Review Article

Development of the Neonatal Intestinal Microbiome and Its Association With Necrotizing Enterocolitis

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ABSTRACT

Purpose: Neonatal necrotizing enterocolitis (NEC) remains the most devastating gastrointestinal disease for premature infants. In the United States alone, NEC affects >4000 premature infants yearly, has a mortality rate of nearly 33%, and costs the health care system > $1 billion annually. Although NEC has been actively researched for several decades, its pathophysiology remains elusive. One potential mechanism suggests that disruption of the normal neonatal intestinal bacterial flora induces a proinflammatory state, allowing translocation of pathogens across the intestinal epithelia. Disruption of the normal intestinal flora (dysbiosis) is associated with many human diseases. Thus, it is a reasonable hypothesis that dysbiosis may play an important role in the development of NEC. This hypothesis is supported by evidence that probiotic use in premature infants can prevent the development of NEC. Although the role of probiotics and NEC is covered in other reviews, this review instead focuses on normal bacterial colonization in both term and preterm infants and on the association of dysbiosis and the development of NEC.

Methods: PubMed was queried with the use of the following key search terms: NEC, neonatal microbiome, fetal microbiome, maternal microbiome, neonatal dysbiosis, and microbiome ontogeny. Relevant literature was reviewed and selected for inclusion in accordance with the objectives of the article according to the authors' discretion. Articles that made key salient points in review articles were further pulled from PubMed.

Findings: Although the onset of NEC is thought to involve bacteria, the mechanisms behind their involvement remain unclear. Research to date has failed to identify a single causative organism, and current theories and data now indicate that a disruption of the host intestinal flora is associated with the onset of disease. Recent reports have found that a bloom of Proteobacteria, specifically Enterobacteriacae species, occurs just before the diagnosis of NEC. Whether this is a causative event or merely a marker of intestinal disease is still unclear.

Implications: Because of the complexity of these interactions, it is vital that we continue to investigate the host-bacterial axis in the developing intestine in both humans and in animal models. (*Clin Ther.* 2016;38:706–715)

Key words: development, microbiome, NEC, neonate, pathogenesis.

Coming together is a beginning; keeping together is progress; working together is success.

Henry Ford

INTRODUCTION

Neonatal necrotizing enterocolitis (NEC) remains the most devastating gastrointestinal disease for premature infants. In the United States alone, NEC affects >4000 premature infants yearly, has a mortality rate of nearly 33%, and costs the health care system >$1 billion annually.[1] The incidence of NEC in extremely low birth weight infants is currently ~7%,[2] but at our institution we found that the infants born at the edge

Accepted for publication January 6, 2016.
http://dx.doi.org/10.1016/j.clinthera.2016.01.005
0149-2918/$ - see front matter

of viability have an even greater risk. Although our overall institutional rate of NEC hovers at ~3.5%, the rate of NEC in infants born at 23 week's gestation is closer to 10%, and the rate of infants born at 22 week's gestation approaches 30%. Although NEC has been actively researched for several decades, its pathophysiology remains elusive. One potential mechanism suggests that disruption of the normal neonatal intestinal bacterial flora induces a proinflammatory state, allowing translocation of pathogens across the intestinal epithelia.[3] The collection of microorganisms residing in our intestinal tract is collectively known as the microbiome. The intestinal microbiome is crucial for human health by contributing to immunity and digestion and by limiting pathogen colonization.[4,5] However, neonates represent a unique population when studying the microbiome.

When neonates are first born, they have relatively few intestinal bacteria. After birth, the intestinal microbiome rapidly expands, steadily progressing toward an adult composition. However, this bacterial maturation is influenced by many factors, including diet, gestation at birth, and antibiotic usage.[6] Disruption of the normal intestinal flora (dysbiosis) is associated with many human diseases. Thus, it is a reasonable hypothesis that dysbiosis may play an important role in the development of NEC. This hypothesis is supported by evidence that probiotic use in premature infants can prevent the development of NEC. Although the role of probiotics and NEC were covered in other reviews,[3,7] this review instead focuses on normal bacterial colonization in both term and preterm infants and the association of dysbiosis in the development of NEC.

METHODS

For development of this review, we queried PubMed with the use of the following key search terms: NEC, neonatal microbiome, fetal microbiome, maternal microbiome, neonatal dysbiosis, and microbiome ontogeny. Relevant literature was reviewed and selected for inclusion in accordance with the objectives of the article according to the authors' discretion. Manuscripts that made key salient points in review articles were further pulled from PubMed.

Bacterial Colonization of the Fetus

Neonatal development marks a transitional period for a fetus's relation with its microbiome. By adulthood, the average human intestine is home to 10 to 100 trillion microbes, or roughly 10 times as many bacteria as there are cells in the human body.[8] This consortium of bacteria contains >150 times more genes than the human genome.[9] However, at birth, the infant has a relative paucity of bacterial flora. In fact, it was traditionally thought that the fetus developed within a sterile environment, was born bacteria free, and began to populate its surfaces only after delivery. This was because of the inability to culture bacteria from amniotic fluid or from surface cultures of infants. However, with the advent of 16S ribosomal RNA sequencing techniques, recent studies have begun to challenge that dogma. With the use of 16s ribosomal RNA sequencing, the presence of commensal bacterial species from the phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria were found to exist within the human placental basal plate (a shared maternal and fetal interface), an area once thought to be sterile.[10] Interestingly, the study also found a similarity between these populations of bacteria living in the placenta and the nonpregnant female oral microbiome.[10] In another study, umbilical blood from scheduled, elective human cesarean deliveries was analyzed and found to contain low numbers of *Enterococcus, Staphylococcus*, and *Streptococcus* species present within the blood.[11] Even meconium from term infants that was tested within 2 hours of life and before the initiation of breastfeeding was found to contain *Enterococcus*, *Staphylococcus*, and *Escherichia coli*.[12] Taken together, these studies show the presence of a placental community of bacteria, bacteria within the infant umbilical blood stream at birth and in the first infant stool, before receiving breast milk. These data and others suggest that the initial colonization of the intestinal tract occurs in utero during fetal development before subsequent delivery or initiation of feeds.

However, the question remains of how these initial colonizers find their way to the fetal intestine. One potential mechanism involves the maternal immune system. Dendritic cells (DCs) are mammalian antigen-presenting cells found on all surfaces of the host that have contact with the external environment. These cells have specialized branched projections on their cell surface that sample the external environment, process antigenic material, and then present it to the T cells of the immune system. Thus, DCs act as a bridge between the innate and adaptive immune systems and play a central role in the creation of