoculation of the probiotic *Bifidobacterium bifidum*.[15] E-cadherin expression has been shown to be decreased in human NEC and experimental NEC, where it was shown to be internalized.[84] Also, the cytoskeletal protein vinculin, known to be associated with adherens junctions, has been found to be decreased in formula-fed neonatal mice compared to dam-fed controls.[85]

Transcellular Permeability

The intestinal epithelium has multiple transport mechanisms for molecules to gain entry into the cellular compartment (Fig. 3). Depending on the specific molecules envolved, it can occur by simple diffusion through the cell membrane, by diffusion through channels and pores, by facilitated diffusion utilizing transport proteins, or by osmosis. Passive transport is driven by concentration gradients. In contrast, active transport is able to move molecules against a concentration gradient and requires the use of energy. Large particles or macromolecules can gain entry into a cell by an active transport process consisting of endocytosis which may or may not be receptor mediated. Transcytosis is the transcellular

transport process that involves endocytosis followed by vesicular transport across the cell to the opposite membrane where exocytosis occurs. Transcytosis thus provides a route of entry from the intestinal lumen to the underlying lamina propria. This process is particulary important in the transport of maternal IgG to the infant, conferring immunologic protection prior to the maturity of their own adaptive immune system.[86] In addition, many pathogens are known to exploit this mechanism leading to dysfunction of the epithelial barrier,[87] and *E. coli* transcytosis has been hypothesized to be an initiating event in necrotizing enterocolitis.[88] Alpha-haemolysin from *E. coli* has been shown to induce focal leaks in colonic epithelial cells which causes increase bacterial translocation.[89] Several strains of commensal bacteria and probiotics have been shown to increase TJ proteins at the cell boundaries and in some cases prevents or reverses the adverse effects of pathogens.[90] In addition, many nutrients have been shown to impact barrier permeability.[90] Luminal antigens that reach the lamina propria have the potential to initiate an immune response. This response includes the production of inflammatory cytokines that may further facilitate transcellular permeability. Specifically, TNF can increase endosomal uptake and enhance transcellular transport.[91] Also, interferron-γ)(IFNγ) which has been shown to play an important role in NEC[92] enhances transcytosis of macromolecules.[93] Increased transcytosis of luminal molecules occurs in conjunction with tight junction reorganization and increased paracellular permeability[91] and barrier function may be affected through alterations of both transcellular and paracellular transport.[94]

Although epithelial cells are able to capture antigens and microbes, transcellular transport is mainly thought to occur at the level of M-cells (M for microfold) which cover isolated lymphoid follicles or Peyer's patches.[87] M-cells are specialized epithelial cells of the follicle-associated epithelium. They take up antigens and microorganisms from the intestinal lumen via transcytosis and present them to dendritic cells and other immune cells such as macrophages and lymphocytes. As their glycocalyx (protective outer cell layer composed of glycoproteins and glycolipids) is thinner than enterocytes, M-cells constitute a functional opening in the intestinal mucosal barrier.[95] During inflammation, there is increased apoptosis of M cells that may contribute to the breakdown of the intestinal barrier.[96] However, not much is known about the developmental maturation of M cells and their potential role in NEC.

Epithelial Cell Layer Integrity

The single layer of epithelial cells is a highly regulated barrier. The cells are replaced approximately every 4–5 days through a process of proliferation, differentiation, migration, and apoptosis. This process is initiated by rapidly cycling epithelial stem cells situated at the base of the small intestinal crypt. Signaling events instruct these newly created immature transit amplifying cells to differentiate into one of four main cell types of the small intestine. Enterocytes are the most numerous and perform the absorptive functions of the barrier. Paneth, goblet, and enteroendocrine cells are of secretory lineage. Paneth cells migrate downward surrounding the stem cells. They are relatively long lived, with a turn-over time of 57 days in mice.[97] The other cell types migrate upward toward the villus tip where they eventually undergo apoptosis and are replaced. During villous tip shedding, MLC gets phosporylated and tight junction reorganization occurs. This process is vital to the normal turnover of enterocytes and does not compromise the barrier.[98] However, when this process is impaired, tight junction function may be affected, impacting barrier permeability. To maintain the integrity of the intestinal epithelial layer, the proliferation and differentiation of intestinal stem cells, and the migration and apoptosis of intestinal epithelial cells are tightly regulated and synchronized.[99] Upregulated apoptotic rate increases permeability and bacterial translocation.[100] Unbalanced epithelial proliferation and apoptosis may be a contributing factor in the loss of the intestinal barrier function and in NEC development. Indeed, NEC has been associated with a decrease in intestinal epithelial cell proliferation and migration and with an increase in intestinal epithelial cell apoptosis.[101] LPS, the ligand of TLR-4, has been shown to play an important role in NEC[102,103] and to inhibit enterocyte migration via increased expression and function of the adhesion molecule alpha 3- and beta-1 integrin[104] and via autophagy.[105] Also, TLR4 expressed on intestinal stem cells regulates enterocyte proliferation and apoptosis and may contribute to the pathogenesis of NEC.[106] Indeed, TLR4 activation has been shown to inhibit beta-catenin signaling via GSK3 β activation thus reducing enterocyte proliferation.[107] An inhibitory interaction between TLR4 and NOD2 signaling in enterocytes leads to the regulation of enterocyte apoptosis and NOD2 may have a protective effect on NEC.[108]

Nonepithelial Influences

Immune Cells and Inflammatory Mediators

Other cell types may affect intestinal barrier permeability indirectly. Indeed, during inflammation, activated macrophages inhibited enterocyte migration and mucosal healing via the release of nitric oxide[109] and the activation of RhoA.[110] IFNγ inhibits enterocyte migration by impairing enterocyte gap junctions, which are intercellular channels composed of connexin43 (Cx43) monomers. Mesenchymal stem cells have been shown to enhance the viability and proliferation of human fetal intestinal epithelial cells following hypoxic injury via paracrine mechanisms.[111]

In the intestine, dendritic cells and CX3CR1^{+} macrophages maintain direct contact with epithelial cells through dendrites that extend from the mucosa to the lumen to sample antigens in the external environment. These cells express tight junction proteins allowing them to span the intercellular space while maintaining an intact barrier.[112,113] The close proximity of epithelial and immune cells facilitates the cytokine and chemokine signaling necessary to initiate an immune response upon a barrier breach.

While the exact mechanism is unknown, the interaction of inflammatory cells with tight junctions may contribute to the TJ restructuring and barrier dysfunction seen in diseases such as NEC. Activated dendritic cells and macrophages secrete a variety of cytokines, which are increased in human and experimental NEC.[114,115] Many of these factors are essential for an appropriate immune response but may also have a negative impact on barrier function. Specifically, TNF at high doses has been shown to induce apoptosis and shedding due to a major redistribution of the tight and adherens junction structures.[116] Low-dose TNF in contrast does not induce cell shedding yet impacts barrier permeability through MLCK activation and endocytosis of occludin.[117] IL-1 β is also known to increase tight junction permeability in an MLCK and NF-κB-dependent pathway without causing apoptosis[118,119] and IL-6 has been shown to increase intestinal permability via the upregulation of claudin 2 mRNA and protein expression both *in vitro* and *in vivo*.[79,120]

During inflammation, effector cells are recruited to the site of injury via the secretion of chemokines such as CCL20 and CCL2 by epithelial and innate immune cells. Recruitment of dendritic cells through the CCL20/CCR6 axis was shown to be responsible for intestinal epithelial damage in a model of NEC.[121] Recruited

T cells secrete Th1 cytokines such as IFNγ and the Th2 cytokine IL-13. Epithelial cells respond to IL-13 by upregulating claudin 2 and thereby increasing permeability.[77] IL-13 also induces epithelial cell apoptosis and decreases proliferation, causing epithelial microerosions and bacterial translocation.[114,122] IFNγ has been shown to increase the intestinal epithelial permeability to macromolecules via the Src kinase pathway. In addition, IFNγ was found to synergize with TNF to induce barrier dysfunction.[123]

Many pro-inflammatory cytokines are under the control of the transcription factor NF-κB, which has been shown to be a major effector molecule in NEC.[124] NF-κB is essential for signaling in both epithelial and immune cells. TNF, IL-1β, and TLR ligands activate the NF-κB signaling pathway and can trigger amplification of the immune response. Also, NF-κB is known to protect the cell against apoptosis and inhibition of NF-κB specifically in epithelial cells leads to a loss of barrier function by inducing apoposis and bacterial translocation.[125] While increased intestinal permeability may be necessary to induce intestinal injury in experimental NEC, it is not sufficient as we found that blocking NF-κB activation in monocytes prevented against NEC without impacting intestinal permeability.[126]

Cytokines produced by immune cells can alter barrier function not only by causing tight junction structure alteration, but also by altering transcytotic mechanisms and by causing apoptotic leaks and mucosal gross lesions.[122]

Commensal Bacteria and Probiotics

Commensal bacteria and probiotics upregulate TJ proteins and may prevent the adverse effects of pathogens on intestinal barrier.[90] The beneficial effect of probiotics such as *Lactobacillus acidophilus* and *B. infantis* in human NEC[127] may be mediated by its effect on intestinal permeability. In human preterm neonates, supplementation of formula with *Bifidobacterium lactis* decreased intestinal permeability at day 30 of life.[128] In a neonatal mouse model of NEC, *B. infantis* has been found to attenuate the increase in intestinal permeability observed at 24 hours and to decrease the incidence of NEC.[5] In this same model, *B. infantis* preserved claudin 2, 4 and occludin integrity at TJ structures and claudin 7 at lateral membranes.[5] *In vitro*, when T84 cells (human colon carcinoma cell line) were treated with *B. infantis* conditioned medium, the expression of claudin 4, ZO-1 and occludin was increased, claudin 2 was decreased and the IFNγ-induced rearrangement of occludin and claudin 1 was prevented.[129] Also, *B. bifidum* reduces intestinal epithelial cell apoptosis in a neonatal rat NEC model.[130] Conditioned medium from *B. infantis* has been found to decrease apoptosis and maintain epithelial cell proliferation in a model of neonatal intestinal inflammation induced by *Cronobacter sakazakii*.[131]

Amniotic Fluid

During development, the intestinal epithelium is exposed to a diversity of bioactive molecules present in the amniotic fluid such as growth factors, which promote mucosal morphogenesis, and cytokines, which have immunomodulatory and anti-inflammatory properties.[132,133] These molecules have protective effects on barrier function, and therefore, postnatal enteral administration of amniotic fluid has been hypothesized to protect against NEC.[134,135] Amniotic fluid has been shown to increase cell migration, proliferation, and cell survival *in vitro* and these effects were dependent on PI3Kinase and were reproduced by HGF treatment.[136] Stem cells isolated from the amniotic fluid have been shown to improve enterocyte cell survival and enhance repair of damaged intestine in NEC via a COX-2 dependent mechanism.[137] Furthermore, isolated amniotic stem cells restored tight junction protein expression in mice.[138]

Breast Milk

Preterm infants who received the majority of feeding as human milk had significantly lower intestinal permeability when compared to infants receiving minimal or no human milk.[139] Breast milk contains many molecules such as trefoil factor[47] and lysozyme[140] that may improve intestinal permeability and protect the neonatal intestine against injury. Several components of breast milk such as hyaluronan 35kD,[141] lactadherin,[61] TIMP-1,[142] and exosomes[143] have been shown to protect TJ proteins. Also, breast milk oligosaccarides protect against NEC by inhibition of TLR4 signaling[144] and were also found to interact with bacterial receptors, inhibiting the binding of pathogenic bacteria with intestinal epithelial cells and preventing bacterial invasion.[145] Breast milk oligosaccharides can also serve as a food source for commensal bacteria promoting their growth and therefore limiting the growth of pathogenic species or preventing intestinal inflammation.[146,147] Heat Shock Protein 70 is induced in enterocytes by exposure to breast milk and has has been shown to preserve barrier function.[148] In addition, breast milk contains many immune cells (such as monocytes and lymphocytes) which downregulate the inflammatory response of the immature intestine.[133]

Growth Factors

Both amniotic fluid and breast milk contain growth hormones which promote the integrity of the intestinal barrier:

Epithelial Growth Factor (EGF)

EGF, which present in breast milk,[149] has been shown to protect against NEC in neonatal rats.[150] There is evidence that some effect of EGF on NEC[150] might be mediated via a protective mechanism on the intestinal barrier. Indeed, in a neonatal rat model of NEC, EGF has been found to abrogate the increase in intestinal occludin and claudin 3 found in the intestine of pups exposed to the NEC model.[3] In caco-2 monolayers, EGF has been found to reverse the increase in epithelial permeability and occludin dephosphorylation and rearrangement induced by bile acids.[151]

Heparin-binding EGF (Hb-EGF)

Hb-EGF is a member of the EGF family produced by macrophages which is present in breast milk.[152] It has been found to increase enterocyte proliferation and migration in a rat model of NEC[153] and has been found to protect against NEC.[154]

Transforming-growth Factor-beta (TGF-beta)

TGF-β is an extracellular peptide which has anti-inflammatory properties and promotes cell differentiation, migration, and cell death in the intestine. TGF-β is present in breastmilk[155] and is secreted by many cell types including immune cells. Monocytes from infants with NEC have reduced TGF-β expression.[156] In rodents, TGF-β administration has been shown to decrease the severity of experimental NEC.[157] TGF-β has been shown to be assosciated with the restoration of intestinal morphology and barrier function in pigs following weaning stress.[158]

Erythropoietin

Erythropoitin is a glycoportein present in breast milk that controls erythropoiesis.[159] It has also been shown to protect epithelial cells

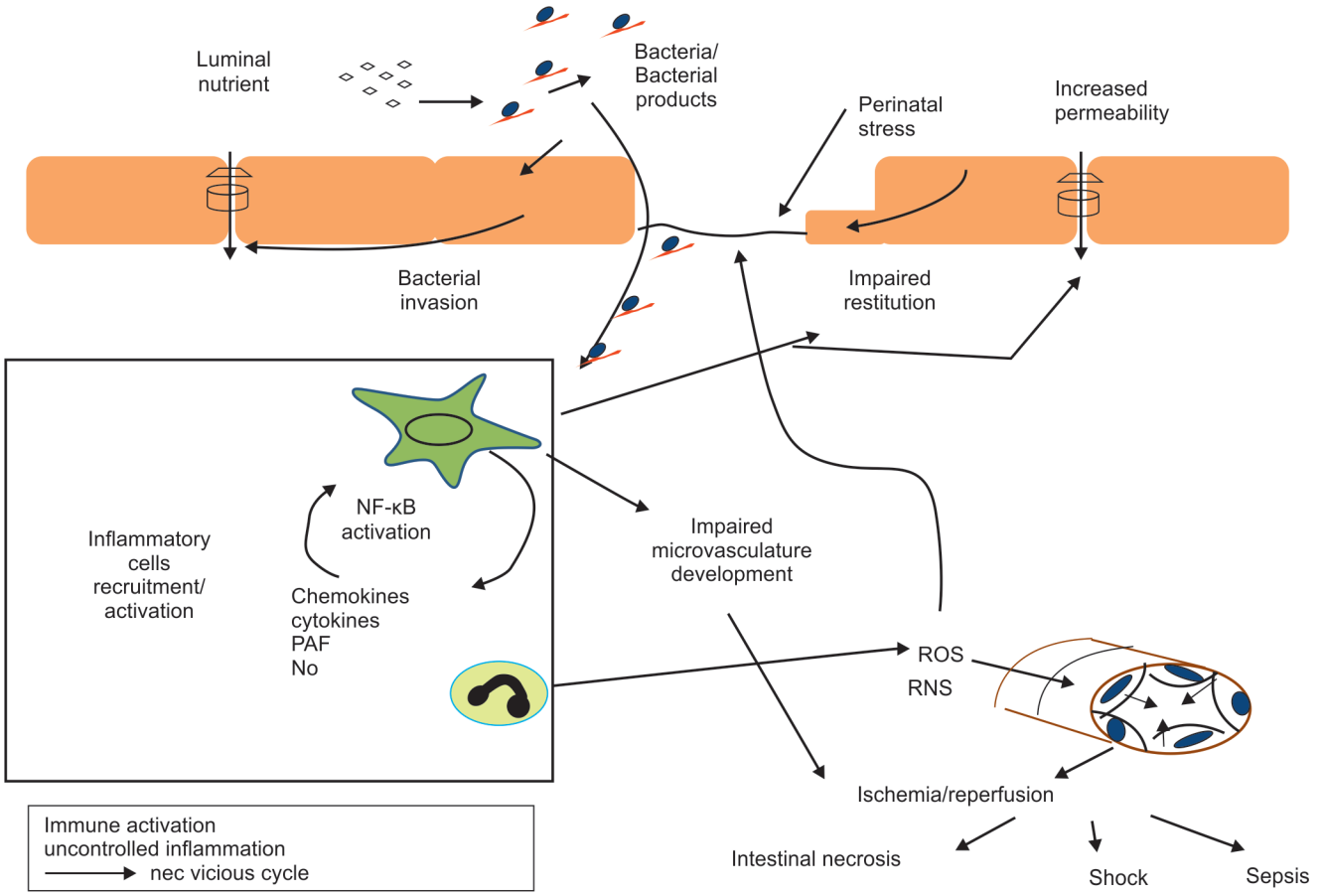

Fig. 4: Illustration of our current understanding of NEC pathogenicity

against autophagy and apoptosis thus preserving intestinal barrier function to protect against NEC.[82,160]

Conclusion

In humans, many mechanisms contribute to tighten the intestinal barrier. In premature infants, several of these mechanisms are immature, which affect the intestinal epithelial barrier function. These include decreased mucus production, decreased amounts of antibacterial peptides, and decreased intestinal motility due to an immature enteric nervous system. This may lead to bacterial translocation, with subsequent activation of NF-κB in lamina propria immune cells, causing them to secrete pro-inflammatory mediators such as chemokines (CXCL2), cytokines (TNF, IL1β), prostanoids, platelet-activating factor, and nitric oxide (Fig. 4). These inflammatory agents further recruit inflammatory cells inducing reactive oxygen species production, and causing further damage to the intestinal barrier resulting in the translocation of bacteria and their products, intestinal epithelial injury, impairment of epithelial cell restitution, apoptosis and mucosal necrosis. In severe NEC, a vicious cycle is thus created where gut barrier failure causes bacterial invasion, immune activation and uncontrolled inflammation, production of reactive oxygen and nitrogen species, vasoconstriction, secondary ischemia-reperfusion injury, intestinal necrosis, sepsis and shock. While the pathogenesis of NEC is multifactorial with involvement of the immune system and the microvasculature, measures aimed at improving barrier function in premature infants may prevent NEC and/or slow down the progression of the disease.

Summary Box

- Intestinal permeability is increased prior to the development of experimental NEC and breakdown of the intestinal barrier is thought to be a critical step in the development and the progression of necrotizing enterocolitis (NEC).
- Immature mucins and decreased antibacterial products such as alpha-defensins, cathelicidins, lysosyme, and secreted PLA2 due to decreased Paneth cell differentiation and maturation may be a predisposing factor for NEC.
- Peri-junctional cytoskeletal condensation occurs at the TJ complex in NEC prior to the development of intestinal injury and tight junction dysfunction may contribute to the increase in permeability preceding NEC.
- Increased apoptosis and decreased epithelial cell restitution may play a role in the altered barrier function seen during NEC.
- Breast milk and amniotic fluid improve intestinal barrier and thus protect the intestinal mucosa against NEC.

References

1. Catassi C, Bonucci A, Coppa GV, et al. Intestinal permeability changes during the first month: effect of natural versus artificial feeding. J Pediatr Gastroenterol Nutr 1995;21(4):383–386. DOI: 10.1097/00005176-199511000-00003.

2. Piena-Spoel M, Albers MJ, ten KJ, et al. Intestinal permeability in newborns with necrotizing enterocolitis and controls: does the sugar absorption test provide guidelines for the time to (re-) introduce enteral nutrition? JPediatrSurg 2001;36(4):587–592. DOI: 10.1053/jpsu.2001.22288.
3. Clark JA, Doelle SM, Halpern MD, et al. Intestinal barrier failure during experimental necrotizing enterocolitis: protective effect of EGF treatment. Am J Physiol Gastrointest Liver Physiol 2006;291(5):G938–G949. DOI: 10.1152/ajpgi.00090.2006.
4. Feng J, El Assal ON, Besner GE. Heparin-binding epidermal growth factor-like growth factor decreases the incidence of necrotizing enterocolitis in neonatal rats. J Pediatr Surg 2006;41(1):144–149. DOI: 10.1016/j.jpedsurg.2005.10.018.
5. Bergmann KR, Liu SX, Tian R, et al. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. Am J Pathol 2013;182(5):1595–1606. DOI: 10.1016/j.ajpath.2013.01.013.
6. Irvine EJ, Marshall JK. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. Gastroenterology 2000;119(6):1740–1744. DOI: 10.1053/gast.2000.20231.
7. Arnott ID, Kingstone K, Ghosh S. Abnormal intestinal permeability predicts relapse in inactive Crohn disease. Scand J Gastroenterol 2000;35(11):1163–1169. DOI: 10.1080/003655200750056637.
8. Johansson ME, Larsson JM, Hansson GC. The two mucus layers of colon are organized by the Muc2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci USA 2011;108:4659–4665. DOI: 10.1073/pnas.1006451107.
9. Johansson ME, Phillipson M, Petersson J, et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA 2008;105:15064–15069. DOI: 10.1073/pnas.0803124105.
10. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 2013;10(6):352–361. DOI: 10.1038/nrgastro.2013.35.
11. Ermund A, Schutte A, Johansson ME, et al. Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. Am J Physiol Gastrointest Liver Physiol 2013;305(5):G341–G347. DOI: 10.1152/ajpgi.00046.2013.
12. Chambers JA, Hollingsworth MA, Trezise AE, et al. Developmental expression of mucin genes MUC1 and Muc2. J Cell Sci 1994;107:413–424. PMID: 7515892.
13. Schaart MW, de Bruijn AC, Schierbeek H, et al. Small intestinal Muc2 synthesis in human preterm infants. Am J Physiol Gastrointest Liver Physiol 2009;296(5):G1085–G1090. DOI: 10.152/ajpgi.90444.2008.
14. McElroy SJ, Prince LS, Weitkamp JH, et al. Tumor necrosis factor receptor 1-dependent depletion of mucus in immature small intestine: a potential role in neonatal necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2011;301(4):G656–G666. DOI: 10.1152/ajpgi.00550.2010.
15. Khailova L, Dvorak K, Arganbright KM, et al. Bifidobacterium bifidum improves intestinal integrity in a rat model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2009;297(5):G940–G949. DOI: 10.1152/ajpgi.00141.2009.
16. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that Muc2 is critical for colonic protection. Gastroenterology 2006;131(1):117–129. DOI: 10.1053/j.gastro.2006.04.020.
17. Tian F, Liu GR, Li N, et al. Insulin-like growth factor I reduces the occurrence of necrotizing enterocolitis by reducing inflammatory response and protecting intestinal mucosal barrier in neonatal rats model. Eur Rev Med Pharmacol Sci 2017;21(20):4711–4719. PMID: 29131241.
18. Jing Y, Peng F, Shan Y, et al. Berberine reduces the occurrence of neonatal necrotizing enterocolitis by reducing the inflammatory response. Exp Ther Med 2018;16(6):5280–5285. DOI: 10.3892/etm.2018.6871.
19. Li B, Hock A, Wu RY, et al. Bovine milk-derived exosomes enhance goblet cell activity and prevent the development of experimental necrotizing enterocolitis. PLoS One 2019;14(1):e0211431. DOI: 10.1371/journal.pone.0211431.
20. Wu RY, Li B, Koike Y, et al. Human milk oligosaccharides increase mucin expression in experimental necrotizing enterocolitis. Mol Nutr Food Res 2019;63(3):e1800658. DOI: 10.1002/mnfr.201800658.
21. Berseth CL. Gestational evolution of small intestine motility in preterm and term infants. J Pediatr 1989;115(4):646–651. DOI: 10.1016/s0022-3476(89)80302-6.
22. Inoue R, Tsuruta T, Nojima I, et al. Postnatal changes in the expression of genes for cryptdins 1-6 and the role of luminal bacteria in cryptdin gene expression in mouse small intestine. FEMS Immunol Med Microbiol 2008;52(3):407–416. DOI: 10.1111/j.1574-695X.2008.00390.x.
23. Ménard S, Förster V, Lotz M, et al. Developmental switch of intestinal antimicrobial peptide expression. J Exp Med 2008;205(1):183–193. DOI: 10.1084/jem.20071022.
24. Mallow EB, Harris A, Salzman N, et al. Human enteric defensins. Gene structure and developmental expression. J Biol Chem 1996;271(8):4038–4045. DOI: 10.1074/jbc.271.8.4038.
25. Kai-Larsen Y, Bergsson G, Gudmundsson GH, et al. Antimicrobial components of the neonatal gut affected upon colonization. Pediatr Res 2007;61:530–536. DOI: 10.1203/pdr.0b013e318045be83.
26. Underwood MA, Kananurak A, Coursodon CF, et al. Bifidobacterium bifidum in a rat model of necrotizing enterocolitis: antimicrobial peptide and protein responses. Pediatr Res 2012;71(5):546–551. DOI: 10.1038/pr.2012.11.
27. Vaishnava S, Behrendt CL, Ismail AS, et al. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci USA 2008;105(52):20858–20863. DOI: 10.1073/pnas.0808723105.
28. Coutinho HB, da Mota HC, Coutinho VB, et al. Absence of lysozyme (muramidase) in the intestinal Paneth cells of newborn infants with necrotising enterocolitis. J Clin Pathol 1998;51(7):512–514. DOI: 10.1136/jcp.51.7.512.
29. Salzman NH, Polin RA, Harris MC, et al. Enteric defensin expression in necrotizing enterocolitis. Pediatr Res 1998;44(1):20–26. DOI: 10.1203/00006450-199807000-00003.
30. Puiman PJ, Burger-Van Paassen N, Schaart MW, et al. Paneth cell hyperplasia and metaplasia in necrotizing enterocolitis. Pediatr Res 2011;69(3):217–223. DOI: 10.1203/PDR.0b013e3182092a9a.
31. Zhang C, Sherman MP, Prince LS, et al. Paneth cell ablation in the presence of Klebsiella pneumoniae induces necrotizing enterocolitis (NEC)-like injury in the small intestine of immature mice. Dis Model Mech 2012;5(4):522–532. DOI: 10.1242/dmm.009001.
32. Chen L, Lv Z, Gao Z, et al. Human β-defensin-3 reduces excessive autophagy in intestinal epithelial cells and in experimental necrotizing enterocolitis. Sci Rep 2019;9(1):19890. DOI: 10.1038/s41598-019-56535-3.
33. Lu WC, Zheng X, Liu JF, et al. Effect of Bifidobacterium on the expression of β-defensin-2 in intestinal tissue of neonatal rats with necrotizing enterocolitis. Zhongguo Dang Dai Er Ke Za Zhi 2018;20(3):224–229. DOI: 10.7499/j.issn.1008-8830.2018.03.012.
34. Dimberg J, Lilja I, Westrom B, et al. Ontogeny of group II phospholipase A2 gene expression in rat stomach and ileum. Biology of the neonate 1995;67(2):113–121. DOI: 10.1159/000244152.
35. Lu J, Pierce M, Franklin A, et al. Dual roles of endogenous platelet-activating factor acetylhydrolase in a murine model of necrotizing enterocolitis. Pediatr Res 2010;68(3):225–230. DOI: 10.1203/PDR.0b013e3181eb2efe.
36. Benveniste J, Chignard M, Le Couedic JP, et al. Biosynthesis of platelet-activating factor (PAF-ACETHER). II. Involvement of phospholipase A2 in the formation of PAF-ACETHER and lyso-PAF-ACETHER from rabbit platelets. Thromb Res 1982;25(5):375–385. DOI: 10.1016/0049-3848(82)90128-1.
37. Hsueh W, Gonzalez-Crussi F, Arroyave JL. Platelet-activating factor-induced ischemic bowel necrosis. An investigation of secondary

mediators in its pathogenesis. Am J Pathol 1986;122(2):231–239. PMID: 3080895.
38. Caplan MS, Hedlund E, Adler L, et al. The platelet-activating factor receptor antagonist WEB 2170 prevents neonatal necrotizing enterocolitis in rats. J Pediatr Gastroenterol Nutr 1997;24(3):296–301. DOI: 10.1097/00005176-199703000-00012.
39. Lalles JP. Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. Nutr Rev 2010;68(6):323–332. DOI: 10.1111/j.1753-4887.2010.00292.x.
40. Whitehouse JS, Riggle KM, Purpi DP, et al. The protective role of intestinal alkaline phosphatase in necrotizing enterocolitis. J Surg Res 2010;163(1):79–85. DOI: 10.1016/j.jss.2010.04.048.
41. Riggle KM, Rentea RM, Welak SR, et al. Intestinal alkaline phosphatase prevents the systemic inflammatory response associated with necrotizing enterocolitis. J Surg Res 2013;180(1): 21–26. DOI: 10.1016/j.jss.2012.10.042.
42. Rentea RM, Liedel JL, Welak SR, et al. Intestinal alkaline phosphatase administration in newborns is protective of gut barrier function in a neonatal necrotizing enterocolitis rat model. J Pediatr Surg 2012;47(6):1135–1142. DOI: 10.1016/j.jpedsurg.2012.03.018.
43. Heath M, Buckley R, Gerber Z, et al. Association of intestinal alkaline phosphatase with necrotizing enterocolitis among premature infants. JAMA Netw Open 2019;2(11):e1914996. DOI: 10.1001/jamanetworkopen.2019.14996.
44. Wong WM, Poulsom R, Wright NA. Trefoil peptides. Gut 1999;44(6):890–895. DOI: 10.1136/gut.44.6.890.
45. Hoffmann W. Trefoil factors TFF (trefoil factor family) peptide-triggered signals promoting mucosal restitution. Cell Mol Life Sci CMLS 2005;62(24):2932–2938. DOI: 10.1007/s00018-005-5481-9.
46. Mashimo H, Wu DC, Podolsky DK, et al. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science (New York, NY) 1996;274(5285):262–265. DOI: 10.1126/science.274.5285.262.
47. Vestergaard EM, Nexo E, Wendt A, et al. Trefoil factors in human milk. Early Hum Dev 2008;84(10):631–635. DOI: 10.1016/j.earlhumdev.2008.04.001.
48. Vieten D, Corfield A, Carroll D, et al. Impaired mucosal regeneration in neonatal necrotising enterocolitis. Pediatr Surg Int 2005;21(3):153–160. DOI: 10.1007/s00383-004-1312-6.
49. Liu J, Yang Q, Chen Z, et al. TFF3 mediates the NF-κB/COX2 pathway to regulate PMN-MDSCs activation and protect against necrotizing enterocolitis. Eur J Immunol 2021;51(5):1110–1125. DOI: 10.1002/eji.202048768.
50. Rognum TO, Thrane S, Stoltenberg L, et al. Development of intestinal mucosal immunity in fetal life and the first postnatal months. Pediatr Res 1992;32(2):145–149. DOI: 10.1203/00006450-199208000-00003.
51. Axelsson H, Johansson BG, Rymo L. Isolation of immunoglobulin A (IgA) from human colostrum. Acta Chem Scand 1966;20:2339–2348. DOI: 10.3891/acta.chem.scand.20-2339.
52. Mehta R, Petrova A. Biologically active breast milk proteins in association with very preterm delivery and stage of lactation. J Perinatol 2011;31(1):58–62. DOI: 10.1038/jp.2010.68.
53. Gopalakrishna KP, Macadangdang BR, Rogers MB, et al. Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. Nat Med 2019;25(7):1110–1115. DOI: 10.1038/s41591-019-0480-9.
54. Weinberg ED. Human lactoferrin: a novel therapeutic with broad spectrum potential. J Pharm Pharmacol 2001;53(10):1303–1310. DOI: 10.1211/0022357011777792.
55. Rahman MM, Kim WS, Ito T, et al. Growth promotion and cell binding ability of bovine lactoferrin to Bifidobacterium longum. Anaerobe 2009;15(4):133–137. DOI: 10.1016/j.anaerobe.2009.01.003.
56. Reznikov EA, Comstock SS, Yi C, et al. Dietary bovine lactoferrin increases intestinal cell proliferation in neonatal piglets. J Nutr 2014;144(9):1401–1408. DOI: 10.3945/jn.114.196568.
57. Sherman MP. Lactoferrin and necrotizing enterocolitis. Clin Perinatal 2013;40(1):79–91. DOI: 10.1016/j.clp.2012.12.006.
58. Pammi M, Abrams SA. Oral lactoferrin for the treatment of sepsis and necrotizing enterocolitis in neonates. Cochrane Database Syst Rev 2011:CD007138. DOI: 10.1002/14651858.CD007138.pub2.
59. Liu Y, Perego M, Xiao Q, et al. Lactoferrin-induced myeloid-derived suppressor cell therapy attenuates pathologic inflammatory conditions in newborn mice. J Clin Invest 2019;129(10):4261–4275. DOI: 10.1172/JCI128164.
60. Pammi M, Abrams SA. Enteral lactoferrin for the treatment of sepsis and necrotizing enterocolitis in neonates. Cochrane Database Syst Rev 2019;5(5):CD007138. DOI: 10.1002/14651858.CD007138.pub4.
61. Shen H, Lei Y, He X, et al. Role of lactadherin in intestinal barrier integrity in experimental neonatal necrotizing enterocolitis. J Cell Biochem 2019;120(12):19509–19517. DOI: 10.1002/jcb.29255.
62. Nusrat A, Turner JR, Madara JL. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. Am J Physiol Gastrointest Liver Physiol 2000;279(5):G851–G857. DOI: 10.1152/ajpgi.2000.279.5.G851.
63. Shen L, Weber CR, Raleigh DR, et al. Tight junction pore and leak pathways: a dynamic duo. Annu Rev Physiol 2011;73:283–309. DOI: 10.1146/annurev-physiol-012110-142150.
64. Colegio OR, Van IC, Rahner C, et al. Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture. Am J Physiol Cell Physiol 2003;284(6):C1346–C1354. DOI: 10.1152/ajpcell.00547.2002.
65. Itoh M, Furuse M, Morita K, et al. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J Cell Biol 1999;147(6):1351–1363. DOI: 10.1083/jcb.147.6.1351.
66. Cunningham KE, Turner JR. Myosin light chain kinase: pulling the strings of epithelial tight junction function. Ann N Y Acad Sci 2012;1258(1):34–42. DOI: 10.1111/j.1749-6632.2012.06526.x.
67. Wang F, Schwarz BT, Graham WV, et al. IFN-gamma-induced TNFR2 expression is required for TNF-dependent intestinal epithelial barrier dysfunction. Gastroenterology 2006;131(4):1153–1163. DOI: 10.1053/j.gastro.2006.08.022.
68. Yang R, Han X, Uchiyama T, et al. IL-6 is essential for development of gut barrier dysfunction after hemorrhagic shock and resuscitation in mice. Am J Physiol Gastrointest Liver Physiol 2003;285(3):G621–G629. DOI: 10.1152/ajpgi.00177.2003.
69. Al-Sadi R, Guo S, Ye D, et al. TNF-alpha modulation of intestinal epithelial tight junction barrier is regulated by ERK1/2 activation of Elk-1. Am J Pathol 2013;183(6):1871–1874. DOI: 10.1016/j.ajpath.2013.09.001.
70. Polak-Charcon S, Shoham J, Ben-Shaul Y. Tight junctions in epithelial cells of human fetal hindgut, normal colon, and colon adenocarcinoma. J Natl Cancer Inst 1980;65(1):53–62. PMID: 6930519.
71. Van IC, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. J Clin Invest 2001;107(10):1319–1327. DOI: 10.1172/JCI12464.
72. Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002;156(6):1099–1111. DOI: 10.1083/jcb.200110122.
73. Holmes JL, Van Itallie CM, Rasmussen JE, et al. Claudin profiling in the mouse during postnatal intestinal development and along the gastrointestinal tract reveals complex expression patterns. Gene Expr Patterns 2006;6(6):581–588. DOI: 10.1016/j.modgep.2005.12.001.
74. Hogberg N, Stenback A, Carlsson PO, et al. Genes regulating tight junctions and cell adhesion are altered in early experimental necrotizing enterocolitis. J Pediatr Surg 2013;48(11):2308–2312. DOI: 10.1016/j.jpedsurg.2013.06.027.
75. Patel RM, Myers LS, Kurundkar AR, et al. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. Am J Pathol 2012;180(2):626–635. DOI: 10.1016/j.ajpath.2011.10.025.

76. Thuijls G, Derikx JP, van Wijck K, et al. Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. Ann Surg 2010;251(6):1174–1180. DOI: 10.097/SLA.0b013e3181d778c4.
77. Prasad S, Mingrino R, Kaukinen K, et al. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. Lab Invest 2005;85(9):1139–1162. DOI: 10.1038/labinvest.3700316.
78. Weber CR, Nalle SC, Tretiakova M, et al. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. Lab Invest 2008;88(10):1110–1120. DOI: 10.1038/labinvest.2008.78.
79. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem 2011;286(36):31263–31271. DOI: 10.1074/jbc.M111.238147.
80. Tamura A, Hayashi H, Imasato M, et al. Loss of claudin-15, but not claudin-2, causes Na+ deficiency and glucose malabsorption in mouse small intestine. Gastroenterology 2011;140(3):913–923. DOI: 10.1053/j.gastro.2010.08.006.
81. Li Q, Zhang Q, Wang C, et al. Disruption of tight junctions during polymicrobial sepsis in vivo. J Pathol 2009;218(2):210–221. DOI: 10.1002/path.2525.
82. Shiou SR, Yu Y, Chen S, et al. Erythropoietin protects intestinal epithelial barrier function and lowers the incidence of experimental neonatal necrotizing enterocolitis. J Biol Chem 2011;286(14):12123–12132. DOI: 10.1074/jbc.M110.154625.
83. Baum B, Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. J Cell Biol 2011;192(6):907–917. DOI: 10.1083/jcb.201009141.
84. Buonpane C, Yuan C, Wood D, et al. ROCK1 inhibitor stabilizes E-cadherin and improves barrier function in experimental necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2020;318(4):G781–G792. DOI: 10.1152/ajpgi.00195.2019.
85. Carlisle EM, Poroyko V, Caplan MS, et al. Murine gut microbiota and transcriptome are diet dependent. Ann Surg 2013;257(2):287–294. DOI: 10.1097/SLA.0b013e318262a6a6.
86. He W, Ladinsky MS, Huey-Tubman KE, et al. FcRn-mediated antibody transport across epithelial cells revealed by electron tomography. Nature 2008;455(7212):542–546. DOI: 10.1038/nature07255.
87. Barreau F, Hugot JP. Intestinal barrier dysfunction triggered by invasive bacteria. Curr Opin Microbiol 2014;17:91–98. DOI: 10.1016/j.mib.2013.12.003.
88. Panigrahi P, Bamford P, Horvath K, et al. Escherichia coli transcytosis in a Caco-2 cell model: implications in neonatal necrotizing enterocolitis. Pediatr Res 1996;40(3):415–421. DOI: 10.1203/00006450-199609000-00009.
89. Troeger H, Richter JF, Beutin L, et al. Escherichia coli alpha-haemolysin induces focal leaks in colonic epithelium: a novel mechanism of bacterial translocation. Cell Microbiol 2007;9(10):2530–2540. DOI: 10.1111/j.1462-5822.2007.00978.x.
90. Ulluwishewa D, Anderson RC, McNabb WC, et al. Regulation of tight junction permeability by intestinal bacteria and dietary components. J Nutr 2011;141(5):769–776. DOI: 10.3945/jn.110.135657.
91. Soderholm JD, Streutker C, Yang PC, et al. Increased epithelial uptake of protein antigens in the ileum of Crohn's disease mediated by tumour necrosis factor alpha. Gut 2004;53(12):1817–1824. DOI: 10.1136/gut.2004.041426.
92. Leaphart CL, Qureshi F, Cetin S, et al. Interferon-gamma inhibits intestinal restitution by preventing gap junction communication between enterocytes. Gastroenterology 2007;132(7):2395–2411. DOI: 10.1053/j.gastro.2007.03.029.
93. Smyth D, Phan V, Wang A, et al. Interferon-gamma-induced increases in intestinal epithelial macromolecular permeability requires the Src kinase Fyn. Lab Invest 2011;91(5):764–777. DOI: 10.1038/labinvest.2010.208.
94. Menard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol 2010;3(3):247–259. DOI: 10.1038/mi.2010.5.
95. Kucharzik T, Lugering N, Rautenberg K, et al. Role of M cells in intestinal barrier function. Ann N Y Acad Sci 2000;915:171–183. DOI: 10.1111/j.1749-6632.2000.tb05240.x.
96. Kucharzik T, Walsh SV, Chen J, et al. Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. Am J Pathol 2001;159(6):2001–2009. DOI: 10.1016/S0002-9440(10)63051-9.
97. Ireland H, Houghton C, Howard L, et al. Cellular inheritance of a Cre-activated reporter gene to determine Paneth cell longevity in the murine small intestine. Dev Dyn 2005;233(4):1332–1336. DOI: 10.1002/dvdy.20446.
98. Bullen TF, Forrest S, Campbell F, et al. Characterization of epithelial cell shedding from human small intestine. Lab Invest 2006;86(10):1052–1063. DOI: 10.1038/labinvest.3700464.
99. Wang L, Zeng X, Ryoo HD, et al. Integration of UPRER and oxidative stress signaling in the control of intestinal stem cell proliferation. PLoS Genet 2014;10(8):e1004568. DOI: 10.1371/journal.pgen.1004568.
100. Bojarski C, Gitter AH, Bendfeldt K, et al. Permeability of human HT-29/B6 colonic epithelium as a function of apoptosis. J Physiol 2001;535:541–552. DOI: 10.1111/j.1469-7793.2001.00541.x.
101. Chokshi NK, Guner YS, Hunter CJ, et al. The role of nitric oxide in intestinal epithelial injury and restitution in neonatal necrotizing enterocolitis. Semin Perinatal 2008;32(2):92–99. DOI: 10.1053/j.semperi.2008.01.002.
102. Jilling T, Simon D, Lu J, et al. The roles of bacteria and TLR4 in rat and murine models of necrotizing enterocolitis. J Immunol 2006;177(5):3273–3782. DOI: 10.4049/jimmunol.177.5.3273.
103. Leaphart CL, Cavallo J, Gribar SC, et al. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. J Immunol 2007;179(7):4808–4820. DOI: 10.4049/jimmunol.179.7.4808.
104. Qureshi FG, Leaphart C, Cetin S, et al. Increased expression and function of integrins in enterocytes by endotoxin impairs epithelial restitution. Gastroenterology 2005;128(4):1012–1022. DOI: 10.1053/j.gastro.2005.01.052.
105. Neal MD, Sodhi CP, Dyer M, et al. A critical role for TLR4 induction of autophagy in the regulation of enterocyte migration and the pathogenesis of necrotizing enterocolitis. J Immunol 2013;190(7):3541–3551. DOI: 10.4049/jimmunol.1202264.
106. Neal MD, Sodhi CP, Jia H, et al. Toll-like receptor 4 is expressed on intestinal stem cells and regulates their proliferation and apoptosis via the p53 up-regulated modulator of apoptosis. J Biol Chem 2012;287(44):37296–37308. DOI: 10.1074/jbc.M112.375881.
107. Sodhi CP, Shi XH, Richardson WM, et al. Toll-like receptor-4 inhibits enterocyte proliferation via impaired beta-catenin signaling in necrotizing enterocolitis. Gastroenterology 2010;138(1):185–196. DOI: 10.1053/j.gastro.2009.09.045.
108. Richardson WM, Sodhi CP, Russo A, et al. Nucleotide-binding oligomerization domain-2 inhibits toll-like receptor-4 signaling in the intestinal epithelium. Gastroenterology 2010;139(3):904–917,17e1–e6. DOI: 10.1053/j.gastro.2010.05.038.
109. Anand RJ, Dai S, Rippel C, et al. Activated macrophages inhibit enterocyte gap junctions via the release of nitric oxide. Am J Physiol Gastrointest Liver Physiol 2008;294(1):G109–G119. DOI: 10.1152/ajpgi.00331.2007.
110. Cetin S, Leaphart CL, Li J, et al. Nitric oxide inhibits enterocyte migration through activation of RhoA-GTPase in a SHP-2-dependent manner. Am J Physiol Gastrointest Liver Physiol 2007;292(5):G1347–G1358. DOI: 10.1152/ajpgi.00375.2006.
111. Weil BR, Markel TA, Herrmann JL, et al. Mesenchymal stem cells enhance the viability and proliferation of human fetal intestinal epithelial cells following hypoxic injury via paracrine mechanisms. Surgery 2009;146(2):190–197. DOI: 10.1016/j.surg.2009.03.031.
112. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2(4):361–367. DOI: 10.1038/86373.

113. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 2005;307(5707):254–258. DOI: 10.1126/science.1102901.
114. Liu Y, Zhu L, Fatheree NY, et al. Changes in intestinal Toll-like receptors and cytokines precede histological injury in a rat model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2009;297(3):G442–G450. DOI: 10.1152/ajpgi.00182.2009.
115. Maheshwari A, Schelonka RL, Dimmitt RA, et al. Cytokines associated with necrotizing enterocolitis in extremely-low-birth-weight infants. Pediatr Res 2014;76(1):48. DOI: 10.1038/pr.2014.48.
116. Marchiando AM, Shen L, Graham WV, et al. The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. Gastroenterology 2011;140(4):1208–1218e1–2. DOI: 10.1053/j.gastro.2011.01.004.
117. Marchiando AM, Shen L, Graham WV, et al. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. J Cell Biol 2010;189(1):111–126. DOI: 10.1083/jcb.200902153.
118. Al-Sadi RM, Ma TY. IL-1beta causes an increase in intestinal epithelial tight junction permeability. J Immunol 2007;178(7):4641–4649. DOI: 10.4049/jimmunol.178.7.4641.
119. Al-Sadi R, Ye D, Said HM, et al. IL-1beta-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-kappa B pathway. Am J Pathol 2010;177(5):2310–2322. DOI: 10.53/ajpath.010.100371.
120. Al-Sadi R, Ye D, Boivin M, et al. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. PLoS One 2014;9(3):e85345. DOI: 10.1371/journal.pone.0085345.
121. Emami CN, Mittal R, Wang L, et al. Recruitment of dendritic cells is responsible for intestinal epithelial damage in the pathogenesis of necrotizing enterocolitis by Cronobacter sakazakii. J Immunol 2011;186(12):7067–7079. DOI: 10.4049/jimmunol.1100108.
122. Schulzke JD, Ploeger S, Amasheh M, et al. Epithelial tight junctions in intestinal inflammation. Ann N Y Acad Sci 2009;1165:294–300. DOI: 10.1111/j.1749-6632.2009.04062.x.
123. Wang F, Graham WV, Wang Y, et al. Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. Am J Pathol 2005;166(2):409–419. DOI: 10.1016/s0002-9440(10)62264-x.
124. De Plaen IG, Liu SX, Tian R, et al. Inhibition of nuclear factor-kappaB ameliorates bowel injury and prolongs survival in a neonatal rat model of necrotizing enterocolitis. Pediatr Res 2007;61(6):716–721. DOI: 10.1203/pdr.0b013e3180534219.
125. Pasparakis M. IKK/NF-kappaB signaling in intestinal epithelial cells controls immune homeostasis in the gut. Mucosal Immunol 2008;1 (Suppl 1) :S54–S57. DOI: 10.1038/mi.2008.53.
126. Managlia ELS, Yan XC, De Plaen IG. Blocking NF-κB activation in intestinal Lysozyme-M+ cells prevents the NEC-induced decrease in Ly6C+ cells in the neonatal intestine. FASEB 2016.
127. Lin HC, Su BH, Chen AC, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005;115(1):1–4. DOI: 10.1542/peds.2004-1463.
128. Stratiki Z, Costalos C, Sevastiadou S, et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. Early Hum Dev 2007;83(9):575–579. DOI: 10.1016/j.earlhumdev.2006.12.002.
129. Ewaschuk JB, Diaz H, Meddings L, et al. Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. Am J Physiol Gastrointest Liver Physiol 2008;295(5):G1025–G1034. DOI: 10.1152/ajpgi.90227.2008. Epub 2008 Sep 11.
130. Khailova L, Mount Patrick SK, Arganbright KM, et al. Bifidobacterium bifidum reduces apoptosis in the intestinal epithelium in necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2010;299(5):G1118–G1127. DOI: 10.1152/ajpgi.00131.2010.
131. Weng M, Ganguli K, Zhu W, et al. Conditioned medium from Bifidobacteria infantis protects against Cronobacter sakazakii-induced intestinal inflammation in newborn mice. Am J Physiol Gastrointest Liver Physiol 2014;306(9):G779–G787. DOI: 10.1152/ajpgi.00183.2013.
132. Rumbo M, Schiffrin EJ. Ontogeny of intestinal epithelium immune functions: developmental and environmental regulation. Cell Mol Life Sci CMLS 2005;62(12):1288–1296. DOI: 10.1007/s00018-005-5033-3.
133. Wagner CL, Taylor SN, Johnson D. Host factors in amniotic fluid and breast milk that contribute to gut maturation. Clin Rev Allergy Immunol 2008;34(2):191–204. DOI: 10.1007/s12016-007-8032-3.
134. Ostergaard MV, Bering SB, Jensen ML, et al. Modulation of intestinal inflammation by minimal enteral nutrition with amniotic fluid in preterm pigs. JPEN J Parenter Enteral Nutr 2013;38(5):576–586. DOI: 10.1177/0148607113489313.
135. Siggers J, Ostergaard MV, Siggers RH, et al. Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates. Am J Physiol Gastrointest Liver Physiol 2013;304(10):G864–G875. DOI: 10.1152/ajpgi.00278.2012.
136. Jain SK, Baggerman EW, Mohankumar K, et al. Amniotic fluid-borne hepatocyte growth factor protects rat pups against experimental necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2014;306(5):G361–G369. DOI: 10.1152/ajpgi.00272.2013.
137. Zani A, Cananzi M, Fascetti-Leon F, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut 2014;63(2):300–309. DOI: 10.1136/gutjnl-2012-303735.
138. Li B, Lee C, Chuslip S, et al. Intestinal epithelial tight junctions and permeability can be rescued through the regulation of endoplasmic reticulum stress by amniotic fluid stem cells during necrotizing enterocolitis. FASEB J 2021;35(1):e21265. DOI: 10.1096/fj.202001426R.
139. Taylor SN, Basile LA, Ebeling M, et al. Intestinal permeability in preterm infants by feeding type: mother's milk versus formula. Breastfeeding Med 2009;4(1):11–15. DOI: 10.1089/bfm.2008.0114.
140. Carvalho EB, Maga EA, Quetz JS, et al. Goat milk with and without increased concentrations of lysozyme improves repair of intestinal cell damage induced by enteroaggregative Escherichia coli. BMC Gastroenterol 2012;12:106. DOI: 10.1186/1471-230X-12-106.
141. Gunasekaran A, Eckert J, Burge K, et al. Hyaluronan 35 kDa enhances epithelial barrier function and protects against the development of murine necrotizing enterocolitis. Pediatr Res 2020;87(7):1177–1184. DOI: 10.1038/s41390-019-0563-9.
142. Bein A, Lubetzky R, Mandel D, et al. TIMP-1 inhibition of occludin degradation in Caco-2 intestinal cells: a potential protective role in necrotizing enterocolitis. Pediatr Res 2015;77(5):649–655. DOI: 10.1038/pr.2015.26.
143. He S, Liu G, Zhu X. Human breast milk-derived exosomes may help maintain intestinal epithelial barrier integrity. Pediatr Res 2021;90(2):366. DOI: 10.1038/s41390-021-01449-y.
144. Sodhi CP, Wipf P, Yamaguchi Y, et al. Insights image for "The human milk oligosaccharides 2′-fucosyllactose and 6′-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling." Pediatr Res 2021;89(1):248. DOI: 10.1038/s41390-020-01184-w.
145. Peterson JA, Patton S, Hamosh M. Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. Biol Neonate 1998;74(2):143–162. DOI: 10.1159/000014020.
146. Holscher HD, Faust KL, Czerkies LA, et al. Effects of prebiotic-containing infant formula on gastrointestinal tolerance and fecal microbiota in a randomized controlled trial. JPEN J Parenter Enteral Nutr 2012;36:95s–105s. DOI: 10.1177/0148607111430087.
147. Fuhrer A, Sprenger N, Kurakevich E, et al. Milk sialyllactose influences colitis in mice through selective intestinal bacterial colonization. The Journal of experimental medicine 2010;207(13):2843–2854. DOI: 10.1084/jem.20101098.

148. Liedel JL, Guo Y, Yu Y, et al. Mother's milk-induced Hsp70 expression preserves intestinal epithelial barrier function in an immature rat pup model. Pediatr Res 2011;69:395–400. DOI: 10.1203/PDR.0b013e3182114ec9.
149. Koldovský O. Is breast-milk epidermal growth factor biologically active in the suckling? Nutrition 1989;5(4):223–225. PMID: 2520295.
150. Dvorak B, Halpern MD, Holubec H, et al. Epidermal growth factor reduces the development of necrotizing enterocolitis in a neonatal rat model. Am J Physiol Gastrointest Liver Physiol 2002;282(1):G156–G164. DOI: 10.1152/ajpgi.00196.2001.
151. Raimondi F, Santoro P, Barone MV, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Gastrointest Liver Physiol 2008;294(4):G906–G913. DOI: 10.1152/ajpgi.00043.2007.
152. Michalsky MP, Lara-Marquez M, Chun L, et al. Heparin-binding EGF-like growth factor is present in human amniotic fluid and breast milk. J Pediatr Surg 2002;37(1):1–6. DOI: 10.1053/jpsu.2002.29415.
153. Feng J, Besner GE. Heparin-binding epidermal growth factor-like growth factor promotes enterocyte migration and proliferation in neonatal rats with necrotizing enterocolitis. J Pediatr Surg 2007;42(1):214–220. DOI: 10.1016/j.jpedsurg.2006.09.055.
154. Yang J, Watkins D, Chen CL, et al. Heparin-binding epidermal growth factor-like growth factor and mesenchymal stem cells act synergistically to prevent experimental necrotizing enterocolitis. J Am Coll Surg 2012;215(4):534–545. DOI: 10.1016/j.jamcollsurg.2012.05.037.
155. Olivares M, Albrecht S, De Palma G, et al. Human milk composition differs in healthy mothers and mothers with celiac disease. Eur J Nutr 2015;54(1):119–128. DOI: 10.1007/s00394-014-0692-1.
156. Pang Y, Du X, Xu X, et al. Monocyte activation and inflammation can exacerbate Treg/Th17 imbalance in infants with neonatal necrotizing enterocolitis. Int Immunopharmacol 2018;59:354–360. DOI: 10.1016/j.intimp.2018.04.026.
157. Maheshwari A, Kelly DR, Nicola T, et al. TGF-beta2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. Gastroenterology 2011;140(1):242–253. DOI: 10.1053/j.gastro.2010.09.043.
158. Xiao K, Song ZH, Jiao LF, et al. Developmental changes of TGF-beta1 and Smads signaling pathway in intestinal adaption of weaned pigs. PLoS One 2014;9(8):e104589. DOI: 10.1371/journal.pone.0104589.
159. Kling PJ, Sullivan TM, Roberts RA, et al. Human milk as a potential enteral source of erythropoietin. Pediatr Res 1998;43(2):216–221. DOI: 10.1203/00006450-199802000-00010.
160. Yu Y, Shiou SR, Guo Y, et al. Erythropoietin protects epithelial cells from excessive autophagy and apoptosis in experimental neonatal necrotizing enterocolitis. PLoS One 2013;8(7):e69620. DOI: 10.1371/journal.pone.0069620.