# Early Development of the Gut Microbiome and Immune-Mediated Childhood Disorders

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## Abstract

The human gastrointestinal tract inhabits a complex microbial ecosystem that plays a vital role in host health through its contributions to nutrient synthesis and digestion, protection from pathogens, and promoting maturation of host innate and adapt immune systems. The development of gut microbiota primarily occurs during infancy and is influenced by multiple factors, including prenatal exposure; gestational age; mode of delivery; feeding type; pre-, pro-, and antibiotic use; and host genetics. In genetically susceptible individuals, changes in the gut microbiota induced by environmental factors may contribute to the development of immune-related disorders in childhood, including atopic diseases, inflammatory bowel disease, irritable bowel syndrome, and necrotizing enterocolitis. Pre- and probiotics may be useful in the prevention and treatment of some immune-related diseases by modulating gut microbiota and regulating host mucosal immune function. The review will discuss recent findings on the environmental factors that influence development of gut microbiota during infancy and its potential impact on some immune-related diseases in childhood. The use of pre- and probiotics for prevention and intervention of several important diseases in early life will also be reviewed.

**Keywords**
- microbiota
- gastrointestinal
- allergy
- asthma
- immune

Human infants are born with a naive and immature immune system. The maturation of immune system during infancy is evidenced by an induction of a pathogen-specific immune response while maintaining immunological tolerance to dietary components and commensal microbes.[1] The gastrointestinal tract is the largest immunological tissue in human body and is colonized with a complex microbial community that induce regulatory T cells (Th3 and Th1 cells) via production of suppressive cytokines, transforming growth factor-β (TGF-β), and/or interleukin-10 (IL-10), which help to guide a balanced T helper 1 (Th1) and T helper 2 (Th2) response.[1,2] Failure to develop a balance between immune tolerance and active immune response is hypothesized to contribute to immune-related disorders, such as allergy (Th2 mediated) and inflammatory bowel disease (IBD) (Th1 mediated).[3,4] In addition, the gut microbiota also stimulates mucosal IgA secretion, produces antibacterial substances, and enhances tight junction of the intestinal barrier, which protect against pathogen invasion in the gut.[5] Thus, cross talk between intestinal epithelium cells and resident microbiota promotes the immune homeostasis and maturation.[6]

Colonization of the infant gut is initially dominated by facultative anaerobes, such as enterobacteria, enterococci, lactobacilli, and streptococci, followed by anaerobic microbes, such as *Bacteroides*, bifidobacteria, clostridia, and eubacteria.[7] By 1 year of age, the infant gut microbiota resembles a complex adult-like pattern.[8] Development of infant gut microbiota is influenced by prenatal exposure; gestational age; mode of delivery; feeding type; pre-, pro-, and antibiotic use; and host genetics. Alteration of gut microbiota by environmental factors may contribute to the development of immune-related disorders in genetically susceptible infants.[9,10] Herein, the environment factors influencing the gut microbiota development (►**Fig. 1**) and the relationship between the

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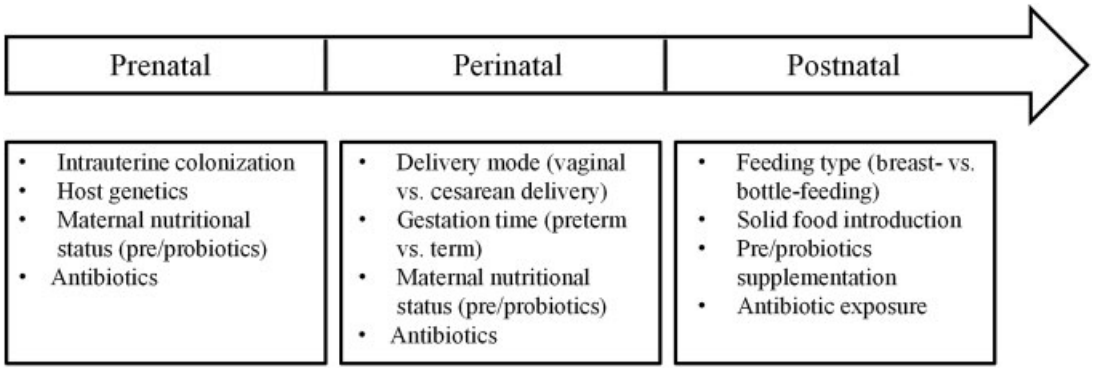


**Figure 1** Factors influencing the development of gut microbiota in early infancy.

gut microbiota and immune-related diseases in childhood (►**Fig. 2** and ►**Table 1**) will be reviewed. In addition, the potential for prevention and intervention of several important diseases that develop in early life by pre/probiotics supplementation will be presented.

## Factors Influencing the Development of the Gut Microbiota

### Prenatal Exposure

It is commonly stated that newborns are born with a sterile gastrointestinal tract, and are rapidly colonized by microbes obtained from their mothers and the surrounding environment during and after birth.[7] However, as early as 1936, Snyder isolated bacteria, such as *Lactobacillus acidophilus*, *Streptococcus pyogenes*, and *S. mitis*, from the first meconium sample of 3 out of 29 infants within 30-minute after delivery.[11] Recently, the presence of bacterial isolates and/or DNA, such as lactic acid bacteria, *Enterococcus*, *Staphylococcus*, and *Bifidobacterium*, has been documented in meconium,[12] amniotic fluid,[13,14] fetal membranes,[15] umbilical cord blood,[16] and placenta[17,18] of the healthy mothers and infants, without any clinical signs of infection or inflammation. These findings suggest that fetuses are not sterile and that prenatal

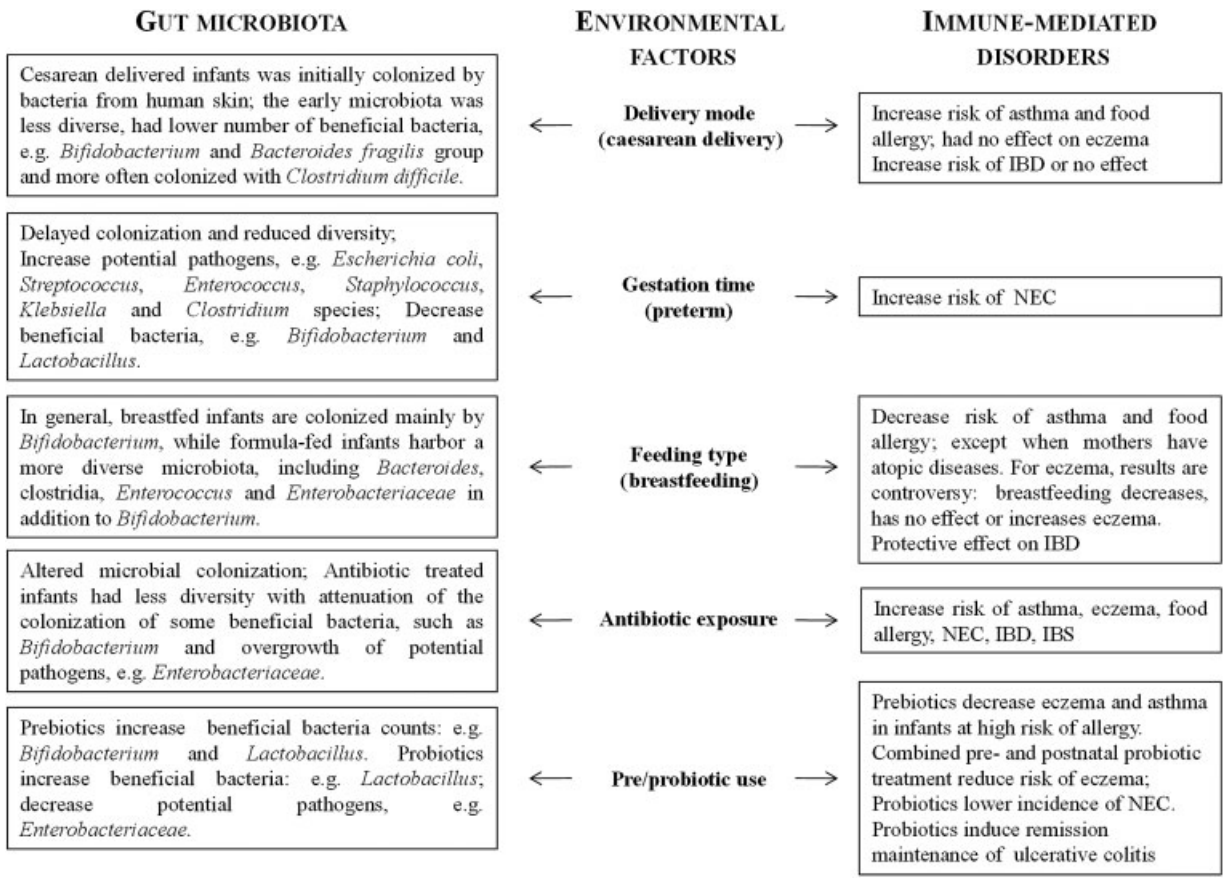


**Figure 2** Impact of environmental factors on the colonization of gut microbiota and immune-related disorders. Abbreviations: IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome; NEC, necrotizing enterocolitis.

**Table 1** Associations between gut microbiota changes and immune-mediated disorders in childhood

| Immune-mediated disorders | Change in gut microbiota |
|---|---|
| Asthma | ↑ *Clostridium difficile*[72]<br>↑ *Bacteroides fragilis* subgroup and *Clostridium coccoides* subcluster XIVa[76] |
| Eczema | ↓ Bacterial diversity[91–93]<br>↑ *B. pseudocatenulatum, Escherichia coli*, and *C. difficile*[72,90] |
| Food allergy (cow milk allergy) | ↑ Total and anaerobic bacteria and ↓ yeast count[100]<br>↑ *C. coccoides* group and *Atopobium* cluster[101]<br>↑ Bacterial metabolic products, e.g., butyric acid and BCFA[101] |
| NEC | ↑ γ-Proteobacteria and ↓ Firmicutes[32]<br>↑ *Citrobacter*-like sequences and *Enterococcus*-like sequences[110]<br>*Enterobacter* associated with NEC[112]<br>No significant differences between NEC and control[111] |
| IBD | ↑ Aerobic and facultative anaerobic bacteria[118]<br>↑ γ-Proteobacteria,[117] e.g., adhesive-invasive *E. coli*[119]<br>↓ Clostridia, e.g., *Faecalibacterium*, *Bacteroides*, e.g., *B. vulgatus* and bifidobacteria[115,116,118] |
| IBS | ↑ γ-Proteobacteria, *Haemophilus*, and *Dorea*[130]<br>↓ *Eubacterium* and *Anaerovorax*[130]<br>↑ *Veillonella*, *Prevotella*, *Lactobacillus,* and *Parasporobacterium* in children with IBS-D[131]<br>↓ *Bifidobacterium* and *Verrucomicrobium* in children with IBS-D[131] |

Abbreviations: IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; NEC, necrotizing enterocolitis; BCFA, branched-chain fatty acids.

transmission of bacteria from mother to fetus exists in healthy pregnancies.[12,19]

Although the mechanism of transfer in humans is still unclear, a genetically labeled *Enterococcus faecium* strain orally inoculated to pregnant mice was detected in amniotic fluid and in meconium of the pups,[12,16] indicating that maternal gut microbes might be the origin of the first colonizers in fetal gut.[12] Additionally, dendritic cells in Peyer patches penetrate the junctions between gut epithelial M cells to directly take up bacteria from maternal gut lumen, and then enter into the lymphatic and blood circulation. Once in the circulation, the bacteria may be transferred to the fetus cross placental barrier, and initiate the first colonization of fetal gut.[19,20] Furthermore, other studies found that bacteria in the maternal mouth could reach the amniotic fluid via the bloodstream, particularly in the presence of gingivitis or periodontitis during pregnancy.[21] Taken together, these studies support the concept that the maternal microbiota seeds prenatal colonization of the human infant gut, which may stimulate the fetal intestinal mucosal immune system in utero.[20,22]

## Gestational Age

Postconceptional age at delivery is another factor affecting infant gut microbial colonization. The gut microbiota of preterm infants is often colonized more slowly, has higher interindividual variability and lower diversity than that of healthy full-term infants.[23,24] The early colonizers in preterm infant gut are often potential pathogens, such as *Escherichia coli*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, *Klebsiella*, and *Clostridium* species.[23,25–27] The beneficial bacteria, for example, *Bifidobacterium* and *Lactobacillus*, which are commonly found in healthy full-term infants, were rarely detected or were present at low numbers in the stool of preterm infants.[25,26,28] The delayed colonization and reduced diversity of gut microbiota in preterm infants might be due to the aseptic environment, the neonatal intensive care unit, and delayed oral feeding.[29,30] In addition, the extensive use of antibiotics in preterm infants may also be an important factor causing the disturbance of gut microbiota,[26,29] and may consequently lead the preterm infant to be more susceptible to gastrointestinal disorders and disease, such as necrotizing enterocolitis (NEC).[31,32]

## Delivery Mode

How an infant is delivered is also associated with the early colonization of microbiota. A recent study compared the fecal microbiota of newborn infants within 24 hours of delivery with the microbiota of their mother' skin, oral mucosa, and vagina by 16S rDNA pyrosequencing[33] and showed that the fecal bacterial composition of vaginally delivered (VD) infants was most similar to that of vaginal communities of the mothers, with *Lactobacillus*, *Prevotella*, and *Atopobium* spp. predominating. In contrast, the fecal bacterial composition of cesarean delivery (CsD) infants was most similar to the maternal skin, with an abundance of *Staphylococcus* and *Corynebacterium.* Thus, initial colonization is determined to a large extent by the first abundant microbes that an infant counters, from maternal vagina, feces, or skin. In addition, the microbiota of CsD infants is less diverse than that of VD infants at 3 days of age.[34] There was an absence of *Bifidobacterium* spp. in the stool of CsD infants, whereas VD infant stool was predominated by *Bifidobacterium longum* and *B. catenulatum*.[34] In another study, CsD infants had lower numbers of *Bifidobacterium* and *Bacteroides fragilis* group, and were more often colonized with *Clostridium difficile* compared with VD

infants.[35] Although differences in early microbiota between CsD and VD infants are well documented, the long-term impact of early colonization on subsequent childhood immune development and disease outcomes are not fully understood and shown to be investigated in a long-term prospective cohort study.

### Diet

Feeding mode is one of the most important determinants of gut microbial diversity; however, its impact on the infant microbiota is often contradictory. Many studies have shown that breastfed (BF) infants are colonized mainly by *Bifidobacterium*, while formula-fed (FF) infants harbor a more diverse microbiota, including *Bacteroides*, clostridia, *Enterococcus*, and *Enterobacteriaceae* in addition to *Bifidobacterium*.[36,37] Other studies have shown that *Bifidobacterium* are found equally often and in similar counts in BF and FF infants.[38] These inconsistencies may arise from the different analytical approaches used in enumerating the microbiota or the variability in the composition of the human milk and infant formula that the infants are consuming and geographically distinct infant groups.

Differences in microbial diversity between BF and FF infants may reflect the prebiotic effects of the large quantity of structurally diverse human milk oligosaccharides (HMO),[39] which are negligible in bovine milk and infant formula.[40] The HMO can function as prebiotics to stimulate the growth of specific gut bacteria, including *Bifidobacterium* and *Bacteroides* species in vitro.[41,42] Moreover, human milk contains bacteria such as *Staphylococcus*, *Streptococcus*, *Bifidobacterium*, and *Lactobacillus*[43] that may serve as a diverse and continuous source of live microbes for the infant gut.

Introduction of solid food causes rapid and sustained alterations in the gut microbiota of infants. Koenig et al catalogued the gut microbiome of one infant over 2.5-year period by 16S rDNA pyrosequencing and found that introduction of table foods to the diet of the BF infant at approximately 5 months of age induced a sustained increase in the abundance of Bacteroidetes.[44] Fallani et al examined the composition of fecal microbiota of 531 infants before weaning and 4 weeks after the introduction of first solid food by fluorescence in situ hybridization (FISH) and reported that weaning significantly increased the proportion of *Clostridium coccoides* and *C. leptum* groups but decreased *Bifidobacterium*, Enterobacteria, *C. perfringens*, and *C. difficile*.[45]

### Pre-, Pro-, and Antibiotics

The composition of the infant's gut microbiota can be influenced by the use of prebiotics, probiotics, and antibiotics. A prebiotic is defined as "a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health."[46] Several groups have reported that supplementation of infant formula with prebiotics affected the composition of the gut microbiota.[47–49] For example, infants fed an infant formula containing a combination of 90% short-chain galactooligosaccharides (scGOS) and 10% long-chain fructooligosaccharides (lcFOS) had increased fecal bifidobacteria and lactobacilli counts.[47,49] Investigations into the effect of prebiotic supplementation during pregnancy on the composition of neonatal microbiota are rare. In a randomized, double-bind, placebo-controlled trail, supplementation of 9 g/d scGOS and lcFOS (9:1 ratio) to mothers in the last trimester of pregnancy resulted in an increase in the relative abundances of bifidobacteria and lactobacilli in the maternal gut, with no effect on the gut microbiota of their offspring.[50] A limitation of the study was that only bifidobacteria and lactobacilli were investigated, and the impact of maternal prebiotic treatment on other bacteria remained unknown. Furthermore, the authors did not separate BF from FF infants; therefore, the lack of effect of the prebiotic supplementation to the mother on infant stool could be due to the HMO consumed by the BF infants.

According to FAO/WHO,[51] probiotics are "live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host." A recent Finnish study investigated probiotic administration to the mother on the infant's microbiota at 6 months of age using FISH and quantitative PCR (qPCR).[52] The gut microbiota of BF infants whose mothers received *Lactobacillus rhamnosus* LPR together with *B. longum* BL999 for 2 months before and 2 months after delivery had higher abundance of lactobacilli, enterococci and lower counts of *Bifidobacterium* compared with infants of mothers receiving a placebo treatment. When the same probiotic combination was administered directly to German FF infants for 4 months, the effect of the probiotics on microbiota composition was minor; however, *Bifidobacterium bifidum* count was lowered compared with the placebo group, which is consistent with the previous study.

In contrast, antibiotics exert detrimental effects on infant microbiota composition. Using terminal restriction fragment length polymorphism (T-RFLP) analysis and qPCR, Tanaka et al[53] monitored the impact of cephalexin exposure in the first 4 days of life on the development of intestinal microbiota. Cephalexin-treated infants had reduced overall diversity, with a specific reduction of *Bifidobacterium* and overgrowth of *Enterococcus* and *Enterobacteriaceae* in the first month of life compared with untreated infants.[53] Whether antibiotic administration to the mother affects the microbiota of their infant has also been studied. If a mother was treated with ampicillin and gentamicin within 48 hours of birth, her infant had a higher proportion of Proteobacteria and a lower proportion of Actinobacteria and genus *Lactobacillus* 4 weeks after the cessation of treatment compared with infants of untreated mothers. Importantly, the Proteobacteria level remained elevated in the infants of treated mothers 8 weeks posttreatment, suggesting long-term effects on microbial populations.[54]

### Host Genetics

The earliest study to show a relationship between host genotype and gut microbiota in children was conducted in 1983 using cultivation-dependent methods, and found that the fecal microbiota of monozygotic (MZ) twin siblings was more similar than that of dizygotic (DZ) twin siblings.[55] Later studies revealed higher similarity of gut microbiota in MZ

twins than in DZ twins and/or unrelated individuals.[56,57] Palmer et al also found that the gut microbiota was more similar in DZ twins than in unrelated children at any stage of development over the first year of life.[8] In contrast, Turnbaugh et al reported that the similarity of fecal microbiota in MZ twin pairs was not different from that in DZ twin pairs as assessed by next generation16S rDNA pyrosequencing.[58] Although the results of twins studies appear equivocal, other studies have confirmed that the gut microbiota of genetically related children is more similar than those of unrelated individuals,[8,56,58] indicating that host genetics must play an important role in the selection and colonization of gut microbes in humans.

Clear evidence linking host genetics and the microbiota has been obtained in mice. Work in a mouse advanced intercross line originating from a cross between C57BL/6J and an ICR-derived outbred line mice demonstrated a relationship between host genetics and the "core measurable microbiota (CMM)" of 64 conserved taxonomic groups that varied quantitatively across most animals.[59] Testing of the CMM abundances for cosegregation with 530 fully informative single nucleotide polymorphism (SNP) markers identified 18 host quantitative trait loci (QTL) that showed significant or suggestive genome-wide linkage with relative abundances of specific microbial taxa. Some of the loci controlled individual microbial species, several controlled groups of related taxa, and others had putative pleiotropic effects on groups of distantly related organisms. Recently, the same group showed that microbial diversity was influenced by both environmental and host genetic factors and was associated with several polygenic diseases in BXD recombinant inbred mice.[60] The gut microbial composition differed among BXD strains and linkage analysis defined QTLs that were either restricted to a particular taxon or that influenced the variation of taxa across phyla. A QTL region on chromosome 4 that overlapped several interferon genes modulated the population of *Bacteroides*, and potentially Bacteroidetes and Firmicutes—the predominant BXD gut phyla. Irak4, a signaling molecule in the Toll-like receptor pathways, was a candidate for the QTL on chromosome15 that modulated *Rikenellaceae*, whereas TGF-β3, a cytokine that modulates intestinal barrier function and tolerance to commensal bacteria, overlapped a QTL on chromosome 12 that influenced *Prevotellaceae*. These findings provide clear evidence for the importance of host genetics in shaping microbiome diversity and contribute to our understanding of the host factors that govern the assemblages of gut microbiota associated with complex diseases in mice.

## Interaction between the Gut Microbiota, Immunity, and Immune-Related Disorders

The gut microbiota plays important roles in shaping the immune system during infancy. The commensal microbes and their products interact with immune cells to create and maintain host tolerance and influence both innate and adaptive immune response.[61–63] Mounting evidence shows that dysbiosis is associated with the development of immune-mediated diseases, such as allergy, irritable bowel syndrome (IBS), NEC, and IBD.

### Allergic Diseases

The prevalence of allergic diseases has been increasing in industrialized countries over the past few decades.[64,65] The dichotomy in the rate of allergic diseases between industrialized and developing countries suggests that environmental changes associated with a western lifestyle are the major factor in the development of allergic diseases.[66,67] In 1988, Strachan first introduced the "hygiene hypothesis," which states that lack of early microbial exposure increases susceptibility to allergic diseases by suppressing natural development of the immune system.[68] Numerous epidemiologic and clinical studies have demonstrated that the composition of the gut microbiota differs between allergic and nonallergic children, even before the emergence of symptoms.[69–72] Gut microbes induce regulatory T cells that influence Th1 and Th2 balance and help prevent aberrant immune response.[73] Therefore, an alternative hypothesis, that is, the "microbiota hypothesis," has been generated, which proposes that alteration of the gut microbiota due to antibiotic use, diet, or lifestyle changes disrupts the normal microbially mediated mechanisms of immunological tolerance, leading to an increase in the incidence of allergic diseases including asthma, eczema, and food allergy in later childhood.[74,75]

Asthma, a chronic inflammatory disorder of the airway, is often thought to be caused by a combination of genetic and environmental factors. A prospective birth cohort study that analyzed nearly 1,000 infants reported that differences in gut microbiota in the first month of life was predictive of subsequent development of recurrent wheeze at 2 years of age, and *C. difficile* was particularly associated with the increase of asthma.[72] In another study, Vael et al identified *B. fragilis* subgroup and *C. coccoides* subcluster XIVa in the gut microbiota of infants as early indicators of risk of asthma later in life.[76]

There is growing interest in exploring which environmental exposures influence the development of asthma via modulation of gut microbiota. For example, CsD infants had a higher risk of asthma compared with VD infants,[77,78] and were more frequently colonized by the asthma-associated pathogen, *C. difficile*.[35] van Nimwegen et al, who first explored the delivery mode–gut microbiota–asthma pathway confirmed that the effect of CsD on asthma development was mediated by *C. difficile* in the gut.[79] Use of antibiotics in early life is another factor associated with the increased risk for wheeze or asthma,[80,81] apparently by suppressing commensal gut bacteria and causing the growth of pathogens, for example, *C. difficile*. In mice, Vancomycin in early life disrupted the microbiota and enhanced susceptibility to ovalbumin-induced asthma.[82] In contrast to the adverse effect of CsD and antibiotics on gut microbiota and asthma, breastfeeding favors beneficial bacteria colonization in the infant gut, for example, *Bifidobacterium*, and protects against asthma in later childhood.[83,84] However, the protection afforded by breastfeeding was not observed in infants whose mother has atopic diseases.[85,86] One possible reason may be related to low amount of bifidobacteria in the breast milk of allergic

mothers, which may have caused their infants to concurrently have lower counts of bifidobacteria in the gut,[87] and possibly greater susceptibility to asthma in later childhood.

Eczema (atopic dermatitis) is a chronic, inflammatory skin diseases, affecting at least 10% of U.S. children, and its prevalence has increased over the past a few decades.[88,89] A study performed in the Netherlands with 957 infants documented that *E. coli* and *C. difficile* in feces at 1 month of age was associated with higher risk of developing eczema,[72] while Gore et al compared *Bifidobacterium* spp. in the feces of infants at 3 to 6 months of age and showed that *B. pseudocatenulatum* was more commonly detected in the feces of children with eczema.[90] Using temperature gradient gel electrophoresis and T-RFLP, Wang et al examined overall patterns of fecal microbial colonization in healthy ($n = 20$) and atopic ($n = 15$) infants and found that infants who developed eczema had significantly lower fecal bacterial diversity at 1 week of age than infants who remained healthy for 18 months.[91] Others have confirmed that a less diverse intestinal microbiota in early life was associated with an increased risk of eczema.[92,93] Thus, diversity of the gut microbiota in early childhood may be more important than an altered prevalence of particular bacterial species for the increasing incidence of eczema. Recently, studies have investigated the association between environmental factors that influences the colonization of microbiota and eczema. For example, a multicenter, multicountry cross-sectional study (Phase III of ISAAC) of 193,412 children showed an association between antibiotic use in the first year of life and symptoms of eczema in 6- and 7-year-old children.[94] The impact of feeding mode on the development of eczema was also studied, but the results are controversial. Several studies showed exclusive breastfeeding for at least 3 months protected against the development of eczema in infants with a family history of atopy.[95,96] Other studies have not found a protective role of breastfeeding and some found that breastfeeding increased the risk of eczema.[97,98]

Food allergy is defined as an adverse immune reaction to a food protein, such as those found in milk, egg, and wheat, which causes dermatitis, gastrointestinal, and respiratory disorders.[99] Several studies have shown an association between altered gut microbiota and the development of food allergy. A prospective study that compared the fecal microbiota of children immediately after diagnosis of cow milk allergy found higher amounts of total and anaerobic bacteria and lower yeast counts[100] compared with healthy children. Furthermore, using FISH combined with flow cytometry, the same group found that the amount of *C. coccoides* group and *Atopobium* cluster and bacterial metabolic products, butyrate and branched-chain fatty acids, were higher in children with cow milk allergy compared with healthy infants.[101] In addition, a germfree (GF) mouse model of cow milk allergy revealed that GF mice were more responsive than conventional mice to oral sensitization and challenge with β-lactoglobulin,[102] while GF mice colonized with an infant fecal microbiota, which was dominated by *Bifidobacterium* and *Bacteroides*, were protected against cow milk allergy via the mechanism of increasing foxp3 gene expression, which is the master regulatory molecule in Treg cell function.[103] It has been proposed that changes in gut microbiota resulting from CsD might correlate to the increased risk of developing food allergy in children. Some studies found that CsD children have a higher risk of food allergy,[61,104] while other studies did not detect significant association between delivery mode and prevalence of food allergy[105] in offspring of atopic mothers.[106,107] Thus, the impact of CsD and other environmental factors on gut microbiota and food allergy should be further investigated in prospective trials.

## Necrotizing Enterocolitis

NEC is a major cause of morbidity and mortality in low-birthweight infants, afflicting 7% of infants weighing less that 1,500 g, with a mortality rate of 20 to 30%.[108] Preterm delivery is the main risk factor for NEC, presumably due to immaturity of gastrointestinal motility, barrier function, and immune defense. Enteral feeding with infant formula constitutes another important risk factor, whereas breast milk is protective against NEC.[108,109] The gut bacteria have been shown to be causative for NEC in animal models and dysbiosis is associative with NEC in preterm infants.[32,110–112] Although several bacteria, viruses, and fungi have been found to cause NEC in human and animal studies, no single change or even pattern of change in the gut microbiota has been consistently identified as a risk factor for NEC.[108] Even investigations conducted by the same research group using the same sequencing approach (454 pyrosequencing) have shown differences in the microbial signatures associated with NEC. For example, Mshvildadze et al detected *Citrobacter*-like sequences only in cases with NEC and an increased frequency of *Enterococcus*-like sequences in cases with *Klebsiella* in control subjects.[110] However, in a subsequent publication,[32] one of the bacterial signatures detected more frequently in NEC cases matched closest to γ-Proteobacteria and the same *Citrobacter*-like sequences were not detected in infants with NEC. In a prospective single-center case–control study, Norman et al mapped the bacterial composition of fecal samples from 20 extremely preterm infants (10 NEC and 10 matched healthy controls). No significant differences were observed between NEC and controls; however, a higher Bacillales and *Enterobacteriaceae* relative abundance was detected at early time points in infants who developed NEC, while healthy controls were more dominated by *Enterococcus*.[111] Stewart et al found that bacterial community structures in infants with NEC and late onset sepsis differed from healthy preterm infants. The presence of Enterobacteria and *Staphylococcus* was associated with the development of NEC and late onset sepsis, respectively.[112] Differences in these studies are likely due to the fact that NEC represents a clinical scenario that can arise from several bacterial etiologies that can vary among neonatal intensive care unit and that most studies involved a limited number of subjects (6–40 cases and controls). Overall, the clinical evidence suggests that NEC does not result from growth of a single causative pathogen, but rather that the disease results from a generalized disturbance of normal colonization patterns in the developing gut.[108] However, findings of prospective studies documented

detectable changes in the microbiota of infants before the onset of NEC.[32,112] Mai et al found phyla-level shifts in fecal microbial signatures of infants who developed NEC that occurred between 1 week and 3 days before the diagnosis of NEC.[32] Using principal coordination analysis to study bacterial community structure, Stewart et al demonstrated that profiles from healthy infants were distributed separately from those infants with NEC and/or late onset sepsis, and samples collected before NEC onset also showed a distinct cluster.[112] These findings suggest that bacterial changes may trigger alterations in host immunity and/or barrier function that subsequently lead to the clinical signs and symptoms of NEC.

### Inflammatory Bowel Disease

IBDs, including Crohn disease (CD) and ulcerative colitis (UC), are chronic inflammation of the small bowel and/or colon leading to recurrent diarrhea and abdominal pain. Complex interactions between host genetics, mucosal immune system, and enteric microbes are thought to underlie the development of IBD.[113] Differences in the composition of the gut microbiota between IBD patients and healthy control have been shown, leading to the hypothesis that dysbiosis could contribute to the etiology and pathogenesis of IBD.[114] Two studies comparing the fecal microbiota of children with CD and UC with healthy controls using culture-based methods, combined with qPCR and T-RFLP, found that the overall fecal microbiota diversity was reduced in CD patients, with specific reductions in the number of commensal bacteria, such as *Faecalibacterium*, *Bacteroides* and bifidobacteria, and increased number of *E. coli* compared with healthy children. No major changes were detected in UC patients, except for a decrease in bifidobacteria in the active state of UC.[115,116] Recently, Michail et al confirmed these results in patients with severe UC. The fecal microbial diversity was lower in UC children with a reduction in clostridia and an increase in γ-Proteobacteria compared with healthy controls.[117] Several studies have examined the mucosal-associated microbiota in subjects with IBD and also showed similar results. Conte et al found a higher number of aerobic and facultative anaerobic bacteria in ileal, cecal, and rectal biopsies from children with IBD and a decrease in *Bacteroides vulgatus*.[118] Negroni et al also detected two adhesive-invasive *E. coli* strains in the inflamed tissues of children with IBD, further supporting their role in the pathogenesis of IBD.[119] Several environmental factors may contribute to the risk of IBD in childhood. A Danish national cohort study showed that CsD was associated with increased risk of IBD at age 0 to 14 years,[120] while a German case–control study including 2,000 children and young adolescents did not find any association between delivery mode and IBD.[121] In terms of early nutrition, two systemic reviews using meta-analysis showed that breastfeeding was associated with lower risk of UC and CD in childhood.[122,123] In contrast, antibiotic use in early life has been shown to associate with a high risk of IBD development,[124–126] although most studies found an association with antibiotic use only in children with CD, not in UC children.[125–127] For additional information on the influence of environmental factors on the development of pediatric IBD, readers are referred to a recent review by Aujnarain et al.[128]

### Irritable Bowel Syndrome

IBS is a functional bowel disorder characterized by abdominal pain or discomfort that is associated with altered bowel habit.[129] The pathophysiology of IBS is poorly understood; however, changes in the composition of gut microbiota have been linked to IBS.[130,131] Saulnier et al compared fecal microbiota of patients with IBS and healthy controls by 16S rDNA pyrosequencing and microarray, and reported that children with IBS had a greater abundance of the class γ-Proteobacteria compared with controls. Furthermore, genera of *Haemophilus* and *Dorea* were increased, while *Eubacterium* and *Anaerovorax* were reduced in IBS children.[130] Rigsbee et al investigated the fecal microbiota of children with diarrhea-predominant IBS. Compared with healthy subjects, higher levels of *Veillonella*, *Prevotella*, *Lactobacillus*, and *Parasporobacterium* and lower levels of *Bifidobacterium* and *Verrucomicrobium* were found in children with IBS.[131] Few studies have examined the link between environmental factors that impacts the colonization of microbiota and IBS; however, a prospective community-based study found that subjects who were given a course of antibiotics were more than three times as likely to report more functional bowel symptoms 4 months later than nonantibiotic-treated controls.[132]

## Prebiotics and Probiotics on the Prevention and Treatment of Immune-Related Disease

### Allergic Disease

The literature surrounding probiotics and allergic diseases is equivocal, which may be related to considerable variations in the probiotic strains used, daily doses administered, and the timing of administration.[133] Several meta-analyses have evaluated probiotics for prevention of eczema in children, and most of studies involved combined pre- and postnatal treatment.[133,134] The majority of combined pre- and postnatal studies demonstrated that probiotics reduced incidence of eczema during first 2 years of life, but a few studies found that probiotics had no beneficial effect on eczema.[133] Most studies examining either prenatal or postnatal probiotic treatment found no eczema-preventative effect,[135–137] except one study that reported a reduced incidence of eczema after postnatal treatment with *L. paracasei* F19.[138] Several studies showed that administration of probiotics was associated with reduced eczema severity (SCORing Atopic Dermatitis, SCORAD) in children with eczema[139,140]; however, other studies did not show any effect of probiotics on the incidence or severity of eczema.[141]

A few studies investigated the role of probiotics on asthma and most of these reported that probiotics had no effect on the prevention or treatment of asthma/wheezing during first 2 years of life.[134] Clinical trials of probiotics for the treatment of food allergy are limited and no protective effect has thus far been reported.[133] A meta-analysis that reviewed the effect of prebiotics given to infants for the prevention of eczema and asthma reported a significant reduction in eczema and

asthma in infants at high risk of allergy up to 2 years of age; however, no protective effect was found when children were not selected for allergy risk.[142] Thus, future randomized, controlled, double-blind studies to examine the efficacy of probiotics for both prevention and treatment of food allergies are warranted.

### Inflammatory Bowel Diseases

Probiotics have been used in the treatment of IBD in children; however, their efficacy is inconsistent. In a small, open-label pilot study, Gupta et al showed that *Lactobacillus* GG (LGG) administered to children with mildly to moderately active, but stable, CD improved gut barrier function and clinical status.[143] However, in a later randomized clinical trial, administration of LGG to children with CD in remission for 2 years did not show any efficacy of LGG compared with placebo treatment.[144] Studies conducted on the effects of probiotics in CD in adults have also shown negative results.[145,146]

In contrast, the efficacy of probiotics in UC seems to be better than in CD. Miele et al[147] conducted the first pediatric randomized, placebo-controlled trial in which children with a newly diagnosed UC received either a highly concentrated mixture of probiotic bacterial strains (VSL#3 containing *L. paracasei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophiles*) or a placebo in conjunction with concomitant steroid induction and mesalamine maintenance treatment. Children treated with VSL#3 had a significantly higher rate of remission and a lower incidence of relapse within 1 year of follow-up compared with the placebo group.[147] The efficacy of VSL#3 in inducing/maintaining the UC remission was also confirmed by an open-label pilot study in children[148] and in clinical trials in adults.[149,150] In addition to oral administration, rectal infusion of *L. reuteri* in children with active distal UC reduced mucosal inflammation and changed mucosal cytokine expression.[151] Thus, a consensus for "Probiotic Use-2011 update" recommended the use of probiotics, such as VSL#3, for maintaining remission of UC,[152] while there is no basis for recommending the use of probiotics in CD.[153]

A few pilot studies have examined the use of prebiotics, such as inulin,[154] germinated barley foodstuff,[155] and FOS[156] in the treatment of IBD in adults and showed similarly positive results; however, no study in children has been reported and the efficacy of prebiotics remains to be further investigated in clinical trials.

### Irritable Bowel Syndrome

The effect of probiotics on the treatment of IBS in adults has been studied extensively, but data in children are limited and the results are inconsistent.[157,158] Several reports indicated that probiotic treatment had no effect on global symptoms of IBS in children[159]; however, recent studies demonstrated improvement of symptoms following probiotics.[160,161] For example, in a randomized, double-blind, placebo-controlled trial in 141 children with IBS or functional pain, LGG significantly reduced the frequency and severity of abdominal pain in children with IBS.[160] In contrast to probiotics, few studies investigated prebiotics on IBS[158] and to our knowledge, no data in children were reported. Several studies suggested that supplementation of low dose of prebiotics, for example, FOS or GOS, may be effective, while high doses may have a negative impact on symptoms. A recent study advised that reducing intake of fermentable carbohydrates, including FOS and GOS, may actually improve symptom in IBS.[162]

### Necrotizing Enterocolitis

Probiotic use to reduce NEC has been the subject of several recent reviews.[163–165] A 2011 Cochrane review based on 16 trials (2,842 infants) concluded that enteral probiotic supplementation to preterm infants less than 2,500 g birth weight significantly reduced the incidence of severe NEC (stage II or more) and mortality.[163] Based on the 2011 Cochrane review, the American Pediatric Surgical Association Outcomes and Clinical trial Committee advised the use of probiotics to decrease the incidence of NEC.[164] However, recently published clinical guidelines from the American Society for Parenteral and Enteral Nutrition did not support the use of probiotics in infants at risk for NEC, and suggested that further studies are necessary to determine the most effective type(s) of probiotic, effective dosage, and duration of treatment.[165] Studies of prebiotics on the prevention of NEC are rare, and, to the best of our knowledge, there is no clinical evidence supporting the use of prebiotics to prevent NEC.[166,167]

## Conclusions and Future Needs

Accumulating evidence provides strong associations between the gut microbiota, dysregulation of mucosal and systemic immune function, and increased risk of immune-related diseases in children. However, these relationships are multifaceted and are mediated through a complex interaction between the child's genetic background, which dictates the child's risk for developing the disorder and the composition of his/her microbiota and pre-, peri-, and postnatal environmental factors. Most of these environmental factors are amenable to clinical intervention, for example, reducing preterm births, CsD, and the use of antibiotics and promoting breastfeeding and potentially pre- and probiotics. However, to maximize the clinical benefit, a richer understanding of the causal relationships among these factors and the incidence and severity of the immune-related disease is needed.

In general, a reduction in overall bacterial diversity, reduced abundance of commensal bacteria, and increased abundance of potentially pathogenic bacteria has been associated with the immune-related disorders reviewed herein. In some cases, specific bacteria have been identified in cases versus controls (►**Table 1**); however, there is a lack of consistency between studies, which may be attributable to the small numbers of subjects included in most studies, differences in analytical approaches; even among 16s rDNA-based approaches (qPCR, DGGE, T-RFLP, 454 Pyrosequencing) and between Pyrosequencing and next generation sequencing technologies. To better define the role of the

microbiota, researchers are encouraged to work together in multicenter prospective trials and to incorporate randomized, placebo-controlled, double-blind study design into future clinical interventions, testing the efficacy of pre- and probiotic interventions. In addition, future investigations should extend beyond descriptions of the composition of the fecal microbiota to incorporate metagenomic and metatranscriptomic analyses, which will provide insight into microbial metabolism. Investigations into the gut virome and fungiome may also provide unique insights into the additional aspects of dysbiosis, underlying disease onset and severity. Additionally, coordinate analysis of host–microbe interactions are needed in both health and disease. For example, using exfoliated epithelial cells, recent work in our laboratory has shown relationship between host intestinal gene expression[168] and bacterial metagenome virulence factors in BF and FF infants.[169] This noninvasive approach facilitates investigations into host–microbiome interactions in infants and children and is also amenable to longitudinal studies of cases of chronic inflammation—for example, relationships between gut gene expression and bacterial composition and gene expression could be investigated during phases of disease activity or clinical remission in IBD or responses before and after pre- or probiotic treatments could be assessed. Finally, based on research in rodent models demonstrating genome-wide linkage with relative abundances of specific microbial taxa, future research investigating specific disease-related QTL and the composition of the microbiota are warranted in children with diseases such as IBD.

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