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REVIEW

# The microbiome in early life: implications for health outcomes

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**Recent studies have characterized how host genetics, prenatal environment and delivery mode can shape the newborn microbiome at birth. Following this, postnatal factors, such as antibiotic treatment, diet or environmental exposure, further modulate the development of the infant's microbiome and immune system, and exposure to a variety of microbial organisms during early life has long been hypothesized to exert a protective effect in the newborn. Furthermore, epidemiological studies have shown that factors that alter bacterial communities in infants during childhood increase the risk for several diseases, highlighting the importance of understanding early-life microbiome composition. In this review, we describe how prenatal and postnatal factors shape the development of both the microbiome and the immune system. We also discuss the prospects of microbiome-mediated therapeutics and the need for more effective approaches that can reconfigure bacterial communities from pathogenic to homeostatic configurations.**



Our knowledge of the microbiome has greatly expanded over the last few years, and growing evidence suggests that aberrant bacterial communities early in life can lead to disease through an altered development of the immune system. Epidemiological studies have established a clear correlation between factors that disrupt the microbiota during childhood and immune and metabolic conditions (**Table 1**). Only recently, however, have we gained an understanding of the assembly of microbial communities during childhood and how its interruption might lead to disease. Recent studies have also described interventions that modify microbial composition, opening the possibility of overcoming microbial imbalances during childhood as a preventive therapeutic approach.

Although we will describe the neonatal microbiome in multiple body sites, the main focus of our review will be on the microbial communities found in the gut. Throughout this review, we will use the expression 'early life' to refer loosely to the first 2 years of life. Young children who are less than 4 weeks of age will be referred to as 'neonates', those older than 4 weeks will be described as 'infant' or 'baby', and, in general, subjects below the age of puberty will be described as 'child'. We first outline the major factors that shape microbial composition at birth and immediately after, including mode of delivery, antibiotics, diet and environmental exposures. We then describe how composition of the early-life microbiota is critical in the development of the immune system, and how deviations from homeostasis can induce disease later in life. Finally, we will summarize the current evidence for a link between early-life microbiota and disease, and discuss opportunities for interventions that modify the microbiome for therapeutic purposes.

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## The neonatal microbiome at birth

**Prenatal factors.** Culture-based and culture-independent studies have questioned the idea of whether the uterus is sterile, and they have suggested that microbes are present in the placenta[1,2], amniotic fluid[3,4], fetal membrane[5], umbilical cord blood[6] and meconium[7,8] (**Fig. 1**). Cultivatable bacteria belonging to the genera *Enterococcus*, *Streptococcus*, *Staphylococcus* and *Propionibacterium* have been isolated from nearly half of the umbilical cord (1–2 $\log_{10}$(colony-forming units (CFU) per 100 µl sample) and from all of the meconium samples (3–9 $\log_{10}$ (CFU per g tissue) in some studies[6,7], as well as DNA from *Bifidobacterium* and *Lactobacillus* from human placental samples[1]. The studies suggest that intrauterine samples harbor bacterial DNA, but not necessarily cultivable bacterial cells. Maternal gut microbiota could be translocated to the fetus via the bloodstream, a hypothesis supported by the detection of labeled *Enterococcus faecium* in the amniotic fluid and the meconium of orally inoculated mice[7]. A recent study has also reported the presence of low levels of bacterial biomass in human placenta and has identified a nonpathogenic microbiota similar to that of the oral cavity (which include members of Firmicutes, Tenericutes, Proteobacteria, Bacteroides and Fusobacteria), and the authors hypothesize that the bloodstream could be the route to deliver maternal oral bacteria to the fetus[2]. However, the presence of bacteria (specifically, *Burkholderia*, and members of the Actinomycelates and Alphaproteobacteria) is associated with pregnancy risks[2], and nearly 25% of preterm infants are born to mothers that had an intrauterine infection and occult microbial invasion of the amniotic cavity[9]. The bacteria detected were in many cases common vaginal residents[10], suggesting that the uterine microbiota derives from vaginal infection, at least in preterm deliveries.

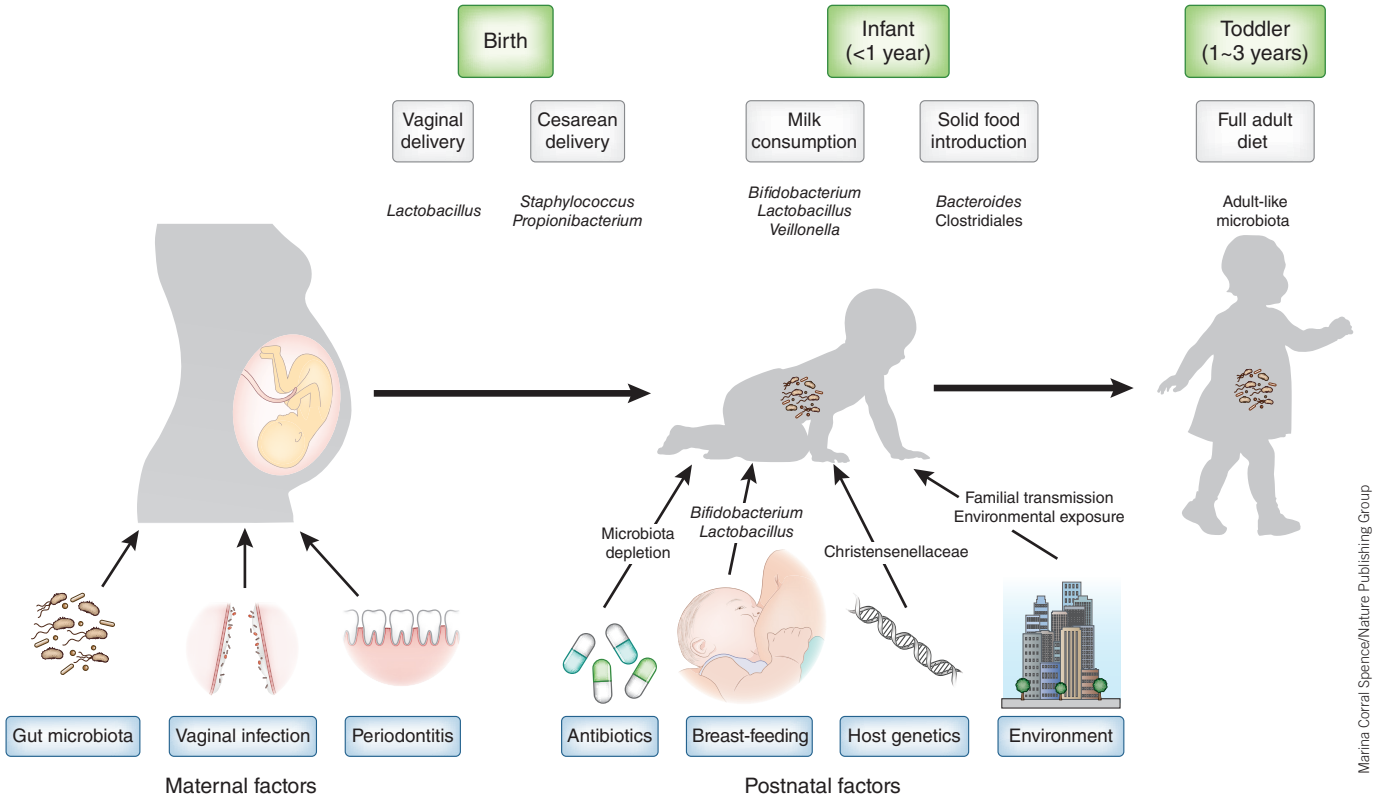


**Figure 1** Factors shaping the neonatal microbiome. Maternal vaginal infections or periodontitis can result in bacteria invading the uterine environment. Gut and oral microbiota could be transported through the bloodstream from the mother to the fetus. Delivery mode shapes the initial bacterial inoculum of the newborn. Postnatal factors such as antibiotic use, diet (such as breast-feeding versus formula, and introduction of solid food), genetics of the infant and environmental exposure further configure the microbiome during early life. As diet diversifies with age, the microbiome gradually shifts toward an adult-like configuration, which is usually reached by age 3. Bacteria associated with the different processes are indicated.

Many of these studies identified bacteria by using quantitative PCR and 16S rRNA sequencing to characterize unculturable bacteria. These methods, however, cannot exactly quantify the number of bacterial cells and, notably, cannot distinguish between free DNA, dead cells, live cells and metabolically active cells (**Box 1**). This makes it difficult to categorically establish the presence of a placental microbiota[11] and to assess whether the number of bacteria in the intrauterine environment is a risk factor during pregnancy. In addition, true controls (such as maternal blood) and absolute bacterial quantification were missing in these studies, a serious limitation given that both vaginal and cesarean section (C-section) delivery are associated with mild levels of bacteremia[12]. Reagents and extraction kits can also contain contaminating DNA, a problem that is particularly acute in samples with low bacterial biomass[13,14]. Further studies will be required to confirm the existence of a viable intrauterine-resident microbiota, quantify its variability and determine how it might affect the future development of the newborn.

**Delivery.** The first major exposure of the newborn to microbes happens during the birthing process and is highly dependent on mode of delivery[15–17]. The skin, gut, and oral and nasopharyngeal cavities of vaginally delivered infants are initially enriched in *Lactobacillus* spp. (**Fig. 1**), which resembles the maternal vaginal microbiota[17]. In contrast, the skin, mouth and gut of children delivered by C-section lack this inoculum and are instead colonized by common skin and environmental microbes such as *Staphylococcus*, *Streptococcus* or Propionibacteria[18,19] (**Fig. 1**). This initial microbiota evolves over time, adapting to the physicochemical and biological characteristics of each body site, and is shaped by the availability of different nutrients[20–22]. Although these differences gradually decrease between vaginally delivered infants and C-section-born infants, a bacterial signal remains associated with C-section-delivered infants until 12–24 months of age[18,23]. This suggests that early colonization provides a competitive advantage to the bacterial communities associated with each delivery mode. Furthermore, C-section delivery has also been shown to delay the colonization of the gut by specific bacterial taxa[23,24]. Although epidemiological studies have shown a relationship between C-section delivery and various diseases (**Table 1**), the causality of this relation remains to be demonstrated[25–27].

To understand the first bacterial inoculum that naturally (vaginally) delivered newborns receive, it is important to characterize the maternal vaginal microbiota. In nonpregnant women, at least six types of vaginal microbiota have been identified[28–30], and five bacterial community state types (CSTs) have been defined[30]. Four of these CSTs are typically found in Asian and white women, and are dominated by *Lactobacillus* species (*L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III) *and L. jensenii* (CST V)). CST IV is often seen in black and Hispanic women, and is characterized by low levels of *Lactobacillus* spp. and increased diversity of various anaerobic bacteria.

Pregnancy results in a number of changes in the vaginal microbiome, which is significantly distinct between pregnant and non-pregnant women[31]. The vaginal microbiota has a lower diversity and a higher stability of bacterial composition during pregnancy[32], and it is generally dominated by *Lactobacillus* species such as *L. crispatus* or *L.iners*[31,33,34]. The dominance of *Lactobacillus* spp. and its stability highlights the importance of this bacterial species in maintaining a healthy vaginal ecosystem[35,36].

In pregnant American women, the vaginal microbiota shifts between

CSTs that are dominated by *Lactobacillus* spp., although it rarely shifts to CST IV[31]. The enrichment in *Lactobacillus* spp. during pregnancy has been confirmed in a cohort of European women, although cases of anaerobic-bacteria-enriched, *Lactobacillus*-negative communities have also been reported[37]. As noted above, the structure and composition of the vaginal microbiota is correlated with ethnicity[30,38], suggesting that host genetics or environmental factors play a role in shaping the vaginal microbiome. Newborns might, therefore, start their life exposed to different bacterial communities, thus stressing the importance of studies that characterize the microbiome of diverse populations.

### Factors influencing the microbiome in early life

**Antibiotics.** Antibiotics have reached widespread prevalence and are among the most commonly prescribed drug for children[39,40]. The use of postnatal antibiotics can, however, disrupt the delicate ecosystem of the neonatal microbiome[41] (**Fig. 1**). The continuous use of antibiotics early in life has also been linked to an increased risk for various conditions (**Table 1**), highlighting the importance of understanding the relation between the neonatal microbiome and the development of asthma[42–44], type 2 diabetes[45–47], inflammatory bowel disease (IBD)[48] or milk allergies[45–47]. The association between antibiotic exposure in early life and development of asthma has, however, been called into question in a more recent study of a Swedish cohort, which suggested shared familial factors and respiratory infections as alternative risk factors[49].

These correlations are not necessarily causal, and it has been reported that children who are exposed to antibiotics in early life might experience more severe viral infections than those who are not, suggesting that impaired viral immunity increases the risk of antibiotic prescription and asthma[50]. The changes that antibiotics induce in the microbiome, both in terms of composition and 'time to return to baseline', depend on body site[51], or the type and dose of antibiotic used[52]. These effects are less understood in infants, leaving an important gap in our understanding of how antibiotics shape the microbiome during this important developmental window.

Mouse models can complement human studies and provide further insights into how disruption of the microbiota through the use of antibiotics during early life can lead to disease. Studies mimicking the effect of either low doses of antibiotics or pulsed antibiotic treatment during childhood have also demonstrated a significant disruption of gut microbiota followed by an increase of total fat mass in mice[53,54]. The antibiotic-mediated reduction in the abundance of *Lactobacillus*, *Allobaculum* and segmented filamentous bacteria further resulted in a blunted induction of a T helper 17 ($T_H17$) cell response in the colon[53,55]. Neonatal mice treated with antibiotics have also been shown to have an enhanced sensitization to food allergens. This phenotype was partially due to members of Clostridia that induced production of interleukin (IL)-22, which prevents food allergens from crossing the intestinal epithelial layer[56].

Antibiotic treatment can also alter the microbial balance among bacteria, viruses and fungi (**Box 2**). For example, antibiotic exposure can lead to an increase in gastrointestinal fungal abundance (*Candida albicans*), resulting in the development of airway diseases caused by allergic responses, owing to the induction of mast cells, IL-5, IL-13 and other inflammatory mediators in mice[57], as well as by impairment of the antiviral immune response in humans[58].

Although mouse models have greatly advance our understanding of how antibiotics modify host microbiome and health outcomes, laboratory mice have clearly distinct immunological profiles from humans, thereby limiting our ability to extrapolate conclusions derived from mouse studies to human disease[59]. However, prolonged antibiotic treatment in humans is uncommon; therefore, the development of humanized mice that better reflect human physiological responses is critical to better characterize the impact of antibiotics in human health.

## Box 1 Tools to characterize the microbiota

The majority of microbiome studies characterize the presence of bacteria in a sample by sequencing a fragment of the 16S rRNA–encoding gene. The relatively low cost of this approach allows for large-scale studies including thousands of samples, although bacterial resolution is usually limited to the genus or species level at best[152]. Shotgun metagenomics and metatranscriptomics have emerged as promising alternatives, as they provide a functional profile of the microbiome while improving taxonomy resolution to the strain level[153,154] and informing on transcriptional activity of the community[143]. DNA-sequencing-based approaches cannot generally distinguish between living and metabolically active, damaged or dead bacterial cells, or free DNA, whereas RNA sequencing analyses can identify living and metabolically active cells but also those that are dying. This is an important distinction, as the human innate immune system can selectively respond to microbial viability[142]. Although metatranscriptomic analysis or methods that combine cell sorting with high-throughput DNA or RNA sequencing have been proposed to address this limitation[143,155,156]; however, their use is still relatively uncommon. Approaches that can simultaneously account for identity and physiology of the bacteria in a community are thus highly desirable, and their use in characterizing the microbiome in early life will provide further insights that complement those derived from current methods.

**Diet.** During the first months of life the infant receives nutrients primarily from maternal breast milk or formula. Breast-feeding, in particular, is associated with numerous benefits for the child (such as increased resistance to infections[60,61], lower risk of obesity[62,63] or decreased risk of allergies[64]) and for the mother, in whom the duration of lactation is associated with a decreased risk of hypertension, hyperlipidemia, cardiovascular disease and type 2 diabetes[65,66]. Although a positive effect of breast-feeding in asthma is still controversial[67], recent evidence has shown that, when adjusting for confounders, prolonged breast-feeding improves lung function regardless of maternal asthma status[68]. The beneficial properties of breast-feeding are thought to be partially mediated through factors that are secreted in breast milk, including immunoglobin (Ig) A, lactoferrin and defensins[69,70]. *Bifidobacterium* and *Lactobacillus* are enriched in breast-fed infants as compared to those who are fed exclusively with formula (**Fig. 1**), which results in a more acidic intestinal content with a higher abundance of short-chain fatty acids (SCFAs)[71]. This decreased gut pH would serve as a defense mechanism against common pathogenic organisms. In the newborn, SCFAs are produced through the bacterial fermentation of oligosaccharides that are present in breast milk. Human milk oligosaccharides (HMOs) are not directly digested by the host[72], but instead serve as an energy source for colonic bacteria. Notably, only *Bifidobacterium* strains found in infants (for example, *B. longum* ssp. *infantis*) can metabolize milk oligosaccharides, whereas species in the adult gut (*B. longum* ssp. *longum*) maintain the ability to ferment complex carbohydrates but not HMOs.

Breast milk can also contain up to $10^7$ bacterial cells/800 ml, including bacteria such as *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Bifidobacterium*, which serve as an inoculum for the newborn[73,74], although the exact origin of these maternal bacteria has yet to be clarified[41]. After an initial contraction in bacterial diversity[19,75], the gradual increase in the diversity of the dietary substrates the growing infant consumes results in a shift in microbial composition and an enrichment of bacterial functions related to carbohydrate utilization and biosynthesis

**Table 1 Summary of studies linking factors disrupting microbial homeostasis during early life and development or protection against diseases**

| Disruptive factor | Study | Cohort characteristics | Outcomes |
|---|---|---|---|
| C-section | Sevelsted *et al.*[25] | 1.9 million Danish term children, ages 0–15 years | Asthma, systemic connective tissue disorders, juvenile arthritis, IBDs, immune deficiencies and leukemia |
| | Huh *et al.*[26] | 1,255 US children, age 3 years | Obesity, higher body-mass index and sum of skinfolds |
| | Eggesbø *et al.*[27] | 2,803 Norwegian children, 0–2 years | Reactions to egg, fish or nuts, and a fourfold increase in egg allergy |
| Antibiotic treatment | Risnes *et al.*[43] | 1,401 US children, ages 0–6 months | Asthma and allergy |
| | Hoskin-Parr *et al.*[44] | 5,780 UK children, ages 0–2 years | Asthma and eczema |
| | Saari *et al.*[150] | 12,062 Finnish children, ages 0–2 years | Overweight and obesity |
| | Schwartz *et al.*[151] | 163,820 US children ages 2–18 years | Weight gain |
| | Kronman *et al.*[48] | 9 million UK children | IBD development |
| Probiotics | Maldonado *et al.*[89] | 215 Spanish children, ages 0–6 months | Reduction in gastrointestinal and upper respiratory tract infections |
| | Braegger *et al.*[76] | ESPGHAN Committee on Nutrition | Reduction in nonspecific gastrointestinal infections |
| Diet supplements | Zimmerman *et al.*[93] | Iron, 139 African children, ages 6–14 years | Intestinal inflammation, lower frequency of colic or irritability |
| Hygiene | Hesselmar *et al.*[96] | 184 children, pacifier cleaning, ages 0–3 years | Lower risk of developing asthma, allergy and sensitization |
| Pets | Virtanen *et al.*[99] | 3,143 Finnish children, ages 0–1 year | Reduction in risk of preclinical type I diabetes |

of amino acids and vitamins[18]. By age 3, the microbiota of the infant has essentially converged to resemble that of adults in his or her population[22].

**Probiotics and dietary supplements.** Probiotics are live microorganisms that are supposed to provide a health benefit to the host, whereas prebiotics are substances that can favor the growth of beneficial microbial organisms. Supplementation of infant formula milk with pre- or probiotics is becoming increasingly common, despite a lack of data supporting their efficacy[76]. The effects of probiotics on pediatric diseases—including allergies, obesity, gastrointestinal infections or colics[76–79]—have been extensively studied, although their benefits remain controversial. Some meta-analyses have found a positive effect for probiotics in the treatment of atopic dermatitis[80–82], whereas others did not find any significant effects on infants <12 months old[83]. The risk of atopic eczema was found to be lowered through administration of *Lactobacillus* alone or in combination with other bacteria in one meta-analysis[84], but the combination was found to be ineffective in another[82]. A meta-analysis of the effect of probiotics in sensitization and asthma (or wheeze) found an overall reduction of IgE and atopic sensitization, but not directly of asthma or wheeze[85].

The most popular probiotic supplements in formula are *Lactobacillus* and *Bifidobacterium* spp. *L. reuteri* DSM 17938 has been evaluated for its effects on infantile colic. After 21 days of administration in newborns who are up to 16 weeks old, a significant increase in lactobacilli and a decrease in *Escherichia coli* and ammonia were observed, as compared to those in controls[86]. *L. johnsonii* La1 was shown to increase total *Lactobacillus* counts after administration, as compared to placebo controls, and was excreted live in 17% of the newborns at least 2 weeks after its administration was discontinued[87]. In a group of infants at risk for allergic diseases, the administration of *B. longum* BB536 and *L. rhamnosus* GG during the first six months of life was not shown to influence the overall composition of the gut microbiota, and the probiotic bacteria did not persist once administration was stopped[88]. Similar results were observed in a study of 6-month-old infants who received formula supplemented with *L. fermentum* CECT5716 plus galactooligosaccharide[89]. Despite a reduction of gastrointestinal infections and upper respiratory tract infections, the administration of this probiotic for 6 months resulted in only higher counts of *Lactobacillus* and *Bifidobacterium*, with no appreciable differences in levels of SCFAs. Human oligosaccharides, which are absent in cow milk, are also often used as a prebiotic to supplement formula. Nondigestible oligosaccharides that are added to formula have shown results similar to those of breast-feeding in reducing the colonic pH and increasing the production of SCFAs, effects that are probably associated with a selective stimulation of *Bifidobacterium* and *Lactobacillus* spp.[90]. Most studies to date, however, suggest that the benefits provided by pre- or probiotics are neither a direct result of the modification of the gut microbiota nor of the establishment of the probiotic as a gut resident.

Nutritional supplements have also been characterized for their effects in microbiome composition and host health. In one study of the gut microbiome of 12-month-old US infants who consumed one of three different diets (pureed meat as the primary complemented food, iron-fortified cereal, or iron- and zinc-fortified cereal[91]), the iron-fortified group had significant differences in bacterial composition at the phylum level, as compared to those in other two groups, as well as a significant decrease of Lactobacillales members. Fish oil, another common dietary supplement, had a more pronounced impact on infants' gut microbiota than sunflower oil, but this effect was limited to infants who were weaned before they were 9 months old[92]. This suggests that long-chain polyunsaturated fatty acids (LCPUFAs), which are present in large amounts in fish oil and are hypothesized to beneficially affect infant development, might only be effective in the absence of breast milk, their natural source. Of note, in a study of 6- to 14-year-old African children, diets supplemented with iron resulted in a significant change in gut community composition, as compared to those in controls, with an increase in the number of enterobacteria, a decrease in the number of lactobacilli and an increase in fecal calprotectin, a surrogate marker for intestinal inflammation[93]. This result emphasizes the need for studies that take into account the differences in gut microbiota composition across various populations[94] before determining the effects of dietary supplements and dietary recommendations.

**Environmental exposure.** The environment surrounding the newborn is also a natural source of microbes that can colonize different body sites (**Fig. 1**). For example, cohabitation increases the probability of bacterial exchange through the use of shared objects, touching of common surfaces, and indoor air[95]. The practice of cleaning a baby's pacifier by sucking it has also been associated with a distinct oral microbiota of the infant and a lower risk of developing allergies[96]. In a study of 60 American families,

it was also shown that family members share a more similar oral, gut and, particularly, skin microbiota than unrelated subjects[97]. Furthermore, cohabiting but genetically unrelated parents still share a higher proportion of their microbiota with their children than with unrelated children[22]. The sequencing of bacterial genomes in family members has further confirmed these findings, with families sharing bacterial strains not found in unrelated subjects[98]. Notably, some of these shared strains were observed in mothers and their adult daughters, suggesting that early exposure to specific strains can lead to a life-long colonization.

Frequent contact with pets and animals early in life is hypothesized to exert a protective effect in the infant through an increased exposure to microbes that help in the development of immune tolerance. In a study of 3,143 children, exposure to indoor dogs (but not to outdoor dogs, cats or farm animals) during the first year of life was reported to be inversely associated with preclinical type I diabetes[99]. Early-life exposure to pets has been shown to reduce the frequency of allergy and asthma[100], although the mechanisms for this observation are not well understood. To investigate this association, a study challenged mice with dust from houses with dogs and found an increase of *Lactobacillus johnsonii* in the gut microbiota of these mice, suggesting that a change in gut microbiota may partially explain the protective effect from exposure to animals[101].

Higher exposure to endotoxin in house dust and a larger family size can also result in a bloom of *Bifidobacterium* in infants and a lower abundance of *Lactobacillus*, *Bifidobacterium adolescentis* and *Clostridium difficile* during the first 2 months of life, a change that was associated with the development of allergies[102]. The study of children living in Finnish and Russian Karelia has also suggested the importance of environmental exposure early in life. These regions are geographically close but have marked differences in socioeconomic status. Although levels of sensitization to pollens and pets were similar in the 1940s, allergies were more common among Finnish children 30 years later[103]. Some of these differences have been attributed to bacteria found in environmental dust and drinking water[104], although further studies will be required for a more comprehensive assessment of the microbiome in these populations. A study of dust in children's bedrooms revealed how children living in farms had higher bacterial and fungal diversity than either those children who visited farms but did not live there or those who never visited farms[105]. The microbial organisms in farm dwellings often correlated with those found in animal sheds, further suggesting that environmental exposure is a potential route for bacterial colonization during childhood.

**Host genetics.** Host genetics plays a major role in human diseases, but it is only recently that we have started to understand how the microbiota interacts with host genetics. For instance, metabolic disorders that are known to have a genetic component[106,107] are also associated with a distinct gut microbiota composition[108–110], suggesting that impaired bacterial regulation by the host is a potential mechanism for pathogenesis. Determining how host genetics and microbiota interact is however notoriously complex, owing to the exponential number of interactions between thousands of bacterial taxa and millions of genetic polymorphysms[111]. Goodrich and colleagues have demonstrated that genetics can partially shape bacterial composition, with members of the Christensenellaceae family seeming to be heritable (**Fig. 1**), whereas colonization by members of Ruminococcaceae, Lachnospiraceae, and Bacteroidetes were mostly environmentally determined[112]. Further studies will be required to determine at what time in life these heritable taxa are acquired, and what is the relative effect of host genetics, as compared to other factors, in shaping early-life microbiota composition.

### The role of the neonatal microbiome in immune development

A balanced relationship between the host's microbiota and the immune system is crucial for a homeostatic response toward pathogenic attack and to prevent aberrant inflammation[113,114] (**Fig. 2**). Multiple studies have reported that dysbiosis in infants correlates with a chronic pro-inflammatory state as seen in obesity[115], IBD[116] or psoriasis[117] in adulthood.

During vaginal delivery infants receive their first bacterial inoculum from the maternal vaginal tract, skin tissue and often from fecal matter, exposing the immature immune system of the newborns to a significant bacterial load[22]. The neonatal innate immune system is biased toward a $T_H2$ phenotype, which is associated with helminth and parasite detection but also with the induction of allergic responses, and is biased against $T_H1$-cell-polarizing cytokines to avoid potentially harmful pro-inflammatory responses[118], allowing microbial homing and colonization. This impaired immune response makes the immunosuppressed neonates more susceptible to opportunistic pathogenic attacks. Hence, following multiple pathogenic encounters, often in a time- and age-dependent manner, there is a transition from $T_H2$ toward $T_H1$ polarization (**Fig. 2**), reducing the neonate's chances of allergy and atopy in adulthood[119,120]. In contrast, an infant's gut in a state of dysbiosis would promote a strong $T_H1$ bias, pushing the immune system to be pro-inflammatory, secreting the cytokines IL-12 and interferon (IFN)-γ. This inflammatory state would promote tissue damage and impair infection resolution and tissue repair, disturbing the normal immune regulatory system, potentially leading to long-term consequences such as IBD[116], allergy and autoimmune diseases.

A recent study in neonatal mice has demonstrated that immune tolerance (the lack of response to certain antigens that allows the immune system to distinguish 'self' from 'non-self') was acquired in vaginally delivered pups but not in C-section-delivered pups[121]. Vaginally delivered mice had spontaneous activation of the intestinal epithelial cells and acquired resistance to lipolysaccharide (LPS)—which are molecules found in the membrane of Gram-negative bacteria that elicit an immune response from the host—shortly after birth. This led to tolerance through the downregulation of genes responsible for immune responses to infec-

## Box 2 Virome and mycobiome

Commensal eukaryotic and bacterial viruses, fungi and archaea are, together with bacteria, also part of the human microbiome. Our knowledge of their role in the health outcomes of infants is, however, more limited, and only recently have we gained a better understanding of these other microbial communities. The virome is the collection of eukaryotic DNA and RNA viruses and bacterial viruses (bacteriophages). In a recent study of the infant gut virome, it was found that co-twins had higher similarity in their virome as compared to unrelated infants. Enterovirus, parechovirus, tombamovirus and sapovirus were the most common eukaryotic viruses, and their abundances seem to depend on environmental exposure. The most abundant bacteriophages belong to the order Caudovirales and to the family Microviridae[21].

Despite its low abundance, the mycobiome (commensal fungal microbes) has an important role in human health. For example, overgrowth of *C. albicans* is associated with antibiotic treatment in immunocompromised individuals[57]. In adults, *C. albincans* is the most abundant species in mucosal sites. *Candida*, *Aspergillus*, *Fusarium* and *Cryptococcus* are common in oral sites of healthy individuals[157], whereas *Malassezia* spp. and *C. albicans* are found in the skin of healthy subjects and those with disease[158,159]. Studies addressing how the infant mycobiome develops and shapes the host immune system will be required for a more comprehensive understanding of the early-life microbiome.

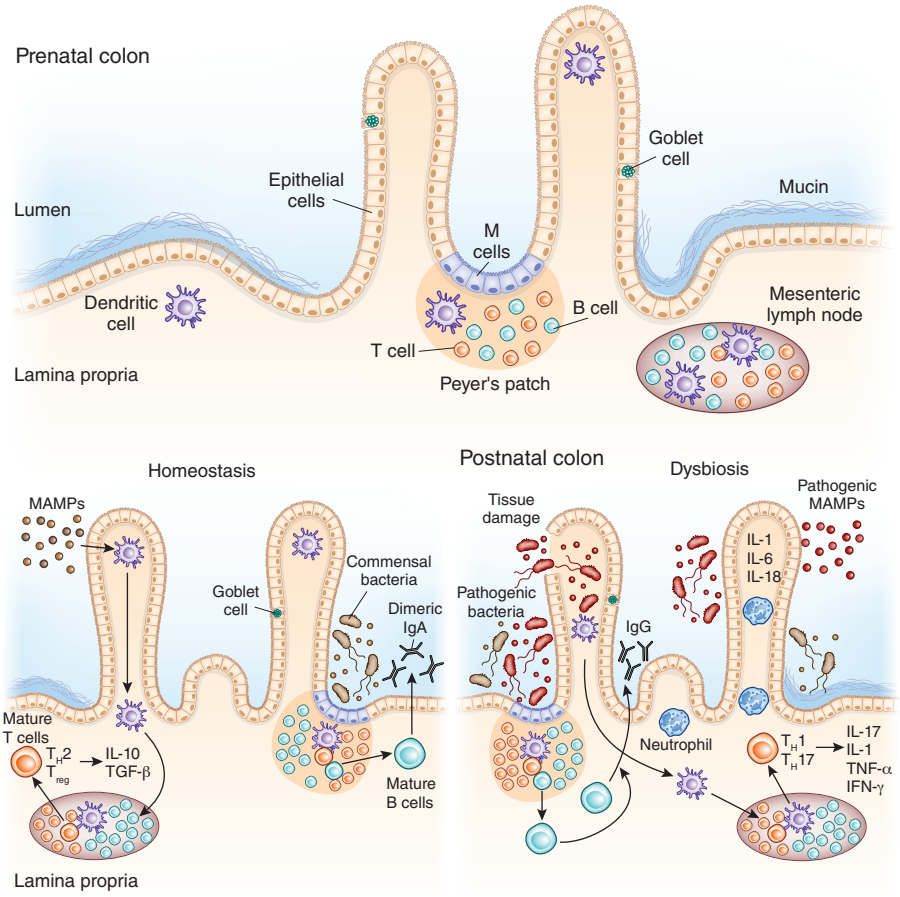


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**Figure 2** Long-lasting effects of early-life interactions between the microbiome and the gut immune system. The development of secondary lymphoid structures, including Peyer's patches and the mesenteric lymph nodes, occurs prenatally before bacterial colonization. Microbial colonization of the gut is established postnatally via interactions between the commensal bacteria and the host's immune system. Microfold (M) cells at the apical surface of Peyer's patches sample luminal antigens and bacteria through endocytosis. Dendritic cells (DCs) then present these antigens to induce T cell–dependent B cell maturation to promote the secretion of dimeric IgA, which play a critical role in defense against pathogens, by plasma cells in the lamina propria. Bacteria can also be transcytosed by DCs in the lamina propria and be presented to T cells in the draining mesenteric lymph node to induce T cell differentiation. In a homeostatic environment (bottom left), the MAMPs associated with commensal bacteria stimulate regulatory cytokine production (IL-25, IL-33, thymic stromal lymphopoietin (TSLP) and transforming growth factor (TGF)-β) by intestinal epithelial cells. The transduction of the signals to DCs induces the development of $T_{reg}$ cells and promotes IL-10 secretion. In a dysbiotic state (bottom right), the reduction of commensal bacteria results in enrichment of pathobionts and pathogens. Pathogenic MAMPs that are sensed by the intestinal epithelial cells induce the secretion of pro-inflammatory cytokines (IL-1, IL-6 and IL-18), prompting the development of effector T cells. These effector T cells differentiate into CD4$^+$ $T_H1$ and $T_H17$ cells, which secrete pro-inflammatory cytokines, such as IL-17, tumor necrosis factor (TNF)-α and IFN-γ, which induce neutrophil recruitment to protect the host against pathogenic infections. B cell maturation in Peyer's patches results in the production of IgG, which is often associated with autoimmunity and allergy.



tion. The role of LPS and microbial composition in early life has been further explored in a cohort of 222 children from Finland, Russian Karelia and Estonia[122]. The microbiome of Finnish and Estonian infants was dominated by *Bacteroides*, and thus the primary source for LPS exposure in these children was probably from this bacterial genus; however, the abundance of *Bacteroides* was significantly depleted in Russian children. LPS from *Bacteroides* was found to be structurally different to that of other bacteria, and notably it also inhibited tolerance *in vitro* and in non-obese diabetic (NOD) mice. These findings suggested a potential explanation for the cause of the higher incidence of autoimmune diseases in Finland and Estonia, as compared to that in Russian Karelia.

Different species of *Bacteroides* can, however, elicit different immune responses. *B. fragilis*, for instance, has been shown to have an anti-inflammatory role, by acting on regulatory T ($T_{reg}$) cells. *B. fragilis* produces surface polysaccharide A (PSA), a microorganism-associated molecular pattern (MAMP) that is recognized by toll-like receptor 2 (TLR2) on $T_{reg}$ cells. Engagement of TLR2 and PSA leads to $T_{reg}$ cell induction and limits the $T_H17$ response, thereby promoting tolerance and immunosuppression in the gut. This protection is only seen in the developing intestinal immunity in neonates and is not observed in adult mice[123].

## The neonatal microbiome and disease

**Association between the early-life microbiome and disease.** As mentioned above, aberrant neonatal microbiota composition is associated with disease during childhood and later in life (**Table 1**). For example, a study of treatment-naive subjects with pediatric Crohn's disease (CD) has shown that members of the Veillonellaceae, Neisseriaceae and Fusobacteriaceae families are enriched in children with CD, as compared to healthy children (who had a higher abundance of *Bacteroides*, *Fecalibacterium* or *Ruminococcus*)[124].

The incidence of asthma has also been linked to an abnormal microbiota in pediatric populations. In a longitudinal analysis of the gut microbiota of 319 children[125], although the bacterial diversity and composition was not significantly different among the groups, those subjects with both atopy and wheeze at 1 year of age had a gut microbiome that was depleted for *Fecalibacterium*, *Lachnospira*, *Rothia* and *Veillonella* at 3 months of age, as compared to control subjects. LPS biosynthesis was also observed to be reduced in this group at 3 months of age, as computationally predicted[126] but also experimentally confirmed, and so were the levels of SCFA acetate.

We have recently shown in a study of 226 children with milk allergy that gut microbiome composition of 3- to 6-month-old children, but not those of 7- to 12-month-old or 13- to 16-month-old children, was associated with milk allergy resolution by 8 years of age[127]. Children younger than 6 months old who resolved their milk allergy had an enrichment of Clostridia and Firmicutes in the gut, whereas persistence was associated with higher levels of *Enterobacter*, *Trabulsiella* and *Salmonella*. This altered microbiota in the younger children was accompanied by a decrease in bacterial fatty acid metabolism, consistent with the observation that healthy infants have lower levels of branched-chain short fatty acids in the gut than infants with milk allergy. This study and others[53,125] suggest

that even transitory disruptions of microbial communities early in life can induce clear phenotypes later in life.

Studies with germ-free mice have allowed the more precise determination of how microbial imbalances can result in disease. For instance, *Fecalibacterium* sp., *Lachnospira* spp., *Veillonella* spp. and *Rothia* spp. (FLVR) are significantly depleted in the gut of children with atopy and wheeze[125]. Inoculating a germ-free mouse model of airway inflammation with these bacteria resulted in an amelioration of symptoms. Similarly, the microbiome that is associated with malnutrition in Malawian twins induces a significant weight loss when transferred to recipient gnotobiotic mice[128]. Noval-Rivas and colleagues also demonstrated how the gut microbiota of a mouse model of food allergies is not only different from that of wild-type (WT) mice, but it can even promote anaphylaxis when inoculated into WT germ-free mice[129]. Taken together, these results clearly demonstrate a causal relation between abnormal microbiome composition during early life and disease, at least in animal models.

**Microbiome-mediated therapeutics.** Approaches to modify the microbiota can be generally classified into three major groups: depletion, modulation, and replacement or restoration. For example, antibiotics are often used to treat conditions not necessarily caused by a specific pathogen, owing to their ability to efficiently deplete the gut microbiota (**Fig. 3**). In pediatric IBD, the disease is sometimes managed through the use of antibiotics either in isolation[130] or in combination[131]; however, the prolonged use of antibiotics in young children carries significant risks[132,133].

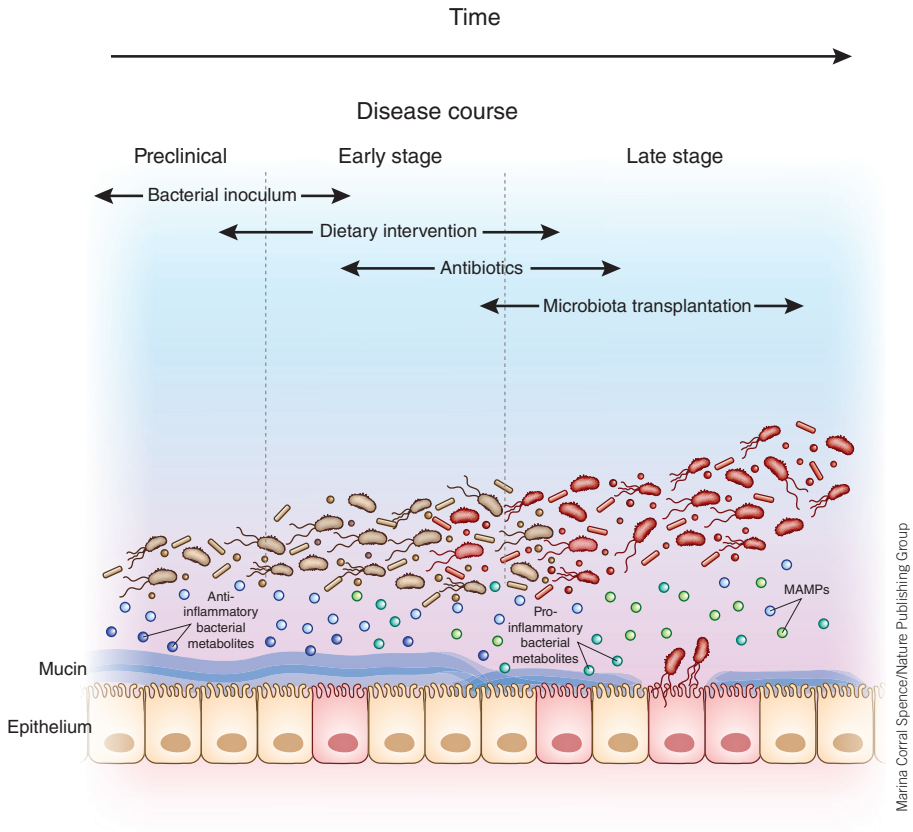


**Figure 3** Microbial therapeutics throughout the course of disease. During preclinical stages the disease has not yet manifested, and symptoms are not apparent, yet subtle biological changes might be already occurring. Approaches such as the use of an inoculum of defined bacterial communities might be most effective at the early stages of disease to prevent the development of disease resulting from early dysbiosis. As the disease progresses, disruption of a homeostatic microbiota results in enrichment of pathobionts (as shown in red), production of pro-inflammatory metabolites and activation of inflammatory pathways (**Fig. 2**). The mucus layer, which protects the epithelium, becomes thinner as damage accumulates owing to an increase in the severity of the disease. Dietary interventions and antibiotics might be used at this stage to manipulate bacterial content more drastically. At late stages, continuous damage leads to further thinning of the mucus layer, allowing for bacteria to break through the epithelial barrier. Aggressive antibiotic therapy combined with fecal microbiota transplantation could restore microbial balance at this point.

Bacterial content can also be modulated through dietary interventions to starve deleterious bacteria or to promote the growth of beneficial ones (**Fig. 3**). Exclusive enteral nutrition (EEN) is a notorious example; EEN replaces normal dietary components by a formula composed exclusively of liquid nutrients that are fed orally or through a feeding tube. EEN is used as a first-line therapy in pediatric Crohn's disease and can induce clinical remission and normalize inflammatory markers[134]. In a recent study of a cohort of 23 children younger than 16 years of age who have active CD, 15 underwent EEN for 8 weeks, and the intervention improved the disease condition while paradoxically increasing the microbiome differences with healthy controls[135]. This unexpected result suggests that the therapeutic mechanism might be due to decreased levels of colonic bacteria and a lower concentration of potentially harmful bacterial metabolites. Despite its success in subjects with pediatric Crohn's disease, the poor palatability and delivery method (tube feeding) makes the prolonged use of EEN problematic.

Studies in malnutrition provide an extreme example of how dietary deficiencies shape gut microbiota during childhood. Smith and colleagues studied a cohort of 317 Malawian twins during their first 3 years of life[128]. Over the course of the study, 43% of the twins became discordant for malnutrition. Although the gut microbiota of the healthy co-twin showed a gradual maturation, the microbiome of the malnourished twin did not progress in a similar manner, even after using a therapeutic food designed to treat malnutrition. These results suggest that current dietary interventions might not efficiently reconfigure the microbiota into a nonpathogenic state, and studies that better address how different diets modify microbial content and health outcomes are pressingly needed.

Highly dysbiotic environments might require more drastic approaches to shape microbial content. Fecal microbiota transplant (FMT) aims at replacing the local gut flora with an exogenous microbiota (**Fig. 3**). FMT is extremely effective in the treatment of refractory *C. difficile* infection (CDI)[136,137], and it has been explored in the treatment of ulcerative colitis in children and young adults (ages 7 to 21)[138]. The efficacy of this therapy in other conditions is, however, lower than that in CDI[139], and further studies will be required to optimize factors (such as dosing, delivery route and formulation) that improve the therapeutic efficacy of FMT. Furthermore, the use of FMT is still undergoing regulatory adjustments, and it is plausible that whole-microbiome transplant will eventually be replaced by designed bacterial communities, provided that efficacy is comparable[137]. Microbial transplant studies in infants will be challenging to design and execute, but given the limited therapeutic options for conditions such as pediatric IBD, we foresee that this will be an application of interest.

Given the influence that early-life microbiota exerts in health outcomes, preventative therapies aimed at restoring microbial homeostasis are highly desirable (**Fig. 3**). We have recently demonstrated that the abnormal microbiota of infants who are delivered by C-section can be partially restored to resemble that of vaginally delivered newborns[19]. By swabbing C-section-born newborns with a gauze that was previously introduced in the maternal vagina for an hour before delivery, a significant enrichment of *Lactobacillus* or *Bacteroides* was observed in the skin, oral and anal microbiomes, and there was a larger similarity in the microbiomes of these C-section-delivered babies to the microbiota of vaginally delivered infants. This proof-of-principle work shows that the transfer of vaginal bacteria to a newborn lacking in them is feasible, although health outcomes of this procedure are as yet undetermined.

## Conclusions and perspectives

An aberrant gut microbiome in early life is associated with an increased risk of disease, although we still lack conclusive evidence that these abnormal bacterial communities are in fact the etiological agent. Mechanistic studies that go beyond mere associations with specific bacteria will provide more valuable information that is translatable to human subjects. The effect of early versus late colonization is, however, only partially understood[140]. Studies in adult animals have shown how immune responses are modulated by bacteria through the fermentation of dietary fibers into SCFA, which can induce $T_{reg}$ cells in the colon[141]. Similar efforts toward understanding how different microbial communities modulate immune development in early life are thus required.

Notably, the interpersonal variability of the microbiome is often neglected during study design and analysis. Longitudinal studies in which each subject serves as its own control could ameliorate this problem, and developing efficient methods to analyze temporal microbiome data will be required. Furthermore, it will be critical to characterize changes in the neonatal microbiome in organs other than the gut to better understand the pathogenesis of conditions affecting those body sites.

We also believe that current methods of estimating microbiome composition through sequencing alone will need to incorporate techniques that can quantify bacterial load and distinguish between viable and dead bacteria (**Box 1**). The magnitude of the immune responses to bacteria depends on cell viability[142], and methods that capture cell viability will provide richer insights on how the microbiome shapes immune and health outcomes. It is also crucial that studies move beyond mere cataloging of bacteria and toward functional characterization and understanding of mechanisms. Metatranscriptomics can provide information for not only what bacteria and bacterial genes are present in a sample but also for transcriptional activity of the community[143]. Metabolomics can also help to determine how bacterial metabolites facilitate interaction with the host and how they might influence health state of the host[144,145].

The structure and diversity of the microbiota also varies greatly among different human populations[22,30,94]. Indeed, strains of *E. coli* or *Helicobacter pylori* are markedly distinct among different populations[94,146]. Genomic differences between strains of otherwise non-pathogenic bacterial species have also been associated with significant differences in disease outcome[147]. Efforts to characterize how newborns from various populations are exposed to different bacteria will therefore be crucial to shed further light on conditions that are associated with microbial disruption.

We envision that microbial interventions might eventually prevent or ameliorate diseases that are associated with aberrant microbial composition in early life. Methods to modify bacterial communities will have to be refined to fulfill this prospect. Studies that modulate the human microbiome can only do so for limited periods of time, with bacterial communities generally returning to baseline quickly after the intervention is discontinued[148]. Furthermore, dietary interventions can quickly modify the microbiome and yet have no health consequences[149]. It is therefore unclear how long these alterations will need to be implemented to permanently alter the microbiome and modify disease course. Recent results from FMT to treat ulcerative colitis seem to indicate that short-term interventions might not be sufficient to have therapeutic value in adults[139]. Studies that test microbial interventions for different amounts of time, at various doses or through different delivery routes will therefore be fundamental in determining how malleable bacterial communities are in early life and how they can be modified for therapeutic purposes. Although these are complex questions that will require significant effort to be answered, the rapid progress that has been made in characterizing the microbiome during childhood will enable the development of improved translational solutions for conditions associated with aberrant microbial communities in early life.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.



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