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Microbial therapeutic interventions

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SUMMARY

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.

Keywords

Microbiome; Prebiotics; Probiotics; Fecal microbial transplant; Microbial ecosystem therapeutics

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

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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- κ B 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 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 as 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 potential to become pathogenic when host defenses are compromised, be it from immune deficiencies or illness [45–48]. The premature infant is considered relatively immunocompromised, and studies reporting increased infant mortality in probiotic-treated

animal models have raised questions as to the prudence of using live bacteria in premature infants [49]. Even the introduction of “beneficial” probiotic bacteria may potentially result in negative repercussions on the gut of a preterm infant if intestinal maturation is not complete. No study has followed long term outcomes. Probiotics are administered to the developing infant and microbiome at a time where long-standing effects are likely. Increasing recognition of the microbiome as foundational to immune system development and long term outcomes demands an understanding of the effect of any microbiome manipulation on long term outcomes before it can be considered safe. Baseline NEC rate is a major factor influencing benefit of probiotic supplementation in a population [50]. Indeed the most recent probiotic trials have shown a very low baseline rate of NEC, so that the statistical decrease in NEC may not be clinically significant or sufficient to outweigh other risks [51].

Furthermore, there is unclear regulatory oversight of probiotic products. Probiotics are regulated as a dietary supplement in many countries including the USA rather than as a pharmaceutical drug or biological agent. Without regulatory agency oversight, there is often no requirement to demonstrate the safety, purity, or potency of the products being sold, which may lead to contamination or discrepancies in the administered dose [52–54]. There have been multiple reports of sepsis with probiotic bacterial strains following supplementation [55–57], and a recent recall of a probiotic product due to fungal sepsis in a preterm infant due to contamination of the product [58].

Given the heterogeneity of the studies and their results in the literature, as well as the potential risks involved with supplementation, further research with well-designed randomized controlled trials needs to be performed. These need to be powered to determine not only whether there is a reduction in NEC or mortality, but also the short term and long term risks of the supplementation. Any supplemental agent for preterm infants must have clear composition and be manufactured to pharmaceutical grade standards. In addition, supplementation with a single or a few species may not provide the diversity necessary for a balanced and healthy microbial ecosystem in the neonates’ developing microbiome.

4. Ecotherapeutics

A large body of literature demonstrates that, in the gut, certain commensal bacteria play beneficial roles, including provision of essential nutrients, competitive colonization against pathogens, and intestinal maturation [59–64]. Normal intestinal homeostasis requires a microbiome that encompasses these key functions. Ecotherapeutics involves replacement of a dysfunctional ecosystem with a healthy ecosystem of “native” intestinal bacteria. Unlike conventional probiotic therapies that are generally limited to a single strain or, at most, a few strains of bacteria, ecotherapeutics utilizes a whole bacterial community that has been derived directly from the human gastrointestinal tract to maintain or restore human health. More than simply a new probiotic treatment, this is an emerging paradigm in medicine that may lead to novel strategies for improvement of preterm infant outcomes [65].

Fecal microbial transplant (FMT) or microbial ecosystem therapeutics (MET) are both used to alter the intestinal microbiome on a larger scale. These methods utilize administration of fecal material consisting of distal gut microbiota from a healthy person to a patient with a

disease or condition related to dysbiosis [66]. In FMT, the transplanted fecal samples have been taken directly from the gastrointestinal tract of healthy donors [1], whereas in MET, select bacteria within the fecal sample are purified and maintained in culture, producing a stable and well-defined ecosystem for transplantation [67]. To date, these therapies have been studied only in older children and adults.

4.1. Fecal microbial transplant

Although FMT has existed for centuries, it is now most widely utilized in the treatment of antibiotic refractory or recurrent *Clostridium difficile* infection (CDI) [67]. Due to the growing prevalence of severe or recurrent CDI, FMT has become increasingly used in clinical practice [66]. Among the published case reports, case series, and the single randomized controlled trial for use of FMT in recurrent CDI, cure rates have been as high as 100% with a mean cure rate of 87–90% [68–70]. FMT is also being used or studied in other conditions related to dysbiosis, such as in metabolic syndrome, obesity, food allergies, IBD, and irritable bowel syndrome (IBS) [66].

FMT is performed by utilizing fecal samples from donors, related or unrelated, that have undergone rigorous screening for infectious diseases. Donors need to be free of conditions that may be transmissible by stool or blood, have had no recent antibiotic treatment, no gastrointestinal illnesses, no autoimmune or allergic illnesses and must not be obese [66]. Following collection, samples are diluted and homogenized into the administration form, and can be given through the upper gastrointestinal tract via endoscopy, or nasogastric or nasojejunal tubes; through the distal colon by enemas, rectal tube or colonoscopy; or concentrated for delivery via oral capsules [66,71]. Although there are advantages and disadvantages of each delivery method within the pediatric and adult patient population, there is no current consensus on which method of delivery is best.

The advantage of FMT via nasogastric or nasojejunal tubes is that it is a minimally invasive and low cost procedure. The disadvantages of this method include an increased risk of aspiration events and vomiting, as well as discomfort to the patient [72]. Upper gastrointestinal tract endoscopy may overcome the risk of aspiration and vomiting, but is a more invasive approach that requires sedation and has higher medical costs. Oral capsules containing fecal samples are non-invasive and low cost, but the effectiveness of this approach needs to be further assessed [66,71].

Retention enemas have the advantages of being inexpensive with little procedural risk, but it can be difficult for the patients to retain the transplant material, therefore requiring multiple treatments. The most widely used method for FMT is colonoscopy, which may be more effective in the treatment of CDI and which allows for direct examination of the colonic mucosa to potentially rule out other pathologies in the setting of CDI. The disadvantages include the need for sedation and the higher procedural costs, but also include the procedural risks of perforation and bleeding.

A disadvantage to FMT is that the processing of human stool often requires biosafety level 2 containment; however, the FDA has approved investigational protocols that allow the processing of fecal materials in patient-care areas under routine “universal precautions” [67].

Additionally, the microbiota varies both between individuals, as well as within individuals over time, making it nearly impossible to standardize the composition of the fecal microbiota that is transplanted in each procedure [67]. The unique advantage of FMT, compared to the other modalities of microbiome modulation discussed, is that FMT promptly restores a diverse composition to the recipient's intestinal microbiome, which is distinctly different from probiotics that only supply a single or limited numbers of species into the intestinal tract [67].

Although the side-effects related to FMT are low, the procedure's use as a treatment modality in CDI is relatively new, limiting the amount of long term data available. Minor, short term adverse events include multiple gastrointestinal symptoms such as abdominal pain, bloating, flatulence, diarrhea, constipation and vomiting [66]. Additional risks include the spread of infectious agents, such as viruses or fungi; allergic reactions; and the potential transmission of microbiota that could contribute to the development of future diseases such as IBD, IBS, colorectal cancers, type II diabetes or metabolic syndrome [67,73]. Although there have been some studies of FMT in children, there are no studies of this modality in preterm infants, and whereas theoretic benefits may exist, identification of an appropriate donor, possible modalities of administration and protocols are all unknown and carry potential risk [74–76].

4.2. Microbial ecosystem therapeutics

Microbial ecosystem therapeutics utilizes defined mixtures of bacteria that are derived from stool samples, rather than transplanting the complete fecal microbiome. This method allows for a known microbial community that can be controlled, recreated, and standardized, without the risk of unknown infectious agents being transmitted to the recipients. In addition, it removes the need for multiple or repeated donations, as the mixtures are maintained in culture following their initial isolation from the donor fecal sample [67]. Two studies have employed this method of transfaunation in the treatment of CDI, developing communities of 10 or 33 bacterial strains [77,78]. In each study, the MET allowed patients to become asymptomatic and cured of CDI, indicating that MET is a viable alternative to FMT for the treatment of CDI [67].

Whereas there are advantages to MET compared to FMT, including a known composition of the transplanted microbiota and ability to reproduce the same sample for transplant, MET encounters other challenges. MET is under strict regulations by the FDA and other regulatory agencies as it is considered both a biologic agent requiring biosafety containment as well as a manufactured product, requiring full genomic sequences of the included microbiota [67]. In addition to regulatory hurdles, there are technical manufacturing challenges faced in maintaining multiple bacterial strains, where losing only a few strains can substantially reduce the efficacy of the transplant [79].

Although microbiome transfaunation procedures have been shown to be beneficial in the treatment of refractory or recurrent CDI in the pediatric and adult populations, and are actively being studied in other gastrointestinal or chronic diseases such as IBD, IBS, and metabolic syndrome, there are no published studies or case reports of transfaunation in infants. The process of transfaunation may be difficult within the neonatal population.

Although most premature infants have nasogastric tubes in place for feeding, the risk of aspiration and the incidence of reflux within this population may preclude upper gastrointestinal modalities from being utilized. Direct visualization with endoscopy or colonoscopy may be mechanically limited due to patient size and to the risk of perforation within this population. There are further risks associated with sedation and its effects on long term outcomes. Retention enemas would also carry a risk of trauma.

Additionally one must consider the naïve nature of the preterm infant intestine. Although the fetal environment is not completely sterile, the neonatal intestines have limited microbiota at birth [10]. The microbiome then develops over time, and is affected by delivery mode, feeding, and environment [10]. It is unknown whether the iatrogenic influences of the NICU affect the preterm microbiome's "normal" development into that of a healthy ecosystem; this requires further investigation. Without knowing what the ideal microbiome of these infants contains, it is impossible to know what would be the proper strains for transplantation via MET. This is further complicated when considering transfaunation with FMT, as age-matched donors would be unlikely, and it may be developmentally inappropriate to transplant a neonate with the microbiome of an adult. Also, without knowing the exact mechanism of NEC and the role of pathogenic bacterial strains, FMT from neonatal samples may actually put the recipient at risk for developing NEC. Other concerns, similar to that of FMT in the adult population, include potential long term health impact of microbiome manipulation.

5. Conclusion

There is a growing understanding of the human intestinal microbiome in relation to health and disease. The developing microbiome affects infants not only within the neonatal time period, but carries the potential for long term health consequences. Protection of the developing microbiome via limited antibiotic use and own mother's milk feeding is preferred; however, when protection of the native microbiome is not possible, options for microbiome modulation exist. These include the use of prebiotics, probiotics, and transfaunation procedures such as FMT or MET.

With the goal of supporting a patient's own bacterial ecosystem, and promoting the growth of beneficial commensal bacteria, the field of prebiotics has blossomed. The theory is to promote the growth and activity of commensal flora by supplying non-digestible food substrates such as oligosaccharides, lactoferrin inulin, or lactalbumin, all products found within human milk, and easily added to infant formulas. The bacteria that metabolize these prebiotics have developed an evolutionary niche within the human intestine. One factor that may limit the optimal effects of prebiotics in preterm infants is the delayed intestinal colonization due to delivery by cesarean section, administration of empiric antibiotics at birth, and delayed initial feedings. Without the initial development of a healthy intestinal microbiome, insufficient numbers of beneficial bacteria are present to utilize the prebiotics given to the patients. However, prebiotics may be of importance in infants with minimal microbial disturbances to further support the growth of beneficial organisms.

Whereas probiotics are the most well-described microbial therapy utilized in the neonatal period, their use is still controversial. Although multiple reports have found that there is a decreased incidence of NEC with the use of probiotics in the neonatal population, long term effects have not been studied. Further studies and regulatory controls are needed before a broad recommendation of probiotic use is made for preterm infants.

Transfaunation procedures, although demonstrated to be both cost-effective and clinically effective for treatment of recurrent CDI in both pediatric and adult populations, have not been studied within the neonatal population. In addition, there is limited evidence for use of FMT/MET for other disease processes. Although the instillation of a fecal microbiome into a neonatal intestinal tract may at first appear to provide a method for developing a “healthy” microbiome in our smallest and sickest of patients, there are many factors to consider. First and foremost, the microbiome of preterm infants varies over time and between patients [3,11]. A clearer understanding of the development of the microbiome in preterm infants is needed, including how environmental and iatrogenic factors affect its development. Without knowledge of the natural progression of a healthy neonatal microbiome, the ideal donor for FMT or bacterial strains to be used in MET are also unknown. With ongoing neonatal microbiome research, the composition of the undisturbed, healthy neonatal microbiome may become better understood. With this knowledge, MET may provide a means to develop and sustain a bacterial community specifically designed and appropriate for the neonatal population, making it a unique and highly specialized technique for microbiome modulation.

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Practice points

- Microbial therapeutic interventions must be evaluated in the context of long term effects on the microbial community, not just short term outcomes.
- Microbial therapeutic interventions include protecting the microbiome: limit empiric antibiotics; promote exclusive breastfeeding.

Research directions

- The role of the microbiome in preterm infant development.
- Components of an optimal microbiome for preterm infants.
- Development of microbial therapeutic agents with known mechanism of action and pharmaceutical grade production.
- Long term effects of microbial therapeutic interventions.