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THE INTESTINAL MICROBIOME OF INFANTS AND THE USE OF PROBIOTICS

Flavia Indrio, M.D. and
University of Bari, Bari, Italy

Josef Neu, M.D.
University of Florida, Gainesville, Florida, USA

Abstract

Purpose of the review—The increasing use of probiotics in neonates deserves scrutiny of the therapeutic as well as potentially harmful effects of these bacteria. In this review we describe the possible application of probiotics in the more common disease in the neonatal period.

Recent finding—Recent advances our capability to identify microbes and their function in the gastrointestinal tract offer exciting opportunities to discover the pathophysiology of enigmatic diseases such as necrotizing enterocolitis (NEC) and late onset sepsis (LOS) in the neonate. The relationship of the resident intestinal microbes to neural and muscular processes such as intestinal motility and neurodevelopment are also being evaluated.

Summary—We focus the possibility of the application of probiotics for disorders of motility in the infant, NEC and LOS Here we will summarize some of the recent advances in these areas as they relate to clinical practice and discuss areas where additional research is needed.

Keywords

Intestinal Microbiota; intestinal mucosa-neural– microbiota interactions; microbial components effect on intestine

INTRODUCTION

Interactions of resident intestinal microbes with the luminal contents and the mucosal surface play important roles in normal intestinal development, nutrition and immunity. The intestine has a large and also fragile surface area covered by a thin monolayer of multifunctional epithelial cells that overlies a highly immunoreactive submucosa. During health, interactions in the lumen between resident microbes, nutrients and the intestinal mucosa promote intestinal development as well as the immune system.(1)Disruption of this barrier may have numerous detrimental consequences including systemic inflammation, autoimmune and allergic disorders. Evidence is rapidly emerging that supports the existence of a brain-gut-intestinal microbiota axis in which specialized intestinal epithelial cells serve as signaling intermediates to neuronal circuits. This has important implications in motility, an important factor in feeding tolerance for neonates, as well as the pathophysiology of acute and chronic gastrointestinal diseases. (2)

Recent advances in molecular microbiota analytic methodology that stems from advances in high throughput sequencing technology have provided us with the tools to determine the

taxonomy of intestinal microbiota in great depth, including the nearly 80% of microbes in the intestine that are very difficult if not impossible to culture by current methodology.(3) In the preterm neonate, application of these techniques to derive a better understanding of the developing intestinal ecosystem may help enhance enteral nutrition and prevent several important diseases including necrotizing enterocolitis (NEC) and late onset neonatal sepsis. This review will provide a brief summary of current knowledge of the developing intestinal microbiota in the term and preterm infant and provide an overview of the interactions of the microbiota and intestinal mucosa and how these interactions may alter physiologic phenomena such as motility, feeding intolerance necrotizing enterocolitis and late onset sepsis.

DEVELOPMENT OF THE INTESTINAL MICROBIOTA IN THE PREMATURE INFANT

The GI tract of a normal fetus is generally thought to be sterile. However, recent studies are suggesting that the fetal intestine may be exposed to microbes via swallowing of colonized amniotic fluid.(4) This remains a largely unexplored area. Nevertheless, during the birth process and rapidly thereafter, microbes from the mother and the surrounding environment colonize the gastrointestinal tract of the infant,(5) eventually leading to a dense and diverse bacterial community.(6)

Whether the infant is born prematurely and requires intensive care or is a term infant who is born without special needs could markedly affect the development of the intestinal microbial core. Although several studies have monitored the bacterial communities in preterm infants, our picture of the microbiota still remains limited; primarily because primarily limited culture-based techniques have been previously used. It is not known whether prematurity itself may influence the intestinal establishment of microbiota. Many preterm infants require intensive care procedures early in life, which may be one of the major factors determining intestinal microbiota development. Many preterm infants receive antibiotics for ill-defined criteria, which are thought to have an influence of bacterial colonization.

In a study that used microbiota profiles determined in 22 infants with denaturing gradient gel electrophoresis (DGGE), unique compositions for each infant were revealed. Although composition changed with time, characteristic bands were observed for each infant at multiple times. There was a trend toward increased diversity with time. No significant associations were detected between microbiota diversity and sex, birth weight, gestational age, prolonged rupture of membranes, maternal intrapartum antibiotics, or mode of delivery when the microbiota were evaluated after the first week of life, although differences were found in meconium samples collected in the first week after birth in terms of intent to breast feed versus formula feed, maternal antibiotic usage and gestational age at delivery.(7) This study also employed a technique that was limited in terms of in-depth taxonomic evaluation. As whole genome sequencing and metagenomic techniques become available and less expensive, the likelihood of a more comprehensive analysis increases.

INTERACTIONS OF THE MICROBIOTA AND THE INTESTINAL BARRIER

The gut-barrier is presented with a major challenge; preventing luminal pathogens and harmful substances from entering into the internal milieu and yet promoting digestion and different architectural units of this barrier. Exposed to trillions of luminal microbes, the intestinal mucosa averts threats by signaling to the innate immune system, through pattern recognition receptors, to respond to the commensal bacteria by developing tolerance (hyporesponsiveness) towards them. This system also acts by protecting against pathogens by elaborating and releasing protective peptides, cytokines, chemokines, and phagocytic

cells. The intestinal mucosa is constantly sampling luminal contents and making molecular adjustments at its frontier. Some of the details of this barrier function are provided in other recent reviews and are beyond the scope of this review. (8, 9)

The Enteric Nervous System and Interaction with Intestinal Microbes: Role in Motility

Brain–intestinal interactions are well known mechanisms for the regulation of intestinal function in both healthy and diseased states. A role of the enteric microbes in these interactions has only been recognized in the past few years. The brain can influence commensal organisms via changes in gastrointestinal motility, secretion, and intestinal permeability, or directly, via signaling molecules released into the gut lumen from cells in the lamina propria (enterochromaffin cells, neurons, immune cells). (2) Enteric microbiota communication occurs via epithelial-cell, receptor-mediated signaling and, when intestinal permeability is increased, through direct stimulation of host cells in the lamina propria. Integral to these communications are enterochromaffin cells, which serve as bidirectional transducers that regulate communication between the intestinal lumen and the nervous system. Disruption of the bidirectional interactions between the enteric microbiota and the nervous system may be involved in the pathophysiology of acute and chronic gastrointestinal disease states, including functional and inflammatory bowel disorders. (2)

Commensal bacteria inhabiting the human intestine participate in the development and maintenance of gut sensory and motor functions, including the promotion of intestinal propulsive activity. On the other hand, intestinal motility represents one of the major control systems of gut microflora, through the removal of excessive bacteria from the lumen. Under normal conditions, the gastrointestinal tract provides a stable habitat for commensal bacteria that supports its structural and functional integrity. Disturbance of normal GI physiology destabilizes the habitat, resulting in changes in its microbial composition. Alternatively, changes in the microbiota, induced by infection or antibiotics, or other events such as stress, perturb physiologic inflammation and gut physiology. (2)

Normal intestinal motility requires the coordination between the extrinsic neurons, enteric motor neurons, interstitial cells of Cajal (ICC) and smooth muscle cells (SMC). The enteric nervous system (ENS) is a complex integrative brain (also called the second brain) which is capable of controlling the GI function. The ENS influences the gut directly with the activity related to the contraction (SMS ICC) and indirectly influencing the cells of the gut immune system and the epithelial cells. This interaction is bidirectional and relies on the mechanisms of neuroimmune interaction, which involves bacterial component activation of Toll-like and other bacterial molecular pattern receptors to trigger innate immune responses and the intestinal neural pathways.

The intestinal microbiota is involved in the development and maintenance of gut sensory and motor functions by the release of bacterial substances, fermentation products and intestinal neuroendocrine factors, and through a close link with the gastrointestinal immune system. (10, 11) The end-products of colonic microbiota fermentation, the short-chain fatty acids (SCFAs), may modulate lower and upper gut motor events via direct and probably via indirect (nervous) pathway. The interplay among all these systems and apparatus is fundamental for the appropriate function of the gut as depicted in Fig 1.

Several studies in animals have evaluated the direct effect of individual probiotics on gastrointestinal motility (Table 1). Of interest is the finding that microbes do not need to be alive to exert an effect. One study aimed explored the effects of live, heat killed, or gamma irradiated *Lactobacillus reuteri* (*L. reuterii*) on cardio-autonomic response and single fiber unit discharge in dorsal root ganglia to colorectal distension in healthy Sprague-Dawley rats housed under conventional conditions. (12) Treatment with live, heat killed, or gamma

irradiated bacteria as well as their products (conditioned medium) prevented the pain response even during the maximum distension pressure at 80 mm Hg.

Rosseaux, et al. (13) reported that oral administration of specific *Lactobacillus* strains (NCFM) induced the expression of μ -opioid and cannabinoid receptors in intestinal epithelial cells, and mediated analgesic functions in the gut, similar to the effects of morphine. This analgesic effect was enhanced as the NCFM dosage was increased from 10^7 CFU/d to 10^9 CFU/d and disappeared 3 d after the last NCFM administration. Another study aimed to determine whether dorsal root ganglion (DRG) somas could be a locus where the antinociceptive probiotic may have an effect.(14) Healthy rats were fed with *L. reuterii* or vehicle control for 9 days whereupon they were anesthetized, and intermittent distal colonic colorectal distention (CRD) at 80 mmHg distension was either performed for 1 h or not. The animals were immediately euthanized and patch-clamp recordings taken after isolation and overnight culture from those DRG that projected to the distal colon. CRD decreased the threshold for action potential generation and increased the number of spikes discharged during a standard depolarizing test stimulus, and this effect was blocked by prior probiotic ingestion.

The same group one year later elucidated the neuronal mechanism of action of *L. reuterii*. (15) In this study *L. reuterii* ingestion alters the motility of the colon segment. decrease the amplitude of contraction at constant luminal pressure and increase the threshold of luminal pressure required to evoke rhythmic contraction.

L. reuterii partially blocked the intermediate conductance calcium dependent potassium channel (IKCa) of AH myenteric neurons.

The major clinical disorders in newborn where the effect of probiotic on gastrointestinal motility have been studied include feeding intolerance, regurgitation, colic and constipation. These are important subject of study for pediatricians and all those diseases might benefit from dietary treatment. A randomized, double blind study demonstrated the beneficial effects of probiotics on clinical and physiological parameters(16). In this study gastrointestinal motility was recorded as a measure of gastric electrical activity, measure of the wall movements, and measure of gastric emptying time. The authors demonstrated that amplitude and percentage of normal slow waves, bradygastria, and tachygastria did not differ in the three groups of preterm newborns. As regard gastric emptying time, a smaller fasting antral area was found in preterm fed with formula added with *L. reuterii* compared to that one fed with formula with placebo and newborns fed with breast milk.. Furthermore, the gastric emptying rate was significantly faster in formula added with probiotics group respect to breast milk and formula plus placebo. In particular it shows that oral probiotic(*L. reuterii*)supplementation in preterm newborns improves feeding tolerance, reduces the crying time and increase stool frequency. Taken together these findings support that this agent reduces gastric residual in newborns.

POSSIBLE ROLE OF INTESTINAL MICROBIOTA IN NECROTIZING ENTEROCOLITIS (NEC)

Necrotizing enterocolitis (NEC) is a devastating disease affecting primarily premature infants. Despite advances in neonatal care, the mortality rate following NEC has not changed significantly in the past 30 years. It remains an enigmatic and potentially devastating condition with high morbidity and mortality in preterm infants. Although the etiology of NEC remains unknown, initial bacterial colonization could play a pivotal role in the development of NEC. Sequencing studies have demonstrated that sequences from NEC patients cluster separately from sequences from control patients. (17) In this study, the

microbial community structure in NEC patients (during the time of NEC and antibiotic use) was distinct based on a significant decrease in diversity of microbial species with an increase in *Proteobacteria* dominance compared to other preterm infants. In another study, (7)microbiota compositions were compared in 6 preterm infants in whom NEC, signs of systemic inflammation, or both developed with matched control subjects by using 16S ribosomal RNA pyrosequencing. In samples taken approximately one week prior to the development of NEC, a 16S ribosomal RNA sequence analysis detected *Citrobacter*-like sequences only in cases with NEC (3 of 4) and a trend toward increased frequency of *Enterococcus*-like sequences in cases and *Klebsiella* in control subjects. The overall microbiota profiles in cases with NEC were not distinguishable from that in control subjects. More studies using similar technologies are required to establish a relationship between specific microbes or microbial patterns and NEC.

PROBIOTICS AND NEC and Late Onset Sepsis (LOS)

Probiotics are microbial strains of human origin, non-pathogenic, adherent to gut epithelium, colonize the intestinal tract, produce antimicrobial substances and modulate immune responses. It is hypothesized that probiotics act to downregulate pathogenic organisms and protect against intestinal inflammation. The lack of adequate colonization in the preterm neonate could place them at an increased risk for neonatal NEC. (18)Therefore, several clinical studies have been performed in premature infants to evaluate the safety and efficacy of probiotic supplementation. A recent meta-analysis suggested efficacy. (19)

Despite these promising initial results, several questions remain that preclude their use as a standard of care for preterm infants. First, there are several species that have been utilized in the various studies, all of which have diverse effects, and the optimal preparation has not been clarified. In addition to the species, little is known regarding optimal dose, dosing strategy and whether live or attenuated probiotics are optimal for this condition. Furthermore, it is suggested that gut colonization with these organisms is important, yet no studies have confirmed that active colonization is necessary for disease prevention. Most importantly, additional information is needed to confirm that this approach is safe in this high-risk population. Although over 1000 preterm infants have been supplemented, the preparations have differed in the specificity of organism, and there is little federal regulation of probiotics when marketed as food additives. This federal regulation is needed as studies have shown that some preparations do not contain the active probiotics reported, and others have pathogenic organisms in the preparation. Finally, long-term effects of this approach should be evaluated, as these organisms can alter immune responses and microbial–epithelial cross talk, and therefore could result in many long-term effects. Nonetheless, probiotics are a promising approach for the prevention of neonatal NEC, and forthcoming studies may confirm the safety and efficacy.

CONCLUSIONS

The intestine serves as a vast interface between our internal and external environments. Evidence is rapidly accumulating that the microbes residing within the intestinal tract play major roles in the development of the immune system, and interact with the intestinal as well as central nervous systems. The implications of these interactions in health and disease are becoming increasingly evident and in some cases manipulations of the microbial ecosystems suggest significant benefit. Most of the studies to date using probiotics to manipulate the intestinal microbiota and to prevent or treat disease have been empiric and much more needs to be learned about the indigenous flora and their interactions with the developing intestinal tract before we can be comfortable in routinely manipulating the intestinal microbial ecosystem.

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Figure 1. NEUROIMMUNE INTERACTION AND MICROBIOTA

Commensal bacteria inhabiting the human intestine (i.e., intestinal microflora) participate in the development and maintenance of gut sensory and motor functions, including the promotion of intestinal propulsive activity. Normal intestinal motility requires the coordination between, enteric motor neurons ICC and smooth muscle cells. The ENS influences the gut directly with the activity related to the contraction (SMC ICC) and indirectly influencing the cells of the gut immune system. The functional bidirectional interaction act via neuroimmune peptide receptor on immune cells and on several receptor for immune mediators expressed on enteric nerves. Immune cells release mediators (cytokines, prostanoids) in response to neural stimuli Enteric and sensory nerves respond to immune stimuli

TABLE 1

In vivo studies demonstrating the effect of specific strain of probiotic on intestinal motility and visceral perception

Study	Probiotic strain	Effect
Kamiya T et al (12)	<i>L. reuteri</i> ATCC 23272	Inhibited cardioautonomic response to colorectal distension
Rousseaux C et al (13)	<i>Lactobacillus acidophilus</i> NCFM	Analgesic effect inducing expression of n-oppiod and cannabinoid receptor in intestinal epithelial cells
Ma X et al (14)	<i>L. reuteri</i> (non specified strain)	Prevent hyperexcitability of colonic DRG neurons induced by noxious stimuli
Wang B et al (15)	<i>L. reuteri</i> ATCC 23272	Enhancing tonic inhibition of colon contractile activity by acting via the IKCa channel current in AH cells