Probiotics and Human Milk Oligosaccharides in Premature Infants

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Education Gap

Understanding of the role of the intestinal microbiota in the premature infant is evolving; this community of microbes is important in the development of necrotizing enterocolitis and sepsis.

Abstract

Intestinal dysbiosis precedes and is a likely causative factor in necrotizing enterocolitis (NEC) and many cases of late-onset sepsis. Randomized controlled trials and observational cohort studies demonstrate decreased risk of NEC, sepsis, and death with the administration of probiotic microbes and decreased risk of NEC and sepsis with feeding of human milk. Animal studies suggest promising mechanisms by which probiotic microbes and human milk oligosaccharides alter the composition of the intestinal microbiota and may prevent disease in premature infants. Inclusion of parents in discussions of the risks and benefits of human milk and probiotics for premature infants is essential.

Objectives After completing this article, readers should be able to:

1. Summarize the translational and clinical evidence that human milk and probiotics decrease the risks of necrotizing enterocolitis, death, and sepsis in premature infants.
2. Summarize known functions of human milk oligosaccharides.

AUTHOR DISCLOSURE Dr Underwood has disclosed that he has received honoraria for lectures on human milk oligosaccharides from Abbott Nutrition and led a clinical trial of probiotic *Bifidobacterium infantis* that was funded by Evolve Biosystems. The funding source for this article is NIH R01 HD059127 and UL1 TR000002. This commentary does contain a discussion of an unapproved/investigative use of a commercial product/device.

ABBREVIATIONS

CMV	cytomegalovirus
GOS	galacto-oligosaccharide
HIV	human immunodeficiency virus
HMOs	human milk oligosaccharides
NEC	necrotizing enterocolitis
RCT	randomized controlled trial
RR	risk ratio

INTRODUCTION

Dysbiosis refers to an alteration of the microbial composition of a given anatomic niche that is associated with disease. Well-recognized acute diseases triggered by intestinal dysbiosis in children and adults include antibiotic-associated diarrhea and *Clostridium difficile* colitis. Increasing evidence suggests that intestinal dysbiosis also plays an important role in the pathogenesis of many chronic diseases of children and adults including allergic and autoimmune diseases. (1)(2) In the premature infant, intestinal dysbiosis appears to be an important trigger for

necrotizing enterocolitis (NEC) and sepsis. Threads of evidence that support this association include:

1) The timing of onset—NEC incidence peaks at 27 to 32 weeks' postmenstrual gestational age, (3) which correlates with the peak predominance of fecal proinflammatory proteobacteria (4)
2) Increased risk of NEC in infants receiving antibiotics or acid-suppressing agents both of which alter the intestinal microbiota (5)(6)(7)(8)
3) Associations between chorioamnionitis/funisitis and increased risk of sepsis weeks later (9)
4) Confirmation of the presence of bacterial species in the feces of preterm infants, which are identical to those isolated from the bloodstream in sepsis (10)
5) Decreased risk of NEC and sepsis with feeding of human milk and/or administration of probiotics, both of which alter the intestinal microbiota (11)(12)(13)(14)
6) Carefully conducted studies of changes in the fecal microbiota that occur before the onset of NEC, most commonly an increase in proteobacteria and decreases in firmicutes and bacteroides. (15)

This article includes a brief review of 1) the evidence that probiotics and human milk prevent NEC, death, and sepsis; 2) the risks associated with probiotics and human milk; and 3) the evidence that human milk oligosaccharides (HMOs) play a role in this protection.

PROBIOTICS

Benefits

Probiotics are dietary supplements that contain live bacteria. Animal and in vitro studies have been useful in elucidating mechanisms by which probiotics exert their beneficial effects, including production of bacteriocins (16)(17); suppression of expression of proinflammatory cytokines (18)(19)(20)(21)(22); stimulation of expression of anti-inflammatory cytokines (23)(24); stimulation of intestinal motility (25)(26); regulation of apoptosis, autophagy, and intestinal premeability (27)(28)(29)(30); production of short chain fatty acids (31); and binding to host mucosa and/or mucus. (32)(33) A recent meta-analysis summarized the probiotic strains most useful in preventing NEC in animal models. (34)

The large number of randomized placebo-controlled probiotic trials in premature infants reporting NEC, death, and sepsis has been the subject of several meta-analyses, results of which are summarized in Table 1. (35)(36)(37)(38)(39)(40)(41)(42)(43) Although several of the individual trials were small and the meta-analyses used varied statistical approaches, the results of each meta-analysis have been similar: administration of probiotics to premature infants decreases the risk of NEC and death, with most but not all meta-analyses demonstrating decreased risk of late-onset sepsis and shorter time to full enteral feeding. The following statement from the most recent update of the Cochrane review is compelling: "Enteral supplementation of probiotics prevents severe NEC and all-cause mortality in preterm infants. Our updated review of available evidence strongly supports a change in practice. Head-to-head comparative studies are required to assess the most effective preparations, timing and length of therapy to be utilized." (44) A recent more extensive meta-analysis that also included non-English manuscripts (51 randomized controlled trials [RCTs] including 11,231 preterm infants) focused on comparisons of benefit by probiotic strain, results of which are summarized in Table 2. (45) A meta-analysis comparing single organism probiotic products to multiple organism products found the latter to be more likely to prevent NEC. (46) A meta-analysis of RCTs reporting outcomes for extremely low-birthweight infants did not find evidence for benefit in this subset that is at particularly high risk for NEC (1,618 extremely low-birthweight infants in 6 RCTs, risk ratio [RR] for NEC stage 2 or 3 of 0.86 (95% confidence interval [CI] 0.64−1.16]). (37) Finally, a meta-analysis of 14 RCTs of probiotic administration to premature infants that reported surgical NEC as an outcome found no difference in stage 3 NEC between groups (3,975 preterm infants, RR 0.74 [95% CI 0.51-1.05]), but did find a lower risk of NEC-related death in the probiotic group (13 studies, RR 0.56 [95% CI 0.34-0.93]). (47)

In addition to these RCTs, 24 observational studies of probiotic administration to premature infants have been published to date. The first 12 and 14 were included in 2 meta-analyses which showed decreases in NEC, death, and sepsis that were similar to those seen in the RCTs. (39)(48) Ten more recent observational studies have been published. Four are of particular interest, in that 2 found no statistically significant benefit in NEC reduction (though 1 did find benefit only in the human milk-fed subgroup) (49)(50); 2 demonstrated an *increase* in NEC during the period of probiotic administration (the only published studies to date to demonstrate a worse outcome in this population) (51)(52); and the other 6 showed benefits similar to those seen in previous studies, including a very large cohort study of 44 NICUs in Germany. (53)(54)(55)(56)(57)(58) The combined unweighted odds ratios for all 24 observational studies are summarized in Table 3.

TABLE 1. **Meta-analyses of Probiotic Studies in Premature Infants**

REFERENCE	YEAR	TRIALS	INFANTS	RR (95% CI)
		NEC (stage 2 or 3)		
AlFaleh and Anabrees (35)	2014	20	5529	0.43 (0.33-0.56)
Yang et al (36)	2014	17[a]	4198	0.34 (0.25-0.45)
Sawh et al (37)	2016	35	10,520	0.53 (0.42-0.66)
Deshpande et al (38)	2017	20[b]	4022	0.46 (0.34-0.61)
Dermyshi et al (39)	2017	29	8535	0.57 (0.47-0.70)
Thomas et al (40)	2017	23	7325	0.57 (0.43-0.74)
		All-cause mortality		
AlFaleh and Anabrees (35)	2014	17	5112	0.65 (0.52-0.81)
Yang et al (36)	2014	14[a]	3583	0.58 (0.46-0.75)
Sawh et al (37)	2016	27	9507	0.79 (0.68-0.93)
Deshpande et al (38)	2017	19[b]	4196	0.73 (0.59-0.90)
Dermyshi et al (39)	2017	27	8156	0.77 (0.65-0.92)
Thomas et al (40)	2017	23	6954	0.72 (0.57-0.92)
		Late-onset sepsis		
Yang et al (36)	2014	17[a]	4043	0.94 (0.83-1.1)
Sawh et al (37)	2016	28	8707	0.88 (0.77-1.0)
Rao et al (41)	2016	37	9416	0.86 (0.78-0.94)
Deshpande et al (38)	2017	18[b]	4062	0.80 (0.71-0.91)
Dermyshi et al (39)	2017	28	7987	0.88 (0.80-0.97)
Aceti et al (43)	2017	20[c]	3402	0.75 (0.65-0.85)
Time to full enteral feedings				Mean difference (95% CI)
Yang et al (36)	2014	9[a]	1626	-1.66 days (−3.6 to 0.27)
Aceti et al (43)	2016	5[d]	719	-3.15 days (−5.3 to −1.1)
Sawh et al (37)	2016	17	4448	-1.2 days (−2.2 to −0.1)
Deshpande et al (38)	2017	13[b]	2154	-1.95 days (−-3.4 to −0.45)

CI=confidence interval; NEC=necrotizing enterocolitis; RR=relative risk.
[a]English and Chinese language publications.
[b]Low and middle income countries only.
[c]Included only studies with exclusively human milk-fed premature infants; 16 studies of exclusive formula feeding showed no significant decrease in late-onset sepsis with probiotic administration (800 premature infants; RR 0.77 (95% CI 0.51-1.17)).
[d]Included only studies with exclusively human milk-fed premature infants; 2 studies of exclusive formula feeding showed no benefit in time to full enteral feeding with probiotic administration.

Risks

In the United States, less than 15% of neonatal intensive care units currently administer probiotics to premature infants. (59) The US Food and Drug Administration does not monitor the purity and viability of probiotic products and does not recommend administration of probiotics without oversight through the Investigational New Drug program. The American Academy of Pediatrics does not recommend probiotic administration because of the lack of products with established safety and efficacy. Safety concerns include contamination of commercial probiotic products with potential pathogens (60) and sepsis caused by translocation of a probiotic organism into the bloodstream. (61) The evidence from the RCTs that death and sepsis are

TABLE 2. **Strain-Specific Summary of Beneficial Probiotics**

PROBIOTIC STRAIN	NO. OF STUDIES	NO. OF INFANTS	RR (95% CI)
	NEC (stage 2 or 3)		
Bifidobacterium lactis Bb12 or B94	5	828	0.25 (0.10-0.56)
Lactobacillus reuteri 55730 or 17938	4	1459	0.43 (0.16-0.98)
Lactobacillus rhamnosus GG	6	1507	0.24 (0.06-0.67)
Bifidobacterium bifidum, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*	2	247	0.25 (0.05-0.89)
B infantis 15697, *L acidophilus* 4356	1	367	0.16 (0.02-1.0)
B infantis Bb02, *B lactis* Bb12, *Streptococcus thermophilus* TH4	2	1244	0.29 (0.07-0.78)
B longum 35624, *L rhamnosus* GG	2	285	0.18 (0.02-0.89)
	All-cause mortality		
B bifidum 1453 + *L acidophilus* 1748	2	494	0.16 (0.02-0.74)
B bifidum, *B infantis*, *B longum*, *L acidophilus*	1	186	0.26 (0.06-0.98)
B infantis, *L acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *L rhamnosus*, *S thermophilus*	1	150	0.09 (0.003-0.70)
	Late-onset sepsis		
B bifidum, *B infantis*, *B longum*, *L acidophilus*	2	247	0.43 (0.18-0.94)
B longum R00175, *Lactobacillus helveticus* R0052, *L rhamnosus* R0011, *Saccaromyces boulardii* CNCM I-1079	3	241	0.34 (0.16-0.66)
	Time to full enteral feedings (days)		Mean difference (95% CI)
L reuteri 55730 or 17938	3	626	−3.3 d (−6.4 to −0.62)
B bifidum, *B infantis*, *B longum*, *L acidophilus*	2	247	−4.7 d (−8.6 to −0.7)
B longum BB536, *L rhamnosus* GG	1	94	−10 d (−16 to −3.6)

Data from van den Akker et al. (45) CI=confidence interval; NEC=necrotizing enterocolitis; RR=relative risk.

consistently either lower or equivalent in the infants receiving the probiotic compared with those receiving the placebo suggests strongly that the risks of probiotic sepsis are very low. Secondary analyses of probiotic trials in preterm infants reporting other outcomes including intraventricular hemorrhage, bronchopulmonary dysplasia, retinopathy of prematurity, and neurodevelopmental outcomes have shown neither benefit nor negative effects, suggesting that probiotics are safe. (62)(63)(64)(65)(66)

HUMAN MILK OLIGOSACCHARIDES

Benefits

Administration of mother's own milk particularly in the first weeks after birth decreases the risk of NEC and sepsis. (11) (67) A meta-analysis of the few RCTs in premature infants comparing formula to donor human milk demonstrated a higher risk of NEC with formula than with donor milk (RR 2.8 [95% CI 1.4-5.5]). (12) Multiple components of human milk are likely responsible for these protective effects, including antibodies (mostly secretory immunoglobulin [Ig] A but also IgG and IgM), lactoferrin, lysozyme, growth factors, enzymes and HMOs. HMOs are nondigestible glycans that are produced in abundance in human milk with a high number of structures and striking diversity of structures among women. The obvious question is why does a mother produce such a large volume of diverse HMOs at great cost if these glycans have no nutritional value to her infant? Three compelling hypotheses are supported by current evidence. First, HMOs are an energy source for a

TABLE 3. **Observational Cohort Studies of Probiotic Administration in Premature Infants**

	NO PROBIOTIC (CASES/N)	PROBIOTIC (CASES/N)	ODDS RATIO (95% CI)
Necrotizing enterocolitis stage 2 or 3	831/16,587 (5.0%)	432/15,111 (2.9%)	0.56 (0.50-0.63)
All-cause mortality	1144/14,165 (8.1%)	981/14,383 (6.8%)	0.83 (0.76-0.91)
Late-onset sepsis	2,083/12,932 (16%)	1,950/13,141 (15%)	0.91 (0.85-0.97)

very limited number of species of bacteria. Unlike commercial prebiotic glycans, such as galacto-oligosaccharides (GOSs), fructo-oligosaccharides, inulin, and lactulose, which can be consumed by a wide variety of gut microbes, intact HMOs can be consumed by only 2 bacterial genera: *Bacteroides* and *Bifidobacterium*, and in fact among the bifidobacteria, only a few species (eg, *Bifidobacterium bifidum, Bifidobacterium breve, B longum* subspecies *longum* and *B longum* subspecis *infantis*) are aggressive consumers of HMOs. (68) Although HMOs play a major role in shaping the microbiota of the term infant, the effect of maternal HMO composition in the preterm infant is more subtle. (69)(70) This is not surprising because 1) neither *Bacteroides* nor *Bifidobacterium* species are common in the premature infant microbiota unless the infant is receiving probiotic organisms, and 2) environmental factors play a major role in shaping the gut microbiota in this hospitalized population. We also found higher variability in HMO composition in the milk of women delivering preterm infants compared with those delivering at term. (71) Thus, the lactating woman invests energy to produce HMOs to shape the intestinal microbiota of her infant, but this appears to have less impact in premature infants than in term infants. Second, HMOs have similar structures as the surface glycans on enterocytes to which intestinal bacteria and viruses are able to adhere. In vitro studies have demonstrated decreased binding of *Campylobacter jejuni* and *Pseudomonas aeruginosa* in the presence of 2 common HMOs (2′fucosyllactose and 3 fucosyllactose) (72) and effective binding of these same HMOs to the site on the norovirus particle that adheres to host enterocytes. (73) Human studies have demonstrated decreased risk of infectious diarrhea in infants whose mothers had higher levels of specific HMOs in their milk. (74) Thus, lactating women produce HMOs to protect their infants from diarrheal illness. Third, HMOs appear to affect the developing immune system. Infants randomly assigned to receive formula containing GOS plus a single common HMO structure (2′fucosyllactose) had lower serum levels of proinflammatory cytokines and a lower incidence of atopic dermatitis than the infants who received a formula with GOS but no added HMO. (75)(76) In a separate study, infants receiving formula with 2 added HMOs (2′fucosyllactose and lacto-n-neotetraose) had fewer parent-reported episodes of lower respiratory infections, fevers, and antibiotic courses than infants receiving a formula without HMOs. (77) Thus, lactating women produce HMOs to influence their infants' immune responses.

Risks

Human milk is recommended as the primary enteral nutrition for premature infants, particularly those with birthweights less than 1,500 g, with donor human milk recommended when available if the volume of mother's milk is insufficient. (78) The major risks of providing unpasteurized mother's own milk are transmission of viral infections. The risk of human immunodeficiency virus (HIV) infection is sufficient to support current recommendations for HIV-infected mothers in developed countries to avoid breastfeeding. (79) The risk of infection with either hepatitis B or hepatitis C through mother's milk is very low, (80) but the risk of cytomegalovirus (CMV) infection in very premature infants is not insignificant. Most recent estimates are quite sobering; prospective cohort studies show infection rates among very preterm infants with CMV-positive mothers of 15% to 100%, with the most premature infants and infants of mothers with the highest DNA load at highest risk. (81)(82)(83)(84) Symptomatic CMV infection and severe sepsislike syndrome are uncommon, with the latter ranging from 0% to 14% of CMV infections (82); however, associations between CMV infection and increased risk of bronchopulmonary dysplasia and retinopathy of prematurity have been reported. (83)(84) Freezing of human milk does not decrease the risk for CMV infection. (85) Pasteurization of donor human milk is highly effective at killing HIV, hepatitis C, and CMV.

The primary risk with donor human milk is poor growth and development. Donor milk is generally provided by mothers who delivered at term and have been breastfeeding for a time. As a result, the milk is often low in protein and must generally be fortified to provide adequate growth. Although current pasteurization techniques denature many of the bioactive proteins and peptides in human milk, HMOs are quite heat resistant and therefore pooled donor human milk is a good source of HMOs.

SYNERGISM BETWEEN HUMAN MILK AND PROBIOTICS

Among the probiotic clinical trials published to date, few have reported how many of the infants in each group received human milk. Among those reporting this important covariate, the infants receiving the combination of human milk and probiotics had a lower risk of NEC than infants receiving probiotics and formula. Researchers at University of California (UC) Davis have hypothesized over the last 15 years that combining administration of human milk (rich in HMOs) with a probiotic that is able to consume HMOs would improve colonization of the gut with the administered probiotic. This was found to be true in premature infants in comparisons of 2 common probiotic bifidobacteria: *Bifidobacterium longum* subsp *infantis* (the most aggressive HMO consumer among the many analyzed) and *Bifidobacterium animalis* subsp *lactis* (a nonconsumer of HMOs). In both formula-fed and human milk-fed premature infants, *B infantis* was a better colonizer than *B lactis* and the greatest increases in fecal bifidobacteria and decreases in fecal proteobacteria were seen with the combination of human milk and *B infantis*. (14) Animal studies have confirmed that combining HMO-consuming probiotics and either synthetic HMOs or bovine milk oligosaccharides improves colonization with the administered organism. (31)(86) In a recent trial among breastfed term infants, administration of *B infantis* for a brief period (from 7-28 days of age) quickly led to domination of the infant fecal microbiota with the administered probiotic to such an extent that the usual differences in the microbiota between infants born via cesarean section and infants delivered vaginally were attenuated. The hypothesis was that once the infants were colonized with this HMO-consuming probiotic, colonization would be maintained as long as the infants were receiving human milk. Indeed, at 2 months of age, *B infantis* remained the dominant organism in the feces, a full month after stopping the probiotic. (87) Whether such an approach will mitigate the risks of chronic diseases associated with cesarean section delivery or intestinal dysbiosis in general remains uncertain.

BRINGING PARENTS INTO THE DISCUSSION

At the first US symposium dedicated exclusively to NEC, held in Davis, CA, in April 2017, the involvement and inclusion of parents whose families have been affected by NEC was powerful. The symposium was cosponsored by the NEC Society (a nonprofit foundation started by 2 mothers whose infants died of NEC) and the Department of Pediatrics at UC Davis. The parents who attended the symposium were united in their profound expressions of appreciation for the research in NEC to date and their frustration and anguish at the relatively little information they received about NEC either before or even after disease onset. The united voice of the parents was that they need more information about NEC prevention early in their infant's life, including discussions about the risks and benefits of mother's own milk, pasteurized donor human milk, probiotics, and avoidance of unnecessary antibiotics and other medications that may increase the risk of NEC. (88) If, after review of the literature and a thoughtful discussion with the parents, a neonatologist decides that the potential benefits of providing a combination of human milk and probiotics outweigh the potential risks, a probiotic product that is manufactured to high standards and has been demonstrated to be effective should be chosen. The NEC Society (NECSociety.org) describes a probiotic quality improvement approach with descriptions of probiotics that meet those criteria and a plea to share quality improvement data. Further comparisons of probiotics to placebo are unlikely to provide new insights; rather, head-to-head comparisons of promising probiotics are needed and will require very large sample sizes.

EVIDENCE

- The benefits of human milk outweigh the risks in premature infants, with the exception of HIV-infected mothers in developed countries and other maternal and infant conditions. The benefits of donor human milk outweigh the benefits of formula for very low-birthweight infants (meta-analysis, strong recommendation).
- The benefits of probiotic administration outweigh the risks in premature infants and justify a careful discussion with parents and a potential practice change (meta-analysis, strong recommendation).

American Board of Pediatrics Neonatal-Perinatal Content Specifications

- Know the pathophysiology of NEC.
- Know the differences between the composition of breast milk of the mother of a preterm infant and that of a full-term infant.
- Know the immunologic and anti-infective constituents in human milk and their physiologic effects.

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1. Alterations in the microbial composition of the gastrointestinal tract (dysbiosis) have been implicated in the pathophysiology of necrotizing enterocolitis (NEC). Which of the following statements does NOT support the role of dysbiosis in the pathophysiology of NEC?
    A. The timing of NEC correlates with the peak predominance of firmicutes.
    B. Infants receiving antibiotics have an increased risk for NEC.
    C. Breastfed infants have a decreased risk for NEC.
    D. Changes in fecal microbiota have been identified before the onset of NEC.
    E. Bacterial species have been identified in stools which are identical to those isolated from blood cultures in preterm infants with sepsis.
2. Probiotics are dietary supplements containing live bacteria that have been shown to be protective against NEC in preterm infants. Which of the following represents a mechanism by which probiotics exert their protective effects?
    A. Production of long-chain fatty acids.
    B. Inhibition of bacterial motility.
    C. Production of bacteriocins.
    D. Inhibition of autophagy.
    E. Binding to proinflammatory cytokines.
3. Several studies have evaluated the use of probiotics in preterm infants and have been included in meta-analyses. These studies support a decrease in NEC rates in preterm infants receiving probiotics. However, there remain questions regarding which preparation is most effective and when and how long to treat. Based on results from these meta-analyses, which statement is CORRECT regarding the use of probiotics in preterm infants?
    A. Probiotic use is most effective in preventing NEC in extremely low-birthweight infants.
    B. Probiotic administration decreases the rate of surgical NEC.
    C. The most recent Cochrane review indicates that while probiotics prevent severe NEC, they do not decrease all-cause mortality in preterm infants.
    D. Products containing multiple strains are more likely to prevent NEC compared with those containing a single organism.
    E. A consistent decrease in the risk of late-onset sepsis is observed with probiotic administration.
4. Human milk feedings have been shown to decrease the risk of NEC and sepsis. Which of the following components of human milk is NOT thought to be responsible for the protective effects of human milk?
    A. Lactoferrin.
    B. Lysozyme.
    C. Immunoglobulin A.
    D. Human milk oligosaccharides (HMOs).
    E. Lactose.

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5. HMOs are nondigestible glycans that are abundant in human milk. A wide diversity in the structure of HMOs as well as large diversity among women has led to several hypotheses regarding the function of HMOs. Which of the following statements regarding the role of HMOs is CORRECT?
    A. Similar to commercial prebiotic glycans, HMOs can be consumed by a wide variety of gut microbes.
    B. Infants receiving formula with added HMOs have a lower incidence of atopic dermatitis.
    C. HMOs have been shown to play a major role in shaping the microbiota of preterm infants, and to a lesser degree, term infants.
    D. There is less variability in the HMO composition of preterm human milk compared with that of term human milk.
    E. In vitro studies indicate that HMOs decrease binding of *Campylobacter jejuni* and *Pseudomonas aeruginosa* but not norovirus.