REVIEW

Building a Beneficial Microbiome from Birth[1,2]

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ABSTRACT

The microbiota has recently been recognized as a driver of health that affects the immune, nervous, and metabolic systems. This influence is partially exerted through the metabolites produced, which may be relevant for optimal infant development and health. The gut microbiota begins developing early in life, and this initial colonization is remarkably important because it may influence long-term microbiota composition and activity. Considering that the microbiome may play a key role in health and disease, maintaining a protective microbiota could be critical in preventing dysbiosis-related diseases such as allergies, autoimmunity disorders, and metabolic syndrome. Breast milk and milk glycans in particular are thought to play a major role in shaping the early-life microbiota and promoting its development, thus affecting health. This review describes some of the effects the microbiota has on the host and discusses the role microbial metabolites play in shaping newborn health and development. We describe the gut microbiota structure and function during early life and the factors that determine its composition and hypothesize about the effects of human milk oligosaccharides and other prebiotic fibers on the neonatal microbiota. *Adv Nutr* 2016;7:323–30.

Keywords: microbiota, human milk oligosaccharides, microbial metabolites, short-chain fatty acids, prebiotics

Introduction

The gut microbiota constitutes a complex ecosystem that harbors >1000 species with ~7000 strains and contains >150 times more genes than the human genome (1). The interaction between the microbial ecosystem and the host represents a long evolutionary symbiosis that is essential for optimal health throughout life. The resident microbiota, with its broad genetic and metabolic diversity, exerts an effect on host metabolism, physiology, and immune system development (2). For this reason, the microbiota is now recognized as a "virtual organ" (3).

The microbiota encompasses 2 predominant bacterial phylotypes, bacteroidetes and firmicutes, with the phyla proteobacteria, actinobacteria, fusobacteria, and verrucomicrobia being present at a relatively lower abundance (1). Bacteroidetes are gram-negative, anaerobic, nonspore-forming bacteria that are enriched with enzymes to degrade carbohydrates; firmicutes are gram-positive, anaerobic, spore-forming bacteria that ferment simple sugars to produce a variety of SCFAs (4). The whole microbial distribution varies along the gastrointestinal tract, with microbial densities and diversities increasing both from the proximal to the distal gut and along the tissue-lumen axis (3). Although newborns were initially thought to be born sterile, it is now believed that the colonization of the gut starts during pregnancy and continues after birth until 2 years of age, when it reaches a relatively stable composition resembling that of an adult (5). Alterations in the normal microbial composition have been described in a whole range of health disorders from infancy to adulthood (2).

In this review, we consider different aspects of the microbiota's influence on health. We summarize factors that contribute to the establishment of a protective microbiota early in life and describe the interactions between human milk oligosaccharides and the microbiota. We evaluate how alterations in early gut microbiota may promote the onset of health disorders. Finally, we provide insights into recent advances in the infant formula field.

Microbiota Axis

The influence of the virtual organ formed by the microbiota extends well beyond the gastrointestinal tract and expands throughout life. It can greatly affect many physiological aspects in the host, interacting with and regulating the activity of distal organs, including mainly the liver and brain. The microbiota exerts a strong influence over the liver and collaborates in maintaining gut-liver axis health. Alterations in the gut-liver axis contribute to the pathogenesis of obesity and nonalcoholic fatty liver disease, among others (6).

Microbiota-gut-brain axis. The gut and brain are in constant bidirectional communication through the gut-brain

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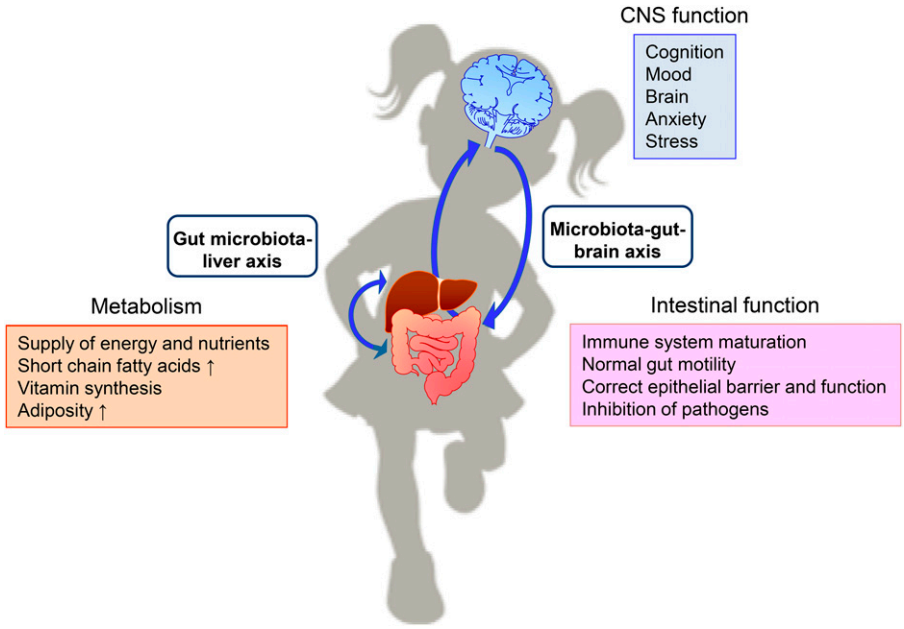

FIGURE 1 Interactions between the gut microbiota and host physiology. The gut microbiota exerts an effect on several aspects of host physiology through the 2 depicted axes. Thus, the microbiota is able to influence metabolism and brain functions and acts locally by modulating intestinal function (2, 3, 7). CNS, central nervous system.

axis, which integrates neural pathways and immune and endocrine mechanisms in a complex relation essential for maintaining homeostasis. It is now evident that endogenous gut bacteria can act as signaling components within this system, and the term "microbiota-gut-brain axis" appeared to designate this communication, which begins early in life to modulate the immune system, central nervous system, and gastrointestinal functions (7, 8) (**Figure 1**).

This axis establishes a bidirectional communication between the brain and gut/microbiota. The latter produces metabolites or cytokines released into the blood stream and sends signals through the vagus nerve. In turn, the brain can influence the composition and function of the gut microbiota either indirectly via changes in gut motility, secretion, or intestinal permeability or directly via signaling molecules released into the gut lumen (8). Early-life events can modify the developing postnatal microbiota, leading to imbalances in the gut-brain communication and thus alterations in brain development and behavior (9). An adequately maturing microbiome during early life and a stable one in adulthood is necessary for appropriate signaling through the gut-brain axis and thereby for maintaining health.

Microbiota Metabolites

The microbiota provides the host with a range of metabolic capabilities that would not be accessible otherwise (Figure 1). It produces numerous metabolites that may act as autocrine or paracrine, thus modulating human health. Many of these microbial metabolites are essential for health and play a major role in regulating normal growth in infants (10). They are largely determined by diet composition and pattern of food intake. In fact, the structure of the microbiota itself is influenced by the diet because certain bacteria may be better adapted for utilizing specific substrates (5). Metabolites produced and/or modified by the microbiota include SCFAs, vitamins, bile acids, and choline, which are required for many aspects of host physiology. This section will cover those especially relevant for infant health.

SCFAs. Nondigestible carbohydrates are fermented in the colon by a subset of anaerobic bacteria, yielding both energy for microbial growth and producing SCFAs, i.e., acetate, propionate, and butyrate. Bacteria that produce SCFAs include *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*, and *Ruminococcus*, among others (11). SCFAs are essential for neonatal intestinal health because they provide energy and maintain gastrointestinal growth and development (12).

Within SCFAs, butyrate is a source of energy for the colonic epithelium, whereas acetate and propionate are carried into the bloodstream and become available to a variety of different organs (2). SCFAs are recognized by receptors expressed in gut endocrine cells that modulate the secretion of hormones involved in appetite control, thus linking microbial SCFAs and food intake (13). SCFAs may also play a role in the stimulation of leptin production by adipocytes by influencing feeding behavior (3). Recent reports have shown that propionate activates intestinal gluconeogenesis via the gut-brain neural circuit (14). Butyrate controls histone acetylation and hence influences host gene expression by remodeling chromatin structure (15). Locally, SCFAs acidify the colon lumen, limiting the growth of potential pathogens (13). Therefore, changes in the production rates of the major SCFAs by the colonic microbiota may be of particular importance if the fluctuations occur early in life, when epigenetic control is vital for function later in life (15).

Tryptophan. Tryptophan is an essential amino acid that is crucial for differentiating and correcting central nervous

system development and regulating normal newborn behavior. Its circulating concentrations are under the influence of the gut microbiota, probably through the modulation of the kynurenine pathway, the main physiological route for tryptophan metabolism (16).

Tryptophan constitutes a precursor of serotonin (5-hydroxytryptamine), a neurotransmitter that regulates gastrointestinal functions, mood, appetite, sleep, and anxiety (16). The gut microbiota can affect serotonin amounts indirectly by stimulating its release from intestinal enterochromaffin cells, thus influencing the gut-brain axis (16, 17).

Vitamins. Certain bacteria of the human gut microbiota can synthesize vitamins essential for neonatal health, such as menaquinone (vitamin K-2), and many of the water-soluble B vitamins. In particular, many *Bifidobacterium* strains have shown the capability of producing vitamins in vitro (18).

Conjugated linoleic acid

CLA refers to a mixture of conjugated isomers of the essential FA linoleic acid, which has been associated with a variety of health benefits regarding obesity, diabetes, and immune function. CLA is produced by certain strains of different bacterial groups, such as *Bifidobacteria*, *Lactobacillus*, *Propionibacterium*, *Enterococcus*, and *Lactococcus*, albeit with different efficiencies, and is important for appropriate newborn growth and development (10).

Neurotransmitters. Some bacteria can produce neuroactive metabolites ranging from serotonin and γ-aminobutyric acid to dopamine and norepinephrine, acetylcholine, and histamine and more newly described neurotransmitters such as agmatine. The modulation of these transmitters in the gut is another possible mechanism of action through which the microbiota could exert its effects on brain development and function (19).

Summary. Metabolites produced by the gut microbiota can affect a wide variety of physiological and metabolic processes in organisms. Consequently, alterations in the host-microbiota relation may disrupt the subtle equilibrium of this symbiosis, resulting in disease—hence the importance of developing and maintaining a healthy microbiota throughout life.

Gut Microbiota Dynamics

Although it was generally considered that the intrauterine environment and newborn were sterile until delivery, there are indications of earlier microbial exposure because bacteria have been found in the umbilical cord, placenta, amniotic fluid, and meconium (20). At birth, however, once the neonate is exposed to several new microbes, the gut undergoes rapid colonization. The earliest colonizers are predominantly facultative anaerobes (*Escherichia coli*, *Enterococcus*), which, as oxygen is consumed, give way to strict anaerobes, including *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, and sometimes *Ruminococcus* (21).

The gut microbiota composition of early infants has low diversity, is dynamic, and continues to develop until it becomes stable and adult-like at 2–3 y of age (21). The factors that influence the gut microbiota include mode of delivery, gestational age, feeding patterns, environment, antibiotic exposure, country of origin, and host genetics (22).

The first main factor that contributes to the colonization of the infant gut is delivery mode (**Figure 2**). Vaginally born infants are colonized with vaginal and fecal bacteria from the mother, whereas cesarean-born infants are mainly colonized by bacteria from the clinical environment (23). The latter, with a less diverse microbiota, harbor lower counts of *Bifidobacterium* spp. and *Bacteroides fragilis* but increased numbers of *Clostridium difficile* (21). These initial differences seem to have long-term effects on infant health, increasing the risk of developing allergy or obesity later in life (22).

Gestational time at birth greatly influences the establishment of the infant gut microbiota, as inferred from comparing fecal microbiota from term and preterm infants (Figure 2). Preterm infants showed higher amounts of facultative anaerobes belonging to *Enterobacteriaceae*, other potentially pathogenic bacteria such as *C. difficile* or *Klebsiella pneumoniae*, and low levels of *Bifidobacterium* and *Bacteroides* (24). In contrast, term infants had higher diversity in their fecal microbiota, with more common genera present, such as *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* (25).

Feeding regimen has a crucial impact on gut microbiota composition (Figure 2) (5). Breast milk has been suggested to be a source of complex bacterial communities in infants who have been breastfed (26) and could contribute to early gut colonization (27). Bacterial transfer from the mother's skin takes place during suckling, but several studies also support the enteromammary pathway hypothesis, in which bacteria from the maternal gut reach the mammary glands through maternal dendritic cells and macrophages. In fact, it has been reported that several gut bacterial species are shared between maternal feces, breast milk, and infant feces (28).

Decades ago, it was broadly accepted that breastfed and formula-fed infants had different microbiotas. The microbiota from infants fed traditional nonsupplemented formulas was reported to be more diverse, with higher proportions of *Bacteroides*, *Clostridium*, and *Enterobacteriaceae* compared with breastfed infants (29). The microbiota in the intestine of breastfed infants was described to contain higher proportions of *Bifidobacterium* and *Lactobacillus* than infants who were formula-fed (21, 29), although other studies found no significant differences (30, 31). Formulas have evolved over the past several years, and the addition of prebiotics has contributed to bringing the microbiota of formula-fed infants closer to that of breastfed infants (32, 33). During weaning, with the introduction of solid foods, infants are exposed to more complex carbohydrates and other nutrients that drive the development of an adult-like microbiota (34). Postweaning changes in the microbiota are more pronounced in breastfed infants, with a decrease in the proportions of *Bifidobacteria*, *Enterobacteria*, and *Clostridium* spp. Proportions of *Bacteroides* do not change and remain one of the most predominant groups in the infant gut microbiota (5).

Pediatric antibiotic use, in particular during prolonged periods, has been linked to disruptions in early microbiota

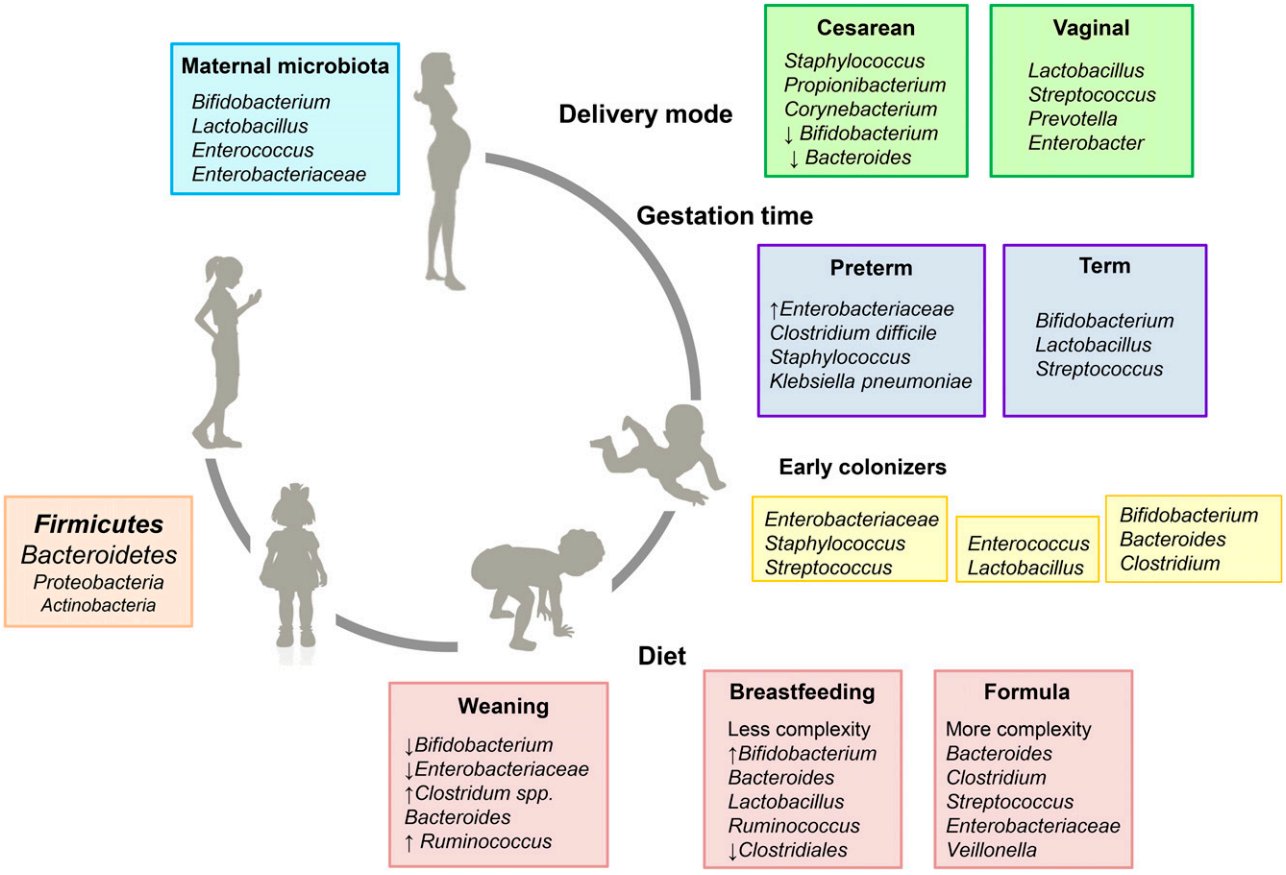

FIGURE 2 Evolution of the early-life gut microbiota and events influencing its composition. Factors such as the maternal microbiota, delivery mode, gestation time, and type of feeding strongly influence the microbiota. Colonization and expansion of the gut microbiota, shaped by diet, results in the establishment of an adult-like microbiota around 2–3 y of age, with firmicutes and bacteroidetes as the predominant phyla. Early life is a susceptible period when modifications in the gut microbiota composition can have long-term effects on health (5, 22).

colonization. Antibiotics can select for an altered community composition in the gut microbiota. Their use can alter the metabolism of the gut microbiota, and they have been described to increase adiposity both in mice and children (35).

Another factor that may influence the infant microbiota is the maternal gut microbiota itself. The mother's microbiota is greatly remodeled during pregnancy (36). In addition, the metabolic health of the mother before or during pregnancy could have an effect on her infant's gut microbiota. Alterations in the microbiota composition in mothers may thus be transferred to their infants and thus lead to an increased risk of metabolic disease in the latter (37). Although it is difficult to determine individual variables, it has been speculated that breastfeeding, natural birth, and lack of hospitalization relate to a more beneficial gut microbiota composition (37).

Bioactive Molecules in Milk

As mentioned previously, breastfeeding is one of the factors that accounts for a beneficial gut microbiota. Human milk constitutes a unique source of nutrients and energy and has been shaped by mammalian evolution to provide not only optimal nutrition but also the bioactive components essential for immune maturation, metabolic and cognitive development, gut maturation, and optimal gut microbial colonization (38). The composition of human milk varies greatly among individuals and during lactation, with maternal lifestyle, nutritional and immunological status, dietary habits, and lactation time influencing its composition and quality (38).

The effects of breastfeeding on the infant intestinal microbiota cannot be attributed to a single compound because several milk factors are speculated to modulate it. There is a wide range of health-promoting constituents, including carbohydrates, human milk oligosaccharides (HMOs), nucleotides, fatty acids, immunoglobulins, cytokines, immune cells, lysozymes, lactoferrin, and other immunomodulatory factors. In particular, lactoferrin promotes the growth of bifidobacteria both in vitro and in animal models (39). Nucleotides, when added to infant formula, were also reported to improve the composition of the gut microbiota in formula-fed infants (40).

Potential Role of HMOs in Supporting the Growth of Beneficial Bacteria

HMOs are the third most abundant component in mature milk (5–15 g/L) (41). HMOs exhibit structural diversity

(**Figure 3**), and their composition and concentration change significantly among different individuals and across lactation (42). They are resistant to digestion and reach the colon intact, where they can be expelled through the feces or fermented by the local microbiota (43). They can then function as prebiotics, selectively promoting the growth of beneficial bacteria in the gut while inhibiting the growth of pathogens (44).

Beyond their prebiotic function, their recent detection in breastfed infants' plasma compartments provides evidence of their systemic effects (45). In vitro studies suggest that HMOs can modulate immune cell responses or exhibit anti-inflammatory properties (46, 47). HMOs can act as decoys, preventing pathogenic bacteria from binding to intestinal cells (48). HMOs are also involved in gut motility through interactions with the enteric nervous system (49). Moreover, they may provide infants with sialic acid, which is important for brain development, and a role for fucosyllactose in learning and memory has recently been described (50).

As classic prebiotics, HMOs selectively promote the growth of specific bacteria (51). Data supporting this function derive from in vitro fermentation studies that used bacteria isolated from infant feces. Clinical data regarding the influence of feeding HMOs on the microbiota are anticipated (52).

The breastfed microbiota was traditionally characterized by a predominance of *Bifidobacterium*, which has long been associated with health (29), although some reports found no differences between breastfed and formula-fed infants (30). The predominance of bifidobacteria in breastfed infants has been partly attributed to the prebiotic effect of HMOs (53). The supplementation of formula with prebiotics, i.e., galactooligosacharides and fructooligosaccharides, has greatly contributed to reducing these differences and increasing *Bifidobacterium* and *Lactobacillus* counts (32, 33).

Only certain bacterial species and strains have developed strategies for utilizing HMOs as a growth substrate, which suggests a coevolution of these microorganisms, the developing infant, and human milk (54). In vitro, only *Bifidobacterium* and *Bacteroides* strains were able to grow on HMOs as the sole carbon source (44). In contrast, other typical commensal bacteria, e.g., *Clostridium* spp., *Enterococcus faecalis*, *Staphylococcus* spp., *Enterobacter* spp., and *E. coli*, were unable to consume HMOs (55). To use HMOs effectively, bacteria harbor various catabolic strategies: some species preferentially import low-molecular-weight HMOs, whereas others export glycosidases for external hydrolysis (51). Some are also able to consume galactooligosacharides from infant formula (56). In addition, the preferential consumption of certain fucosylated or sialylated HMOs varies among different bacteria (57). The catabolism of the major HMOs by *Bifidobacterium longum* subps. *infantis* produces lactate and SCFAs, which reduces the pH significantly and diminishes the growth of putative pathogenic bacteria, therefore confirming the prebiotic effect of these glycans (58).

Health Benefits Derived from a Protective Microbiota

There are important interactions between human milk, the developing intestinal tract, and the gut microbiota. The intestinal epithelium of a newborn is immature, and changes leading to maturation are triggered by microbial colonization and linked to the diet and microbiota-derived metabolites (59). Breastfeeding is associated with a reduced risk of necrotizing enterocolitis (NEC), an inflammatory intestinal disorder affecting preterm infants (5). NEC has been associated with low gut microbial diversity, increased Proteobacteria, and decreased firmicutes numbers, although not all studies have corroborated such an association (60). HMOs have been suggested to reduce the incidence of NEC either by generating a protective microbiota or reducing the incidence of NEC on its own (61).

The balance between the microbiota, immune response, and tolerance mechanisms is essential for newborn gut

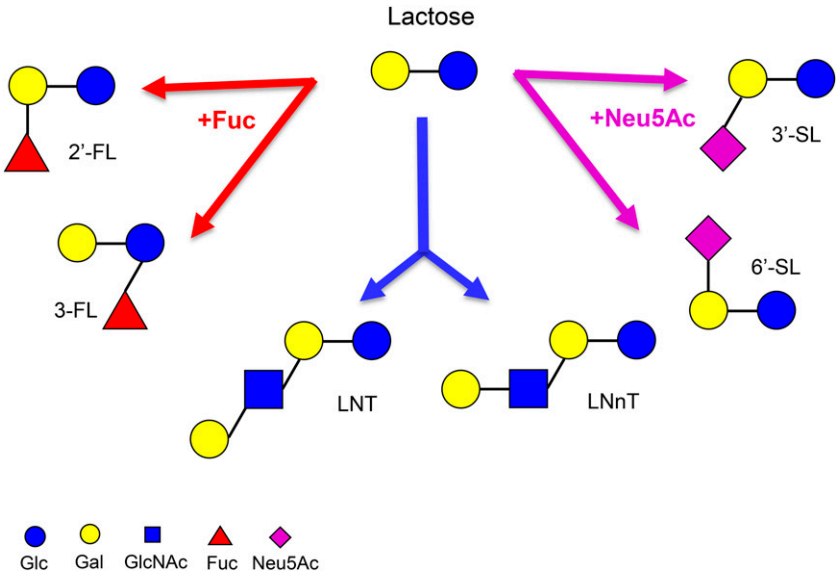

FIGURE 3 Selected HMO structures. HMOs are composed of 5 monosaccharides. Lactose can be fucosylated or sialylated to generate the following trisaccharides: the fucosyllactoses 2′-FL and 3-FL and the sialyllactoses 3′-SL and 6′-SL. It can be further elongated to generate tetrasaccharides, e.g., LNT and LNnT. Chains can be further elongated, fucosylated, and/or sialylated to generate more complex structures. FL, fucosyllactose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; HMO, human milk oligosaccharide; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; Neu5Ac, *N*-acetylneuraminic acid; SL, sialyllactose.

health and for preventing gastrointestinal disease in adulthood. Alterations in normal bacterial colonization patterns early in life may change the immune development and cause predisposition to several diseases. Thus, lower microbial diversity in early infancy seems to lead the way for the development of allergic manifestations (62). In addition, changes in gut microbiota composition may influence the development of several autoimmune disorders in children, including type 1 diabetes and Crohn and celiac disease, as increasing evidence suggests (63). Early-life changes in gut microbiota composition can alter susceptibility to developing obesity later in life (64).

It remains to be demonstrated whether dysbiosis is really the cause or the consequence of a specific disease. Most studies agree on recognizing dysbiosis as a risk factor associated with many pediatric diseases. This highlights the importance of establishing a healthy microbiota early in life as well as maintaining a healthy microbial composition to prevent the onset of diseases during childhood and later in life.

Infant Formula

Considering that bovine milk constitutes the base for infant formula, unsupplemented formulas have a much lower content of bioactive molecules, e.g., oligosaccharides. HMOs are characterized by their great diversity, complexity, and abundance, whereas milk oligosaccharides in other mammals are present at smaller amounts and with less diverse structures (65). Bovine milk contains oligosaccharides, albeit at a lower concentration than human milk (0.05 g/L) and with less complexity and diversity (66). Contrary to HMOs, sialylation is predominant (70%) in bovine milk oligosaccharides, and fucosylated structures are present at much lower concentrations (67). Hence, formula-fed infants do not currently benefit from the advantages conferred by the HMO structures present in human milk.

Some years ago, the supplementation of infant formula with HMOs was unfeasible because of the lack of a large-scale synthesis at an affordable price. Recent advances in glycan synthesis have yielded industrial amounts of specific structures (the smallest but more abundant) at a reasonable cost. The more structurally complex HMOs still remain unavailable.

The understanding of human milk composition continues to evolve and helps to guide infant formula research. Since the late 1970s, researchers have observed that formula feeding influenced fecal microbial populations in a way that was different from breast milk (68). Efforts were thus directed to adding technologies, e.g., prebiotics, that would enhance the bifidogenic effect of formulas (69). At that time, the main prebiotics available were galactooligosacharides and fructooligosaccharides, which were able to improve bacterial communities in formula-fed infants (70). In fact, the addition of galactooligosacharides, fructooligosaccharides, or their combination had a stimulating effect on the growth of *Bifidobacterium* (32, 33, 71–73),

It is worth considering that studies that evaluated infant gut microbiota and the effects of prebiotic formula supplementation have sometimes rendered contradictory results. Differences may depend on laboratory methodology, formula composition, infant population, and individual intestinal microbiomes (72). With the appearance of high-throughput methods to study the microbiota, analyses became more sensitive and accurate than traditional culture-dependent methods (74). In summary, there are reasons to believe novel ingredients and techniques within the dairy industry may further contribute to minimize differences between formula and human milk.

Conclusions

The gut microbiota behaves like an organ, exerting a wide range of effects that extend beyond the gastrointestinal tract. Microbiota metabolites such as SCFAs, tryptophan, vitamins, and neurotransmitters may be important for newborns by supporting growth and development.

The gut microbiota is established early in life and matures during the first 2–3 y, whereupon it reaches an adult-like profile. Alterations in early microbiota are closely related to disease not only during childhood but also later in life. Because early life is a critical period when alterations may have a more pronounced long-lasting effect in health, this stage may provide a window of intervention for disease prevention.

Breast milk is one of the main factors driving the proliferation of a protective gut bacterial community enriched in bifidobacteria. Bioactive factors in human milk may promote the growth of beneficial bacteria and therefore ameliorate infant health. Although breast milk is considered the ideal nutrition for newborns, formulas represent an alternative for those who are unable or choose not to breastfeed.

Formula composition has greatly improved within the past decade. The addition of prebiotics to infant formula has shown beneficial effects on the gut microbiota. Novel ingredients may further contribute to minimize differences between breastfed and formula-fed infants.

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