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The first thousand days – intestinal microbiology of early life: establishing a symbiosis

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Abstract

The development of the intestinal microbiota in the first years of life is a dynamic process significantly influenced by early-life nutrition. Pioneer bacteria colonizing the infant intestinal tract and the gradual diversification to a stable climax ecosystem plays a crucial role in establishing host–microbe interactions essential for optimal symbiosis. This colonization process and establishment of symbiosis may profoundly influence health throughout life. Recent developments in microbiologic cultivation-independent methods allow a detailed view of the key players and factors involved in this process and may further elucidate their roles in a healthy gut and immune maturation. Aberrant patterns may lead to identifying key microbial signatures involved in developing immunologic diseases into adulthood, such as asthma and atopic diseases. The central role of early-life nutrition in the developmental human microbiota, immunity, and metabolism offers promising strategies for prevention and treatment of such diseases. This review provides an overview of the development of the intestinal microbiota, its bidirectional relationship with the immune system, and its role in impacting health and disease, with emphasis on allergy, in early life.

Our microbial world

We live in a microbial world. Micro-organisms were among the very first life forms and still today form the greatest biomass on this planet (1). They hardly exist as single cells in nature, but rather live in complex communities coevolved and adapted to the habitats they colonize. Surveys of these complex communities are taking great advantage from the use of high-resolution cultivation-independent methods such as phylogenetic microarrays or next generation sequencing (2). In particular, the study of human intestinal microbial species of which approximately 70% have not been isolated, cultivated, or sequenced, due to the inability to reproduce necessary growth conditions in the laboratory. Sequencing of PCR amplified 16S ribosomal RNA (16S rRNA), a conserved gene routinely used for phylogenetic identification of bacteria, and whole genome surveys (metagenomic sequencing) are now starting to reveal the true microbial diversity of the human intestine and their role in health and disease. The human intestinal tract is colonized with about ten times more microbial cells than human body cells and contain about 150 times more microbial genes than the human genome (2). The intestinal microbiota is coexisting in a homoeostatic relationship with the host (3). This host–microbial relationship is maintained in a bidirectional manner with the immune system. The intestinal microbiota benefits from a stable environment and nutrient supply that are provided in the intestinal tract, while the host gains products from microbial fermentation conversion of host indigestible components (dietary fibres) into short-chain fatty acids (SCFA; mainly acetate, propionate and butyrate) contributing to an estimated 10% of our energy requirement (4), vitamin K and B12 production (5, 6), and protection against potential pathogens through competitive exclusion (7, 8). The importance of the human microbiota is particularly clear as alterations of the intestinal microbiota have been associated with short- and long-term health and disease issues, such as intestinal bowel disease (IBD), allergy, diabetes, obesity and autism (9).

The development of the intestinal microbiota is a dynamic process in the first years of life (10–12), a time frame that is also a critical period of gut and immune development and maturation (13). Indeed, the pioneer bacteria colonizing the infant intestinal tract and the gradual diversification to a stable

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climax ecosystem play a crucial role in establishing host–microbe interactions essential for optimal symbiosis; and this colonization process may profoundly influence health throughout life (8). Future research should focus on the analyses of longitudinal data that may identify the patterns of early intestinal microbiota and functionality of not yet-cultivated species that could affect health later in life (14).

Microbial pioneers

Theodor Escherich (1857–1911) pioneered the study of intestinal microbiology in early life (15). In 1886, Escherich published his 177-page postdoctoral thesis entitled, 'The Intestinal Bacteria of the Infant and Their Relation to the Physiology of Digestion' (16). Escherich demonstrated that meconium was sterile and that bacterial intestinal colonization is attributable to the infants' environment (including milk) within 3–24 h after birth, and emphasized the value of breastfeeding.

The rise of molecular biology in the second half of the 20th century and the more recent revolution in sequencing technologies identified the key players of the developmental intestinal microbiota in more detail. The majority is assigned to four phyla, namely the Actinobacteria (with genera like *Bifidobacterium* and *Colinsella*), the Bacteroidetes (with genera like *Bacteroides* and *Prevotella*), the Firmicutes (with genera like *Lactobacillus*, *Clostridium, Eubacterium* and *Ruminococcus*) and the *Proteobacteria* (e.g. *Enterobacter* spp.) (17). Another phylum identified throughout life is the Verrucomicrobia consisting of one major species, the mucin-degrading *Akkermansia muciniphila* (7).

The composition of the microbiota changes substantially at two stages in early life: from birth to weaning, and from weaning to adulthood driven by further diversification of diet (4). The pioneer species in neonates are facultative anaerobic bacteria, such as *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Enterobacter* spp., and these bacteria create an anaerobic environment that promote the growth of obligate anaerobes, such as *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Eubacterium* spp., predominating after one or 2 wk. Escherichs' observation of sterile meconium and thus supposed sterile intestine at birth has only recently been opposed with molecular surveys suggesting that microbial exposure may start before birth and that infants may already receive microorganisms from the mother during gestation (18, 19). Right after birth, the early settlers are derived from the maternal microbiota (vaginal, faecal, human milk, mouth, skin) and the environment (20–22). Human milk forms an important continuous inoculum, while bacterial strains found in breast milk have also been detected in faecal samples from the corresponding infants (21, 23). These bacteria are postulated to translocate from the mothers' intestine to the mammary gland via the mesenteric lymph nodes, suggesting a possible route of inducing immunologic tolerance to these commensals (24). Another possible or contributing route may include the establishment of the mothers' skin microbiota and infants' oral microbiota into the mammary gland (25). Host genotype, gestational age, medical practices (i.e. antibiotic use), mode of delivery (caesarean section vs. vaginal delivery), geographic origin and linked to that, cultural traditions, especially regarding diet, are factors profoundly influencing the microbiota development (26, 27). Breastfed infants typically have a microbiota dominated by *bifidobacteria*, while formula-fed infants have a more diverse microbiota. Infants born preterm or by caesarean section show a reduced diversity and a delayed colonization by *bifidobacteria* compared to infants born at term or vaginally (26, 28). Some studies applying PCR amplification and sequencing did not reproduce the early predominance of *Bifidobacterium* (12); however, efficient DNA extraction and careful selection of PCR primers have proven to be critical to effectively detect this genus (29).

Introduction of first solid foods around 4–6 months of age impacts the infant microbiota considerably. Although still 'infant-like', with decreased but still dominating levels of *bifidobacteria*, a gradual diversification is seen towards more adult-type species, mainly *Bacteroides* spp. and *Clostridium* clusters IV and XIV; the latter two clusters are known to contain numerous butyrate producers (10, 30). Interestingly, the factors influencing the early colonization process strongly influence the post-weaning colonization pattern. Early diversification, as observed under formula-feeding not containing prebiotics, promotes earlier acquisition of an adult-type microbiota (10, 30). Further diversification of diet gradually increases diversity and abundance of Bacteroidetes and Firmicutes towards adult levels and generally low abundant levels of *Bifidobacterium* (27). Healthy adults have a stable microbiota; unique for individuals though sharing a core microbiome with other individuals, which may change and destabilize only at older age again (4, 31). Although low abundant in adults, *Bifidobacterium* species still play important metabolic roles in adults (32). On top of this, ageing is generally associated with a significant decrease in *bifidobacteria*, along with other rearrangements and decreasing stability, all together associated with increased susceptibility to infections in elderly (31). The exact age at which a stable adult community is established is unclear, but is thought to be reached around 3 yr of age (11, 27). Changes in the genetic capacity of the microbiome with human development include changes in the abundance of genes involved in access to host-derived glycans (in human milk and intestinal mucosa) and vitamin biosynthesis, i.e. infants having more genes that encode enzymes involved in folate biosynthesis and adults more encoding for vitamin B12 (27). The influence of early colonization patterns on the composition of the adult microbiome is not yet fully understood. However, these patterns have been shown to influence gut maturation, immune development and host metabolism (8), and differences in composition driven by environmental factors in infancy may affect susceptibility to metabolic (e.g. obesity), immunologic (e.g. IBD and allergy) and even behavioural (e.g. autism) disorders into adulthood (Fig. 1), diseases which are increasingly prevalent in developed countries (9). The central role of diet in influencing the human microbiota, immunity and metabolism offers promising strategies for prevention and treatment of such diseases.

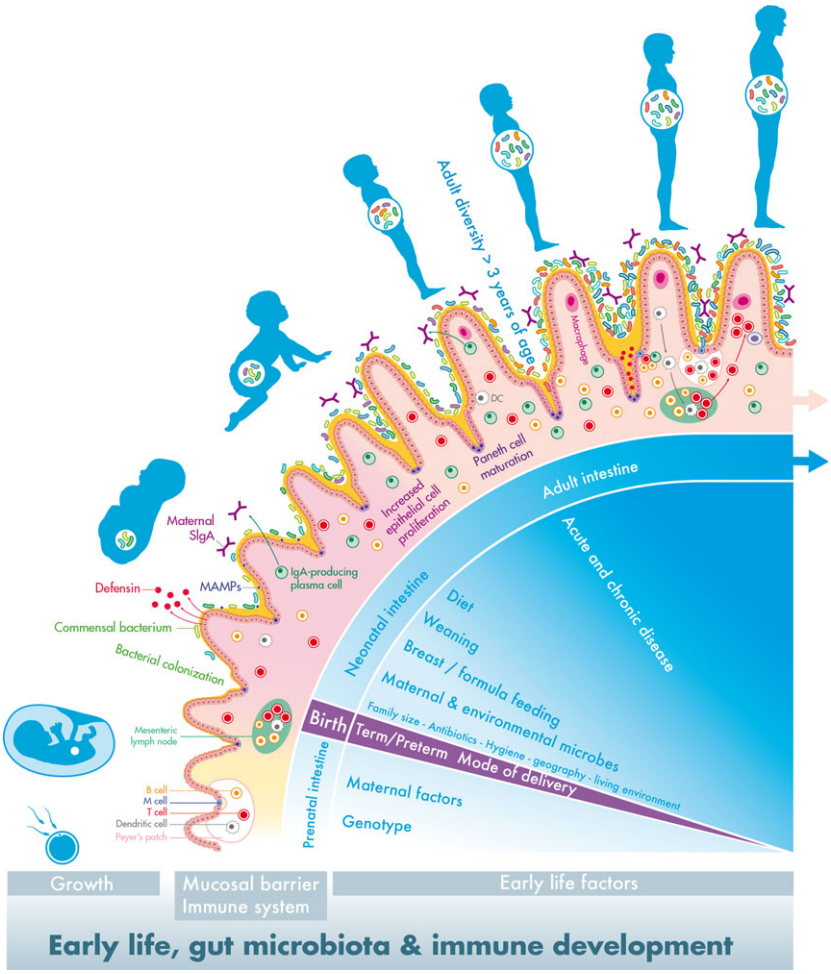

Figure 1 Early life, gut microbiota and immune development – establishing a symbiosis. The establishment of the intestinal host–microbiota symbiosis is driven by both developmental and environmental signals especially in early life, profoundly influencing health throughout life. The prenatal intestine is thought to be sterile, and development depends most importantly on genotype as well as on maternal factors, including nutrition and health status. The cryptopatches and lymphoid tissues (mesenteric lymph nodes and Peyer's patches) with dendritic, T and B cells develop in preparation of the exposure to the extrauterine world. During birth, infants are inoculated with maternal and environmental microbes, and the type and patterns strongly depend on birth mode and gestational age. The gut microbial development in the neonatal period is influenced by several early-life factors and especially diet (type, composition and timing) drives the further diversification towards an adult complexity, which is reached around 3 years of age. This postnatal colonization process provides several signals, known as microbe-associated molecular patterns (MAMPs), affecting the maturation of the immune system and the mucosal barrier, accompanied with increased mucus secretion. These signals also result in the proliferation of intestinal epithelial cells in crypts and the cryptlocated Paneth cells, resulting in their increased depth and the production of antimicrobial peptides (defensins), respectively. Specialized epithelial cells (M cells) reside above Peyer's patches and facilitate direct interaction of the luminal content with the underlying lymphoid cells to stimulate mucosal immunity. SIgA is the most abundant immunoglobulin on mucosal surfaces, and maternal SIgA is provided by human milk during the early postnatal period along with the initiation of the infants own SIgA.

Establishing a healthy symbiosis

Neonates have a limited capacity to initiate immune responses and both innate and adaptive immune responses are not yet fully functional. In the months and years after birth, the immune system gradually matures (8), concurrent with the infants' microbiota development. The largest immune component in the body, the mucosal immune system, comprised of the gut-associated lymphoid tissue (GALT); the mucosal lamina propria; and the mucosal surface, plays a central role in this developmental process. The mucosal immune system is providing protection from the external environment and directly interacts with the environmental antigens and commensal bacteria (13). The epithelial layer in neonates shows a higher permeability in both the respiratory and gastrointestinal tracts, and secretion of proteases and antimicrobial peptides have not fully developed (33). The epithelial production of mucus forms an important first line of defence against microbes. The

thickness and continuity of the intestinal mucus layer increase from the small intestine towards the colon correlating with increasing bacterial loads (13). Mucin glycans are nutrients for some constituents of the microbiota, such as *Bifidobacterium*, *Bacteroides* spp. and *Akkermansia muciniphila*, giving them an ecologically advantage to reside in the outer mucous layer close to the intestinal epithelial cells (IECs) (34). Niche occupation by such commensals is not only establishing a physical barrier excluding potential pathogens, but also the production of acetate and lactate forms an effective chemical barrier toxic for potential pathogens (35, 36). Levels of faecal SCFA of human milk fed infants are characterized by relatively higher proportions of acetate and lower proportions of propionate and almost complete absence of butyrate, when compared to adults. Also, lactate is more commonly detected in the faeces of infants, while undetected in healthy adults due to immediate onward conversion by lactate-utilizing bacteria. These high levels of acetate and lactate in human milk fed infants are reflecting the dominance of *bifidobacteria* and *Lactobacilli* (37). Although faecal levels of butyrate are generally low in human milk fed infants, acetate and lactate may in turn be used to gradually establish butyrate producers within the Firmicutes (4, 38), which have recently been shown to be less abundant in colicky infants at 2 wk of age at the expense of increased levels of potential pathogenic members of the *Proteobacteria* (39). Many of the direct effects of SCFA on epithelial cells associated with maintenance of the epithelium relate mostly to their role as an energy source and their inhibition of histone deacetylases; the latter is directly impacting human gene expression and, e.g., shown to downregulate inflammation in patients with ulcerative colitis (40). The SCFA have also been shown to influence immune function beyond the gut by signalling through G-protein-coupled receptors (GPR) on IECs. Mice deficient of GPR43 has exacerbated and poorly resolving inflammation in inflammatory models of arthritis, allergic airway inflammation, and colitis (41).

An important extra layer of innate mucosal defence in neonates is derived from human milk. In addition to a unique mix of human milk oligosaccharides (HMO), and antimicrobial proteins that influence the ecology of the neonatal microbiota, human milk provides abundant secretory immunoglobulin A (sIgA), the specificities of which have been shaped by the maternal digestive system and microbiota (13). Secretory immunoglobulin A is the most abundant immunoglobulin on mucosal surfaces, where it neutralizes harmless food and microbial antigens and prevents them from penetrating the epithelium. However, IgA can also function in high-affinity modes for neutralization of toxins and pathogenic microbes, and as a low-affinity system to contain the dense commensal microbiota within the intestinal lumen (42). Next to sIgA, maternal IgG-antigen complexes also play a major role in shaping the infants' immune system. Antigen bound to IgG will be very efficiently transferred across the gut barrier using the neonatal Fc receptor (43). Therefore, both maternal sIgA and IgG may be important in the development of non-responsiveness to harmless commensals and food antigens, i.e. induction of oral tolerance (44). The developmental microbiota is essential for the initiation of an infants' own sIgA, while germ-free mice show drastically reduced mucosal IgA-secreting cells. Studies using prebiotics or synbiotics (combination of pre- and probiotics) treatment given for 6 months to infants showed increased levels of faecal sIgA (45, 46) and is linked to reduced risk of allergy before 2 yr of age in one of these studies (46).

More evidence is mounting in how commensal bacteria directly influence adaptive immunity and oral tolerance and is focusing on the mechanisms involved in the cross-talk between the intestinal microbiota and the host. This cross-talk is mediated through pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), specifically recognizing conserved microbial molecular structures, called microbe-associated molecular patterns (MAMPs) (8). Recognition of these patterns may promote pro-inflammatory responses or repress them and seem to depend on by whom (i) and where (ii) they are triggered (13). First (i), subtle differences between commensal bacteria, probiotic and pathogenic micro-organisms may mediate different host responses (47), and second (ii), apical signalling normally promotes intestinal homoeostasis; however, basolateral signalling, implicating intestinal barrier disruption and infection, initiates inflammatory responses of innate and adaptive immune cells (13) and can lead to exacerbate intestinal inflammation (48). Pro-inflammatory responses are counterbalanced by specialized T cells known as regulatory T (Treg) cells and play a crucial role in maintaining immune homoeostasis. Treg cells are characterized by production of IL-10, one of the main immunoregulatory cytokines required for immune tolerance of the intestinal microbiota (13). The role of TLR in sensing the microbiota in this process is evident by the absence of colonic inflammatory disease in germ-free IL-10-deficient mice and mice deficient for both IL-10 and myeloid differentiation factor 88 (MyD88). Remarkably, IL-10-producing T cells can be induced to develop in response to specific commensals or their products. This was first shown for a common commensal, *Bacteroides fragilis* through its polysaccharide A (PSA) mediating through the TLR2–MyD88 pathway (49). More human symbionts are thought to exert comparable mechanisms to induce mucosal tolerance, i.e. a probiotic *Bifidobacterium breve*, but not a *Lactobacillus casei* strain, induced development of IL-10-producing Treg cells and were shown to prevent inflammation in a colitis model (50).

In these and other studies, MyD88-dependent TLR signalling has proven to be a key mediator for maintenance of intestinal homoeostasis, requiring active communication among epithelial cells, immune cells, and the intestinal microbiota (48). Hill et al. (51) showed that antibiotic-mediated disruption of the microbiota is sufficient to predispose mice to allergic disease. The authors showed that the commensal microbiota modulates B-cell production of IgE antibody in a MyD88-dependent manner and that perturbation leads to high circulating levels of basophils and high serum IgE concentration. Exposure of antibiotic-treated mice with DNA motifs specific for bacteria (unmethylated cytosine-guanosine CpG oligonucleotides), a known Toll-like receptor 9 (TLR9)-dependent microbial ligand, was sufficient

to reduce serum IgE as well as the frequency and total number of circulating basophils. These findings identify intriguing links between the adaptive immune system interacting with the intestinal microbiota and will further elucidate the specific microbes involved in promoting a healthy host–microbiota symbiosis.

Early microbiota and allergy

The prevalence of atopic manifestations (atopic dermatitis, food allergy, allergic rhinitis and asthma) has been increasing worldwide, predominantly in the western world and particularly among children (52). Expression of an allergic phenotype is dependent on the interaction between two major factors: a genetic predisposition and gene-environment interactions (e.g. lifestyle, diet). Infants suffering from atopic dermatitis and or food allergy are more susceptible to develop other allergies like allergic asthma later in life, a process known as the atopic march (53). There is mounting evidence that modifications in the pattern of microbial exposure early in life represents a critical factor underlying the development of an allergic phenotype (54–57), such as the protective effects observed for exposure to siblings or a farming environment (58, 59). Accumulating preclinical studies start to reveal pathways linking aberrant microbial patterns to atopic diseases (51, 60). The classical explanation for the increasing prevalence of allergies in western countries, and a possible role of 'early' microbes, was postulated in the hygiene hypothesis in 1989 (61). This hypothesis focuses merely on decreased exposure to infectious agents under improved hygiene standards to explain the hypersensitive reaction of the immune system towards normally harmless substances in the environment. The supposed mechanism proposes a lack of shifting of allergen-specific responses from the T helper 2 to the T helper 1 phenotype, because of reduced exposure to infectious agents in early childhood (62). More recently, the possible implication of the resident human intestinal microbiota in developing allergy has been suggested to play a crucial role in the development of mucosal immunologic tolerance. The discovery of Treg cells and their role in immune suppression and self-tolerance (63) lead to an important explanatory mechanism of reduced activity of Treg to a loss of microbial symbionts, which may partly explain the increasing prevalence of other western diseases, like IBD, obesity and diabetes (9, 62). The role of the endogenous microbiota in developing allergy under this extended hygiene hypothesis is supported by the positive correlation of environmental factors, known to impact microbial colonization, and allergic manifestations (i.e. antibiotic use and caesarean section), correlations with an altered microbiota composition and increasing evidence of successful prevention or reduction of allergy through microbiota modulating diets (52). Altered microbial composition and activity between healthy and atopic children have been shown in several cross-sectional epidemiologic studies and have been extensively reviewed up to January 2007 by Penders et al. (64). Table 1 gives an overview of observational studies summarized in a similar approach, from 2007 onwards, considering type of atopic disease under study, the study population, design and methodology to examine the intestinal microbiota. Interestingly, differences in the intestinal microbiota composition often precede the manifestation of atopic symptoms and atopic sensitization (65–69), although two studies reported no meaningful differences between groups (70, 71), possibly explained, as the authors suggest, by the application of cultivation methods overlooking the unculturable bacteria. Reduced bacterial diversity in the early microbiome has been associated with developing atopic disease by several comparative studies (54, 65, 66, 72, 73). Abrahamsson et al., applying 16S rRNA sequencing, linked reduced bacterial diversity at 1 month to IgE-associated eczema in infants at 2 yr of age, which was subsequently confined to developing asthma at 7 yr of age (74), supporting the importance of pioneer microbes in early immune maturation. This early reduced diversity was mainly attributed to a decreased diversity of *Bacteroides* spp., within the Bacteroidetes phylum. At 12 months of age, a decreased diversity of *Proteobacteria* was observed and a tendency of higher levels of the phylum Firmicutes in atopic infants, a phylum indicating development towards a more 'adult-type' microbiota. Nylund et al., applying a phylogenetic microarray, reported increased diversity at 18 months of age, but not at 6 months, in eczematous vs. healthy infants. This increased diversity at 18 months of age was associated with higher abundances of *Clostridium* clusters IV and XIVa, members of the Firmicutes phylum. At this age, healthy infants showed increased abundance of members within Bacteroidetes (57), a group of bacteria which may have been underestimated in early life, due to molecular bias (75). Notably, species within the Bacteroidetes have been shown, next to *Bifidobacterium* spp., to be efficient fermenters of human milk oligosaccharides in contrast to species within the Firmicutes phylum (76). Interestingly, a recent study showed that colonization of germ-free mice with the faecal microbiota of a healthy infant rich in *Bifidobacterium* spp. and *Bacteroides* spp. protected against the development of cow's milk allergy following sensitization to β-lactoglobulin (77). The genera within the phyla Bacteroidetes and Firmicutes linked to allergy in both observational studies may thus play important roles in the gradual succession of an infant-type microbiota, dominated by *bifidobacteria*, towards a stable adult-type microbiota.

The observations of both decreased and increased bacterial diversity linked to allergic manifestations may seem contradictory, but were made at different stages of early life and development of allergic disease. Bacterial diversity as such is difficult to interpret in early childhood, while the early microbiota is highly dynamic with high inter-individual variation. Also, bacterial diversity gradually increases towards adulthood reaching adult levels no earlier than around 3 yr of age (11, 27). There is an ongoing debate whether low total diversity of the gut microbiota in early childhood is more important than the altered prevalence of particular bacterial species in allergy development (78), but more likely the combination of both may lead to identifying the key microbial signatures for developing allergy and response to nutritional strategies.

Table 1 Human observational studies on the association between the gut microbiota composition and atopic diseases. Studies applying next generation sequencing or microarray technologies as microbial analysis tool are indicated in bold

Allergic phenotype	Study design and population (country)	Faecal microbial analysis tool	Allergic vs. non-allergic (ref)
Ecz (Williams' criteria) and/or sIgE+ until 18 m	PC: 324 infants at risk for allergy (SE, UK and IT)	Cultivation	No differences observed (70)
Allergic manifestations (ISAAC questionnaire) until 2 yr	PC: 15 infants (JP)	qPCR	Increased abundance of Bacteroidaceae at the ages of 1 and 2 m of age (67)
Any allergic manifestation or SPT+ until 6 m	CC: 10 allergic and 16 non-allergic infants (JP)	PCR	Higher prevalence of *Bifidobacterium catenulatum* group at 1 m and higher prevalence of *B. bifidum* at 6 m of age (88)
Ecz (PD) until 6 m	CC: 9 allergic and 12 non-allergic infants (USA)	DGGE	Lower diversity at 1 and 4 m of age (73)
Ecz (PD) until 6 m	CC: 37 allergic and 24 non-allergic infants (NZ)	TTGE, FISH	Higher prevalence of *Bifidobacterium pseudocatenulatum* (89)
API: wheezing + Ecz/ wheezing + allergic heredity until 3 yr	PC: 117 infants (B)	Cultivation	Higher prevalence of *Bacteroide fragilis* at 3 wk of age (90)
Ecz (Williams' criteria) and/or sIgE+ until 18 m	CC: 15 allergic and 20 non-allergic infants (SE, UK, IT)	T-RFLP, TTGE, qPCR	Lower diversity at 1 wk of age (72), same cohort as (70)
Allergic manifestations and at least one SPT+ until 5 yr	CC: 16 allergic and 31 non-allergic infants (SE)	qPCR	Lower prevalence of *Lactobacilli*, *Bifidobacterium adolescentis* and *Clostridium difficile* during first 2 m of life (91)
Ecz (PD) until 2 yr	CC: 3 allergic and 5 non-allergic, C-section (USA)	**16S rRNA sequencing**	Lower abundance of *Bifidobacterium*, higher abundance of *Enterococcus*, *Klebsiella* and *Shigella* in 1st yr of life (92)
Allergic manifestations (PD) until 1 yr	CC: 24 allergic and 72 non-allergic, VLBW (NL)	FISH	Lower prevalence of *Bifidobacterium* at 1 yr of age (93)
SPT+ and sIgE+ and/or allergic manifestations (PD) until 6 yr	PC: 411 infants with maternal history of asthma (DK)	Cultivation, DGGE	Low diversity at 1 and 12 m with SPT+/sIgE+ and allergic rhinitis, but not with asthma or AD (65)
Allergic manifestations and SPT+ and/or sIgE+ until 5 yr	CC: 16 allergic and 19 non-allergic infants (SE)	qPCR	Lower prevalence of *Lactobacilli* (*L. casei, L. paracasei, L. rhamnosus*) in 1st 2 m, lower prevalence of *Bifidobacterium bifidum* in 1st wk of life (56)
Allergic manifestations (ISAAC questionnaire) until 2 yr	CC: 11 allergic and 11 non-allergic infants (JP)	**16S rRNA sequencing**	Higher abundance of *Bacteroides*, lower abundance of *Clostridium* and *Proteobacteria* (other than *Klebsiella*) at 1 m and higher abundance of *Klebsiella* at 1 and 2 m (94). Same cohort as (67)
sIgE+ and/or Ecz (UK Working Party criteria) until 2 yr	PC: 94 infants (NO)	qPCR	Lower abundance of *Escherichia coli* at 4 m and 1 yr, higher abundance of *Bifidobacterium longum* at 1 yr and lower levels of *Bacteroides fragilis* at 2 yr of age associated with sIgE+ not with Ecz (95)
CMPA with SPT+, sIgE+ and DBPCFC+ for cow's milk, age 2–12 m	CC: 46 allergic and 46 non-allergic (SP)	FISH	Increased abundance of *Clostridium coccoides* group and Atopobium cluster, increased concentrations of butyric acid and branched-chain SCFA (96)
API: wheezing + Ecz/wheezing and allergic heredity until 3 yr	PC: 110 infants (B)	DGGE	Association of *Clostridium coccoides* XIVa species and *Bacteroides fragilis* species at 3 wk (68). Same cohort as (90)

Table 1 (Continued)

Allergic phenotype	Study design and population (country)	Faecal microbial analysis tool	Allergic vs. non-allergic (ref)
SPT+ and/or positive atopic patch test and/or radioallergosorbent test, age from 6 to 24 m	CC: 10 allergic and 20 non-allergic infants (FR)	Cultivation, TTGE, PCR-based fingerprinting	No differences observed in bacterial groups cultivated nor in the *bifidobacterial*-specific fingerprinting (71)
Ecz with SPT+ and/or sIgE+ until 2 yr	CC: 20 allergic and 20 non-allergic infants (SE)	**16S rRNA sequencing**	Lower microbial diversity at 1 m of age, linked to reduced *Bacteroides* spp. diversity (54)
Allergic manifestations (PD) and/or sIgE+, age from 4 to 14 yr	CC: 19 allergic children and 12 non-allergic (IT)	**16S rRNA microarray, qPCR**	Decreased abundancies of Clostridium cluster IV with *Faecalibacterium prausnitzii, Akkermansia muciniphila* and increased abundance of *Enterobacteriaceae* (97)
Ecz (PD) with or without SPT+ until 12 m	PC: 98 high-risk infants (AUS)	T-RFLP	Low microbial diversity at 1 wk of age with Ecz development but not with SPT+ or parental allergic status (both/single) (66)
Allergic manifestations and SPT+ until 7 yr	CC: 47 infants (SE)	**16S rRNA sequencing**	Low microbial diversity at 1 wk and 1 m in infants having SPT+ associated Ecz in first 2 yr of life, subsequently developing asthma at 7 yr of age (74), same chort as (54)
Ecz (PD) with or without SPT+ until 2 yr	CC: 15 allergic and 19 non-allergic high-risk infants (FI)	**16S rRNA microarray, qPCR**	Higher diversity at 18 m, increase of *Clostridium* clusters IV and XIVa and lower abundance of Bacteroidetes members (57)
Ecz (PD) and/or sIgE+ until 3 yr	PC: 606 high-risk infants (DE)	qPCR	Increased prevalence of *Clostridium* cluster I at ages 5 and 13 wk associated with Ecz, but not sIgE+ (69)
FA and SPT+, food challenge and/or sIgE+, age from 2 to 11 m	CC: 34 allergic and 45 non-allergic infants (CN)	**16S rRNA sequencing**	Lower abundance of phyla Bacteroidetes, *Proteobacteria*, and Actinobacteria and increase of Firmicutes (98)

CMPA, cow's milk protein allergy; CC/CS/PC, case-controlled study/cross-sectional study/prospective cohort; DBPCFC+, positive for double-blind placebo control food challenge; DGGE/TTGE, denaturing gradient gel electrophoresis/temporal temperature gel electrophoresis, method to separate 16S rRNA gene amplicons on a gel to create a fingerprint of the microbial community; Ecz, eczema; FA, food allergy; FISH, fluorescent *in situ* hybridization, method to fluorescently label bacterial cells and quantify using 16S rRNA-targeted oligonucleotide probes; ISAAC, International Study of Asthma and Allergies in Childhood; (q)PCR, (quantitative) polymerase chain reaction, method to amplify a limited amount of specific DNA copies across several orders of magnitude and either assess qualitatively or quantify in real-time (qPCR/real-time PCR); sIgE+, positive serum-specific IgE; SPT+, positive skin prick test; T-RFLP, terminal-restriction fragment length polymorphism, method to digest 16S rRNA gene amplicons using restriction enzymes and subsequently separate them on a gel to create a fingerprint of the microbial community; VLBW, very low-birth-weight infants.

Early microbiota and nutrition

The initial bacterial colonizers of our gastrointestinal tract may determine the composition of our intestinal microbiota throughout life. Furthermore, this early development occurs concomitantly to the development of our metabolism, cognitive and immune systems, which have been described to be closely linked to the intestinal microbiota. Knowing that the microbiota can significantly interfere with the human metabolic, cognitive and immune systems, the initiation of symbiosis seems a crucial step for preparing optimal health later in life. Consequently, understanding the early interaction between the intestinal microbiota and the human body opens new avenues for important nutritional innovations, particularly for infants and young children. Human milk is the natural source of nutrition in early life, and exclusive breastfeeding is recommended for at least 6 months by WHO. In allergy, breastfeeding is thought to be protective because of both the presence of numerous allergens in human milk that are absent from artificial milks and their tolerogenic presentation due to human milk feeding-related factors such as antigen handling by maternal gut, allergens found in immune complexes in milk, the presence of tolerogenic immune mediators in milk, increased gut maturation and a microbiota favouring tolerance induction in breastfed infants (44). The latter is linked to the HMO, naturally present in human milk. Consequently, a significant number of studies have been performed with different types of prebiotic oligosaccharides, defined as non-digestible carbohydrates that reach the colon intact and are known for their ability to selectively stimulate the growth and or activity of intestinal bacteria that impact health positively as postulated by Gibson & Roberfroid (79). Interestingly, intervention with infant milk formulas containing a specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS, 9:1 ratio, 8 g/l) till 6 months of age reduced the risk of atopic dermatitis and some allergic manifestations in infants with a familiar history of atopy not only at 6 months, but also at two and 5 yr of age (80–82) and reduced the number of infectious episodes in healthy term infants (83) or infants with a high risk of developing allergy (80), underlining the importance of early nutrition on

infant health. Efforts to prevent or manage atopic dermatitis and food allergy may prevent the onset of other atopic manifestations such as allergic asthma later in life. A study applying synbiotics gives indications that this may actually be possible. The combination of scGOS/lcFOS (9:1) and *Bifidobacterium breve* M16-V in a 12-week intervention in infants around 5 months of age showed reduced severity of atopic dermatitis in a subgroup of infants with elevated IgE levels but not in the whole study group. However, at 1 yr of age, it was found that the synbiotic group showed attenuated use of asthma medication and lower prevalence of asthma-like symptoms in the whole study group suggesting long-term effects of the intervention early in life (84). Microbial analysis of the dominant bacterial groups affected in this 12-wk intervention showed an increase in bifidobacteria at the expense of mainly adult-type clostridial clusters XIV and clostridial clusters containing potential pathogens *C. difficil*e and *C. perfringens* (85). Establishing such infant-type microbiota and reducing the adult-type clusters may lead to a more gradual diversification, while, e.g., clostridial cluster XIV has been associated with atopic manifestations later in life (57). The exact mechanism of this synbiotic concept remains to be elucidated. Recently, induction of galectin-9 (a soluble-type lectin expressed by IEC exhibiting binding specificity for β-galactosides) by this synbiotic concept has been suggested to be involved in suppression of IgE-mediated allergy (86). Galectin-9 was shown to neutralize IgE and to induce Th1- and Treg-type immune responses and was indeed enhanced in serum of the synbiotic treated infants. The exact mechanism underlying induction of galectin-9 expression remains to be clarified; however, the synergy shown for the combination of scGOS/lcFOS and *Bifidobacterium breve* M-16V in enhancing serum galectin-9 levels in mice suggest a possible interaction between microbe-induced TLR signalling and direct interaction of scGOS/lcFOS with IECs. Recently, *in vitro* studies confirmed that galectin-9 is secreted by IEC apically exposed to TLR9 ligand (either synthetic or DNA derived from *B. breve* M-16V) in the presence of scGOS/lcFOS that is involved in inducing Th1 and Treg immune responses (87). These results give important mechanistic insights and may be a promising target to prevent or treat allergic disease.

Concluding remarks

Clearly, the first 1000 days in life are very important, since this is the period where we encounter external stimuli for the first time and the body is trained to respond to these stimuli. Longitudinal studies of this critical period are limited and include several confounding factors that complicate the identification of specific microbes associated with, e.g., atopic disease. In the light of the recent revolution of next generation sequencing technologies, we can gain important new insight how early-life events like type of feeding, mode of delivery, genetic background or geographic differences may interfere with the colonization pattern and therefore determine a predisposition to disease later in life. The challenge will be to go from taxonomic mapping to functionality of the microbiota. Omics-technologies, like transcriptomics, proteomics or metabolomics, will certainly catalyse our further understanding of the intestinal microbiota. Our genome is more or less fixed, but still the environment can have a major impact on the development. Processes like epigenetics are particularly interesting, and we are just starting to understand how DNA methylation and histone modification mechanisms can regulate gene expression and can confer phenotypical changes. And where our genome is fixed, we can still influence the epigenome and our microbiota. Knowing the importance of the intestinal microbiota for human physiology, the incredible development of infants in the first years of life and the concurrent colonization of the body with microbes makes it reasonable to believe that the intestinal colonization of early life may be very important for health also in later life. Whether immunologic, metabolic or neurologic, all these systems are developing at this period. Therefore, it is important to understand the impact of factors like early-life nutrition, but also the increase in caesarean deliveries or the increasing use of antibiotics. Disturbances in early life may lead to altered growth, immune diseases like allergy, metabolic diseases like obesity or cardiovascular diseases and maybe even brain and behavioural problems. Nutrition in early life and acquiring the essential microbes is probably a critical factor in this process.

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