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Invited Article

The Perinatal Microbiome: Implications for Future Health

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Abstract The importance of the association between the human host and microbes during early life is receiving increased attention because this relationship is crucial to subsequent health. Recent studies have demonstrated that the maternal-fetal unit is not sterile and microbes are present in placenta, amniotic fluid, as well as the babies' first stools, the meconium. The interactions between the mother, fetus and the microbes continue after the infant leaves the uterus via breast-feeding by the mother, as well as close postnatal contact. We are just beginning to discover that perturbations of this relationship by antibiotics and various other drugs, maternal and neonatal diet, may result in life-long consequences. This article offers a brief introduction and should provide an appreciation of these early host-microbial interactions as they pertain to subsequent health and disease.

Key words Development; Fetus; Microbiome; Neonate

Introduction

The notion that the microbes are essential to human health is becoming increasingly recognised, especially during early childhood. Furthermore, the widely accepted concept that the fetus resides in a sterile environment and acquires a set of associated microbes only after birth is now recognised as incorrect.[1,2] Recent studies have demonstrated microbes present in placenta,[3] amniotic fluid,[4,5] as well as the babies' first stools, the meconium.[6-8] Furthermore, microbes residing in various niches of the mother, such as her gastrointestinal tract, mouth, vagina, and skin may also affect the fetus in various ways. The fetal maternal unit should no longer be considered simply a dyad but a triad consisting of a microbial-maternal-fetal "holobiont".[9] The interactions between these three components are continued after the infant leaves the uterus via breast-feeding by the mother, as well as close postnatal contact.[10] Here we will discuss some of the prenatal, perinatal and postnatal microbial interactions that may affect subsequent health.

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The Microbiome during Pregnancy

It has been known for several decades that even without ruptured amniotic sac, there are significant levels of bacteria present in amniotic fluid.[11] Whether these microbes are present as commensals and symbionts rather than pathogens has not been extensively evaluated, but is likely to be the case. The fact that the microbes are present in meconium[6-8,12] supports the notion that these microbes may be acquired through swallowing of amniotic fluid[13] that contains these microbes. The origin of the microbes found in the fetus, amniotic fluid and placenta is not clear, but where some studies suggest they are of vaginal origin,[14] others suggest a maternal oral origin,[3] and still others suggest an origin from the maternal gastrointestinal tract.[15]

Studies evaluating meconium of preterm versus term infants have demonstrated that the taxonomy of these microbes differ depending on gestational age.[7,8] These microbes exhibit greater similarity to the taxonomy of microbes in amniotic fluid than in the vagina.[7] The fact

that these microbes differ depending on gestational age suggests an association between the types of microbes present and potential causality for preterm delivery. Although many cases of preterm delivery can be traced to anatomic or related problems, a relationship between inflammation and the induction of "spontaneous" preterm labor has clearly been demonstrated.[16]

The maternal gastrointestinal tract harbors a vast variety of microbes. These microbes can exert immunologic as well as metabolic roles. Recent studies have shown that the maternal gastrointestinal microbes differ depending on the stage of pregnancy.[17] When microbes from the pregnant mother in the first trimester are inoculated into germ-free animals a normal phenotype is subsequently exhibited.[17] However, if microbes from the third trimester are inoculated into the gastrointestinal tract of germ-free animals, these animals subsequently develop a phenotype of insulin resistance and obesity similar to the metabolic profile of the mother during the third trimester of pregnancy.[17] There is evidence to suggest that microbes in the amniotic fluid and fetal gastrointestinal tract can be derived from the maternal gastrointestinal tract via translocation of bacteria.[18] Studies in rodents have shown that labeled microbes derived from breast milk inoculated into a pregnant mouse can be found in the amniotic fluid of the fetus and subsequent studies have also shown that these microbes can be found in the meconium of the animal inoculated with these, labeled microbes.[12]

It has been demonstrated that numerous circulating molecules found in the blood arise exclusively in the presence of gut microorganisms.[19] The fact that microbes produce large quantities of metabolically active substances that can pass into the maternal blood and can then subsequently affect the fetus is an area that is not very well investigated. The interaction of microbes with the maternal gastrointestinal mucosal immune system during pregnancy may also exert significant distal effects on the mother's neuroendocrine system, as well as the placenta, thereby resulting in "programming" effects on the fetus.[20] This is another area that is ripe for investigation.

Perinatal Factors

Cesarean section versus vaginal delivery may play a significant role in the development of the infants' intestinal microbial environment. This is especially important given the fact that cesarean section deliveries have been increasing markedly in various geographic locations. It is likely that this high rate of cesarean section delivery is not all due to medical indications and may actually result in health issues for the individual as they mature. For example epidemiologic studies have suggested greater odds of certain illnesses such as celiac disease, type I diabetes and inflammatory bowel conditions in individuals who were born by cesarean section versus vaginal delivery.[21] Other studies have suggested a significant increase in the odds ratio of development of obesity in individuals who were delivered by cesarean section.[22] This is a very difficult area to evaluate a major role of mode of delivery because of confounding factors such as the high rate of antibiotic usage associated with cesarean section versus vaginal delivery. It is, however, clear that microbial differences do exist and these may persist years[23-25] after birth.

From the perspective of the development of obesity as well as some of the autoimmune diseases and allergic diseases that are seen after C-section versus vaginal delivery, pathogenic mechanisms for these have not yet been elucidated. Whether short chain fatty acid, folate, amino acids and other potentially epigenetically active metabolites may play a role still is under investigation. Whether the interaction of the microbes with the intestinal mucosal immune system, where they may induce T-cell effector versus a tolerising regulatory response provides the foundation for hypotheses that remain to be tested.

The Preterm Infant Microbiome

Several studies have evaluated the intestinal microbial environment of preterm infants during their hospital stays and have also evaluated the microbial ecology of the gastrointestinal tract of these infants prior to the development of the diseases such as necrotising enterocolitis (NEC) and sepsis.[6,26-31] The microbial ecology of the gastrointestinal tract of the preterm infant depends at least partially on the initial keystone colonisers which may actually be present prior to delivery[7,32] and in some studies have shown to be important in playing a role in the development of subsequent sepsis.[33] The microbial environment of the preterm stools infant's has been shown to differ prior to the development of NEC[34] and late onset sepsis.[30] Although no specific single microbes has been found to differ prior to the development of the NEC, there appears to be an imbalance between the Proteobacteria compared to the Firmicute phyla and shifts have been seen in these major phyla prior to the development of NEC.[29,34,35] Additional studies are needed to clarify these responses and

to evaluate not only this as an association but also to evaluate causality.

Medications Used in Prenatal and Neonatal Care

The widespread use of antibiotics in mothers who do not have rupture of membranes threatening to deliver a baby preterm should raise concern, especially since several studies do not support their benefit in prevention of preterm delivery or other neonatal outcomes.[36] Evidence also suggests that intrapartum antimicrobial prophylaxis, even though having significantly reduced the incidence of early-onset neonatal infection, the risk of amoxicillin-resistant *E. coli* infection was significantly increased.[37] The widespread use of the antibiotics in preterm neonates shortly after birth is also of concern. Commonly used reasons for this widespread use of antibiotics[38] is that one cannot tell the difference between respiratory distress syndrome, pneumonia, or other causes of respiratory distress. Another reason is that chorioamnionitis is a common antecedent for preterm delivery, but many of the so-called cases of "chorioamnionitis" are diagnosed simply based on fever in the mother rather than by true histologic criteria. The actual prevalence of positive blood cultures in those very preterm infants who are treated with antibiotics in the first couple of days in neonatal intensive care units related to preterm delivery is only approximately 2%.[39] Thus, many infants are treated with antibiotics that probably do not benefit from them. Antibiotic use in preterm infants has been related to NEC with an increased odds ratio that correlates with the days of antibiotic usage.[40,41] Even one dose of antibiotic given to a mother can alter the intestinal microbial environment of the newborn at 30 days after delivery.[42]

There are several other pharmacologic agents that may affect the developing newborn. Included among these are of the antacid agents such as proton pump inhibitors and the histamine 2 (H2) blockers. Use of the H2 blockers has been associated with increased NEC, death and subsequent infections in preterm infants.[43] Related to this are studies showing that there are major shifts in the phyla of intestinal microbes in the gastrointestinal tract of these preterm infants related to the use of these H2 blockers and proton pump inhibitors.[44] Thus the acid-base environment of the preterm infant's gastrointestinal tract induces alterations in the microbial ecology that has detrimental consequences.

Although not extensively investigated in the human preterm, the lack of enteral feeding and provision of total parenteral nutrition (TPN) may also alter the gastrointestinal microbial environment. Studies in animals show a higher predominance of Proteobacteria compared to Firmicutes in animals given TPN versus enteral feedings.[45] The Proteobacteria include a high prevalence of pathogens such as *E. coli*, *Klebsiella Pseudomonas*, etc., which may have detrimental consequences in the gastrointestinal tract including increased permeability. Such increased permeability can subsequently lead to greater stimulation of the highly immunoreactive sub epithelium of the intestine, which can result in systemic inflammation which can affect all organ systems of the body.

Summary

We are beginning to recognise that the newborn does not arise from a sterile intrauterine environment and actually acquires a microbiota prior to birth. The effect of acquisition of some of these very early microbes is not fully understood, but they may exert effects for the lifetime of the individual. This is an exciting time to study the microbiota in the pregnant mother, fetus and newborn largely because there is potential for learning not only direct effects of the microbes on the host, where microbe-derived epigenetically active metabolites may affect the host as well as subsequent offspring. Much is being learned but we are still just scraping the tip of the iceberg when it comes to studies of the microbiota during early life and its effects on subsequent health and disease.

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