



Patterns of Early-Life Gut Microbial Colonization during Human Immune Development: An Ecological Perspective

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Alterations in gut microbial colonization during early life have been reported in infants that later developed asthma, allergies, type 1 diabetes, as well as in inflammatory bowel disease patients, previous to disease flares. Mechanistic studies in animal models have established that microbial alterations influence disease pathogenesis *via* changes in immune system maturation. Strong evidence points to the presence of a window of opportunity in early life, during which changes in gut microbial colonization can result in immune dysregulation that predisposes susceptible hosts to disease. Although the ecological patterns of microbial succession in the first year of life have been partly defined in specific human cohorts, the taxonomic and functional features, and diversity thresholds that characterize these microbial alterations are, for the most part, unknown. In this review, we summarize the most important links between the temporal mosaics of gut microbial colonization and the age-dependent immune functions that rely on them. We also highlight the importance of applying ecology theory to design studies that explore the interactions between this complex ecosystem and the host immune system. Focusing research efforts on understanding the importance of temporally structured patterns of diversity, keystone groups, and inter-kingdom microbial interactions for ecosystem functions has great potential to enable the development of biologically sound interventions aimed at maintaining and/or improving immune system development and preventing disease.

Keywords: microbiome, early-life events, immune development, microbial ecology, diversity, keystone taxa

INTRODUCTION

Recent advances in immune-mediated disease research have provided a considerable body of proof revealing the importance of the early gut microbiome for neonatal immune system development and disease pathogenesis [see Ref. (1) for a review]. The drastic increase of allergies and other immune-mediated diseases in industrialized countries has been hypothesized to be a result of deficiencies in the exposure to microbial organisms and their products, resulting in impaired immune system development, a concept first introduced as the hygiene hypothesis (2, 3). Pioneer work has identified the first 6 months after birth as a “window of opportunity” (4–7) during which contact with specific microbe-associated molecular patterns (MAMPs) triggers a cascade of reactions crucial for infant gut maturation (8–10). Disrupting early gut community succession may lead to dysbiosis, a state of ecological imbalance ensuing when the community loses key taxa, diversity, and/or metabolic capacity. This state can lead to a reduction of colonization resistance, allowing for a subsequent bloom in

opportunistic pathogens [(11); for a definition of relevant ecological concepts refer to **Table 1**]. Concomitantly, microbial dysbiosis during infancy may also lead to health-related consequences in the neonatal stage or later in life. Preterm neonates can develop necrotizing colitis (NEC), a life-threatening disease strongly associated with microbial dysbiosis (12). Infants may also experience an elevated risk of developing inflammatory diseases such as asthma and allergies (13, 14), type 1 diabetes (15, 16), celiac disease (17), inflammatory bowel disease (18, 19), and obesity (20, 21) when exposed to a microbial dysbiosis early in life. Thus, studying the patterns of microbiome assembly and how disturbances to this process reflect in the developing immune system is of utmost importance to understand the origin of human diseases responsible for enormous health and economic burden to societies.

The infant gut microbiome is a complex ecosystem involving a great number and diversity of members (e.g. bacteria, phages, fungi, viruses, protozoans) that interact in a spatially and temporally structured environment (26–28). The neonatal

gut microbiota can be considered a complex adaptive system in which both low-level local interactions and selection mechanisms combine to create high-level patterns (22). Complex adaptive systems are non-linear (output not proportional to the input, thus impeding predictability) in that they are heavily influenced by stochastic temporal events that result in a plethora of variable outcomes (22). The infant gut microbiome supports a set of emergent properties contributing to host physiology, including nervous, metabolic, and immune development (29–31), as well as tissue differentiation (32, 33). The emergent properties of a complex adaptive system are considered to be supported by combinations of taxonomic and/or functional diversity, as well as key taxonomic and/or functional groups, both of which insure community resilience (22), and increase the difficulty of attributing a cause–effect relationship to unique features or groups. Therefore, including community ecology theory to study the temporal dynamics of the infant gut microbiome has the potential to provide key information about its influence on the host immune system maturation.

Until 2 years of age, the human infant microbiome remains highly heterogeneous and lacks stability (34), being influenced by temporally structured environmental factors such as (1) maternal factors (35–37), (2) birth (38–41), (3) neonatal nutrition (27, 42, 43), and (4) other non-temporally structured factors, such as antibiotic treatments (41, 44, 45). The initial intestinal bacterial community composition of vaginally born infants involves higher levels of a multitude of bacterial groups (e.g., *Atopobium*, *Bacteroides*, *Clostridium*, *Escherichia coli*, *Streptococcus* spp. and *Prevotella*), while the community of infants born by C-section is dominated by skin-related taxa including *Staphylococcus* spp. (38). Key bacterial groups are also transferred to the infant by breastfeeding: *Bifidobacterium* and *Lactobacillus* (46–48). The multiple studies that have shown how intestinal dysbiosis can lead to detrimental immune-mediated outcomes (e.g., asthma, allergies, NEC, etc.) [see Ref. (30) for a review] suggest that the human immune system relies on an evolutionary conserved temporally structured succession of microbiome assembly. Unraveling the links between the temporal mosaics of the gut microbiome (structured succession patterns) with the emergent properties of this ecosystem (e.g., taxonomic and functional diversity, resilience, etc.) is key to improve our understanding of the importance of the infant microbiome for the development of the immune system.

The successful identification of the mechanisms linking the infant gut microbiome and immune development depends on our capacity to disentangle the relative effects of multiple factors (host genetics, environmental factors), key actors (e.g., *Bacteroidetes*, *Bifidobacterium*, etc.) and their interactions. Resilience, the ability of a system to adjust its activity to retain its basic functionality after a disturbance, is a crucial property of complex adaptive systems (49) and could be a key characteristic protecting the infant gut microbiome from reaching a dysbiotic state. Here, we review the recent findings on the links between infant gut microbiota and immune system maturation. Our review highlights the reliance of the neonate immune system development on a complex set of host-specific, environmental, temporal, and self-organizing characteristics of the infant gut

TABLE 1 | Definition of selected ecological concepts.

Concept	Definition
Complex adaptive system	A system composed of a multitude of autonomous and interdependent actors that share a variety of interactions, and behave as a unified whole in reacting and adjusting to changes in the environment (22)
Emergent property	A system's property that its components lack individually
Stochasticity	The unpredictable fluctuation of environmental conditions
Temporally structured ecosystem	An ecosystem in which emergent properties (e.g., taxonomic and functional diversity, resilience) rely on a conserved succession of events ordered in time
Richness	The number of "species" in a community
Alpha-diversity	The number of "species" and their abundance within a community or the mean in a collection of communities (i.e., Shannon index)
Beta-diversity	The absolute turnover in community composition often measured as communities' pair-wise dissimilarity in microbial ecology, also defined as the ratio between regional and local species diversity
Taxonomic diversity	The number and the relative abundance of species or taxa in a community
Functional diversity	The variety of processes or functions in a community that are important to its structure and dynamic stability
Resilience	A system's or community's capacity to promptly return to its initial state after a perturbation
Resistance	A system's or community's capacity to resist or impede changes in its state while withholding a perturbation
Selection	A key evolutive process in which genetic and environmental pressures determine which organisms succeed at survival and reproduction
Keystone species	An exceptionally important species whose presence is crucial in maintaining the organization and diversity of the ecological community (23, 24)
Succession	A pattern of changes in specific composition of a community after a radical disturbance or after the opening of a new patch in the physical environment for colonization (25)

microbiome. We propose that future studies should consider multi-level dynamics of the infant gut microbial community by disentangling the ecosystem reliance on (1) temporally structured patterns of alpha- and beta-diversity, both taxonomic and functional; (2) keystone species or microbial groups; and (3) inter-kingdom interactions. This will require a conceptual framework based on the understanding that the infant gut harbors a complex and diverse set of microbial species interacting in a temporally structured, multi-level, and non-linear network. Rightfully recognizing these structural characteristics has the potential to enable the identification of disturbance thresholds threatening the healthy development of the infant gut microbiome and its role in immune system training.

AGE-DEPENDENT IMMUNE SYSTEM DEVELOPMENT

Multiple studies and comprehensive reviews discuss how the maturation of the immune system relies on the exposure to MAMPs (50–52). Here, we discuss the recent findings demonstrating that the efficiency of microbial exposure in immune system training can be age dependent, suggesting the importance of microbial composition and infant gut microbiome temporal succession patterns.

The gastrointestinal tract is already anatomically and functionally developed at birth in full-term infants, yet important aspects of its maturation occur postnatally and depend on exogenous stimulations with microbial cells, metabolites, hormones, growth factors, and antigens (53, 54). Recent studies in murine models have revealed that several aspects of immune development are more permissive to microbial-mediated changes during early life, and that certain microbial taxa are crucial in these interactions. For instance, oral administration of *Bifidobacterium breve* was effective in inducing proliferation of FoxP3-positive regulatory T cells (FoxP3⁺ Tregs) only if administered during the pre-weaning stage in mice (55). This age-dependent promotion of an important tolerogenic immune cell was also shown to be species specific, thereby suggesting that the tolerogenic gut immune response may have adapted to respond to specific—and important—bacterial taxa. *Bifidobacterium* species and subspecies are dominant members of the infant gut microbiome (56) and are strong modulators of the immune response (57). Their role as keystone taxa of the infant gut is proposed later in this review. Another microbial species that cause an age-dependent immune effect is the *Helicobacter pylori*, which ameliorated airway hyperresponsiveness more effectively when administered before weaning in two relevant mouse models (58), although it remains unclear if and when this bacterium colonizes the infant gastrointestinal tract.

While age-dependent modulation of the host's immune response can be attributed to specific microbial taxa, most studies point to global changes in the microbial community (diversity shifts, and metabolites of poly-microbial origin) as drivers of immune development. Cahenzli et al. (59) showed that regulation of IgE responses and amelioration of antigen-induced oral anaphylaxis is dependent upon increased microbial diversity during early life. Their work thus suggests that there may be a

diversity threshold necessary for proper maturation of these Th2 immune mechanisms. Furthermore, several other studies have demonstrated the immune consequences of the disruption of the early-life gut microbial community using antibiotics. Antibiotics induce drastic compositional and diversity shifts that lead to changes in crucial immune functions, including Treg proliferation (60, 61), IgE response (60, 62), Th-17 response (61, 63), and basophil-mediated Th2-cell responses (62). Given the influence exerted by these immune functions on widespread tissues and systems, it is not surprising that antibiotic-induced immune alterations during early life in animal models aggravate autoimmune diabetes (61, 64), allergic lung inflammation (60, 62, 63), inflammatory chronic colitis (65), and obesity (20, 21).

Early-life immune development is also reliant on the actions of a group of bacterial metabolites known as short-chain fatty acids (SCFAs). These compounds are direct by-products of bacterial colonic fermentation and are produced at very high rates (66). Acetate, propionate, and butyrate are the SCFAs produced in highest concentrations in the human gut, and are rapidly taken up by the gut epithelium through passive and active transport mechanisms (67). SCFAs are essential energy sources for colonocytes cells in the mammalian gut, and are precursors for gluconeogenesis, liponeogenesis, and protein and cholesterol synthesis (68). Among many of their immune functions [reviewed in Ref. (66)], SCFAs have been shown to induce extrathymic proliferation of Foxp3⁺ T cells (68–70), which orchestrate peripheral tolerance in mucosal tissues. This critical immune function of SCFA has been shown to be relevant for the offspring even if exposure occurred before birth. Oral administration of acetate during pregnancy was sufficient for the priming of FoxP3⁺ Treg cells and preventing allergic airway inflammation in the adult offspring (36), suggesting that *in utero* exposure to maternal gut microbial metabolites contributes to the development of immune functions in the airways of the offspring.

In addition to interactions with the developing immune system, a recent study by Kim et al. (71) suggests that the early gut microbiome confers colonization resistance through the production of bacterial metabolites resulting from age-dependent colonization with key bacterial taxa. Clostridial species from *Clostridium* clusters IV and XIVa, which increase in abundance with age, induced colonization resistance to intestinal mouse pathogens *Salmonella enterica* subsp. *typhimurium* and *Citrobacter rodentium*. Interestingly, the conferred mechanism of resistance is unrelated to immune adaptors MyD88 and TRIF, and independent of B and T cell function. The settlement of *Clostridia* in the gut of GF mice was also greatly reduced by the absence of neonatal bacteria, which may help explain the increased susceptibility of newborns and young infants to these GI infections.

Collectively, these studies constitute compelling evidence that key taxa, microbial community diversity, and bacterial metabolites constitute modulatory triggers of host immune function maturation. Although considerable research effort has been made, a great deal of the age-dependent processes through which microbial exposure drives immune system development remains to be identified. The infant gut microbiome temporal succession patterns, driven by birth, weaning, and introduction of solid foods, match marked changes in host immune function (72, 73).

Therefore, future studies designed during these events, such as human longitudinal cohorts, hold great potential to improve our understanding of the dynamics at play.

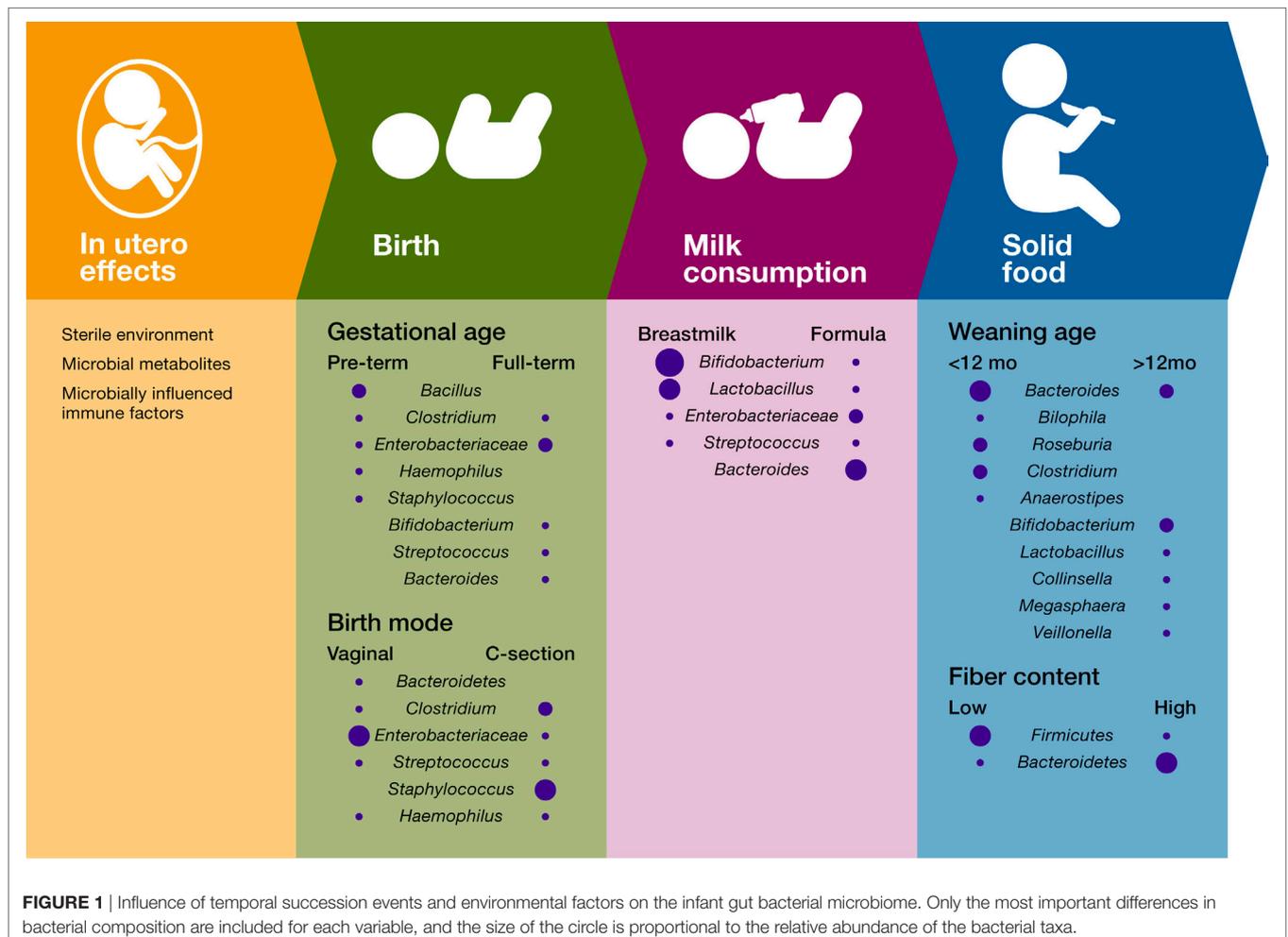
TEMPORALLY STRUCTURED ENVIRONMENTAL FACTORS

Succession in ecology is defined as the pattern of changes in a community after a disturbance or after the opening of a new patch to colonization (74). Correspondingly, succession in the infant gut microbiome starts with the arrival of pioneer species that transform the gut habitat and enable the settlement of first succession species. The identity of the infant gut pioneer and first succession species is influenced by factors such as maternal factors (e.g., body weight and stress) (35–37), delivery mode (38–41), and type of milk consumption [(27, 42); **Figure 1**]. The temporal structure of these environmental factors contributes to the identity and dynamics of the infant gut microbiome and plays a role in the immune system training.

Prenatal Life

Even before birth, fetal immune development relies on microbial products present in the placenta. In an experimental system in

which germ-free mice were transiently colonized with genetically engineered *E. coli* HA107, maternal gut colonization influenced the offspring's immune system by increasing the intestinal group 3 innate lymphoid cells and F4/80⁺CD11c⁺ mononuclear cells (iMNCs), and strongly altering the offspring's intestinal transcriptional profiles (37). These early shifts in the offspring immune system improved the capacity of the pups to avoid inflammatory responses to MAMPs and intestinal microbes' penetration, thus suggesting that microbial training of the immune system starts *in utero* (37). Despite some reports suggesting that fetal colonization may begin *in utero* (75, 76), lack of appropriate contamination controls and failure to show bacterial viability in these studies yields this work inconclusive and inadequate to disprove the currently accepted view of the placenta as a sterile environment (77). More importantly, several studies have shown that early colonization of the infant gut is strongly driven by mode of birth (39–41, 78), thus suggesting that direct colonization of the infant gut most likely begins after membrane rupture, during labor and birth. For example, Backhed et al. (39, 40) showed that the gut microbiome of vaginally born infants exhibited an enrichment in *Bifidobacterium*, *Bacteroides*, *Escherichia*, and *Parabacteroides*. In comparison, the gut microbiome of infants born through cesarean sections (C-sections) was enriched in



microbes associated with the skin, the mouth, and the surrounding environment.

Birth

The infant's gut habitat changes rapidly after birth with facultative anaerobes species (e.g., *E. coli*, *Staphylococcus*, and *Streptococcus*) colonizing first and consuming the available oxygen (79). A longitudinal study following 39 infants from birth demonstrated that mode of delivery impacts *Bacteroides* populations in the infant's gut microbiota between 6 and 18 months of age (41). Yassour et al. (41) showed that, in comparison with vaginal-birth, most infants born by C-section lacked the presence of the *Bacteroides* genus until about 6–18 months of age. Their work also showed that a higher abundance of *Bifidobacterium* species, both in C-section and vaginally delivered infants, was detected concomitantly with a lower abundance of *Bacteroides*, suggesting that infant gut microbial communities are also influenced by microbe–microbe interactions. Delayed colonization with *Bacteroides* species was also associated with cesarean sections in a study of 24 infants (80), a finding that was linked to lower levels of Th-1-associated chemokines CXCL10 and CXCL11 in blood. *Bacteroides* species are important and extremely common members of the human gut microbiome, capable of fermenting a variety of fibers in the colon (81) and modulating the immune system (their potential as a keystone taxa and role in immunomodulation is discussed later in this review). Hence, vertical transmission during vaginal birth is likely a structured environmental factor that promotes colonization by members of this influential bacterial group.

Gut microbiome differences driven by mode of birth have been reported in almost all microbiome infant studies that recorded this variable (28, 38, 82–87). Although the cumulated evidence points to the mode of birth being a major influence in the gut pioneer microbiome, one recent study performed on 115 infants showed no differences in the meconium microbial communities between both mode of birth (C-section and vaginal delivery) (88). Unfortunately, key bio-statistical parameters of their analyses are missing from the paper, crucial information to assess the robustness of their results. What still remains unclear is how long these differences last, with only a few reports showing differences beyond early childhood (82). Nonetheless, changes in important microbial groups, community diversity, or functions during this critical and permissive window of immune development are likely to induce immune alterations that may remain beyond the age at which these taxonomic differences are no longer detectable.

Intriguingly, the taxonomic identity of pioneer colonizers not only depends on birth but also on gestational age. A 1-month longitudinal study of 58 preterm infants in a neonatal intensive care unit showed that time post-conception can also impact the type of early colonizers in the premature gut yet not the pattern of bacterial succession (89). Members of the *Bacilli* class appear as the initial colonizers in premature infants, which contrasts with the initial colonization with *Enterobacteriaceae* members in most term babies (Figure 1). In addition, this study showed that gut microbiome follows a progression strongly determined by host biology factors, suggesting that, during the first month

after delivery, the genetic and physiologic characteristics of the preterm infant gut drive a conserved pattern of succession in gut microbiome.

Milk Consumption

In addition to mode of birth and post-conceptual age, diet during early infancy strongly impacts community structure and diversity. Comparisons between breastfed and formula-fed infants have shown that *Bifidobacterium* spp. and *Lactobacillus* spp. predominate in breastfed infants whereas formula-fed infants exhibit higher proportions of *Bacteroides* spp., *Clostridium* spp., *Streptococcus* spp., *Enterobacter* spp., *Citrobacter* spp., and *Veillonella* spp. (39, 40, 90–93). Breast milk can modulate the infant gut microbiome through different mechanisms. First, human breast milk contains a significant number of bacteria that is passed to the infant constantly during the first months of life (46, 47, 94–98). Besides being a direct source of microbes, human milk contains a group of unconjugated glycan resistant to human enzymatic digestion known as human milk oligosaccharides (HMOs). These compounds act as prebiotics for key infant gut taxonomic bacterial groups including *Bifidobacterium* (99–103) and *Bacteroides* species (104). Importantly, fermentation of HMOs results in the production of SCFAs (102, 103), increases secretory immunoglobulin A (sIgA) production, and improves gut microbiome resistance to pathogens (105, 106).

Breastfeeding also influences the training of the infant immune system through the presence of antimicrobial compounds in the human milk (lactoferrin, lysozyme) and immune effectors [sIgA, immune cells, and cytokines; (107)]. Bridgman et al. (108) demonstrated that sIgA abundance is associated with breastfeeding status in a cohort of 47 4-month-old infants. sIgA is critical for the infant gut mucosal immune defense [see Ref. (109) for a review] mainly through a process known as immune exclusion, where sIgA adheres to bacterial cells and antigens and prevents their access to the gut epithelium (110). Although this antibody is initially acquired through breastfeeding, the infant gut microbiota will ultimately stimulate its local production through the maturation of B cells (111). Notably, the risk of developing atopy is increased if B cells maturation is delayed (112–115), stressing the importance of breastfeeding in infant gut microbiome and immune development.

Solid Food Introduction and Weaning

The introduction of solid foods constitutes the last step in early-life microbiome succession events, which leads to the consolidation of a gut microbial community that remains largely stable for the remainder of childhood and adult life. Due to the availability of new fiber sources and other substrates, transition to solid foods results in an increase of diversity and the enrichment of *Bacteroides* spp., *Clostridium* spp., *Ruminococcus* spp., *Faecalibacterium* spp., *Roseburia* spp., and *Anaerostipes* spp., as well as the reduction in *Bifidobacterium* spp. and *Enterobacteriaceae* (39, 40, 116, 117). Functionally, solid food introduction increases SCFA production, vitamin biosynthesis, and xenobiotic degradation (34, 39, 40). Notably, these changes coincide with important aspects of digestive development (e.g., pancreatic function and intestinal nutrient absorption) and shifts in immune development, some of

which are driven by microbes. For instance, the expression of the epithelial antimicrobial granule protein, Angiogenin-4 (Ang4), and of epithelial fucosylated glycans is markedly increased during weaning in conventional but not in germ-free mice. Remarkably, colonization with *Bacteroides thetaiotaomicron*, a bacterial commensal that increases in abundance post-weaning, was able to induce both Ang4 expression and fucosylated glycan reprogramming [Ang4; (118, 119)], strongly suggesting that specific host functions have adapted to rely on microbial signals that arrive in a temporally structured manner.

Furthermore, it has been suggested that cessation of breast-feeding, rather than solid food introduction, drives the main compositional shifts that result in an “adult-like” gut microbiome. In a longitudinal study of 98 infants, an early weaning age (under 12 months) was associated with an increase in *Bacteroides* spp., *Bilophila* spp., *Roseburia* spp., *Clostridium* spp., and *Anaerostipes* spp. In comparison, breastmilk supplementation beyond this age favored a more “immature” community composition, characterized by *Bifidobacterium* spp., *Lactobacillus* spp., *Collinsella* spp., *Megasphaera* spp., and *Veillonella* spp. (39, 40).

INFANT GUT COMMUNITY DIVERSITY: AN INDICATOR OF HEALTH?

The impact of early-life dysbiosis on the risk of developing several human diseases has led to the hypothesis that there is a critical window during which changes in the gut microbiome are most influential in immune development. During this “window of opportunity,” the infant gut harbors a highly variable and increasingly diverse microbial community of low resilience, which renders it easily disrupted by disturbances such as antibiotic treatments (41). During this period of time, a loss of diversity or change in community composition has the potential to disrupt the development of certain aspects of neonate immune system and to promote a bloom of pathogens, thus increasing the risk of developing immune-mediated and infectious diseases. However, it remains unclear if community diversity *per se* represents a robust indicator of infant gut microbiome disruption, especially since (1) there could be a threshold to be crossed for the gut ecosystem to suffer a significant loss of function; and (2) diversity as a diagnostic tool provides no information on the gut microbial community composition or functional properties.

Many studies have argued that a loss of community diversity could indicate a disruption of the natural infant gut microbiome community. After birth, both the taxonomic and functional diversity of the infant bacterial microbiome have been shown to increase (88). Life-threatening diseases such as NEC have been suggested to occur as an effect of disruption of the natural succession in the infant gut microbiome after antibiotic treatment (120, 121), lowering community diversity and creating an opportunity for other bacterial groups (e.g., Gammaproteobacteria) to dominate the normal bacterial community (122, 123). At that stage, a loss of community diversity can also hinder the training of the immune system by reducing its ability to recognize commensal bacteria [see Ref. (52) for a review]. Recent studies have confirmed that a significant loss in gut microbial diversity

is indicative of an increased risk of developing autoimmune diseases (80, 124). In addition, a loss of diversity can promote a long-term increase in IgE levels, which has been suggested to trigger immune-mediated disorders in mice (59).

However, it remains to be determined if the link between the development of immune diseases and the loss of microbial diversity is caused by a reduction of microbial species alone or, more precisely, by a loss of key taxonomic or functional microbial groups essential to the development of the infant immune system. The work of Arrieta et al. (13) on 319 infants in a longitudinal cohort, showed no significant relationship between fecal microbial alpha-diversity and the risk of developing asthma. Yet, four bacterial taxa (*Faecalibacterium*, *Lachnospira*, *Rothia*, and *Veillonella*), fecal acetate and deconjugated bile acids were significantly altered in babies at risk of asthma. By contrast, Kostic et al. (15) identified that a significant reduction in infant gut community alpha-diversity is a characteristic condition of the T1D state in a cohort of 33 infants predisposed to type 1 diabetes. This loss in alpha-diversity was combined with an alteration of the metabolic pathways and microbial community phylogenetic structure (15). These studies suggest that both subtle and global changes in community composition may lead to immune impairment and disease development, and that functional dysbiosis can occur independently of significant changes in community alpha-diversity.

Community alpha-diversity may also not be a reliable indicator across all human populations given its geographic variability (27). In a study comparing European to Burkina Faso children, De Filippo et al. (125) showed that the latter group had a greater gut microbial diversity and shift in community composition, potentially associated with their high fiber diet. However, other lifestyle factors and environmental exposures may also explain these differences. In addition, bacterial alpha-diversity fluctuates significantly during the first year of life, making it an unreliable ecosystem measurement unless studies are strictly age- and population matched. Further, an opposite relationship between alpha-diversity and health status occurs during the first weeks of life, where lower alpha-diversity and a predominance of a few subspecies of *Bifidobacterium longum* is associated with better growth (126).

Another factor that is rarely taken into account when assessing microbiome alpha-diversity is the impact of other non-bacterial microbes. In a unique study targeting both infant gut bacterial and fungal communities, Fujimura et al. (14) showed that infant gut bacterial alpha-diversity increased with time while the fungal alpha-diversity decreased in reciprocal correlation. This finding suggests that microbial diversity *per se* might naturally fluctuate depending on the targeted organism and that currently unexplored inter-kingdom gut microbial associations may influence these dynamics. Most interestingly, their work demonstrated that the fungal beta-diversity better predicted atopy risk than bacterial beta-diversity. Therefore, fluctuations in infant gut fungal community composition could play a role in influencing infant's susceptibility to childhood allergies and asthma.

The increase in both taxonomic and functional diversity of the infant bacterial gut microbiome in the few months after birth

appears to be associated with multiple aspects of the immune system development, providing further evidence that the immune system relies on a temporally structured succession of the gut microbiome. However, the infant gut microbial diversity *per se* might not be an indicator conveying enough information to be considered as a diagnostic tool. Notwithstanding, studies to date do suggest that the training of the immune system relies on a particular pattern of microbial diversity increasing from birth until 3 years old, and that disrupting this pattern can increase the risk of developing immune-mediated disorders. Future research disentangling the relative impact of species richness, community taxonomic, and functional composition on the retention of infant gut ecosystem emergent properties (e.g., infant immune system development) will provide key information for the development of diagnostic tools.

KEYSTONE GROUPS

In community ecology, the concept of a keystone species or group of species is described as an actor of a community that is so important to its organization and diversity that losing it provokes a massive cascade of extinctions and loss of ecosystem function (23, 24, 127). In other words, a keystone species has a remarkable impact in relation to its abundance (128). In an ecosystem, keystone species can belong to any trophic levels, from low-level species providing the resources on which a plethora of other species depends, to high-level species applying top-down regulation on the community. Keystone taxa of the infant gut microbiome contribute significantly to the ecosystem by (1) contributing to the establishment of other species; (2) by producing important metabolites including SCFAs (e.g., butyrate) that trigger local trophic cascades; (3) by improving ecosystem resistance against invading pathogenic species; and (4) by aiding in sustaining a balanced symbiosis with the host, which will in turn favor the stability of the microbial ecosystem. Because of the high inter-individual [i.e., Ref. (7)] and temporal (27, 34, 39, 40) variability of the infant gut ecosystem, identifying keystone taxa is a great challenge. Here, we discuss the potential for *Bifidobacterium* and *Bacteroides* to be keystone taxa and their role on infant immune system training.

Bifidobacterium

Bifidobacteria are dominant members of the infant gut microbiome, have a large repertoire of genes for the digestion of HMOs (104, 129), and have been isolated from maternal feces, human milk, and infant feces (130, 131), demonstrating how well adapted they are to the transmission routes and growth conditions in the infant gut. *B. longum* is the predominant species in the human gut, but several *B. longum* subspecies have different levels of adaptability and functionality in the infant gut. *B. longum* subsp. *infantis* (*Bifidobacterium infantis*), *B. longum* subsp. *longum* (*B. longum*), and *B. longum* subsp. *breve* (*B. breve*) are commonly isolated from healthy breastfed infant feces, while formula-fed infants are also colonized with *Bifidobacterium adolescentis* (132–134). Of these subspecies, *B. infantis* has the largest gene repertoire to digest all HMO structures in human milk (129). In addition, when administered as a probiotic

to preterm neonates, *B. infantis* colonizes better than other subspecies (135), which may explain why clinical trials using *B. lactis* or *B. breve* as a probiotic strain in the prevention of NEC have been unsuccessful (136, 137), while 5 out of 6 trials using *B. infantis* have shown to be effective in decreasing NEC incidence in neonates (138–143).

Bifidobacterium species decrease the intestinal luminal pH through the production of lactate and acetate, which is considered a crucial strategy in increasing intestinal nutrient absorption (144). Acetate accounts for more than 80% of the SCFA production in the infant gut (13) [compared to over 50% in the adult gut (145)] and is a key metabolite in the early establishment of colonization resistance, by preventing infections with enteropathogens (146, 147).

Through a process known as metabolic cross-feeding, where the metabolic products of a species or group of species provide growth substrates for other populations, *Bifidobacterium*'s production of lactate and acetate sustains the growth of other species, such as *Roseburia*, *Eubacterium*, *Faecalibacterium*, and *Anaerostipes* (148–151). In addition to this strong influence of microbe–microbe interactions, the sustained growth of other microbial species also enables the subsequent production of butyrate (152, 153). Notably, the lower abundance of colonization with *Bifidobacterium* in formula-fed babies is associated with a lower concentration of lactate and a higher gut luminal pH compared to breastfed babies (93, 154), and likely accounts for one of the root causes of the striking microbiome discrepancies observed between breastfed and formula-fed infants.

Bifidobacteria also play an exceptionally important role through its direct interactions with the developing immune system. Besides preventing enteropathogenic infections, *Bifidobacterium* species also protect the infant gut by modulating mucosal barrier function and promoting immunological and inflammatory responses (155, 156). The dominance of the infant gut microbiome by *Bifidobacterium* spp. was associated with an improved T-cell-mediated response to oral and parenteral vaccines and with lower neutrophilia at 15 weeks of age (126). *B. breve* has also evolved a mechanism to be protected from the immune system response by synthesizing a specific exopolysaccharide that increases its competitive power for space and colonization in the mouse gut (157, 158).

Collectively, *Bifidobacterium* species possess important strategies that insure their colonization at high abundance in the infant gut, prevent the growth of competing species that disfavor host fitness, and promote immune development. Due to the very high microbial inter-individual variation, and the number of subspecies found in the infant gut, it remains unclear if *Bifidobacterium* is a biomarker of infant gut health, yet the sub-species *B. infantis* may be a likely candidate.

Bacteroides

Together with *Bifidobacterium*, *Bacteroides* are the only groups known to use HMOs as a primary nutrient source (102, 103, 159). In addition, *Bacteroides* species are considered *generalists*—organisms with a great capacity to switch dietary nutrient sources or host-derived substrates (151). In an elegant study that followed the transcriptional profile of the human and murine

symbiont, *B. thetaiotaomicron*, and the structure of murine cecal glycans, it was demonstrated that this bacterium has the gene encoding capacity to switch from digesting food sugars to foraging host mucus glycans (160). The metabolic plasticity of this species likely improves their adaptability to the fluctuating luminal conditions of the developing infant gut, especially after weaning and introduction of solid foods. Importantly, colonization with *Bacteroides* species is heavily reliant on natural events that drive succession patterns, such as vaginal birth and breastfeeding (41, 80), suggesting that *Bacteroides* spp. transmission is advantageous for both the host and members of this taxa, and that it is highly coevolved.

Certain symbionts are thought to have evolved mechanisms through which they influence the host immune system maturation in a way that is beneficial for them. An example of these mechanisms is the development of specific metabolic capacity by *B. thetaiotaomicron* (119), a microbial species previously linked with angiogenesis in the postnatal intestine development (161). This species influences the gut microbial community by regulating the epithelial glycan synthesis (162), therefore creating a specific niche for itself and for other microorganisms with similar nutrient biochemical capacity.

Another species involved in immune system development is *Bacteroides fragilis*. Its production of polysaccharide A has been shown to suppress inflammation by downregulating interleukin (IL)-17 (163). Monocolonization of germ-free mice by *B. fragilis* has been shown to balance Th1 and Th2 responses (164). In addition, these monocolonized mice showed an increase in the conversion of CD4⁺ T cells into IL-10-producing Foxp3⁺ Treg cells, which induced a strong anti-inflammatory effect during gut inflammation (165). *B. fragilis* was also demonstrated to be negatively associated with the expression of toll-like receptor-4 and with lipopolysaccharide (LPS)-induced production of multiple inflammatory cytokines and chemokines (166).

Intriguingly, recent findings on the links between *Bacteroides* and immune system training suggest that, although they are important members of the early gut microbiome, an overabundance of *Bacteroides* spp. and a corresponding increase of exposure to their LPS, result in improper stimulation of the innate immune system and in inhibition of LPS tolerance in non-obese diabetic mice. This mechanism was proposed to explain the disparity in type 1 diabetes incidence in Northern Europe, where Russian children have reduced *Bacteroides* spp. abundance and lower disease rates, compared to Finnish and Estonian children (16). This study highlights the importance of attaining a balanced stimulation of the immune response early in life and how specific gut microbes have evolved to do so in a temporally structured manner. It also underlines the complexity of disentangling the effects of particular bacterial species and higher phylogenetic groups on the emergent properties of the infant gut ecosystem and host fitness.

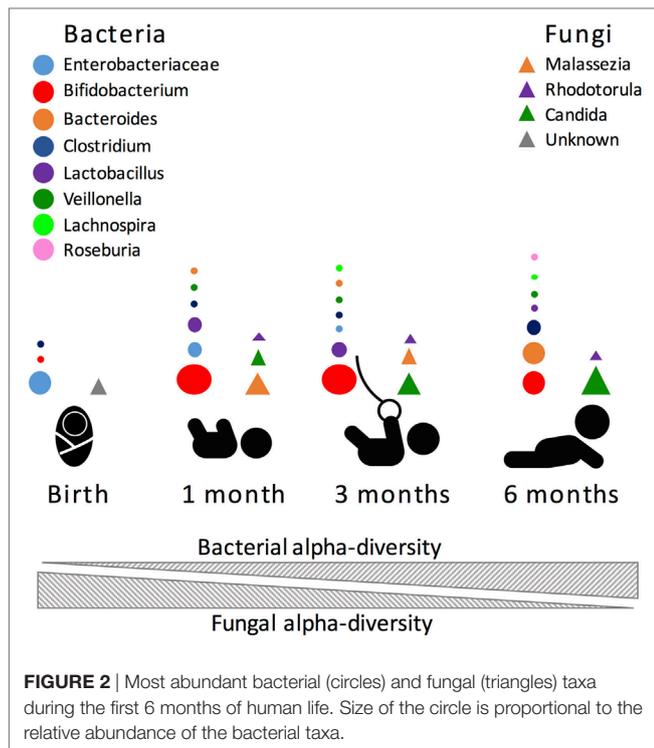
FUTURE RESEARCH

At its beginning, complexity theory suggested that ecosystems exhibiting a higher complexity were more stable when sustaining disturbances such as species loss (167, 168). However, mathematical

model simulations of food webs led to the proposal that instead of focusing on the stability of individual populations within an ecosystem, a better comprehension of complex systems could be gained from studying emergent properties such as productivity, resilience, and biomass (169, 170). From this point, studies have employed multiple properties to characterize ecosystems including species richness, taxonomic composition, functional profile, the level of interactions between species of the ecosystem, and the strength of these interactions. This transition in community ecology theory mirrors the improvement of our comprehension of complex ecosystems shifting from a singular to a multi-level perspective.

In this review, we advocate that the infant gut microbiome should be considered as a complex adaptive system crucial to the maintenance of various emergent properties (e.g., infant immune system training). These ecosystem properties are hardly attributable to a single group, instead they seem to rely on a temporally structured pattern of bacterial diversity increase after birth and the succession of particular keystone groups. The properties of complex adaptive systems highlight the great challenges faced by studies of the infant gut microbiome: a system far-from equilibrium dynamics, characterized by permanent novelty and incessant adaptation, dispersed multi-level interactions, and the absence of a global controller (171). The emergent properties of this ecosystem highlight the necessity of prospective, longitudinal infant gut microbiome studies, both taxonomic and functional, which will eventually allow us to identify the critical points at which this system loses its emergent properties and reaches a state of dysbiosis, impeding adequate immune system development. In addition, there is a need to disentangle the influence of loss of taxonomic and functional diversity, as well as of shifts in keystone taxa on immune system training and subsequent disease development. From past studies, we now understand that the maturation of the immune system relies on a temporally structured dynamic, starting *in utero* with maternal effects, influenced by environmental factors (delivery mode, type of milk consumption, and solid foods) and host biology, and depending heavily on auto-correlated local interactions between microbial groups. Further understanding of this complex adaptive system will also require (1) sampling a variety of geographically distinct human populations, (2) carrying out longitudinal cohorts that sample numerous times during the first 12 months, and (3) combining amplicon-based surveys with functional assays, such as metagenomics and metabolomics.

Another important influence in gut microbiome composition that remains vastly unexplored is the role of non-bacterial microorganisms. The role of the virome, the collection of viruses colonizing the host, has been previously explored in adult animals. Similarly to the bacteriome, the virome strongly interacts with the host immune system, with both positive and negative consequences for host health [see Ref. (172–174) for reviews]. However, it remains unknown what role the virome has during early-life immune development. Further, fungi, protozoans, and helminths, which are traditionally excluded from culture- and non-culture-based studies, are important and immunomodulatory members of the gut microbiome, albeit in



smaller proportions than bacteria. Nonetheless, it was recently shown that fungi species are present at much higher diversity in the first months of life, compared to later months, and that this change in diversity inversely correlates with bacterial diversity [(14); **Figure 2**]. Future studies directed at exploring inter-kingdom gut microbial associations during early life and how

these associations influence the host will provide a more global understanding of the microbial triggers influencing immune system development.

Eventually, the identification of the critical events and factors that influence microbiome resilience and function will enable the development of effective interventions aimed at maintaining and/or improving immune system development and disease prevention. Although an astounding amount of work has been carried out to understand the reliance of the immune system on the infant gut microbiome, much remains to be elucidated on the particular mechanisms responsible for this training. Improvements in our understanding will arise from continuing multidisciplinary joint efforts between immunologists, microbiologists, clinicians, bioinformaticians, and ecologists.

AUTHOR CONTRIBUTIONS

M-CA formulated the concept for this review, and IL-L wrote the first draft. Both authors co-wrote and revised the entire review article.

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