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REVIEW

Bacterial colonization and intestinal mucosal barrier development

Xiao-Zhong Huang, Li-Bin Zhu, Zhong-Rong Li, Jing Lin

Xiao-Zhong Huang, Li-Bin Zhu, Zhong-Rong Li, Jing Lin, Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China
Jing Lin, Division of Newborn Medicine, Department of Pediatrics, Kravis Children's Hospital of the Icahn School of Medicine at Mount Sinai, New York, NY 10029-6574, United States
Author contributions: Huang XZ wrote the first draft; Zhu LB and Li ZR contributed some sections of the first draft; Lin J initiated the project and finalized the manuscript.
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Correspondence to: Jing Lin, MD, Division of Newborn Medicine, Department of Pediatrics, Kravis Children's Hospital of the Icahn School of Medicine at Mount Sinai, Box 1508, One Gustave L. Levy Place, New York, NY 10029-6574, United States. jing.lin@mssm.edu
Telephone: +1-212-2416186 Fax: +1-212-5345207
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Abstract

The intestinal tract is colonized soon after birth with a variety of ingested environmental and maternal microflora. This process is influenced by many factors including mode of delivery, diet, environment, and the use of antibiotics. Normal intestinal microflora provides protection against infection, ensures tolerance to foods, and contributes to nutrient digestion and energy harvest. In addition, enteral feeding and colonization with the normal commensal flora are necessary for the maintenance of intestinal barrier function and play a vital role in the regulation of intestinal barrier function. Intestinal commensal microorganisms also provide signals that foster normal immune system development and influence the ensuing immune responses. There is increasingly recognition that alterations of the microbial gut flora and associated changes in intestinal barrier function may be related to certain diseases of the gastrointestinal tract. This review summarizes recent advances in understanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. Disruption in the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including inflammatory bowel disease, nosocomial infection, and neonatal necrotizing enterocolitis.

Key words: Bacterial colonization; Intestinal barrier; Intestinal microflora; Microbiota; Neonatal necrotizing enterocolitis; Nosocomial infection; Premature infants; Short chain fatty acids

Core tip: This review summarizes recent advances in understanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. There is increasingly recognition that the stimulation of initial intestinal microbial colonization is important for proper maturation of the innate immune system and continued regulation and maintenance of intestinal barrier function. Disruption of the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including inflammatory bowel disease, nosocomial infection, and neonatal necrotizing enterocolitis in premature infants.

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INTRODUCTION

The human gut is home to a large collection of micro-

organisms, the composition of which varies along the intestinal tract. The important role of normal microbial intestinal colonization in human health has gained increased recognition over the past several decades. The gut microflora, which is composed of approximately 10^{14} bacteria, or approximately 10 times the number of body cells, is now considered as a functional human organ[1]. New studies such as the Human Microbiome Project are revealing how the gut microflora manipulates and complements physiology in ways that are important for the host[2-4]. The normal gut microflora provides protection against infection, educates the immune system, ensures tolerance to foods, and contributes to nutrient digestion and energy harvest. In addition to these important functions, normal microbial colonization of the intestine is important in the induction of the host innate response and plays a vital role in the regulation and maintenance of intestinal barrier function. Disruption in the establishment of a stable normal gut microflora may be associated with or even contribute to the pathogenesis of diseases including inflammatory bowel disease (IBD), nosocomial infection, and neonatal necrotizing enterocolitis (NEC)[5]. This review summarizes recent advances in understanding the complex ecosystem of the gut microflora and the roles of gut microflora in regulating intestinal barrier function as well as a few common pediatric diseases which may be related to an altered gut microbiota.

ESTABLISHMENT OF NORMAL INTESTINAL MICROBIAL COLONIZATION IS ESSENTIAL FOR THE POSTNATAL INTESTINAL BARRIER MATURATION

Before birth, the intestine is sterile. The intestinal tract becomes colonized soon after birth with a variety of ingested environmental and maternal microorganisms. This process is influenced by many factors including mode of delivery, diet, environment, and the use of antibiotics[6,7]. For example, a breast-fed full-term infant normally has an intestinal microbiota in which bifidobacteria predominate over potentially harmful bacteria, whereas in formula-fed infants, enterococci, bacteroides and clostridia predominate[8]. In premature infants, the immature intestinal mucosa is even more sensitive to gut colonizing bacteria. Host defenses can be improved by feeding the breast milk which helps the immature intestinal mucosal immune system to develop and respond appropriately to the highly variable bacterial colonization[8].

The dense communities of bacteria in the intestine are separated from body tissues by a monolayer of intestinal epithelial cells. Therefore, normal intestinal function depends on the establishment and maintenance of the mucosal epithelial barrier which prevents the invasion of host tissues by resident bacteria. The assembly of the multiple components of the intestinal barrier is initiated during fetal development and continues during early postnatal life; thus the intestinal barrier has not completely developed soon after birth, particularly in preterm infants[9]. Several studies indicate that the normal bacterial colonization process may be important for postnatal intestinal barrier development. By using a newborn piglet model, Kansagra *et al*[10] demonstrated that intestinal barrier function was significantly less developed in full term newborn piglets receiving total parental nutrition compared to those receiving enteral nutrition. Even in the mature intestine, lack of enteral nutrition is associated with loss of intestinal epithelial barrier function which can lead to bacterial translocation and subsequent sepsis[11]. In a rodent model, replacing enteral nutrition with parenteral nutrition can lead to bacterial translocation from the gut[11,12]. Total parenteral nutrition significantly increases intestinal permeability, which can be ameliorated by enteral feeding and especially with a fiber enhanced diet[13]. All of these results suggest that enteral feeding and colonization with the normal commensal flora are necessary for the maintenance of intestinal barrier function.

Recent studies have demonstrated that certain commensal bacteria increase intestinal epithelial cell survival by inhibiting the activation of the epithelial cell pro-apoptotic pathway associated with pathogenic bacteria[14]. The intestinal commensal flora is also involved in maintenance of barrier function by inducing increased epithelial cell proliferation and enhancing intestinal epithelial integrity, through translocation of the tight junction proteins and up-regulation of genes involved in desmosome maintenance[15,16]. Fermentation products of commensal bacterial have been shown to enhance the intestinal barrier function by facilitating the assembly of tight junctions through the activation of AMP-activated protein kinase[17]. On the other hand, the deletion of all detectable commensal flora in the gut by a four-week course of orally administration of vancomycin, neomycin, metronidazole, and ampicillin leads to a more severe intestinal mucosal injury in a dextran sulfate sodium induced mouse colitis model[18]. The importance of normal bacterial colonization in the development and maintenance of the intestinal barrier is further supported by the observations that the gastrointestinal tract gene expression profile and intestinal barrier development are altered by early treatment with broad-spectrum antibiotics or withholding enteral feeding[19].

INTESTINAL MICROFLORA STIMULATES THE MATURATION OF THE MUCOSAL INNATE IMMUNE SYSTEM

The colonization with normal gut microflora protects against infection from pathogenic bacteria. This long-known but poorly understood protection provided by commensal flora against pathogens is commonly referred to as colonization resistance[20-24]. Several mechanisms have been proposed to explain colonization resistance including a direct competition for nutrients, prevention of access to adherence sites, limitation of pathogen proliferation

through production of inhibitory substances or conditions, and stimulation of host natural immune defenses[21,25].

Before birth, the fetus is protected from microbial exposure, and postnatal stimulation by initial intestinal microbial colonization is important for the proper maturation of the innate immune system. Exposure to immunostimulatory microbial constituents may trigger activation of the infant's mucosal innate immune system as shown recently in the gnotobiotic (germ-free) mouse model[26-28]. For example, when compared to mice with normal intestinal microbial colonization, gnotobiotic mice need 30% more calories to maintain their body weight, exhibit an enhanced susceptibility to infection with enteropathogenic bacteria, and have an immature immune system[28].

Remarkably, intestinal mucosal homeostasis is maintained despite the large surface area continuously being exposed to different bacterial species[29,30]. To face the menace represented by intimate contact with a huge concentration of bacteria, the intestinal epithelium has evolved into a highly regulated barrier that can recruit immune cells of hematopoietic origin and produce mucus and a diverse arsenal of antimicrobial proteins that directly kill or inhibit the growth of microorganisms[30,31]. The intestinal epithelium comprises several cell lineages. Enterocytes constitute the most abundant epithelial cell type, and secrete several antimicrobial proteins. Paneth cells are unique to the small intestine and secrete abundant quantities of antimicrobial proteins, such as α-defensins. Finally, goblet cells secrete mucin glycoproteins and trefoil factors that assemble to form a thick mucus layer overlying the epithelium[32].

The development of the gut immune system is initiated before birth by a genetic program that drives the formation of Peyer's patches and mesenteric lymph nodes, but its postnatal maturation depends on the establishment of a balanced indigenous microbiota[28]. Intestinal commensal microorganisms provide signals that foster normal immune system development and influence the ensuing immune responses[33]. Signals delivered by these commensal microorganisms drive the development of isolated lymphoid follicles, stimulate maturation of Peyer's patches and initiate the migration of IgA producing plasma cells and mature T lymphocytes into the mucosa[33-35]. Among the first colonizing organisms evident in the intestine of newborn infants are strains of *Escherichia coli* (*E. coli*) derived from the maternal gastrointestinal tract[36-38]. It has been demonstrated in animal models that commensal *E. coli* strains can inhibit invasive *E. coli* O157:H7 growth in the intestine[39]. These studies prove that the gut commensal microflora clearly have important effects on the development of normal immunity.

INTERACTION BETWEEN INTESTINAL MICROFLORA AND IMMUNE HOMEOSTASIS

The intestinal microflora regulates immune homeostasis through many different mechanisms. Gut epithelia actively sense enteric bacteria and play an essential role in maintaining host-microbial homeostasis at the mucosal interface. Many innate immune responses are regulated by Toll-like receptors (TLRs), a conserved family of innate immune receptors that recognize microbial-derived molecules, including lipopolysaccharide, lipoprotein, RNA, and methylated DNA. Experiments in mice demonstrate that the beneficial effects of commensal bacteria are mediated *via* TLRs[40]. Intestinal epithelial cells and mucosal immune cells express pattern-recognition-receptors such as TLRs, enabling them to respond to distinctive microbial-associated molecular patterns[41]. TLRs are therefore critical for the specific detection of microbe-associated patterns, allowing differentially regulated responses to commensal versus pathogenic flora. The recognition of commensal bacterial-derived molecules by TLRs represents a critical component of the symbiosis between the host and indigenous microflora and is important for protection against gut injury and associated mortality[42]. Deregulated interactions between commensal bacteria and TLRs have been reported to promote chronic inflammation and tissue damage, such as that seen in IBD[43]. TLRs may also direct expression of the MyD88-dependent antimicrobial response. Paneth cell-intrinsic MyD88 activation limits translocation and dissemination of microbes across the mucosal barrier, while having little impact on luminal microbial numbers[44]. These results highlight the essential role of TLR-dependent pathways in compartmentalization of enteric commensal bacteria[45].

TLR-dependent signals mediate important regenerative signals to maintain intestinal mucosal integrity[46]. TLR-mediated protection may works through both constitutive and damage-induced production of protective factors by TLRs expressed on colonic epithelium[40]. Stimulation of TLRs results in activation of multiple signaling cascades that control expression of a wide range of innate immune response genes[47,48]. Recent evidence indicates a role for CpG DNA, the bacterial agonist of TLR9, in mediating some of the beneficial effects of probiotics in the intestine, and more generally, to modulate the immuno-physiological status of the gut[49]. Furthermore, TLR2 has been demonstrated to be responsible for the effects of bacteroides fragilis. This bacterium possesses an unusual capsular polysaccharide A that exerts potent immunoregulatory effects and can dampen intestinal inflammation in several models of colitis[50,51]. Systemic administration of flagellin, a bacterial protein that stimulates TLR5 protects mice from infection with vancomycin-resistant enterococcus (VRE)[52].

Another immune regulatory effect of commensal bacteria involves the inhibition of the nuclear factor-kappaB (NF-κB) pathway through the stabilization of IγBα[53]. IγBα is a central inhibitor of the NF-κB pathway, which acts by retaining the inactive NF-κB dimers in the cytosol. Most pro-inflammatory signals trigger the NF-κB pathway by inducing the phosphorylation of IγBα, which targets the molecule for degradation by the

ubiquitin/proteasome system[54]. Incubation of epithelial cells with nonpathogenic Salmonella has been shown to induce the accumulation of IγBα through the down-regulation of the protein's ubiquitination[53]. Similarly, it has been recently reported that the probiotic bacteria Lactobacillus casei also inhibits the NF-κB pathway by targeting the degradation of IγBα[55].

The specialized intestinal epithelial cells are capable of secreting proteins into the lumen of the intestinal tract which enhance epithelial barrier function and/or interact with the bacterial flora resident in the intestine. Goblet cells are highly polarized exocrine epithelia that secrete proteins apically into the lumen of the small and large intestine through the release of secretory granules. One particular class of glycoproteins produced by goblet cells, known as mucins, forms a viscoelastic protective gel that covers the intestinal epithelium[56]. Another class of secretory peptides, now designated as trefoil factors, is also normally produced by goblet cells and is important for the maintenance and repair of the gut mucosal barrier[57]. Mucins interact with trefoil factors and perform a defensive role during establishment of the intestinal barrier. Mucin oligosaccharides influence the bacterial milieu of the intestinal tract by enhancing the ability of certain bacteria to colonize the intestinal tract while inhibiting the adherence of others[58]. Mucins are also a direct source of carbohydrates and peptides that can promote the growth of bacteria[59]. To further enhance the symbiotic relationship between gut bacteria on the host, bacteria can alter mucus synthesis, secretion, and chemical composition[60]. Changes in mucin profile in response to bacterial colonization suggest a potential role as a protective mechanism against pathogenic invasion of the intestinal mucosa during early development[61].

The mucosal barrier is also reinforced by secretory immunoglobulin A (sIgA) and sIgM generated through external translocation of locally produced dimeric IgA and pentameric IgM. The dimeric IgA and pentameric IgM, containing a small polypeptide called joining chain to form sIgA and sIgM, can be actively exported by secretory epithelia. This external transport is mediated by the polymeric Ig receptor (pIgR), also known as membrane secretory component (SC)[62]. Notably, pIgR/SC knock-out mice that lack secretory IgA and IgM antibodies exhibit reduced epithelial barrier function with aberrant mucosal leakiness[63]. sIgA and sIgM form the first line of defense against pathogens as well as other potentially dangerous agents. Therefore, secretory immunity is of great importance for the intestinal epithelial barrier. In newborn infants, only a few IgA-secreting cells circulate in the blood. However this number is remarkably increased after 1 mo of age mainly due to the progressive microbial stimulation of gut-associated lymphoid tissues[64]. A much faster establishment of secretory immunity is often seen in infants from developing countries where there is exposure to a heavy microbial load, and an associated lower incidence of atopy[65]. Altogether, the secretory immune system is critical for the mucosal barrier function and the intestinal epithelial barrier maturation depends on exposure to microbial factors from the environment and the indigenous microbiota.

DISEASES ASSOCIATED WITH AN ALTERED INTESTINAL MICROFLORA AND ABNORMAL BARRIER FUNCTION

Alterations of the microbial gut flora and changes in intestinal barrier function are associated with certain diseases of the gastrointestinal tract. There is growing evidence that changes of the intestinal flora composition may play a pathogenic role in IBD, nosocomial infection, and NEC[66-68]. It has been proposed that a genetic predisposition causes IBD patients to have a deregulated immune response against harmless antigens derived from intestinal commensal bacteria and changes of the intestinal flora composition have been described in patients with IBD[66]. Several studies found an enhanced number of Proteobacteria and Actinobacteria but decreased numbers of Firmicutes (particularly the species Faecalibacterium prausnitzii) in stool samples of IBD patients as compared to healthy controls[69,70]. In biopsies of pediatric IBD patients, higher numbers of mucosa-associated aerobic and facultative-anaerobic bacteria were found, whereas bacterial species of the normal anaerobic intestinal flora such as Bifidobacteria were reduced[71,72].

It is well recognized that nosocomial infection is frequently a consequence of gut derived organisms. The infections with highly antibiotic-resistant bacteria are usually acquired during hospitalizations. Destruction of the normal flora by antibiotic administration disinhibits antibiotic-resistant members of these bacterial families, leading to their expansion to very high densities[73]. Reintroduction of a diverse intestinal microbiota to densely VRE-colonized mice eliminates VRE from the intestinal tract[74]. Characterization of the fecal microbiota of patients undergoing allogeneic hematopoietic stem cell transplantation demonstrated that intestinal colonization with Barnesiella confers resistance to intestinal domination and bloodstream infections with VRE[74]. Furthermore, there is an increased incidence of septic complications in patients receiving parenteral as opposed to enteral nutrition and this, in some cases, is due to alterations in intestinal barrier function predisposing to bacterial translocation[75].

In premature infants, colonization with abnormal gut flora increases the risk of hospital acquired infection or late-onset sepsis (LOS)[76]. Prolonged use of broad spectrum antibiotics, reduced bowel motility, immature epithelial host defenses, lack of enteral feeding, and parenteral nutrition are common risk factors for an altered microbial gut flora and abnormal mucosal barrier function. The possible subsequent bacterial translocation from the gastrointestinal tract may be an important pathway initiating LOS in premature infants[76]. Mai *et al*[77] analyzed stool samples that had been prospectively

collected from ten preterm infants with LOS and from 18 matched controls. A 16S rRNA based approach was utilized to compare microbiota diversity and identify specific bacterial signatures that differed in their prevalence between cases and controls. They found that the types and distributions of bacteria that initially colonize the intestine in premature infants differ in those with LOS compared to uninfected control babies. Therefore, it was proposed that a distortion in normal microbiota composition, and not an enrichment of potential pathogens, is associated with LOS in preterm infants. This may suggests that administration of probiotics may protect high-risk neonates and infants from developing sepsis. However, currently there is no clinical evidence regarding the usefulness of probiotics or prebiotics for the prevention of nosocomial sepsis in preterm infants[78].

Failure of the postnatal developmental of the intestinal barrier in the immature intestine plays an important role in the pathogenesis of NEC, a devastating disease seen mainly in preterm infants[68,79,80]. A major component of the pathophysiology of NEC is the nature of the interaction of bacteria with the premature gut. The pattern of bacterial colonization in the intestine of the premature neonate is quite different from that of the healthy full-term infant. Infants requiring intensive care acquire intestinal organisms slowly, and the establishment of bifidobacteria flora is retarded. A delayed bacterial colonization of the gut with a limited number of bacterial species tends to be virulent. Indeed, several clinical observational studies have shown that the duration of antibiotic exposure including prenatal exposure to antibiotics is associated with an increased risk of NEC in preterm infants[81-83]. Therefore, an aberrant colonization of the bowel of the premature infant has been proposed to contribute to the development of NEC[84]. By using non-culture-based microbial analyses of feces, Wang *et al*[85] studied fecal samples of ten preterm infants with NEC and ten matched controls and found that patients with NEC had less bacterial diversity and an increased abundance of γ-proteobacteria in the stools. Similar findings were presented in another study by Mai *et al*[86], in which one of the bacterial signatures detected more frequently in NEC patients matched closest to γ-proteobacteria. These observations suggest that abnormal patterns of microbiota contribute to the cause of NEC. However, a study by Mshilddazde *et al*[87] using the same technology demonstrated that the overall microbial profiles in patients with NEC were not different from those of control infants. Thus to date, molecular methods have not clarified the bacterial pathogenesis of NEC.

None of the clinical studies to date has been able to fulfill Koch's postulate linking NEC to a particular pathogen. Nevertheless, we proposed a hypothesis that excessive production/accumulation of short chain fatty acids (SCFAs) due to bacterial fermentation of undigested formula or abnormal bacterial colonization contributes to the pathogenesis of NEC[88,89]. There is substantial indirect clinical evidence to support the theory that bacterial fermentation is involved in the development of NEC in premature infants[90]. Further, in two separate studies, increased breath hydrogen excretion (an indicator of bacterial fermentation and an indirect measurement of SCFAs production) was found in NEC patients even prior to the onset of clinical symptoms[91,92]. The well-known finding of pneumatosis intestinalis (gas in the bowel wall) in NEC patients is also thought to be secondary to hydrogen gas produced by bacterial fermentation[93]. Recent reports of several cases of premature infants who developed NEC after they were fed SimplyThick®, a xanthan gum-based thickener used in the management of dysphagia, is another example[94,95]. Increased production of hydrogen and SCFAs as the consequence of accumulation of luminal carbohydrates and fecal bacteria fermentation of xanthan gum were proposed as the main mechanisms for NEC[94]. Both probiotics and prebiotics have been proposed to promote a healthy gut microbiota in human, and oral probiotics have been proven to be effective in reducing the incidence of NEC in premature infants in several clinical trials[96-98]. On the other hand, there is no evidence showing that prebiotics can effectively reduce the incidence of infection or NEC in premature infants. Therefore, there is insufficient evidence to recommend the use of oligosaccharides as prebiotics in formula for premature infants since these may prove to be unsafe.

In summary, this review summarizes recent advances in understanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. There is increasingly recognition that the stimulation of initial intestinal microbial colonization is important for proper maturation of the innate immune system and continued regulation and maintenance of intestinal barrier function. Disruption of the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including IBD, nosocomial infection, and NEC in premature infants.

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