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Review

Microbial therapeutic interventions

Nicole G. Grady [a], Elaine O. Petrof [b], Erika C. Claud [a, *]

[a] University of Chicago, Chicago, IL, USA
[b] Queen's University, Department of Medicine, Kingston General Hospital, Kingston, ON, Canada

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S U M M A R Y

The microbiome comprises all the microbes living in and on the human body. Human cells are greatly outnumbered by bacterial cells; thus human health depends on the health of the microbial ecosystem. For the immature preterm infant, the microbiome also influences intestinal and immune system development. This has implications for short term morbidities such as neonatal necrotizing enterocolitis and sepsis, but also long term health outcomes. Optimization of the preterm infant microbiome is a growing topic of interest. The microbial world is not one of good versus evil, but rather one of community; thus optimization includes not only minimizing pathogens, but also enhancing beneficial organisms. Options for optimization include judicious antibiotic use, administration of supplements such as prebiotics or probiotics, and transfaunation procedures such as fecal microbial transplant or microbial ecosystem therapeutics. Potential for benefit as well as risk for each of these options will be discussed.

1. Introduction

Human health depends on the health of the microbiome; thus it is important to understand the microbial perspective. The microbiome is an ecosystem. A healthy community has a high diversity of species resulting in balance, functional redundancy, and resistance to disease. In contrast, low species diversity is associated with a sick ecosystem, imbalance, functional disability, and susceptibility to disease. Dysbiosis is a state of imbalance in the gut microbial system with overgrowth of some organisms and loss of others [1]. It is the foundation of many diseases; thus restoring microbial balance may be a therapeutic option that, to date, has remained largely unexplored. The preterm infant is unique due to the confluence of a developing host as well as a developing microbiome [2]. The microbiome of all preterm infants in the process of development has low diversity, but, unlike full term infants, it matures under the influence of neonatal intensive care unit (NICU) environmental pressures such as a hospital environment, instrumentation, and empiric antibiotic exposure [3].

The development and health of the intestinal microbiota of the preterm infant has most often been studied in the context of neonatal necrotizing enterocolitis (NEC). NEC is an inflammatory bowel disease of preterm infants associated with intestinal immaturity, bacterial colonization, enteral feeding, and altered intestinal blood flow [4]. Multiple studies have demonstrated an altered microbial community associated with NEC that appears several weeks prior to the onset of disease and is associated with a further decrease in diversity and a bloom of Gammaproteobacteria [3,5]. However, microbiome development may affect not only the infant clinical course while in the NICU, but also long term health outcomes. There has been increasing recognition that alterations in the microbiome during early infancy may be linked to the risk of developing asthma, obesity, atopy, and inflammatory bowel disease (IBD) [6–9]. In addition, the microbiome has been associated with the development of the immune system, behavior, and cognitive function [10].

We have previously demonstrated that there is a temporal component to microbiome development in healthy preterm infants with clustering prior to 2 weeks of life, at 3–5 weeks of life and >6 weeks of life [11]. This potentially necessary progression must be considered in addition to the microbiome taxonomic composition and functional content at any single point in time. When investigating the functional effects of early microbiota (<2 weeks of life) we found that different microbial communities could influence intestinal innate immune system development including baseline tight junction formation and nuclear factor-κB activation [2]. This was associated with differences in systemic cytokine production and infant growth [2]. As much of the morbidity of prematurity is inflammatory in nature, increased systemic release of

* Corresponding author. Address: University of Chicago, 5841 S. Maryland Ave. MC6060, Chicago, IL 60637, USA. Tel.: +1 773 702 6210; fax: +1 773 702 0764.
E-mail address: eclaud@peds.bsd.uchicago.edu (E.C. Claud).

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inflammatory cytokines may affect other developing organs including the lung, brain, and eye. Understanding microbiome development could thus influence multiple preterm infant outcomes.

Development of a healthy intestinal microbiome, containing a healthy bacterial community with a balanced and diverse population, should be a goal in neonatal practice. One way to support a healthy bacterial community is through protecting the innate microbiome by limiting therapies known to cause alterations such as antibiotics, and by encouraging therapies known to be beneficial such as own mother's milk feeding. Indeed, clinical studies have demonstrated an increased risk of NEC with increased number of days of initial empiric antibiotics [12], and a decreased incidence of NEC in a dose–response relationship to amount of mother's milk as a percentage of total feeds in the first two weeks of life [13]. Alternatively, options exist for manipulating the intestinal microbiota to restore balance and prevent disease. These options include prebiotics, probiotics and ecotherapeutics such as fecal microbial transplant and microbial ecologic therapeutics.

2. Prebiotics

Prebiotics are "non-digestible food ingredients that selectively stimulate the growth or activity of anaerobic/microaerophilic flora (*Bifidobacterium*/*Lactobacillus*) in the colon of mammals" [14]. Prebiotics do not supplement with actual organisms, but rather support the growth of the bacteria already established within the intestinal tract. Oligosaccharides are the most frequently occurring prebiotics, and are prevalent in human milk [15], as are lactoferrin and lactalbumin milk proteins that promote specific growth of bifidobacteria and are described as "bifidogenic factors" [16].

Oligosaccharides, including those found in human milk, are resistant to digestion within the gut, as the human small intestine produces no luminal enzymes to hydrolyze these molecules [17,18]. However, many intestinal bacteria express glycosidases that are capable of metabolizing the human milk oligosaccharides [19]. Genomic studies of intestinal bacteria, such as *Bifidobacteria*, *Bacteroides*, and *Actinobacteria* species, found that these bacteria have adapted to the intestinal environment with their ability to metabolize these sugars and therefore occupy a specific ecological niche in breastfed neonates [20–22].

Additional studies have found that prebiotics may improve intestinal motility and gastric emptying times, which may improve feeding tolerance in preterm neonatal populations [23,24]. In studies performed by Indrio and colleagues, formula-fed infants who received prebiotics of oligosaccharides had gastric emptying times and motility similar to that of breast-fed infants, which were improved compared to formula-fed infants receiving placebo treatments [23]. Whereas there is evidence for improved feeding tolerance through improved intestinal motility, softer stool consistency, and increased numbers of *Bifidobacteria* in fecal samples with prebiotic supplementation, there is no evidence to suggest that the prebiotics inulin, lactulose, fructo-oligosaccharides or galacto-oligosaccharides reduce the incidence of NEC or reduce the time to reach full feeds [23,25–31].

Advantages of using prebiotics include ease of oral administration and support of the selective growth of beneficial bacteria that are endogenous to the gastrointestinal tract rather than the introduction of new bacterial species [23]. Prebiotics are a native constituent of human milk, and are now being added to many commercially available infant formulas. Committee statements based on large reviews have found that whereas prebiotic additives do not raise safety concerns in healthy infants in regards to growth or adverse effects, there are insufficient data to recommend routine use [32]. A similar review has additionally found insufficient evidence to demonstrate that prebiotics improve growth or clinical outcomes in formula-fed preterm infants [33].

Although prebiotic therapy has not been related to any serious adverse effects in previous studies, it has been associated with several gastrointestinal side-effects, including bloating, diarrhea, and flatulence, which all stopped with termination of treatment [34]. Prebiotics such as inulin, lactulose, and short chain fructo-oligosaccharides and galacto-oligosaccharides are not well studied in the neonatal and preterm patient populations with respect to their use, efficacy, or safety [14].

Prebiotics may be a beneficial method to improve the microbiome balance in infants whose natural colonization has not been significantly altered. However, if the bacterial communities that benefit from prebiotics have already been eliminated due to iatrogenic dysbiosis (altered initial colonization, cesarean section delivery, antibiotic use, hospital environment), the addition of a prebiotic supplement may have no impact on the development of a healthy microbial community, as the substrates will not be utilized.

3. Probiotics

Whereas prebiotics provide no actual organisms to the patients, probiotics are supplements that contain viable micro-organisms. These supplements most often contain one to a few species of bacteria and directly alter the microbiome of the host, with the potential to provide missing bacterial species and confer a health benefit. As premature infants have delayed or disrupted acquisition of their commensal bacteria, notably bifidobacteria, these oral supplements may provide the ability to transition to an intestinal microbiome that contains potentially beneficial bacteria [15,35]. Both bifidobacteria and lactobacilli are widely used probiotics. In trials for preterm infants, they are usually initiated within the first week of life, and continued beyond a month of life or until discharge from the NICU [15]. There are multiple mechanisms proposed to explain the effects of probiotics on the neonatal microbiome and disease susceptibility. These mechanisms include competitive exclusion of pathogenic bacteria, improvement of the epithelial barrier function within the intestines, secretion of bacteriocins, and direct anti-inflammatory effects on epithelial signaling pathways [14,15,36–38].

Probiotics have been investigated for improvement in the growth and enteral feeding of preterm infants, as well as other feeding-related symptoms such has colic and reflux [39,40]. However, the most compelling reason for use of probiotics in preterm infants is prevention of NEC. NEC is the most frequent gastrointestinal emergency in preterm infants. It carries significant morbidity and mortality, and there is no cure. Probiotics may prevent NEC in a multifactorial manner through intestinal maturation and normalization of gastrointestinal colonization [15]. A recent Cochrane review evaluated the benefit of probiotics in sepsis or NEC [41]. Twenty-four randomized controlled trials including preterm infants <37 weeks gestational age and <2500 g birth weight were reviewed. Studies varied in terms of baseline risk of NEC, probiotic used, and protocols of administration. A meta-analysis revealed decreased incidence of severe NEC and mortality, but no difference in risk of sepsis. There appeared to be no risk associated with the probiotic preparations, defined as sepsis during the course of study.

However, the safety and efficacy of probiotic usage within the neonatal period remains controversial. Studies are heterogeneous in all aspects, including probiotic strain(s) utilized, the doses administered, and inclusion and exclusion criteria employed with enrollment of study participants [14,42]. No study has been powered to evaluate safety with regard to possible risks of the probiotic treatment, including sepsis [43,44]. Live probiotic bacteria have the

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