Key Words
Microbiota · Probiotics · Prebiotics · Health

Key Messages
• A healthy microbiota preserves and promotes host wellbeing and absence of disease, especially in the gastrointestinal tract
• Initial colonization with 'pioneer bacteria' is enhanced by both bacteria and galacto-oligosaccharides in breast milk and the microbiota of the mother. These pioneer bacteria direct the later microbiota succession which forms the platform for a healthy gut microbiota throughout one’s lifetime
• The microbiota resembles that of adults by 1–2 years of age
• Bifidobacterial numbers in children often remain higher than in adults
• A disturbed microbiota succession during early infancy has been linked to an increased risk of developing infectious, inflammatory and allergic diseases later in life
• Intestinal microbial colonization and its modulation through dietary means are important considerations during the first years of life

Initial Establishment of Microbiota

Source of Original Microbiota
The microbiota of a newborn is acquired from the mother at birth and develops rapidly thereafter. It is initially strongly dependent on the mother’s microbiota, mode of delivery and birth environment [1, 2]. The microbiota of the mother is determined by genetic and environmental factors. Stress and dietary habits during later pregnancy have a significant impact on the microbiota at delivery, thus influencing the quality and quantity of first colonizers of the newborn. Subsequently, feeding practices (formula or breastfed) and the infant’s home environment influence the succession microbiota at the genus and species level, as well as species composition and numbers of bacteria.

Succession of Microbial Communities
The establishment of microbiota in the newborn occurs in a stepwise fashion. Studies in mice have shown that the first bacteria to colonize the newborn intestine ('pioneer bacteria') can modulate gene expression in host intestinal epithelial cells. This results in an altered intestinal microenvironment which influences the nature of subsequent intestinal colonization.

In the newborn, initial colonization with facultative anaerobes, enterobacteria, coliforms, lactobacilli and streptococci is rapidly followed by colonization with anaerobic genera such as Bifidobacterium, Bacteroides, Clostridium, and lactic acid bacteria. Molecular analyses demonstrate significant differences in the microbiota of formula-fed and breastfed infants with respect to bifidobacterial numbers and species composition. In breastfed infants, bifidobacteria constitute
from 60 to 90% of the total fecal microbiota, while lactobacilli comprise less than 1% [3]. The most common bifidobacterial species in breastfed infants are *B. breve*, *B. infantis* and *B. longue* [4]. In formula-fed infants the microbiota is more complex and influenced by the formula composition. The lactic acid bacteria composition in breastfed and formula-fed infants is similar, with *Lactobacillus acidophilus* group microorganisms such as *L. acidophilus*, *L. gasseri* and *L. johnsonii* being most common. Microbiota differences between breastfed and formula-fed infants have lessened with improved infant formulas.

**Gut Microbiota in the First 6 Months of Life**

Breastfeeding for 4–6 months may assist in the development of healthy gut microbiota by providing bifidobacteria and lactic acid bacteria which reinforce colonization, and by supplying galacto-oligosaccharides that promote a healthy microbiota composition. Breastfeeding also facilitates the exchange of microbes between mother and infant via skin contact and exposure to microbiota in the immediate environment. Every individual has a unique characteristic microbiota during later phases of breastfeeding that comprises a dynamic mixture of microbes typical to each individual. Weaning, introduction of solid foods, and antimicrobial drug treatment will break the constant supply of oligosaccharides and microbes from the mother, thus affecting intestinal microbiota development.

Molecular analysis of bacterial communities in healthy babies during the first 10 months of life demonstrated progression from a simple profile in the first days of life to a more complex diverse profile with members of the genera *Bifidobacterium*, *Ruminococcus*, *Enterococcus*, *Clostridium*, and *Enterobacter* identified by 6 months of age [3]. *Bifidobacterium* and *Ruminococcus* species dominated the intestinal microbiota with high level stable expression over time. A pilot study in 6-month-old infants reported higher bifidobacterial levels and lower clostridial numbers in breastfed infants than infants receiving either formula or formula with prebiotics. Ongoing improvements in formulae have lessened these differences [5].

The healthy intestinal microbiota in infancy is characterized by a large gram-positive bacterial population and significant numbers of bifidobacteria, mainly *B. longum*, *B. breve* and *B. infantis*. Lactic acid bacteria may play a role in providing the right intestinal environment for bifidobacteria to dominate. A healthy microbiota during infancy is particularly important as this establishes the basis for healthy gut microbiota later in life.

**Gut Microbiota in Infants from 6 Months Onwards**

After the first 6 months of life, the microbiota becomes more diverse [6]. Several studies have examined the progression of microbiota from 6–24 months of life (summarized in fig. 1). Weaning is associated with increased *Escherichia coli*, enterococi, bacteriodes and anaerobic gram-positive cocci and decreased enterobacteria. Differences between breastfed and formula-fed infants disappear.

By 1–2 years of age the microbiota resembles that of adults, although levels of bifidobacteria and enterobacteria in children (16 months to 7 years) remain higher than in adults.

**The Importance of a Healthy Microbiota: Biological Effects**

The intestinal microbiota is crucial for normal development of the gut-associated lymphoid tissue (GALT), and has important effects on intestinal mucosal barrier function and other aspects of intestinal function.
**Immune Development**

Microbial colonization of the newborn intestine is required for normal immune development, which in turn is important for regulation of gut inflammatory responses and oral tolerance induction. The mucosal immune system of the gastrointestinal tract is constantly challenged by diverse antigens including microbial and food antigens. Such priming of the gut-associated lymphoid tissue is important for two opposing functions: mounting a response to pathogens and maintaining hyporesponsiveness to innocuous antigens. Mice raised in a germ-free environment fail to develop oral tolerance and have a persistent Th2-dependent antibody response [7]. This immune deviation can be corrected by reconstitution of intestinal microbiota, but only if this occurs during the neonatal period [7].

An important question is how the microbiota is altered by the significant changes in diet during the first years of life and how this impacts upon intestinal immune development. The host–microbe cross-talk during and after breastfeeding is critical in this regard. The strains of healthy gut microbiota are likely to stimulate local and systemic immune responses via pattern recognition molecules such as toll-like receptors providing the host with an anti-inflammatory stimulus and directing the host–microbe interaction towards immune tolerance. In particular, the bifidobacteria-dominated environment in childhood may provide a more anti-inflammatory stimulus than bacteria from adults which have been shown to be more proinflammatory. A complex microbial community is required to achieve a healthy microbiota that exhibits powerful anti-pathogenic and anti-inflammatory capabilities.

**Intestinal Function**

An absent or inadequate intestinal microbiota has been shown to cause defects in intestinal barrier function. The microbiota may also influence other intestinal functions. Before weaning, formula-fed infants have a greater ability to ferment complex carbohydrates than breastfed infants, probably due to the presence of a more complex microbiota. Following weaning these differences disappear. Breastfed infants have delayed establishment of mucin-degrading microbiota, but this increases in both groups between 6 and 9 months. Conversion of cholesterol to coprostanol commences after 6 months of age, and levels of ammonia, phenol, β-glucosidase and β-glucuronidase activity increase after weaning.

**Fig. 1.** Relative changes in gut microbiota composition suggested by culture-dependent and culture-independent studies. The numbers of bifidobacteria can be influenced by diet, probiotics and prebiotics.
β-Glucuronidase activity is often higher in formula-fed infants; however, this difference resolves after weaning.

**Maintenance and Modulation of the Individually Optimized Healthy Microbiota**

The healthy gut microbiota created during early life must be maintained throughout life. Deviations in microbiota associated with disease can be redirected to the healthy balance by dietary means, for instance by using probiotics or prebiotics. Probiotics are defined as viable microbes which through oral administration produce health benefits to the host. Probiotics are members of the healthy gut microbiota that mimic the healthy microbiota of both a breastfed infant and the healthy infant, and are generally regarded as safe [8, 9]. Prebiotics are oligosaccharides that promote expansion of specific microbes with potential to maintain health. A prerequisite for the efficacy of prebiotics is that such strains are already present in the gut. Carefully designed combinations of probiotics and prebiotics may offer an optimal means for creating and maintaining a healthy microbiota, as this would mimic the mother–infant relationship of offering both microbes and oligosaccharides to the newborn infant.

It is important to recognize that individual probiotic bacterial strains can have distinct and specific effects [9]. Therefore, the effects of one probiotic strain cannot be generalized to another, and the individual properties of a probiotic strain must be evaluated prior to clinical application. For example, in a double-blind placebo-controlled trial, *Lactobacillus rhamnosus* GG (LGG) but not a mixture of 4 probiotic strains (LGG, *L. rhamnosus* LC705, *B. breve* Bbi99, *Propionibacterium* JS) was effective for the treatment of eczema [10]. LGG has also been shown to enhance IgA responses against rotavirus, which is not found with different strains of the same species [11]. Furthermore, in addition to species/strain specific effects of probiotics, the timing of probiotic administration may also be important. For example, in separate studies, LGG (alone or in combination with other probiotics and a prebiotic) and *L. reuteri* administered prenatally to mothers in the last 2–4 weeks of pregnancy and to the infant in the first 6 months of life have been reported to reduce the risk of developing eczema in childhood up to age 7 years [12–15]. In contrast, a bacterium that had not been characterised in preclinical studies, *L. acidophilus* LAVRI-A1, administered only to infants from 4 weeks to 6 months of life did not have any effect on eczema risk, suggesting that prenatal administration may be requisite for efficacy in the prevention of allergic disease [16]. These results highlight the different effects of specific probiotics, which are further supported by genomic studies.

Interestingly, a recent study of LGG administered prenatally to mothers from 36 weeks gestation and to infants for the first 6 months of life conducted in Germany failed to demonstrate a protective effect against the development of allergic disease [17]. This may reflect reduced power of the study (study population 94 as compared to 159 in the original Kalliomaki et al. study [12]), or geographic/population specific differences.

Similarly, prebiotic oligosaccharides have different microbiota-modifying properties. Although, most prebiotic components have been shown to enhance the bifidobacterial microbiota, detailed investigation of specific effects is required. A wide variety of galacto-oligosaccharides are found in breast milk, and have documented bifidogenic and health-promoting effects in the infant gut. However, some fructo-oligosaccharides have been reported to enhance levels of unknown microbes in the human gut, thus potentially facilitating untoward effects in infants. Therefore, when evaluating a probiotic or prebiotic for clinical use, the safety and clinical benefit of that specific product must be documented to verify efficacy before it can be recommended for clinical application.
Conclusions

- The healthy human microbiota is metabolically active and provides an important defense mechanism for the host. Deviations in its composition are related to multiple disease states.

- Evidence supports a crucial role for the infant microbiota and the first colonization steps in later health. Bifidobacteria play a key role in this process.

- The mother–infant contact has an important impact on initial microbiota development, providing the critical first inoculum at birth, promoting the bifidogenic environment through prebiotic galacto-oligosaccharides and microbes in breast milk and introducing environmental bacteria through contact with the infant.

- Both the succession of microbial communities during the first years of life and the sequelae of these events need to be clarified in more detail.

- The potential application of specific probiotics and/or prebiotics to influence microbiota development for the treatment and prevention of disease also warrants further evaluation.

References