Evolution of Human Microbiota


Microbial Composition of the Initial Colonization of Newborns

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Abstract
Early-life interaction with indigenous intestinal microbes is a prerequisite for healthy immune and metabolic maturation. Human infants acquire their gut microbiota predominantly from the mother. A considerable inoculum of microbes is received by the neonate during vaginal delivery. Recent observations suggest that human gut colonization may be initiated prenatally by microbes in amniotic fluid, but the significance of this phenomenon remains unknown. After birth, neonatal gut colonization is guided by human milk factors, which selectively promote the growth of specific microbes, as well as by live microbes present in human milk. Aberrant gut colonization in early life has been associated with an increased risk of noncommunicable diseases in later life. Epidemiological and experimental studies suggest a causal relationship between early-life gut microbiota perturbations and disease risk. Perinatal antibiotic exposure, cesarean section delivery, postnatal antibiotic administration, and formula feeding, which may disrupt intestinal microbiology, have been associated with disease development in later life. The modulation of gut microbiota in the perinatal period by pre- and probiotics, for example, may offer a means to reduce the risk of chronic diseases.

Introduction
At birth, the neonate enters a world inhabited by a myriad of microorganisms. While certain bacteria, viruses, and fungi represent a threat to health and well-being as potential pathogens, humans, among all other species on our planet, have evolved to live and thrive in an environment densely inhabited by
microbes. During the past decades, it has become apparent that rather than mere cohabitation, our existence in the microbial world takes the form of mutually beneficial symbiosis. Indeed, except for erythrocytes, every human cell contains mitochondria, which are thought to originate from bacteria trapped inside our eukaryotic ancestors to form an essential part of our energy metabolism. Furthermore, our skin and mucosal surfaces harbor complex indigenous microbiota, which appears to be specific to both the anatomical site and the individual. The microbial community in the gastrointestinal tract and particularly the distal gut is currently most comprehensively understood. The predominant species of the gut microbiota are not frequently encountered in the environment, and it is therefore apparent that we obtain our indigenous microbes from other humans and, for the most part, from the mother. The vertical microbial transmission and colonization of the infant gut is a stepwise process (Fig. 1) disturbances of which may have deleterious consequences on health in infancy and beyond [1].

The contribution of commensal microbes for healthy immune and metabolic maturation has been the subject of rigorous scientific research over the past decade. It has become apparent that aberrant composition of the indigenous gut microbiota during early life is associated with the risk of developing immune-mediated and inflammatory disorders, including atopic disease, inflammatory bowel disease, type 1 diabetes mellitus, and obesity [reviewed in 1]. Perinatal and early-life factors, which may affect neonatal gut colonization, have also been linked to the risk of disease development (Table 1) [1]. Consequently, elucidating the origin and optimal composition of the gut microbiota during the first days, weeks, and months of life may be assumed to have significant clinical relevance.

**Fig. 1.** The development of early gut microbiota.
Gut Colonization at Birth

The human neonate enters the extrauterine world through the birth canal, which is heavily populated with microbes. It is well established that maternal vaginal and intestinal microbes provide an important inoculum to the neonatal gut. Vaginal lactobacilli have been shown to transiently colonize the newborn intestine only to be replaced by bacteria from other maternal sources [2, 3]. The details on and the significance of this brief interaction with vaginal bacteria are not known. Based on studies comparing subjects born by vaginal or cesarean section (CS) delivery, it is evident that microbial contact during birth has a profound effect on gut colonization. In the immediate neonatal period, infants born by vaginal delivery harbor microbiota resembling that of the maternal vagina characterized by species belonging to *Lactobacillus, Prevotella, and Sneathia*, whereas subjects born by CS are colonized by bacteria typically found on the skin [3]. It is evident that the maternal gut is also an important source of colonizing microbes during vaginal delivery, since 72% of the early colonizers have been reported to match the species in the maternal gut in vaginally delivered newborns as compared to 41% in neonates born by CS [4]. The gut microbiota of vaginally delivered neonates are reportedly enriched by *Bacteroides, Bifidobacterium*,

| Table 1. Association between early gut microbiota composition and the risk of diseases |
|-----------------------------------------------|-------------------------------|
| Characteristics of early gut microbiota preceding disease | Evidence of causality |
| Atopic disease | ↓ Diversity  |
| | ↓ Bifidobacteria  |
| | ↓ Enterococci  |
| | ↑ *Escherichia coli*  |
| | ↑ *Clostridium difficile*  |
| | Experimental animal models  |
| | ↑ After early AB  |
| | ↑ After CS  |
| | ↓ By probiotics  |
| Obesity and overweight | ↓ Bifidobacteria  |
| | ↑ *Bacteroides fragilis*  |
| | Experimental animal models  |
| | ↑ After AB  |
| | ↑ After CS  |
| Necrotizing enterocolitis | ↓ Fimbrites  |
| | ↑ Proteobacteria  |
| | ↑ Clostridia  |
| | Experimental animal models  |
| | ↑ After early AB  |
| | ↓ By breast milk  |
| | ↓ By probiotics  |
| Infantile colic | ↓ Diversity  |
| | ↓ Bifidobacteria  |
| | ↓ Lactobacilli  |
| | ↑ Proteobacteria  |
| | ↓ By probiotics  |

AB, antibiotic administration; CS, cesarean section.

Parabacteroides, and Escherichia/Shigella species in contrast to those born by CS, in whom bacteria typically encountered in the skin and mouth as well as the environment are frequently detected [4]. Later in infancy, infants born by CS reportedly display low bacterial richness and diversity [5] and delayed colonization by Bacteroidetes [6]. Differences in gut microbiota composition between vaginally and CS-delivered children have been detected until the age of 7 years [7].

There is compelling epidemiological evidence to suggest that birth by CS is associated with a significantly increased risk of obesity [8] and immune-mediated diseases such as asthma, inflammatory bowel disease, and arthritis in later life [9]. It is plausible that these detrimental long-term health effects of CS are at least partially attributable to aberrant gut colonization patterns, since the gut microbiota composition in early life has also been associated with the development of these disorders [1]. Still, it is conceivable that hormonal and immuno-modulatory exposure during labor may also modulate disease risk in the offspring, or that confounding factors, such as maternal obesity, increase both the likelihood of CS as mode of birth and the risk of disease in the next generation. Meticulously conducted epidemiological studies and meta-analyses are needed to address these issues. It has recently been shown that the increase in the risk of asthma is particularly pronounced following CS conducted before rupture of membranes [10], which may possibly suggest a causal role for microbes in the process.

After the recognition of the significance of the microbial contact during delivery for the development of a healthy immune system and the risks associated with CS, an intriguing notion of inoculating maternal vaginal microbes to the neonate has been introduced [11]. The procedure is based on the general hypothesis that seeding vaginal microbes to the newborn infant directly following birth by CS might result in early colonization resembling that of vaginally delivered neonates and, consequently, reduce the risk of long-term health problems related to CS. The vaginal seeding procedure naturally also carries the risk of transmitting pathogenic microbes such as group B streptococci or the herpes simplex virus to the neonate, and measures need to be taken to minimize these risks. Furthermore, as discussed above, the neonate is also exposed to maternal intestinal microbes during vaginal delivery, and the significance of vaginal microbes for infant gut colonization remains largely unknown. Nonetheless, an interesting report from a preliminary study of 18 infants has recently been published [11]. In the 4 neonates inoculated with maternal vaginal microbes after CS delivery, the anal, oral, and skin microbiota of the infants at 1 month of age appeared to resemble that of vaginally delivered subjects more closely than infants born by CS without inoculation. Whether these changes in
early colonization are associated with long-term differences in the indigenous microbiota or improved health outcomes remains to be determined by larger clinical trials.

Is the Fetal Gut Colonized by Microbes?

Several independent reports indicate that meconium, the first stool passed by the neonate but formed during fetal life, is not sterile [reviewed in 1]. Bacteria belonging predominantly to the phylum Firmicutes as well as species representing the genera *Enterobacterium*, *Bifidobacterium*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and *Enterococcus* have been detected in low abundance in meconium using both traditional culture methods and culture-independent molecular techniques [12, 13]. The origin of the bacteria in meconium is currently not known. It is possible that the bacteria detected in meconium are not present in the intestine in utero but introduced at or after birth or even after passage. Nonetheless, data from experimental animal studies indicate that the fetal mouse gut harbors viable bacteria [14]. The intrauterine origin of the microbes in meconium is also corroborated by data indicating that the duration from rupture of membranes before delivery or the time from passage to analysis does not affect meconium bacterial counts [15].

It has been suggested that the bacteria in meconium are derived from amniotic fluid swallowed during fetal life [16]. This notion was originally suggested based on a comparison of microbial communities detected in meconium and previous reports on microbiota in amniotic fluid and at other sites [16]. We have recently provided data demonstrating similarities between meconium and amniotic fluid microbiota from the same mother-neonate pairs [13]. The amniotic fluid samples in the study were collected during sterile elective CS delivery to minimize the possibility of contamination. Several bacterial genera, including *Bacteroides*, *Lactobacillus*, *Prevotella*, and *Peptostreptococcus*, were detected in both amniotic fluid and meconium [13]. If the hypothesis of fetal gut colonization is further corroborated, the dogma of the sterile intrauterine compartment needs to be revised.

Small but detectable numbers of bacteria have been reported in the amniotic fluid and the pregnant and nonpregnant uterus outside the context of clinical infection using both conventional culture and molecular methods [1, 13]. A distinct microbiota dominated by enterobacteria has been reported in the placenta by Aagaard et al. [17]. Given the novelty of the hypothesis of a microbial community in the intrauterine compartment, the possibility of artifact or contamination needs to be carefully ruled out. False signals originating from...
the contamination of the reagents used in DNA purification may be problem-
atic particularly when analyzing samples with very low microbial abundance. A
recent study using quantitative PCR failed to detect consistent results distin-
guishing placenta samples from controls [18]. In contrast, we have published
data corroborating the presence of microbes in both placenta and amniotic
fluid using both conventional culture and denaturing gradient gel electropho-
resis PCR and sequencing of the 16S rRNA gene [13]. To date, the bacterial
genera detected in amniotic fluid or placenta include among others Propioni-
bacterium, Enterococcus, Staphylococcus, Citrobacter, and Lactobacillus [13,
19]. Furthermore, intriguing experimental animal studies demonstrate that la-
beled Enterococcus faecium introduced into pregnant mice may be detected in
the fetal gut [14]. These data suggest bacterial transfer from the mother to the
fetal intestine, but our understanding of prenatal bacterial contact and its po-
tential significance for subsequent gut colonization or later health is still virtu-
ally nonexistent.

Gut Microbiota in the Neonatal Period and Early Infancy

Human Milk as a Modulator of Gut Colonization

After birth, the most significant modulator of neonatal gut colonization is breast
milk. In healthy newborns, initial neonatal gut microbiota characterized by
Escherichia coli, enterococci, streptococci, and clostridia is rapidly followed by
anaerobes including Bifidobacterium and Bacteroides species [20–22]. It is well
established that the gut microbiota of breastfed infants is dominated by bifido-
bacteria [23, 24], which may be detectable already during the first days of life. In
contrast, formula-fed infants harbor a more diverse gut microbiota [25] resem-
bling that of older children. Breast milk contains a large array of nondigestable
oligosaccharides (human milk oligosaccharides, HMO), one of the functions of
which is to selectively promote the growth of specific intestinal bacteria and par-
ticularly bifidobacteria [reviewed in 26]. Bifidobacterium longum subspecies in-
fantis is capable of utilizing a variety of HMOs [27] and, consequently, B. longum
is almost universally detectable in the stool of breastfed infants from various
geographical locations, including Northern Europe, Brazil, and Malawi [28, 29].
The role of HMOs in modulating gut colonization and human health is exten-
sively reviewed elsewhere in this volume. It is of note, however, that in addition
to HMOs, breast milk contains glycoproteins, which may also act as a source of
selective substrates for specific bifidobacteria [30].

Human milk has been demonstrated to harbor a unique microbial commu-
nity the composition of which is modulated by factors such as obesity, maternal
immune-mediated disease, and mode of birth [reviewed in 31]. The origin of the bacteria in human milk remains elusive, but even the nonlactating mammary gland is reportedly colonized by bacteria [31]. It is likely that many of the microbes detected in milk originate from the skin. It is of note, however, that certain microbes typically detected in human milk, including lactic acid bacteria, are also characteristic of the human gut microbiota. Intestinal origin of milk microbes is suggested by observations according to which maternal intestinal microbes may be detected in peripheral blood immune cells and breast milk during lactation [32]. Based on these data, it has been suggested that maternal gut permeability is increased during lactation and that specific intestinal microbes are transported to the mammary gland by immune cells [32]. Consistently with this notion, the probiotic *Lactobacillus reuteri* has been detected in breast milk after maternal consumption in a clinical trial [33]. The physiological function of the bacteria in breast milk is not well understood, but it is possible that they function as a source of colonizers to the neonatal and infant gut. In accordance with this hypothesis, the neonatal gut microbiota shifts to bear resemblance to the microbial community in maternal milk during the first week of life [13], and there are data suggesting that breast milk microbes are transferred to the neonatal gut [34].

From an evolutionary point of view, the energy investment on HMOs on the mother’s part, the profound impact of breastfeeding on early gut microbiota composition, and the unique predominance of bifidobacteria in the gut microbiota during breastfeeding are likely to provide a survival benefit for the infant. In line with this notion, epidemiological studies have linked disturbances in early gut microbiota composition and particularly reduced numbers of bifidobacteria to an increased risk of developing immune-mediated or inflammatory disorders such as atopic disease and obesity [reviewed in 1] (Table 1). Moreover, there are data to suggest that breastfeeding exerts a protective effect against a number of chronic, noncommunicable diseases [reviewed in 35]. It is possible but not proven that some of these beneficial effects of breastfeeding may result from the promotion of intestinal bifidobacteria.

**Antibiotic Exposure**

Exposure to antibiotics is known to exert a major effect on intestinal microecology and is relatively often followed by the development of diarrhea. It is alarming that antibiotic exposure in early life may also be associated with an increased risk of noncommunicable diseases, including asthma and obesity, in later life [reviewed in 1, 36]. While it is difficult to dissect the causal relationships between antibiotic exposure, the infections against which the antibiotics have been administered, and the development of chronic disease, based on both
epidemiological and sophisticated experimental data, it has been suggested that aberrant gut microbiota composition resulting from early antibiotic exposure plays a causal role in the pathogenesis of obesity [reviewed in 36]. Given the significance of the neonatal period in gut colonization discussed above, it is of note that antibiotics are administered to 33–39% of mothers during delivery to prevent bacterial infection in the mother and the neonate [37, 38]. To date, relatively little is known about the effect of perinatal antibiotic exposure on gut colonization. A study based on 84 mother-infant pairs suggests that antibiotic prophylaxis administered because of maternal colonization with group B streptococci is associated with lower numbers of bifidobacteria detected by quantitative PCR in neonatal stool samples at the age of 7 days [39], but no differences were observed at the age of 30 days.

Early-onset neonatal sepsis is a devastating disease initially presenting with nonspecific symptoms or signs often followed by rapid deterioration. According to current guidelines, all infants with signs or symptoms suggesting sepsis as well as certain asymptomatic individuals with a high risk of infection based on the presence of factors such as chorioamnionitis should be subjected to empirical antibiotic therapy [36]. A large proportion of neonates (approximately 5%), therefore, receive broad-spectrum antibiotics during the first days of life [38].

Neonatal exposure to antibiotics has been reported to result in an increase in fecal Proteobacteria and particularly Enterobacteriaceae during the first weeks of life [40–42]. In addition, significant decreases in microbial diversity and in the abundance of *Bifidobacterium* have both been reported in neonates subjected to antibiotic interventions [40]. Our unpublished data suggest that gut microbiota perturbations caused by neonatal antibiotic exposure persist at least until the age of 6 months, but whether perinatal antibiotic exposure has an impact on the risk of disease in later life is currently not known.

**Prematurity**

Preterm newborns treated in neonatal intensive care units exhibit an aberrant gut microbiota composition in early life. Low diversity of the gut microbiota and delayed colonization with bifidobacteria have been associated with being born preterm [43, 44]. These disturbances may result directly from intestinal and immunologic immaturity. On the other hand, detrimental exposures including CS delivery, formula feeding, and early antibiotic exposure tend to cluster in preterm infants. Our unpublished observations suggest that while mode of birth, antibiotic exposure, and formula feeding, all have an impact on fecal bifidobacteria in late preterm infants, prematurity per se independently modulates *Bifidobacterium* colonization. Aberrant gut colonization in preterm infants has
been suggested to be causally related to the risk of developing necrotizing enterocolitis [45] (Table 1), but whether the increase in metabolic risk factors associated with preterm birth is mediated by perturbations of early gut colonization remains unknown.

Conclusions

The composition of the early human gut microbiota has been associated with the development of noncommunicable diseases in later life [reviewed in 1]. Both reduced diversity and altered abundance of specific members of the intestinal ecosystem have been linked with various disorders ranging from atopic disease and obesity to infantile colic and necrotizing enterocolitis (Table 1). In addition, perinatal factors including CS delivery, antibiotic exposure, and formula feeding have been linked to both aberrant gut colonization patterns and an increased risk of chronic diseases later in childhood (Fig. 1; Table 1). Data from clinical and experimental studies may be interpreted to suggest that the link between early gut microbiota composition and disease risk may at least in part be causal, but our understanding of the interactions between the indigenous microbial community and ourselves, the host, are by no means complete. It is to be hoped that rigorous basic, translational, and clinical research will unravel these complex phenomena.

The potential causal role of early gut microbiota composition in the development of disease underscores the importance of supporting healthy gut colonization by reducing CS rates, prudent use of antibiotics, and promotion of breastfeeding. In addition, the intriguing possibility of reducing disease risk by modulating early microbial contact by prebiotics or probiotics deserves to be assessed. Thus far, the most convincing scientific evidence has been obtained from studies assessing the efficacy of early probiotic interventions in reducing the risk of atopic dermatitis [46] and necrotizing enterocolitis [47] in high-risk infants. Future studies will show whether prebiotics or probiotics are effective in the prevention of other chronic conditions, including obesity.

Disclosure Statement

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References


