

REVIEW ARTICLE



Intestinal epithelium in early life

Lauren C. Frazer¹ and Misty Good¹

© The Author(s), under exclusive licence to Society for Mucosal Immunology 2022

Rapid development of the fetal and neonatal intestine is required to meet the growth requirements of early life and form a protective barrier against external insults encountered by the intestinal mucosa. The fetus receives nutrition via the placenta and is protected from harmful pathogens in utero, which leads to intestinal development in a relatively quiescent environment. Upon delivery, the intestinal mucosa is suddenly tasked with providing host defense and meeting nutritional demands. To serve these functions, an array of specialized epithelial cells develop from intestinal stem cells starting in utero and continuing postnatally. Intestinal disease results when these homeostatic processes are interrupted. For preterm neonates, the most common pathology resulting from epithelial barrier dysfunction is necrotizing enterocolitis (NEC). In this review, we discuss the normal development and function of the intestinal epithelium in early life as well as how disruption of these processes can lead to NEC.

Mucosal Immunology (2022) 15:1181–1187; <https://doi.org/10.1038/s41385-022-00579-8>

INTRODUCTION

The intestinal epithelium balances a multiplicity of roles in early life. It facilitates the digestion and absorption of nutrients and forms a barrier against potential pathogens in the intestinal lumen. These functions must be maintained during a period of rapid growth and intestinal development. When these processes are interrupted, infant health is negatively impacted. Clinically significant intestinal pathologies that arise when the barrier function of the intestine is compromised in the neonatal period include necrotizing enterocolitis (NEC), sepsis, and infectious diarrheal illness.

In this review, we discuss intestinal morphogenesis, mechanisms of epithelial cell differentiation from intestinal stem cells (ISCs), and the function of the predominant epithelial cell subtypes in the intestine. We also discuss how disruption of the intestinal epithelium is implicated in neonatal disease with a focus on NEC, the most common intestinal pathology in preterm neonates.

Formation of crypt-villus intestinal architecture

Mature intestinal structure develops through a highly regulated process that begins in utero. The small intestine is composed of crypts and villi lined by a continuous epithelial cell monolayer. Structural maturation of the intestine involves lengthening of villi, deepening of crypts, and localization of proliferating ISCs in the crypt base (Fig. 1). Intestinal crypts and villi develop in human fetuses from 8–24 weeks gestation with an increase in crypt depth and villus height with advancing gestational age¹. Similarly, at 9 weeks gestation, proliferating cells are detected throughout the intestine, but these cells localize to their final destination of the crypt base as gestation progresses¹. Development of the crypt-villus structure is influenced by a complex pattern of growth factors provided by surrounding mesenchymal cells and amniotic fluid^{2–4}. Mechanical distension has also been implicated in intestinal morphogenesis. For example, flow of amniotic fluid through the intestinal lumen in utero is required for the formation

of normal intestinal structure, as the development of crypts and villi is impeded in human fetuses with intestinal obstructions such as esophageal or intestinal atresia^{1,5}. Development of the crypt-villus architecture leads to the formation of unique microenvironments that allow for the differentiation of intestinal stem cells (ISCs) into multiple epithelial subtypes, including enterocytes (also named intestinal epithelial cells, IECs), Paneth cells, goblet cells, enteroendocrine cells, tuft cells, and microfold (M) cells. These processes will be discussed in detail below.

Intestinal stem cells (ISCs)

ISCs are the precursor from which all other intestinal epithelial cells differentiate^{1,6,7}. ISCs are located at the base of intestinal crypts and identified by the expression of Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) once mature intestinal architecture is achieved^{1,6,7}. Upon differentiation from ISCs, most epithelial subtypes migrate from the crypt up the intestinal villus⁶. The exception to this rule is Paneth cells, which migrate toward to crypt base⁶. A complex interplay of signaling factors from a variety of cell types, such as Paneth cells, mesenchymal cells, and CD4+ T cells is required to determine epithelial cell fate upon differentiation from ISCs^{8–10}.

Although mature ISCs express Lgr5, the gene expression pattern of ISCs in the fetal intestine had not been analyzed until recently¹¹. Single-cell RNA sequencing of human fetal intestinal tissue provided new insight into the identity of epithelial cell precursors in utero¹¹. At 10 weeks gestation, *Lgr5* expression is found throughout the intestine, not specifically in intestinal crypts, and enterocytes differentiate instead from a “stem-cell like” population of progenitor cells high in *vitronectin*, *transferrin*, and *gata4* expression and low in *Lgr5* expression¹¹. *Lgr5* expression becomes increasingly localized in the crypt at 12 weeks, and Lgr5+ progenitors increase in frequency from 8 weeks to 19+ weeks gestation¹¹. Interestingly, Lgr5+ cells compose as much 18–22% of epithelial cells at 19+ weeks gestation, which is

¹Division of Neonatal-Perinatal Medicine, Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. email: mistygood@unc.edu

Received: 2 July 2022 Revised: 30 October 2022 Accepted: 3 November 2022

Published online: 15 November 2022

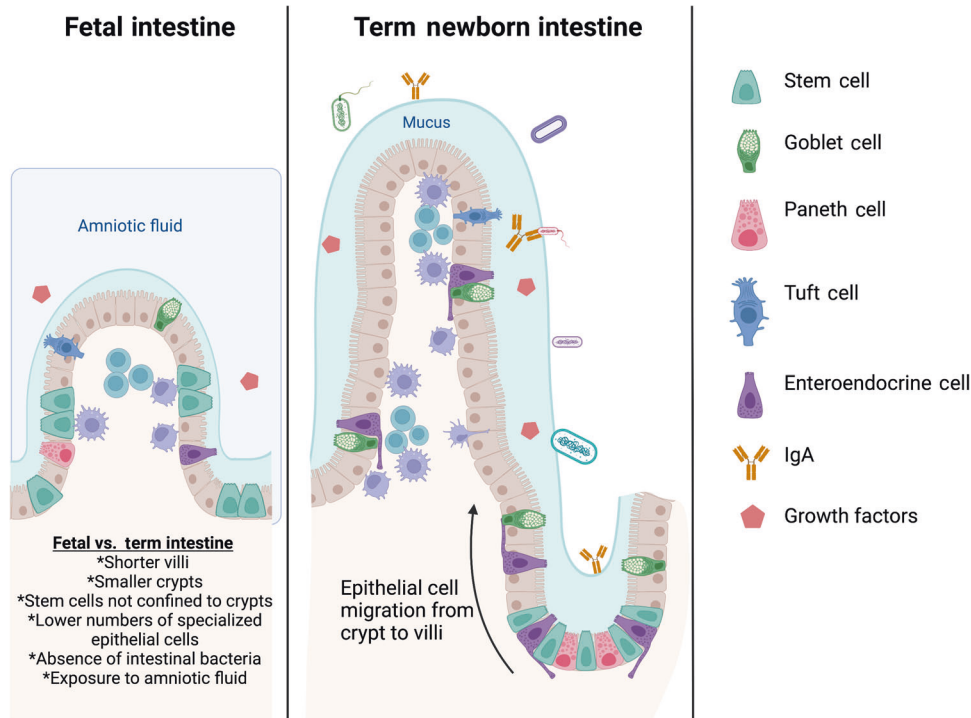


Fig. 1 Comparison of the small intestinal epithelium in the fetus and term neonate. In utero, the intestinal epithelium is exposed to amniotic fluid, which contains growth factors essential for proper intestinal development. The crypt and villus structures mature with increasing gestational age and Lgr5+ intestinal stem cells transition from localization throughout the intestine to the intestinal crypt. Goblet cells and Paneth cells also increase in number throughout fetal development and early life, leading to increased mucus formation and antimicrobial peptide production. Both beneficial and pathogenic microorganisms can be found in the intestinal lumen as the microbiome develops after delivery. Secretory IgA provided by breast milk is a central component of the immune repertoire of the developing intestine and is crucial for establishing control of intraluminal bacteria. Figure created with Biorender.com.

significantly higher than the 3–4% they represent in the adult colon¹¹. The abundance of ISCs in the first and second trimesters is necessary to provide adequate numbers of precursors to form the epithelial lining of the rapidly developing crypt-villus architecture. Once this structure is achieved, the signaling niches required for the development of each epithelial subtype begin to form.

Extremely preterm infants are delivered during a period of intestinal development characterized by an abundance of ISCs and reduced numbers of mature specialized epithelial cells, which places them at risk for a myriad of complications related to inadequate intestinal epithelial function.

Enterocytes

Enterocytes (IECs) are the most abundant epithelial cell in the intestine and serve as the primary barrier for the regulation of transport between the intestinal lumen and the circulation¹². Barrier function provided by these IECs is formed by tight junctions, which are protein complexes between epithelial cells¹³. Tight junctions are initially observed between epithelial cells at approximately 10 weeks gestation¹⁴. After delivery, gestational age and receipt of breast milk feeds impact intestinal permeability^{15–17}. For example, comparison of intestinal barrier function in neonates based on gestational age and the number of days after delivery indicates that intestinal barrier permeability is increased in preterm neonates compared to term infants (> 37 weeks gestation) during the first 2 days after delivery¹⁵. By 3–6 days, intestinal permeability decreases in both term and preterm neonates and is similar between infants across gestational ages¹⁵. Receipt of breast milk feeds is associated with reduced intestinal permeability over the first 30 days of life in preterm neonates, with the most benefit derived from a higher percentage of breast milk feeding (>75% of diet), although

receiving volumes as low as 25% is likely beneficial during that time frame¹⁷. In vitro studies using human intestinal organoids found that human milk increased epithelial cell differentiation and expression of the tight junction protein occludin, which was associated with reduced permeability¹⁸. These studies highlight the importance of providing human milk feeds to neonates. In addition to improving intestinal barrier function, human milk provides numerous factors that provide protective immune functions including immunoglobulins, which will be discussed below.

Due to the restriction of movement across the intestinal epithelium imposed by the epithelial monolayer and tight junctions, specialized transporters are required for the passage of larger molecules such as immunoglobulins between the intestinal lumen, lamina propria, and systemic circulation. One such transporter, the neonatal Fc receptor (FcRn), is expressed by the intestinal epithelium and transfers immunoglobulin G (IgG) across the epithelial monolayer in a bidirectional fashion¹⁹. This process facilitates immune cell activation by transporting antigen-antibody complexes formed in the intestinal lumen to the lamina propria and dendritic cells²⁰. The importance of FcRn-mediated IgG transport in the intestine of human infants is uncertain given the robust transplacental transfer of maternal IgG to the fetal circulation; however, FcRn-mediated transfer of maternal breast milk-derived IgG from the intestinal lumen is important in protection from enteric pathogens in mice and may be of higher importance in the preterm infant who is born prior to transplacental transfer of IgG that occurs in the 3rd trimester^{21,22}. In addition to FcRn, intestinal epithelial cells express a polymeric immunoglobulin receptor (pIgR), which facilitates secretory IgA (sIgA) and IgM transfer across the intestinal epithelium²³. sIgA is the most abundant antibody present in human breast milk and

has a vital role in early host defense²⁴. Mice lacking this receptor or who lack exposure to maternal breast milk-derived sIgA have decreased mucosal barrier integrity and an altered microbiome as adults^{23,25}.

The healthy developing intestinal epithelium, although immature, forms a functional barrier. There are also mechanisms in place for the transport of immune mediators and nutritional factors between the lamina propria and intestinal lumen. Achieving a balance between these opposing roles is critical for intestinal homeostasis, and one of the first challenges faced by the intestinal epithelium is provided by the developing microbiome.

Microbiome in IEC development

The intestinal microbiome develops rapidly upon delivery, and these bacteria can directly impact the development of the intestinal epithelial monolayer. Bacteria abundant in the intestine of breastfeeding term neonates, such as *Bifidobacterium* spp., metabolize human milk oligosaccharides in breast milk and produce biologically active compounds such as short chain fatty acids (SCFAs)^{26,27}. SCFAs can act directly on intestinal epithelial cells and immune cells²⁸. When infants are born prematurely, their microbiome is instead predominated by potentially pathogenic bacteria such as *Staphylococcus*, *Klebsiella*, *Escherichia*, or *Enterococcus* spp²⁹. The presence of these microbes in stool samples has been associated with the development of bacteremia and sepsis in preterm neonates³⁰. Given the negative impacts of microbial dysbiosis on neonatal health, there are ongoing discussions regarding whether routine administration of probiotics to infants in the NICU should occur in an effort to modify the microbiome³¹.

Preterm and critically ill neonates are frequently exposed to antibiotics in the NICU due to their lability and risk of death from infection³². These antibiotics directly impact the composition of the intestinal microbiome^{29,30}. In addition, mechanistic studies in mice have demonstrated how antibiotic administration impacts intestinal development. For example, treatment of neonatal mice with the commonly used antibiotics ampicillin and gentamicin for 10 days resulted in significantly shorter villus length and crypt depth relative to untreated controls, as well as increased intestinal permeability and inflammation³³. These findings were associated with decreased intestinal epithelial cell proliferation within the crypts as well as lower numbers of goblet and Paneth cells³³. In another study examining the impact of the microbiome on intestinal development, germ-free mice were colonized with the microbiome of preterm human infants born at 27 weeks gestation who had weight gain of either < 10 g/kg/day (inadequate growth) or >10 g/kg/day (normal growth). The microbiome from infants with normal growth resulted in the development of a significantly longer small intestine as well as longer villi and deeper crypts in recipient mice³⁴. This improved intestinal growth was associated with increased numbers of proliferating stem cells and transit amplifying cells as well as goblet cells, and Paneth cells³⁴. Mice that received the microbiome from infants with poor weight gain were found to have increased intestinal and systemic proinflammatory gene expression³⁴. Improving growth and reducing inflammation are two additional reasons to strive towards promoting a healthy microbiome in neonates by providing human milk feeds and avoiding unnecessary antibiotic administration.

Activation of intestinal epithelial cells

Bacteria in the intestinal lumen can induce inflammation by stimulating innate immune receptors such as Toll-like receptors (TLRs), which are located on intestinal epithelial cells and immune cells. TLRs are a class of innate immune receptors that recognize components of bacteria and viruses^{35,36}. Stimulation of TLRs activates an inflammatory cascade that can lead to the elimination of an invading pathogen and/or tissue damage^{35,36}. Studies

utilizing reporter mice that express fluorescent proteins in conjunction with TLRs found similar levels of TLR2, 4, 7, and 9 in both neonatal and adult small intestines and colons³⁷. Studies in fetal mice detected higher expression of TLR4, the receptor for bacterial lipopolysaccharide (LPS), and lower levels of TLR9, a receptor for bacterial DNA, in the intestine of mice in utero relative to the expression at the time of delivery³⁸. This expression pattern has been linked to the pathogenesis of NEC, which will be discussed below in more detail³⁸. TLR5, which recognizes bacterial flagellin, is expressed throughout the intestinal epithelium in neonatal mice³⁷. This level of expression decreases until weaning/adulthood, when TLR5 is primarily localized to Paneth cells³⁷. Increased TLR5 signaling in neonatal mice has been associated with the early establishment of the microbiome and controlling flagellated intestinal bacteria³⁹. Expression of TLR3, the receptor for double stranded RNA, is also developmentally regulated in mice. PCR analysis of gene expression on intestinal epithelial cells found that TLR3 expression gradually increased from P7 to P28³⁷. Studies in mice indicate that increased susceptibility of neonates to infection with Rotavirus, a cause of severe diarrhea in infants, may be in part due to the reduced TLR3 expression on IECs of neonatal mice⁴⁰. Although more mechanistic studies are needed, it is clear that changes in TLR expression over the course of intestinal development are a critical mechanism underlying age-based differences in susceptibility to inflammatory and infectious GI diseases.

The innate immune defense mechanisms of the small intestine extend beyond TLR signaling in the intestinal epithelium. Two important epithelial cell subtypes involved in innate immunity include Paneth cells and goblet cells. These cells will be discussed in detail below.

Paneth cells

Paneth cells produce antimicrobial peptides (AMPs), a diverse family of molecules that serve as an initial line of defense against potentially harmful pathogens in the gut^{41,42}. Murine experiments indicate that Paneth cells also impact Lgr5+ ISC development via growth factor production⁴³.

Paneth cell numbers increase in the human intestine with advancing gestational age (Fig. 1)^{44,45}. In human intestinal biopsy samples, Paneth cells are observed in very low numbers at 17–23 weeks gestation but increase dramatically at term⁴⁴. Similarly, in the murine small intestine, Paneth cell numbers increase linearly from P1 to P28, with low numbers found in the first 7 days after birth^{44,46}. Paneth cell-produced AMPs also have a gestational age-dependent expression pattern^{44,45,47}. For example, the expression of the AMPs regenerating islet-derived protein 3, lysozyme-1, and alpha defensin increase with increasing Paneth cell numbers in mice and humans⁴⁴. When infants are delivered preterm, their Paneth cell and AMP levels are reduced compared to term infants, which likely increases their risk for sepsis resulting from bacterial translocation from the gut. In contrast to the other AMPs, the expression pattern of the antimicrobial peptide cathelin-related antimicrobial peptide (CRAMP) is highest shortly after birth and subsequently decreases with age. IEC expression of CRAMP is detectable in the intestine of neonatal mice until P14 and then becomes undetectable at P21 and P28⁴⁷. This may be clinically significant as neonatal CRAMP knockout mice exhibit decreased control of oral *Listeria monocytogenes* infection⁴⁷. In addition, CRAMP has dose-dependent antimicrobial activity against a variety of commensal bacteria as well as potential neonatal pathogens⁴⁷. These findings have led to the hypothesis that CRAMP may have an important role in the initial establishment of the neonatal intestinal microbiome⁴⁷.

Goblet cells

Goblet cells produce mucus, secrete AMPs, and deliver antigens to intestinal immune cells via goblet cell-associated antigen passages

(GAPs)⁴⁸. In utero, goblet cell numbers are minimal at 8–10 weeks gestation but increase between 12 weeks to 19+ weeks¹¹. Mucin 2 (muc2) expressing goblet cell numbers remain stable between 17 weeks and term gestation⁴⁴. A similar pattern is observed in the murine intestine from P1 to P28⁴⁴. Disruption of goblet cell function and mucus production can lead to reduced protection of IECs from intraluminal gut bacteria, which can subsequently lead to increased inflammation³³. The mucus produced by goblet cells is an important component of the intestine's innate immune defenses and barrier function. In contrast, GAPs have been associated with the development of late onset sepsis from *Escherichia coli* in a neonatal model⁴⁹. Epidermal growth factor (EGF) from breast milk led to reduced GAP formation and decreased *E. coli* translocation from the intestine⁴⁹. GAPs are important in providing antigens to antigen-presenting cells, which leads to immune activation. In neonates, there is a delicate balance between providing immune education and preventing dissemination of potentially harmful bacteria. Bacterial species that evade mucosal defense mechanisms, such as the invasive *E. coli* described above, are a particularly important threat to neonatal health. Another intestinal cell type involved in providing antigens from the intestinal lumen to underlying immune cells is Microfold (M) cells.

M cells

M cells are a specialized type of epithelial cells found in close association with gut-associated lymphoid tissue⁵⁰. These cells serve as a route of transfer of microbial products from the intestinal lumen to antigen-presenting cells, which then modulate the immune response⁵⁰. In mice, M cell numbers are minimal in the first few days after birth, with increasing numbers noted after P8⁵¹. In human fetal tissue, M cells have been identified as early as 17 weeks in the small intestine⁵².

Functionally, sIgA production in the intestine requires appropriate M cell development due to the role of M cells in intestinal microbial antigen delivery to underlying immune cells⁵³. For example, recently weaned mice with an M cell maturation defect were found to have reduced concentrations of reduced of commensal bacteria-specific IgA and IgA bound bacteria in their stool^{53,54}. The importance of M cells in sIgA production prior to weaning is likely minimized due to the abundance of sIgA provided by human milk⁵⁵. This is supported by findings of significantly lower levels of intestinal bacteria bound by sIgA in the feces of formula fed preterm human infants relative to those receiving maternal milk⁵⁶. Thus, sIgA provided by maternal milk likely predominates above M cell mediated mechanisms in early life.

Enteroendocrine cells

Enteroendocrine cells are primarily known to produce hormones in the gut in response to nutritional signals, which subsequently aid in digestion and metabolism⁵⁷. Quantification of enteroendocrine cells by staining tissue sections for α -chromogranin over the course of fetal development found similar percentages in the small intestine from 17 weeks gestation to term⁴⁴. Mice have a similar developmental pattern with a constant number between P1 and P28⁴⁴. Analysis of serum levels of gut hormones found that the impact of gestational age at delivery and the number of days since birth on hormone levels is variable⁵⁸. For example, serum leptin levels are higher in term neonates and decrease rapidly postnatally, whereas glucagon-like peptide-1 (GLP1) is inversely correlated with gestational age and increases with time after delivery⁵⁸. Although rare, enteroendocrine cells are essential for effective nutrient absorption. For example, patients with disrupted enteroendocrine cell formation due to mutations in neurogenin-3 develop severe malabsorptive diarrhea leading to a dependence on parenteral nutrition⁵⁹.

Tuft cells

Tuft cells are another rare intestinal epithelial cell subtype and are characterized by apical microvilli extensions that express receptors traditionally associated with the detection of taste in addition to the receptor for the short chain fatty acid succinate^{60–62}. Tuft cell numbers in mice increase after weaning and the transition to solid food⁶². In human fetal intestinal tissue, tuft cells can be identified as early as 16 weeks gestation⁵². Studies in mice indicate that tuft cells and group 2 innate lymphoid cells (ILC2) are involved in a homeostatic signaling loop whereby tuft cells' secretion of interleukin (IL)-25 leads to increased ILC2 numbers in the small intestine and enhanced ILC2 production of IL-13^{63,64}. IL-13 then acts on epithelial progenitors in the intestinal crypt to promote the expansion of both tuft and goblet cell populations^{63,64}. In the presence of a parasitic infection, this cycle is magnified, leading to tuft cell hyperplasia^{63,64}.

Overview of necrotizing enterocolitis

Normal intestinal development is abruptly interrupted upon preterm delivery, and stress is placed on the immature intestinal epithelium in the setting of nutritional demands, microbial challenges, and inflammation resulting from critical illness (Fig. 2). These factors contribute to the development of NEC, which is a severe intestinal disease that impacts approximately 7% of preterm neonates born at <1500 g annually⁶⁵. The sequelae of NEC are numerous and include sepsis, prolonged hospitalization, developmental delay, short bowel syndrome, and death⁶⁶. The mechanisms underlying NEC are incompletely understood, but we will discuss the role of each subtype of specialized epithelial cell that has been implicated in the pathophysiology of NEC.

Intestinal stem cells and NEC

Mechanistic studies in mice indicated that NEC-like intestinal injury leads to impaired intestinal healing associated with increased IEC apoptosis and decreased ISC proliferation⁶⁷. Activation of the innate immune receptor Toll-like receptor 4 (TLR4) on Lgr5+ ISCs has been implicated in this process^{67,68}. Conversely, amniotic fluid inhibits TLR4 signaling in utero, and administration of amniotic fluid decreases NEC severity in mice via activation of epidermal growth factor signaling in the intestinal epithelium⁶⁹. This subsequently protects against the decrease in enterocyte proliferation seen with TLR4 activation and development of NEC-like intestinal injury⁶⁹. Similarly, administration of lactoferrin, a component of breast milk, during experimental NEC has also been associated with reduced disease severity, increased *lgr5* expression, and improved epithelial cell proliferation⁷⁰. In the setting of intestinal injury, it is crucial for ISCs to replicate and the monolayer of enterocytes lining the intestinal crypts and villi to heal. If this process is interrupted, impaired intestinal barrier function can lead to sepsis, bowel necrosis, and death.

TLR signaling and NEC

Epithelial cell TLR4 signaling has been associated with the development of NEC in animal models and human infants⁷¹, and reviewed in detail by Mihi et al.⁷². Resected intestinal samples from infants with NEC exhibit increased TLR4 expression^{71,73,74}. In addition, expression of TLR2 and the TLR-associated signaling molecules myeloid differentiation primary response 88 (MyD88), TNF receptor-associated factors (TRAF), and nuclear factor-kappa B (NFkB) are increased in fetal intestinal samples and in resected tissue from infants with NEC while negative regulators of inflammation such as single Ig IL-1-related receptor (SIGIRR), toll-interacting protein (TOLLIP), and tumor necrosis factor- α (TNF- α)-induced protein 3 (TNFAIP3) are reduced⁷⁴. In the absence of intestinal epithelial cell expression of TLR4, the severity of experimental NEC in mice is significantly reduced⁷¹. Several mechanisms have been identified for the TLR4-mediated role in NEC. Murine studies have found that the absence of TLR4

The Intestinal Epithelium during Necrotizing Enterocolitis

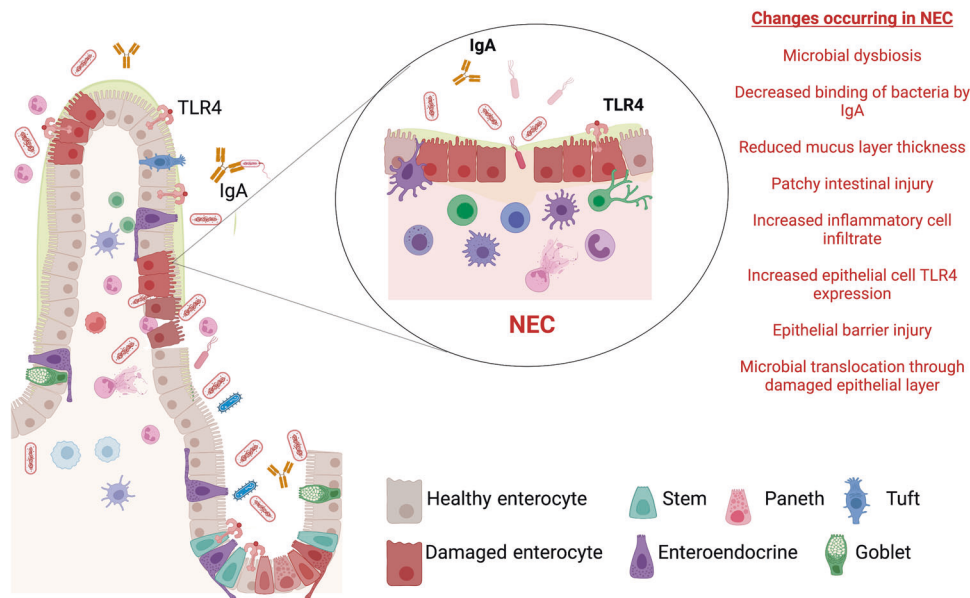


Fig. 2 The neonatal intestinal epithelium during necrotizing enterocolitis. Injury to the developing intestinal epithelium underlies the pathophysiology of necrotizing enterocolitis (NEC, circular inset). Preterm neonates are at risk for NEC due to a myriad of factors related to their stage of intestinal development and critical illness. When an infant is delivered preterm, their intestinal epithelium is composed of lower numbers of mature specialized epithelial cells relative to term infants. For example, lower goblet and Paneth cell numbers are associated with associated impairments in mucus and antimicrobial peptide production. These deficits lead to an increase in potentially pathogenic Gram-negative bacteria in the intestinal lumen. These bacteria can activate TLR4 signaling on the intestinal epithelium leading to the induction of an inflammatory cascade. Inflammation subsequently results in patchy intestinal epithelial injury, reduced barrier integrity, and translocation of bacteria from the intestinal lumen. When this occurs, infants are also at risk for sepsis, irreparable intestinal injury, and death. Figure created with Biorender.com.

expression during experimental NEC is associated with reduced IEC apoptosis and increased IEC migration⁷³. In addition, goblet cell differentiation is enhanced, and numbers of goblet cells are increased⁷¹. TLR4 activation in intestinal epithelial cells also induces autophagy, and levels of autophagy are increased in mice during experimental NEC as well in human intestinal tissues from infants with NEC⁷⁵. TLR4 activation has also been implicated in the induction of endoplasmic reticulum stress and in the impairment of ISC regeneration⁶⁸.

Paneth cells and NEC

In preterm neonates, Paneth cells are immature, and present at reduced numbers relative to term neonates, as discussed above^{44,45}. Paneth cell numbers are also decreased in intestinal samples from infants with NEC^{76–81}. Depletion of Paneth cells by dithizone administration or using genetic deletion strategies in neonatal pups (P14–16) followed by gavage with *Klebsiella pneumoniae* leads to NEC-like intestinal injury and increased mortality in mice^{78,82}. Similarly, in neonatal rats, depletion of Paneth cells with dithizone administration followed by enteral *E. coli* gavage leads to significantly increased *E. coli* burden, NEC-like intestinal injury, and death⁸³. It has been hypothesized that the decreased antimicrobial properties of Paneth cells in preterm neonates contributes to microbial dysbiosis, intestinal inflammation, loss of intestinal barrier function, and NEC⁷⁶.

Goblet cells and NEC

Goblet cell numbers are reduced in the intestine of neonates with NEC^{71,79,80}. In mice, decreased numbers of goblet cells and reduced mucus production have been associated with increased NEC severity^{33,71,84}. Antibiotic treatment of mice has been associated with impaired goblet cell development, thinning of the mucus layer, increased proximity of gavigated *K. pneumoniae* to

the intestinal epithelium, and worsened intestinal injury in a model of NEC³³. In contrast, the promotion of goblet cell differentiation in mice via inhibition of Notch signaling reduces NEC severity⁷¹. The protection provided by augmented goblet cell development is likely directly related to an improved mucus layer and decreased activation of innate immune receptors by intraluminal gut bacteria.

Enteroendocrine cells and NEC

Enteroendocrine cells have not been studied in detail in the context of NEC; however, they are detected in reduced numbers in intestinal resections from infants with NEC relative to those without NEC⁸⁰. This decrease is likely reflective of damage to ISCs and the intestinal epithelium and not due to a prominent causative role in the pathophysiology of NEC.

CONCLUSION

The intestinal epithelium provides a dynamic barrier that prevents invasion by dangerous pathogens while also permitting nutrient metabolism and absorption. This epithelial layer is constantly being replenished by ISCs during both homeostasis and tissue injury. The specialized epithelial cells that develop from ISC progenitors have unique functions that contribute to the diverse requirements for proper intestinal function. Interruption of the cycle of rapid replacement of the epithelium from ISCs can lead to intestinal diseases such as NEC. Developmental regulation of specialized epithelial cell numbers and function can also place the preterm neonate at increased risk of intestinal injury. Current research efforts are focused on furthering our understanding of how the immature intestine functions during homeostasis and how it can be protected from potentially devastating injury such as that which occurs during NEC.

REFERENCES

1. Condino, A. A. et al. Abnormal intestinal histology in neonates with congenital anomalies of the gastrointestinal tract. *Biol. Neonate* **85**, 145–150 (2004).
2. McCarthy, N. et al. Distinct mesenchymal cell populations generate the essential intestinal BMP signaling gradient. *Cell Stem Cell* **26**, 391–402.e395 (2020).
3. Kwon, O., Han, T. S. & Son, M. Y. Intestinal morphogenesis in development, regeneration, and disease: the potential utility of intestinal organoids for studying compartmentalization of the crypt-villus structure. *Front Cell Dev. Biol.* **8**, 593969 (2020).
4. Dasgupta, S., Arya, S., Choudhary, S. & Jain, S. K. Amniotic fluid: source of trophic factors for the developing intestine. *World J. Gastrointest. Pathophysiol.* **7**, 38–47 (2016).
5. Trahair, J. F., Harding, R., Bocking, A. D., Silver, M. & Robinson, P. M. The role of ingestion in the development of the small intestine in fetal sheep. *Q. J. Exp. Physiol.* **71**, 99–104 (1986).
6. Barker, N. et al. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* **449**, 1003–1007 (2007).
7. Sato, T. et al. Single *Lgr5* stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009).
8. Farin, H. F., Van, E. J. H. & Clevers, H. Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. *Gastroenterology* **143**, 1518–1529.e1517 (2012).
9. Beumer, J. & Clevers, H. Cell fate specification and differentiation in the adult mammalian intestine. *Nat. Rev. Mol. Cell Biol.* **22**, 39–53 (2021).
10. Schreurs, R. et al. Human fetal TNF- α -cytokine-producing CD4(+) effector memory T cells promote intestinal development and mediate inflammation early in life. *Immunity* **50**, 462–476.e468 (2019).
11. Fawcner-Corbett, D. et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. *Cell* **184**, 810–826.e823 (2021).
12. Snoeck, V., Goddeeris, B. & Cox, E. The role of enterocytes in the intestinal barrier function and antigen uptake. *Microbes Infect.* **7**, 997–1004 (2005).
13. Suzuki, T. Regulation of the intestinal barrier by nutrients: the role of tight junctions. *Anim. Sci. J.* **91**, e13357 (2020).
14. 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.* **65**, 53–62 (1980).
15. van Elburg, R. M., Fetter, W. P., Bunkers, C. M. & Heymans, H. S. Intestinal permeability in relation to birth weight and gestational and postnatal age. *Arch. Dis. Child Fetal Neonatal Ed.* **88**, F52–55 (2003).
16. Shulman, R. J. et al. Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. *Pediatr. Res.* **44**, 519–523 (1998).
17. Taylor, S. N., Basile, L. A., Ebeling, M. & Wagner, C. L. Intestinal permeability in preterm infants by feeding type: mother's milk versus formula. *Breastfeed. Med.* **4**, 11–15 (2009).
18. Noel, G. et al. Human breast milk enhances intestinal mucosal barrier function and innate immunity in a healthy pediatric human enteroid model. *Front. Cell Dev. Biol.* **9**, 685171 (2021).
19. Shah, U. et al. Distribution of the IgG Fc receptor, FcRn, in the human fetal intestine. *Pediatr. Res.* **53**, 295–301 (2003).
20. Yoshida, M. et al. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity* **20**, 769–783 (2004).
21. Zheng, W. et al. Microbiota-targeted maternal antibodies protect neonates from enteric infection. *Nature* **577**, 543–548 (2020).
22. Fouda, G. G., Martinez, D. R., Swamy, G. K. & Permar, S. R. The Impact of IgG transplacental transfer on early life immunity. *Immunohorizons* **2**, 14–25 (2018).
23. Johansen, F. E. et al. Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J. Exp. Med.* **190**, 915–922 (1999).
24. Atyeo, C. & Alter, G. The multifaceted roles of breast milk antibodies. *Cell* **184**, 1486–1499 (2021).
25. Rogier, E. W. et al. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc. Natl Acad. Sci. USA* **111**, 3074–3079 (2014).
26. Lawson, M. A. E. et al. Breast milk-derived human milk oligosaccharides promote Bifidobacterium interactions within a single ecosystem. *ISME J.* **14**, 635–648 (2020).
27. Tsukuda, N. et al. Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME J.* **15**, 2574–2590 (2021).
28. Parada Venegas, D. et al. Short Chain Fatty Acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* **10**, 277 (2019).
29. Rao, C. et al. Multi-kingdom ecological drivers of microbiota assembly in preterm infants. *Nature* **591**, 633–638 (2021).
30. Lee, C. C. et al. Gut dysbiosis, bacterial colonization and translocation, and neonatal sepsis in very-low-birth-weight preterm infants. *Front. Microbiol.* **12**, 746111 (2021).
31. Poindexter B, COMMITTEE ON FETUS AND NEWBORN. Use of probiotics in preterm infants. *Pediatrics* **147**, e2021051485 (2021).
32. Flannery, D. D. et al. Temporal trends and center variation in early antibiotic use among premature infants. *JAMA Netw. Open* **1**, e180164 (2018).
33. Chaaban, H. et al. Early antibiotic exposure alters intestinal development and increases susceptibility to necrotizing enterocolitis: a mechanistic study. *Microorganisms* **10**, 519 (2022).
34. Yu, Y., Lu, L., Sun, J., Petrof, E. O. & Claud, E. C. Preterm infant gut microbiota affects intestinal epithelial development in a humanized microbiome gnotobiotic mouse model. *Am. J. Physiol. Gastrointest. Liver Physiol.* **311**, G521–532 (2016).
35. Fitzgerald, K. A. & Kagan, J. C. Toll-like receptors and the control of immunity. *Cell* **180**, 1044–1066 (2020).
36. Gong, T., Liu, L., Jiang, W. & Zhou, R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat. Rev. Immunol.* **20**, 95–112 (2020).
37. Price, A. E. et al. A map of toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. *Immunity* **49**, 560–575.e566 (2018).
38. Gribar, S. C. et al. Reciprocal expression and signaling of TLR4 and TLR9 in the pathogenesis and treatment of necrotizing enterocolitis. *J. Immunol.* **182**, 636–646 (2009).
39. Fulde, M. et al. Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition. *Nature* **560**, 489–493 (2018).
40. Pott, J. et al. Age-dependent TLR3 expression of the intestinal epithelium contributes to rotavirus susceptibility. *PLoS Pathog.* **8**, e1002670 (2012).
41. McElroy, S. J. & Weitkamp, J. H. Innate immunity in the small intestine of the preterm infant. *Neoreviews* **12**, e517–e526 (2011).
42. Vaishnav, S., Behrendt, C. L., Ismail, A. S., Eckmann, L. & Hooper, L. V. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc. Natl Acad. Sci. USA* **105**, 20858–20863 (2008).
43. Sato, T. et al. Paneth cells constitute the niche for *Lgr5* stem cells in intestinal crypts. *Nature* **469**, 415–418 (2011).
44. Stanford, A. H. et al. A direct comparison of mouse and human intestinal development using epithelial gene expression patterns. *Pediatr. Res.* **88**, 66–76 (2020).
45. Heida, F. H. et al. Paneth cells in the developing gut: when do they arise and when are they immune competent? *Pediatr. Res.* **80**, 306–310 (2016).
46. Pandey U. & Aich P. Postnatal intestinal mucosa and gut microbial composition develop hand in hand: a mouse study. *Biomed. J.* S2319-4170(22)00033-6 (2022).
47. Menard, S. et al. Developmental switch of intestinal antimicrobial peptide expression. *J. Exp. Med.* **205**, 183–193 (2008).
48. Yang, S. & Yu, M. Role of goblet cells in intestinal barrier and mucosal immunity. *J. Inflamm. Res.* **14**, 3171–3183 (2021).
49. Knoop, K. A. et al. Maternal activation of the EGFR prevents translocation of gut-residing pathogenic *Escherichia coli* in a model of late-onset neonatal sepsis. *Proc. Natl Acad. Sci. USA* **117**, 7941–7949 (2020).
50. Dillon, A. & Lo, D. D. M. Cells: intelligent engineering of mucosal immune surveillance. *Front. Immunol.* **10**, 1499 (2019).
51. Zhang, K. et al. Age-dependent enterocyte invasion and microcolony formation by *Salmonella*. *PLoS Pathog.* **10**, e1004385 (2014).
52. Moxey, P. C. & Trier, J. S. Specialized cell types in the human fetal small intestine. *Anat. Rec.* **191**, 269–285 (1978).
53. Rios, D. et al. Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunol.* **9**, 907–916 (2016).
54. Kimura, S. et al. Sox8 is essential for M cell maturation to accelerate IgA response at the early stage after weaning in mice. *J. Exp. Med.* **216**, 831–846 (2019).
55. Rio-Aige, K. et al. The breast milk immunoglobulinome. *Nutrients* **13**, 1810 (2021).
56. Gopalakrishna, K. P. et al. Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. *Nat. Med.* **25**, 1110–1115 (2019).
57. Gribble, F. M. & Reimann, F. Function and mechanisms of enteroendocrine cells and gut hormones in metabolism. *Nat. Rev. Endocrinol.* **15**, 226–237 (2019).
58. Kawamata, R. et al. Gut hormone profiles in preterm and term infants during the first 2 months of life. *J. Pediatr. Endocrinol. Metab.* **27**, 717–723 (2014).
59. Wang, J. et al. Mutant neurogenin-3 in congenital malabsorptive diarrhea. *N. Engl. J. Med.* **355**, 270–280 (2006).
60. Ting, H. A. & von Moltke, J. The immune function of tuft cells at gut mucosal surfaces and beyond. *J. Immunol.* **202**, 1321–1329 (2019).
61. Schutz, B. et al. Chemical coding and chemosensory properties of cholinergic brush cells in the mouse gastrointestinal and biliary tract. *Front. Physiol.* **6**, 87 (2015).
62. Schneider, C. et al. A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. *Cell* **174**, 271–284.e214 (2018).

63. von Moltke, J., Ji, M., Liang, H. E. & Locksley, R. M. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* **529**, 221–225 (2016).
64. Howitt, M. R. et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **351**, 1329–1333 (2016).
65. Alsaied, A., Islam, N. & Thalib, L. Global incidence of Necrotizing Enterocolitis: a systematic review and Meta-analysis. *BMC Pediatr.* **20**, 344 (2020).
66. Bazacliu, C. & Neu, J. Necrotizing Enterocolitis: long term complications. *Curr. Pediatr. Rev.* **15**, 115–124 (2019).
67. Neal, M. D. 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.* **287**, 37296–37308 (2012).
68. Afrazi, A. et al. Toll-like receptor 4-mediated endoplasmic reticulum stress in intestinal crypts induces necrotizing enterocolitis. *J. Biol. Chem.* **289**, 9584–9599 (2014).
69. Good, M. et al. Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. *Proc. Natl Acad. Sci. USA* **109**, 11330–11335 (2012).
70. Liu, J. et al. Lactoferrin reduces necrotizing enterocolitis severity by upregulating intestinal epithelial proliferation. *Eur. J. Pediatr. Surg.* **30**, 90–95 (2020).
71. Sodhi, C. P. et al. Intestinal epithelial Toll-like receptor 4 regulates goblet cell development and is required for necrotizing enterocolitis in mice. *Gastroenterology* **143**, 708–718.e705 (2012).
72. Mihi, B. & Good, M. Impact of Toll-like receptor 4 signaling in necrotizing enterocolitis: the state of the science. *Clin. Perinatol.* **46**, 145–157 (2019).
73. Leaphart, C. L. et al. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. *J. Immunol.* **179**, 4808–4820 (2007).
74. Nanthakumar, N. et al. The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. *PLoS ONE* **6**, e17776 (2011).
75. Neal, M. D. et al. A critical role for TLR4 induction of autophagy in the regulation of enterocyte migration and the pathogenesis of necrotizing enterocolitis. *J. Immunol.* **190**, 3541–3551 (2013).
76. Lueschow, S. R. & McElroy, S. J. The Paneth cell: the curator and defender of the immature small intestine. *Front Immunol.* **11**, 587 (2020).
77. McElroy, S. J., Underwood, M. A. & Sherman, M. P. Paneth cells and necrotizing enterocolitis: a novel hypothesis for disease pathogenesis. *Neonatology* **103**, 10–20 (2013).
78. Zhang, C. 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.* **5**, 522–532 (2012).
79. McElroy, S. J. 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.* **301**, G656–G666 (2011).
80. Lanik, W. E. et al. Microfluidic device facilitates novel in vitro modeling of human neonatal necrotizing enterocolitis-on-a-chip. Preprint at *bioRxiv* <https://doi.org/10.1101/2020.11.29.402735> (2020).
81. Coutinho, H. B. et al. Absence of lysozyme (muramidase) in the intestinal Paneth cells of newborn infants with necrotizing enterocolitis. *J. Clin. Pathol.* **51**, 512–514 (1998).
82. Lueschow, S. R. et al. Loss of murine Paneth cell function alters the immature intestinal microbiome and mimics changes seen in neonatal necrotizing enterocolitis. *PLoS ONE* **13**, e0204967 (2018).
83. Sherman, M. P., Bennett, S. H., Hwang, F. F., Sherman, J. & Bevins, C. L. Paneth cells and antibacterial host defense in neonatal small intestine. *Infect. Immun.* **73**, 6143–6146 (2005).
84. Martin, N. A. et al. Active transport of bile acids decreases mucin 2 in neonatal ileum: implications for development of necrotizing enterocolitis. *PLoS ONE* **6**, e27191 (2011).

ACKNOWLEDGEMENTS

L.C.F. is supported by a Thrasher Research Fund Early Career Award and a UNC Children's Development Early Career Investigator Grant through the generous support of donors to UNC. M.G. is supported by National Institutes of Health (NIH) grants R01DK124614, R01DK118568, and R01HD105301.

AUTHOR CONTRIBUTIONS

L.C.F. and M.G. reviewed the relevant literature, drafted, revised, and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Misty Good.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.