Manipulation of the Intestinal Microbiome in Newborn Infants¹,²

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ABSTRACT

The mammalian gastrointestinal tract harbors a highly diverse microbial population termed the microbiome, which plays a major role in nutrition, metabolism, protection against pathogens, and development of the immune system. It is estimated that at least 1000 different bacterial species cohabit the human intestinal tract. Herein we provide a brief review of the developing intestinal microbiome, with the understanding that its development often begins before birth and that disturbance in the microbiome during fetal life, birth, and shortly thereafter may result in adverse consequences. Postnatally, numerous environmental factors including premature delivery, mode of delivery, antibiotic usage, and diet can play an important role in how the intestinal microbiome of infants is shaped. The fact that human milk contains microbes is likely to have important ramifications. We discuss where these microbes come from and their potential role.

The Developing Microbiome

With the advent of the Human Microbiome Project, where new high-throughput technologies are used to evaluate the taxonomy and function of populations of microbes residing in various regions of the body, there is increasing recognition of the scope of the intestinal microbiota. The total number of bacterial cells residing in the human intestine far exceeds the number of host cells. More than 3 million genes have been identified in this “microbiome,” which is >100-fold of our own human genes (1). But interestingly, most of the microbiome in the human intestine is restricted to 4 dominant phyla: Firmicutes, Bacteroides, Actinobacteria, and Proteobacteria.

Although commonly thought to be related to disease as pathogens, the majority of intestinal microbes are commensals and symbionts that provide beneficial effects in terms of nutrition, development of the immune system, and postnatal maturation of the intestine. They perform functions that cannot be performed by the human host itself.

The amniotic fluid (AF)³ from mothers of infants born prematurely is often colonized by microbes. The quantity of microbial DNA correlates inversely with gestational age, and directly with IL-6 and white blood cells in AF (2,3). Although evidence supports that the majority of microbes found in AF are of vaginal origin, seeding the AF via translocation through the chorioamniotic intracellular junctions (4), other origins such as maternal intestinal translocation or hematogenous spread from periodontal tissue are also possible. Figure 1 shows a broad depiction of the most commonly studied modes of transfer of microbes from mother to infant. The infant’s ultimate microbiome is influenced by many factors, which can be categorized into prenatal, perinatal, and postnatal periods (Table 1).

The fetus swallows large quantities, ~400 mL/d during the last trimester of pregnancy, of such microbe-containing AF (6–8), which subsequently reaches the distal small intestine, an organ that was initially found in rodents and later in humans to be significantly more sensitive to inflammatory stimuli than postnatal infant intestine (9). An injection of endotoxin or IL-1 into AF invokes a strong inflammatory response in the ileum of the 125-d ovine fetus (10,11). The fetal intestine responds to bacterial components such as LPS more robustly than to mature intestine when isolated in culture (2). Fetal intestinal inflammation and fetal inflammatory response syndrome induced by ureaplasma is blunted with intraamniotic injection of IL-1β receptor antagonist (IL-1RA) (10,11). Thus, the fetal intestine is a likely origin of fetal inflammatory response syndrome that is responsible for triggering preterm labor.
AF is difficult to obtain routinely for the purpose of research investigations. However, meconium and infant stools are readily available, and collection is noninvasive. The first stool in the newborn (meconium) reflects the in utero fetal intestinal environment. If microbes in AF enter the intestine of the fetus and cause an inflammatory response, then microbial remnants as well as some of the markers of inflammation would be expected to be present in the meconium of these infants. Indeed, in our ongoing studies in premature infants at risk of necrotizing enterocolitis (NEC), we found significant quantities of microbial DNA in the meconium of these infants (12). We speculate that a relation exists between the fetal microbiome as evaluated in meconium and spontaneous preterm labor. The mechanisms of this relation remain poorly understood, but research in the next few years is likely to yield information that should lead to microbe-host based interventions for prevention.

In a recent review it was shown that mode of delivery, cesarean section or vaginal, is thought to play a role. In the United States, the rate of cesarean delivery (CD) has increased by 48% since 1996, reaching a level of 31.8% in 2007 (13). This trend is reflected in many parts of the world, with the most populous country in the world, China, approaching 50% (14), and some private clinics in Brazil approaching 80% (15). Although a significant number of CDs are performed for obstetric indications, some are simply due to maternal request or convenience for the physician and family. In some cases, CD is due to wanting a child born on a certain date thought to be auspicious. Concurrent with the trend of increasing CDs, there has been an epidemic of autoimmune diseases such as type 1 diabetes, Crohn disease, and multiple sclerosis and allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis (16,17).

The mechanism of this relation between CD and increased risk of these diseases remains poorly understood. However, during vaginal delivery, the contact with the maternal vaginal and intestinal microbiota is an important source for the start of the infant’s colonization. During CD, this direct contact is absent, and nonmaternally derived environmental bacteria play an important role in infants’ intestinal colonization (18,19). There is accumulating evidence that intestinal bacteria play an important role in the postnatal development of the immune system (20). Thus, the development of the immune system depends on the intestinal microbiota, which, if altered from its usual condition due to CD rather than vaginal birth, may incur aberrations in its normal development.

Obesity has also been shown to be more prevalent in infants born by CD (21). Whether this is due to differences in intestinal microbiota remains speculative, and investigations are ongoing to determine potential mechanisms (22).

### The Microbiome in Late-Onset Sepsis and NEC

Late-onset sepsis (LOS) is typically defined as the identification of pathogenic organisms from blood culture acquired...
after the third day of life and represents a common complication of prematurity (23). Many of these cases of LOS involve direct bacterial translocation (24,25). In 1 study in premature infants, stool samples were obtained weekly and 16S ribosomal RNA was amplified by high-throughput pyrosequencing, comparing participants with and without sepsis (26). The meconium in these infants was not sterile and had a predominance of *Lactobacillus, Staphylococcus*, and *Enterobacteriales*. Infants who developed LOS began life with low microbial diversity and acquired a predominance of *Staphylococcus*, whereas healthy infants had more diversity and predominance of *Clostridium, Klebsiella*, and *Veillonella*.

In another study, a matched case-control analysis identified microbiota differences in very premature infants with LOS (27). A higher proportion of organisms in the *Firmicutes* phylum in LOS cases were detected 2 wk before diagnosis. These interesting distortions before the development of LOS are intriguing, but whether they represent causation and the actual mechanisms requires further investigation.

NEC is among the most common and devastating diseases in neonates (28). The excessive inflammatory process initiated in the highly immunoreactive intestine in NEC extends the effects of the disease to distant organs such as the brain and places affected infants at substantially increased risk of neurodevelopmental delays (29,30). A microbial dysbiosis appears to be involved in most of the cases of NEC seen in preterm infants. In studies by our group (12,13), a significant increase in * Proteobacteria* and a decrease in *Firmicutes* in NEC cases were detected between the first week and <72 h before the onset of NEC.

Although providing the taxonomy of microbes before the onset of a disease suggests an association between these microbes and the disease, this does not prove causality. One recent study used metabolomics to evaluate effects that may be incurred by an altered microbiome and found that urinary metabolite analysis showed a high urinary alanine:histidine ratio that was associated with the microbial characteristics seen in NEC patients (31). It is possible that this and similar metabolic findings may serve as early biomarkers for the development of the disease.

**Human Milk as a Source of Microbes**

Before the 1900s, human milk was the only source of infant nutrition. It is thought to be the ideal food for the majority of infants, with a composition commensurate for optimizing infant health. The protective role of human milk seems to be the consequence of a synergistic action of the wide range of health-promoting components such as carbohydrates, nucleotides, FAs, immunoglobulins, cytokines, immune cells, lysozymes, lactoferrin, and other immunomodulatory factors (32). Formula and donor breast milk contain many ingredients similar to an infant's own mother's breast milk, but most notably, because of heat treatment, they both lack live immune cells and microbes that provide the infant both direct and indirect protection against disease (33).

It is widely accepted that the intestinal microbiota of breast-fed infants provides anti-infective properties and is an important stimulating factor for the postnatal development of the immune system. The synergy provided by the interaction between the contents of human milk amplifies the health benefits, specifically with increased absorption and immune stimulation. In contrast, mixing ingredients into formula does not guarantee they will act together the way they do in human milk. Human milk also contains a group of bacteria called the human-milk microbiome (34). The bacteria found in human milk were initially perceived as harmful to the mother and infant. With the advent of culture-independent techniques and the development of the “omics” approaches, the concept of the human-milk microbiome has developed further. Culture-dependent techniques can identify from 2 to 18 different bacterial species in an individual milk sample. Some of the most commonly cultured species include *Staphylococcus epidermidis, Staphylococcus aureus, Lactobacillus salivarius, Lactobacillus fermentum, Bifidobacterium breve*, and *Bifidobacterium bifidum* (34). Two of these bacterial groups, *Lactobacillus* and *Bifidobacterium*, are recognized to have health-promoting properties and are present in commercial foods such as yogurt and used in pharmaceutical probiotics to enhance protective gut microbiota, thus improving intestinal microbial balance.

The newer culture-independent techniques using 16S ribosomal RNA have allowed a more comprehensive assessment of the bacterial diversity of human milk (34–36). These culture-independent techniques have identified additional bacteria such as gram-negative bacteria not identified by the culture-dependent techniques. The human-milk microbiome is thus a complex ecosystem with a greater diversity than previously anticipated. Although many bacterial variations exist in different samples of breast milk, it has been discovered that there are a few core bacteria that are present consistently in most milk samples in different lactating mothers. In a study by Hunt et al. (35), which longitudinally analyzed breast-milk samples from 16 healthy women, there were 9 bacterial genera consistently present in all samples. These included *Streptococcus, Staphylococcus, Serratia, Pseudomonas, Cornybacteria, Ralstonia, Propionibacterium, Sphingomonas*, and *Bradyrhizobiaceae*. Although these bacteria were present in all samples, the percentages of each varied between the lactating women in the study. Further studies revealed that human milk has a microbiome that tends to be stable over time, and it is highly personalized. Hunt et al. (35) did not find *Lactobacillus* or *Bifidobacteria*. These differences from previous studies suggest technical limitations with some of the molecular techniques to study bacterial communities. Research that evaluates the functional metagenome of human milk shows the presence of immunomodulatory motifs that may be important in gut colonization and immunity (37). Furthermore, the composition of microbes changes over time and is influenced by several factors, including maternal weight, that skew its composition (38).
Despite the convincing evidence of a human-milk microbiome, the mechanism by which bacteria reach the mammary gland remains uncertain. Three hypotheses have been proposed (34). The first hypothesis is that hormonal changes during pregnancy cause the gastrointestinal tract of the mother to be more permeable, which facilitates bacterial uptake by the bloodstream, which transports the bacteria to the mammary gland. The second hypothesis is known as the contamination theory. This postulates that human-milk microbes come from direct contamination of the mother’s skin and oral secretions from the infant. The third hypothesis involves active migration of bacteria with the aid of dendritic cells. This final hypothesis is thought to be the most likely mechanism based on the current research and is important for future research that may lead to ways to manipulate the transport of certain favorable bacterial strains from the maternal gut to improve maternal and infant health.

Clinical Implications

Currently, the American Academy of Pediatrics Committee on Breastfeeding recommends that all preterm infants receive human milk (39). According to these recommendations, a mother’s own milk, fresh or frozen, should be the primary diet and should be fortified appropriately for infants born weighing <1.5 kg. If the mother’s own milk is unavailable despite significant lactation support, pasteurized donor milk should be used (39). Currently in level 3 neonatal intensive care units, preterm infants receive enteral feeds with mother’s own milk, donor milk, or formula. A Cochrane review suggested that donor breast milk is associated with a lower risk of NEC but slower growth in the early postnatal period, but the quality of the evidence is limited (40). Further research is needed to confirm these findings and measure the effect of fortified or supplemented donor breast milk (40). Whether the lack of bacteria in donor milk may measure the effect of fortified or supplemented donor breast milk remains speculative.

There is a broad consensus that the intestinal microbiota plays an important physiologic role for the host. This has significant implications for very low birth weight infants. The neonatal microbiome is likely to be influenced by the human-milk microbiome, and if we can optimize beneficial microbes this may lead to numerous benefits including decreased mortality, less antibiotic use and thus less antibiotic resistance, decreased rates of NEC and sepsis, and both direct and indirect cost savings.

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Literature Cited


