Probiotics in the Prevention of Necrotizing Enterocolitis

Kriston Ganguli, MD and W. Allan Walker, MD

Abstract: Necrotizing enterocolitis (NEC) is a common gastrointestinal inflammatory necrosis affecting almost exclusively premature infants usually after oral nutrition has been started, for example, 10 day plus postpartum. Although the pathogenesis is incompletely understood, major risk factors include prematurity and incomplete bacterial colonization. Evidence has been shown that the premature infant because of rapid passage through the birth canal or because of delivery by cesarean section has an inadequate initial ingestion of maternal colonic and vaginal flora and therefore, an inadequate initial colonization with less diversity of bacteria phylla and fewer species of bacteria in the microbiota. As a result, they are more susceptible to environmental pathogens. In addition, prematures have immature intestinal defenses (glycocalyx, tight junctions, innate immune response, etc.) resulting in excessive inflammation in response to luminal stimuli. Recently, we reported that genes mediating the innate inflammatory immune response are developmentally expressed with an increase in toll-like receptors, signaling molecules and transgenic factors and decreased negative regulators of inflammation, which undoubtedly contribute to an excessive inflammatory response. Several clinical studies have suggested that the use of probiotics and ingestion of expressed maternal breast milk containing probiotics can help to stabilize colonization and to reduce the incidence and severity of NEC when given to premature infants at risk. Meta-analyses of multiple small studies strongly suggest a protective effect in the use of probiotics. A multicenter study in Taiwan suggests that *Bifidobacteria infantis* and *Lactobacillus acidophilus* in combination may prevent NEC. These meta-analyses suggest that these probiotics should be used in routine care of premature infants. Other clinicians, however, suggest caution, holding out for a single protocol multicenter trial before routine use can be suggested.

Key Words: probiotics, necrotizing enterocolitis, premature infants

(*J Clin Gastroenterol* 2011;45:S133–S138)

Necrotizing enterocolitis (NEC) is a potentially devastating disease, characterized by severe intestinal inflammation and necrosis, which occurs primarily in preterm infants. Although it is the most common gastrointestinal emergency of neonates, advances in the prevention and management of this disorder, compared with global advances in neonatal care, in general, have been relatively limited.[1,2] The risk of developing NEC is inversely related to gestational age and birth weight. However, a small percentage of cases occur in full-term infants, and may represent a distinct disease entity.[3] According to data in the United States and Canada, the mean prevalence of NEC in neonates between 500 and 1500 g at birth is approximately 7%.[4–7] Neonates younger than 28 weeks gestation and of extremely low birth weight (< 1000 g) are particularly susceptible.[8]

The pathogenesis is poorly understood despite decades of research, and therefore, continued scientific investigation in this area has become a priority.[9,10] An exaggerated inflammatory response of the immature intestine occurs during a complex interplay between bacterial colonization, initiation of enteral nutrition, and hypoxic-related intestinal injury. In a susceptible host, this process may contribute to the development of intestinal necrosis, and ultimately NEC.[9] Intestinal innate immune responses mature throughout gestational development, therefore prematurely born infants are required to interact with the extrauterine environment with ill-equipped intestinal immunity.

Abdominal distention, feeding intolerance, and bloody stools comprise the classic triad of signs and symptoms described in NEC. Regardless of gestational age, NEC often presents after 8 to 10 days of age. The initial signs and symptoms may be subtle and nonspecific, emphasizing the importance of meticulous monitoring once enteral feeds have been initiated. Ominous clinical progression includes intestinal perforation, peritonitis, and hemodynamic instability. Radiographic findings such as fixed, dilated small bowel loops, extraluminal air, paucity of intestinal gas, pneumatosis intestinalis, and portal venous gas, suggest severe disease. Although some neonates respond to aggressive medical management, others require acute surgical intervention as well. The effective management option depends upon the baseline clinical condition of the neonate and the extent and severity of disease at the time of diagnosis. There are a number of NEC-like syndromes described in the literature, which may occur in preterm or term infants. The relatively older preterm infant may develop a disorder of intestinal inflammation and necrosis, which seems clinically similar to NEC, typically during the first week of life. This may represent a unique pathogenesis, as comorbid conditions such as congential heart disease, intestinal anomalies and perinatal stress, and specific risk factors such as intrauterine illicit drug exposure, have been associated with these cases.[11,12] Spontaneous intestinal perforation in preterm infants is likely an unrelated entity as the pathognomonic findings of intestinal inflammation and necrosis are very mild.[4,13,14]

The mortality rate associated with NEC is approximately 20% to 30% in all neonates, with those requiring surgical intervention facing an increased risk.[15] Morbidity is due to the intestinal sequelae of the disease process, such as intestinal strictures, and surgical outcomes, such as short bowel syndrome after intestinal resection. In addition, the extension of the inflammatory effects systemically may result in extraintestinal morbidity, particularly chronic neurodevelopmental impairment. This is particularly true of extremely low birth weight infants.[16,17] In addition to birth weight, the degree of neurodevelopmental impairment is also associated with the severity of NEC. Although

From the Department of Pediatric Gastroenterology, Harvard Medical School, Mucosal Immunology Laboratory, Massachusetts General Hospital for Children, Boston, MA.

Walker is supported by grants from the National Institutes of Health (R01 HD012437; R01 DK070260; P30 DK040561; P01 DK033506).

The authors declare that they have nothing to disclose.

Reprints: W. Allan Walker, MD, Harvard Medical School, Mucosal Immunology Laboratory, Massachusetts General Hospital for Children, 114 16th Street (114-3503), Charlestown, MA 02129-4404 (e-mail: wwalker@partners.org).

infants with NEC requiring surgical intervention without associated late bacteremia had an increased risk of psychomotor developmental delay, odds ratio (OR) = 2.7 (range, 1.2 to 6.4), those with associated late bacteremia had a markedly increased risk of diparetic cerebral palsy, OR = 8.4 (range, 1.9 to 39), and microcephaly, OR = 9.3 (range, 2.2 to 40). Infants with medically managed NEC and late bacteremia were twice more likely to develop diparetic cerebral palsy than those with late bacteremia alone (6% vs. 3%).[18]

Initiation of enteral nutrition is a prerequisite to the development of NEC. As a preventive strategy, several institutions have established prolonged parental nutrition protocols, which have lead to increased infectious risk and delayed hospital discharge.[19] Delays in enteral nutrition may promote atrophic changes of the intestine and therefore may prolong the process of enteral nutrition initiation and advancement later. The expense of neonatal care provided to infants with NEC in the United States is estimated to be $500 million and $1 billion annually. Hospitalizations are prolonged by 20 to 60 days on average compared with the length of stay for neonates without NEC. The most common chronic consequence of surgically managed NEC, short-bowel syndrome, incurs $1.5 million for 5 years of continued outpatient care per patient.[4,20]

ESTABLISHMENT OF INTESTINAL MICROBIOTA

Before intestinal colonization, the newborn intestine is sterile. Several host and microbial factors affect the process of intestinal microbiota acquisition and ultimately the bacterial composition.[21] The critical process of bacterial colonization is altered by mode of delivery, antibiotic exposure, neonatal environment, and source of nutrition. Bacterial strains known to colonize the newborn intestine include beneficial strains such as *Bifidobacterium* and *Lactobacillus,* those which are potentially harmful, such as *Escherichia coli* and *Bacteroides* and opportunists, including *Clostridia, Staphylococcus,* and *Pseudomonas aeruginosa*.[22] Newborns delivered vaginally are colonized with beneficial bacterial strains earlier than those delivered by cesarean section. In addition, increased colonization of *Klebsiella, Enterobacter,* and *Clostridia* is associated with cesarean delivery.[23–25] Breast-fed infants are primarily colonized with *Bifidobacterium*, with minor populations of *Lactobacillus* and *Streptococcus*. In contrast, formula-fed infants are primarily colonized with *Bacteroides* and *Bifidobacterium*, with minor populations of *Clostridia, Staphylococcus, and Escherichia coli*.[25–27] The process of bacterial colonization is delayed in premature infants, which may be due to relative environmental sterility and antibiotic use (Fig. 1). In addition, the risk of colonization with pathogens is increased in this population compared with full-term infants.[28] A critical relationship exists between the various enteric bacterial populations and the underlying balance of this relationship is necessary for host health. An imbalance, or dysbiosis, is responsible for compromised host health and ultimately clinical disease expression.[29] The effects of pathogenic bacterial dominance include diarrhea, enteric infections, liver disease, carcinogenesis, and intestinal putrefaction.[30] In contrast, the effects of beneficial bacteria, which promote host health, may include inhibition of pathogenic bacterial growth, promotion of immune function, diminution intestinal gas production, and improvement of digestion and absorption.

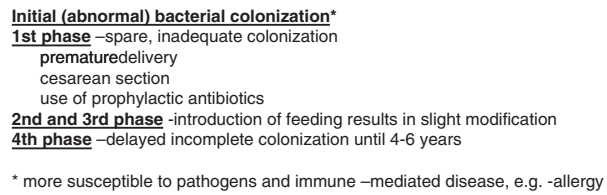

FIGURE 1. Sequence of abnormal colonization in prematures with cesarean section or excessive use of perinatal antibiotics. Complete colonization is delayed for several years during which time the infant is susceptible to both infection and immune-mediated diseases such as allergy.

When the process of weaning is complete, the established intestinal microbiota approaches that of an adult.[30]

HOST IMMATURITY OF THE PRETERM INFANT

The fetal immune system has evolved to avoid maternal rejection rather than to process potential pathogens.[31] Therefore, after delivery, significant alterations of the neonatal immune system occur to allow postnatal tolerance of the extrauterine environment.[32] The development of postnatal immune function, characterized by a shift from a Th2-predominant to Th1-predominant phenotype, has been shown to be strongly influenced by the intestinal microbiota.[33,34] After the initial stages of postnatal colonization, intestinal immune responses adapt to the increased microbial stimulation.[35,36]

There are several functional characteristics of the mature intestine, which limit the ability of bacteria to enter the intestinal lumen and prevent bacterial adherence and translocation. In addition, the innate and adaptive immune responses to luminal bacteria are closely regulated in the mature intestine by negative feedback inhibition of toll-like receptor (TLR) activation. Negative inflammatory regulators, such as MyD88, IRAKM, ST2, and SOCS1, allow a controlled inflammatory response.[37]

Relative intestinal immaturities of the preterm infant include abnormal peristalsis, unique adhesion site glycoconjugates,[38,39] intestinal mucus composition,[40] increased intestinal permeability, secretory IgA levels,[41–43] lower gastric acidity, trefoil factor deficiency,[44–46] and alterations of defensins.[47,48] An exaggerated inflammatory response to both commensal and pathogenic bacteria has been demonstrated in human immature enterocytes, compared with mature enterocytes.[49,50] This may be due to aberrant inflammatory regulator expression in the immature intestine. Developmentally regulated changes result in decreased IkB expression and increased TLR4 expression in immature enterocytes, allowing a more robust inflammatory response.[49,51,52] In addition, the expression of several positive and negative regulators of intestinal inflammation was shown to be distinctly different between immature and mature human enterocytes. Immature human enterocytes and intestinal xenografts showed increased expression of TLR4, TLR2, MyD88, TRAF-6, and NFkB1 and decreased expression of SIGIRR, IRAK-M, A-20, and TOLLIP (Fig. 2). This inflammatory regulator profile was further altered in NEC enterocytes.[53] The intestinal immaturity and unique environmental factors of the preterm infant may alter the interaction between colonizing bacteria and the developing enteric immune system, further compounding the risk of NEC.

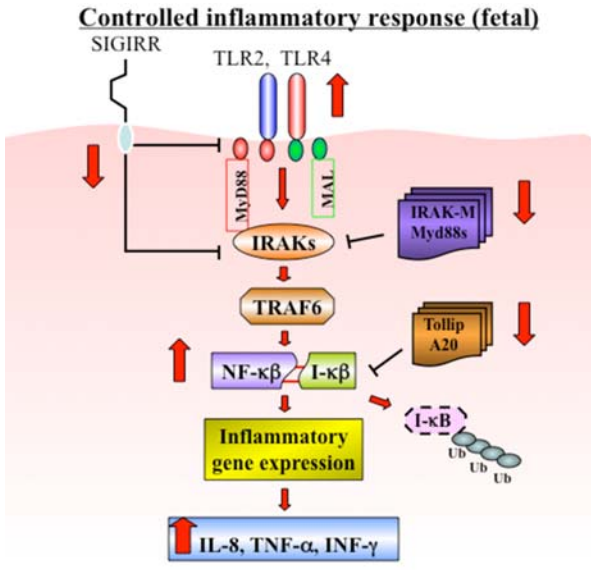

FIGURE 2. A diagram of the innate inflammatory immune response in the intestinal epithelium of premature infants. Unlike the mature enterocyte, premature enterocytes have an increased expression of toll-like receptors, their signaling molecules, and transcription factor genes and a decreased expression of negative regulator genes, which turn off inflammation. This developmentally regulated expression of innate inflammatory response genes may explain the excessive inflammatory response to luminal stimuli seen in the premature infant.

ROLE OF INTESTINAL MICROBIOTA IN NEC

Clinical trials demonstrating a reduction in the incidence of NEC in premature infants receiving probiotic supplementation emphasize the importance of commensal bacteria colonization in the early postnatal period.[54–56] The interaction of the immature intestine with the extrauterine microbial environment after delivery is a critical risk factor in the pathogenesis of NEC. The inability to induce a NEC-like disease process in germ-free in vivo models supports the importance of luminal bacteria in the pathogenesis of this disease. NEC typically develops after 8 to 10 days of life, which parallels the timeline required for intestinal colonization by anaerobes.[57,58] Using 16s rRNA sequencing, the fecal bacterial strain diversity is significantly decreased in preterm infants who develop NEC, compared with those who do not.[59] Molecular bacterial analysis of fecal samples of preterm infants have shown that atypical species and reduced diversity is associated with an increased risk of developing NEC.[59,60] Decreased fecal microbial diversity of the gut microbiome is common to a variety of clinical conditions that may be related to dysbiosis, including NEC.[61] A rich intestinal microbial diversity protects against colonization with potential pathogens, and therefore the lack of this diversity, as seen in preterm infants, is considered an additional contributing factor to the pathogenesis of NEC.[4,57]

ROLE OF PROBIOTICS IN NEC

Given the scientific understanding of NEC pathogenesis and the abnormal colonization patterns of preterm infants, strategies to favorably alter the intestinal microbiota could offer an effective preventive approach against this devastating disease. Probiotic bacteria may promote a healthy intestinal microbial balance and enhance intestinal immune function, which would provide an element of protection against NEC in the neonate. The mechanisms by which probiotics protect against intestinal disease include optimizing microbial balance, competitive exclusion of pathogens, promotion of mucus secretion, production of bacteriocins, enhancement of barrier integrity, and maturation of intestinal immunity.[61] The mechanism of protection depends upon the specific probiotic strain and disease model used in investigation.[61] In clinical trials investigating probiotics as a preventive strategy for NEC, commensal strains known not to translocate or cause mucosal injury to the host are typically chosen.[62] Not all probiotic strains have the same immunologic effects, even between bacteria of the same species.[63,64] However, selection of the optimal probiotic strain or combination regimen is critical in obtaining desired protective effects, and the mechanisms of probiotic reduction of NEC are not well understood.[63–65]

At the time of our last published review of probiotic use in NEC in 2008, there were 5 clinical trials investigating the use of orally administered probiotics in the prevention of neonatal NEC.[66–70] These studies varied in size, enrollment criteria, probiotic strain, timing of intervention, probiotic dose, and specific outcome measures. Of the 3 studies, which showed a statistically significant reduction in the incidence of NEC,[66,68,70] 2 were prospective, randomized, controlled trials.[66,70] The first three studies, by Hoyos[68] included all newborns admitted to a neonatal intensive care unit in Colombia, regardless of size or gestational age. *L. acidophilus* and *B. infantis* administration decreased the incidence of NEC from 6.6% to 2.9%, compared with historical controls. The 2 subsequent studies in 2005, by Lin et al[70] in Taiwan and Bin-Nun et al[66] in Israel, enrolled only preterm infants < 1500 g and investigated 2 different combination probiotic regimens using a prospective, randomized, control trial design. They showed a significant reduction in the incidence of NEC with combined *L. acidophilus* and *B. infantis* in the former, and combined *B. infantis*, *Streptococcus thermophilus,* and *Bifidobacterium bifidus*, in the latter.

Deshpande et al[71] published a systematic review of 11 randomized, controlled trials between 1997 and 2009, from the Cochrane Central register, which included 2176 neonates. Trials selected for this meta-analysis included any randomized controlled trail which investigated the use of an orally administered probiotic regimen, started within the first 10 days of life, continued for ≥ 7 days in preterm very-low-birth-weight neonates (< 34 wk gestation and birth weight < 1500 g) and included stage II-III (severe) NEC as an outcome measures, using Modified Bell staging criteria.[66,67,69,70,72–78] This meta-analysis included the 2 randomized controlled trails discussed in our 2008 review. The data from these trials reported an incidence of severe NEC as 6.56% in the control group compared with 2.37% of the probiotic group. A fixed-effects analysis estimated a decreased risk of severe NEC in neonates supplemented with probiotics [relative risk (RR): 0.35; 95% confidence interval (CI), 0.23-0.55; $P < 0.00001$]. Heterogeneity between trials was minimal and the NNT to prevent 1 case of severe NEC was 25 (95% CI, 17-34). Data analysis of the 10 trials, which included culture-positive sepsis as an outcome measure, showed no significant difference between the 2 groups, however, significant trial design heterogeneity was noted.[66,67,69,70,72,73,75–78] Data from 9 trials showed a reduced

risk of all cause mortality in the probiotic group compared with the control group (RR: 0.42; 95% CI, 0.29-0.62; $P < 0.00001$), with no significant trial heterogeneity.[66,67,69,70,73,75–78] In addition, the risk of NEC-associated mortality was similar between groups (RR: 0.30; 95% CI, 0.08-1.081), with no significant trial heterogeneity.[66,67,69,73,76]

The most recent Cochrane Collaboration Review, by AlFaleh et al,[56] was published in 2011. This analysis included randomized controlled trials, which enrolled infants < 37 weeks gestation and/or < 2500 g at birth, investigated an orally administered probiotic regimen and reported severe NEC (stage II-III) and positive blood or cerebral spinal fluid cultures after 5 days of age.[66,67,69,70,72–80] Two new trials completed since the Deshpande et al[71] review qualified for inclusion in the AlFaleh analysis.[79,80] The 2011 Cochrane Review reported a significantly reduced incidence of severe NEC in the probiotic groups of 13 qualified studies, regardless of birth weight (RR: 0.35; 95% CI, 0.24-0.52; NNT 25) and similar findings in the neonates < 1500 g at birth, from 12 of these studies (RR: 0.34; 95% CI, 0.23-0.50).[66,67,69,70,72,73,75–80] Four of the 13 studies were considered superior, based on standard Cochrane Collaboration methods of determining methodological quality.[67,70,76,79] The data from these trials showed a significant reduction in the incidence of NEC in the probiotic group compared with controls (RR:0.25; 95% CI, 0.13-0.49). Ten studies reported all-cause mortality,[66,67,69,70,73,76–79,81] which showed a significantly reduced incidence in the probiotic group compared with controls (RR: 0.40; 95% CI, 0.27-0.60) and 5 studies reported NEC-associated mortality,[66,67,69,76,80] which also showed a significantly reduced incidence in the probiotic group (RR: 0.31; 95% CI, 0.10-0.94). Sepsis was reported in all 13 trials, and was not different between groups (RR: 0.90; 95% CI, 0.76-1.07). Although the data included extremely low birth weight infants, data specific to this subgroup could not be determined.

Since the publication of the 2011 Cochrane Review, an additional prospective, double-blind, randomized, controlled clinical trial was conducted by Braga et al[82] Preterm infants with a birth weight between 750 to 1499 g received either human milk supplemented with *Bifidobacterium breve* or *L*actobacillus *casei* or human milk alone, initiated during the second day of life. The primary outcome was stage ≥ 2 NEC by Bell modified criteria, which was noted in 3.6% of the control group and not noted in the probiotic group ($P = 0.05$). In addition, there were no significant differences in the incidence of sepsis or mortality between groups.

CONCERNS

Although data supports the efficacy of probiotics in the prevention of NEC, concerns regarding the safety of probiotic prophylaxis in neonates remain. Given the relative immune compromise and specific immaturities of intestinal function in preterm infants, probiotic translocation and subsequent sepsis is of concern. There was an increased risk of sepsis in neonates receiving orally administered probiotics in the randomized, controlled clinical trial by Lin et al,[76] especially in the most vulnerable neonates with birth weights < 750 g. However, none of the positive blood cultures grew *Lactobacillus* or *Bifidobacterium* spp. Although several trials included infants < 1000 g, the data is insufficient to allow analysis of this subpopulation alone. All trials included in the 2010 Deshpande et al[71] meta-analysis and 2011 Cochrane Review by AlFaleh et al[56] showed no cases of probiotic sepsis, however, this has been reported in neonates and immunocompromised hosts before these reviews.[83–87]

In addition, given design variability of the trials showing efficacy of probiotics in NEC prevention, the optimal probiotic strain, duration of administration, and host selectivity remain unclear. Although data demonstrate strain-specific immunologic effects, a consistently decreased risk of NEC in trials using variable probiotic regimens suggests strain nonspecific protection. Whether intact, live bacteria are required for their immunoprotective effects in neonates remains unanswered. Data suggest that bacterial components or secreted bioactive factors may provide effective, novel preventive strategies without the risk of probiotic-associated morbidity, such as sepsis.

CONCLUSIONS

Despite advances in neonatal care and improved survival of premature infants, NEC continues to be devastating intestinal disorder, which afflicts this vulnerable population. Prematurity is the primary risk factor for NEC. Immaturity of the intestinal immune system, leading to exaggerated inflammatory responses, leaves the neonate ill equipped to tolerate the extrauterine microbial environment. The role of intestinal microbes in the pathogenesis of disease has been established and appropriate bacterial colonization can promote intestinal maturation and health. Therefore, postnatal acquisition of a beneficial microbiota balance and variety is critical to newborn health and administration of probiotics may provide an opportunity to modify the microbiota toward an advantageous balance. Several randomized, controlled clinical trials have shown a statistically significant reduction in the incidence of NEC in preterm infants with orally administered probiotics; however, consensus on the optimal strain, dose, timing of administration, and host characteristics remains unclear due to heterogeneity of trial design. A theoretical risk of probiotic-associated morbidity exists in the neonatal population, particularly given the immaturities of intestinal function and immune status. Despite this risk, probiotic-associated sepsis occurred very infrequently in the randomized, controlled clinical trials included in this review. Repeated studies using a single design protocol to demonstrate reproducibility, safety, and efficacy would significantly strengthen the existing data. Data stratification of the previous clinical trials may be helpful in guiding the most promising protocol for future pursuit. These studies should also be powered to allow sufficient data from the most vulnerable neonates, those < 1000 g at birth. Further research to demonstrate the exact mechanisms by which specific probiotics protect against intestinal disorders is also still necessary, and will ultimately allow data-supported, disease-specific clinical application in the future.

REFERENCES

1. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotizing enterocolitis in preterm neonates with very low birth weight: a systematic review of randomized controlled trials. *Lancet*. 2007;369:1614–1620.
2. Obladen M. Necrotizing enterocolitis—150 years of fruitless search for the cause. *Neonatology*. 2009;96:203–210.
3. Ng S. Necrotizing enterocolitis in the full-term neonate. *J Paediatr Child Health*. 2001;37:1–4.
4. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011;364:255–264.

5. Holman RC, Stoll BJ, Curns AT, et al. Necrotising enterocolitis hospitalisations among neonates in the United States. *Paediatr Perinat Epidemiol*. 2006;20:498–506.
6. Guillet R, Stoll BJ, Cotten CM, et al. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*. 2006;117:e137–e142.
7. Horbar JD, Badger GJ, Carpenter JH, et al. Trends in mortality and morbidity for very low birth weight infants, 1991-1999. *Pediatrics*. 2002;110:143–151.
8. Rowe MI, Reblock KK, Kurkchubasche AG, et al. Necrotizing enterocolitis in the extremely low birth weight infant. *J Pediatr Surg*. 1994;29:987–990.
9. Hsueh W, Caplan MS, Qu XW, et al. Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. *Pediatr Dev Pathol*. 2003;6:6–23.
10. Grave GD, Nelson SA, Walker WA, et al. New therapies and preventive approaches for necrotizing enterocolitis: report of a research planning workshop. *Pediatr Res*. 2007;62:510–514.
11. Martinez-Tallo E, Claure N, Banclari E. Necrotizing enterocolitis in full-term or near-term infants: risk factors. *Biol Neonate*. 1997;71:292–298.
12. Raboei EG. Necrotizing enterocolitis in full-term neonates: is it aganglionosis? *Eur J Pediatr*. 2009;19:101–104.
13. Stark AR, Carlo WA, Tyson JE, et al. Adverse effects of early dexamethasone in extremely low birth weight infants. *N Engl J Med*. 2001;344:95–101.
14. Watterberg KL, Gerdes JS, Cole CH, et al. Prophylaxis of early adrenal insufficiency to prevent bronchopulmonary dysplasia: a multicenter trial. *Pediatrics*. 2004;114:1649–1657.
15. Fitzgibbons SC, Ching Y, Yu D, et al. Morality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg*. 2009;44:1072–1075.
16. Lin PW, Stoll BJ. Necrotising enterocolitis. *Lancet*. 2006; 368:1271–1283.
17. Schulzke SM, Desphande GC, Patole SK. Neurodevelopmental outcome of very low birth weight infants with necrotizing enterocolitis—a systematic review of observational studies. *Arch Pediatr Adolesc Med*. 2007;161:583–590.
18. Martin CR, Dammann O, Allred EN, et al. Neurodevelopment of extremely preterm infants who had necrotizing enterocolitis with or without late bacteremia. *J Pediatr*. 2010;157:751–756.el.
19. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002;110:285–291.
20. Spencer AU, Kocevich D, McKinney-Barnett M, et al. Pediatric short-bowel syndrome: the cost of comprehensive care. *Am J Clin Nutr*. 2008;88:1552–1559.
21. Ley R, Bäckhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA*. 2005;102:11070–11075.
22. Claud EC, Walker WA. Bacterial colonization, probiotics, and necrotizing enterocolitis. *J Clin Gastroenterol*. 2008;42: S46–S52.
23. Gronlund MM, Lehtonen OP, Eerola E, et al. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr*. 1999;28:19–25.
24. Chen J, Cai W, Feng Y. Development of intestinal bifidobacteria and lactobacilli in breast-fed neonates. *Clin Nutr*. 2007; 26:559–566.
25. Conroy M, Shi HN, Walker WA. The long-term health effects of neonatal microbial flora. *Curr Opin Allergy Clin Immunol*. 2009;9:197–201.
26. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr*. 2000;30:61–67.
27. Wold AE, Adlerberth I. Breast feeding and the intestinal microflora of the infant—implications for protection against infectious diseases. *Adv Exp Med Biol*. 2000;478:77–93.
28. Morowitz MJ, Poroyko V, Caplan M, et al. Redifining the role of intestinal microbes in the pathogenesis of necrotizing enterocolitis. *Pediatrics*. 2010;125:777–785.
29. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune reponses during health and disease. *Nat Rev Immunol*. 2009;9:313–323.
30. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*. 1995;125:1401–1412.
31. Trowsdale J, Betz A. Mother's little helpers: mechanisms of maternal fetal tolerance. *Nat Immunol*. 2006;7:241–246.
32. Schroeder HJ, Zhang L, JR Philips. Slow programmed maturation of the immunoglobulin HCDR3 repertoire during the third trimester of fetal life. *Blood*. 2001;98:2745–2751.
33. Salzman N, Underwood M, Bevins C. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. *Semin Immunol*. 2007;19: 70–83.
34. Morein B, Blomqvist G, Hu K. Immune responsiveness in the neonatal period. *J Comp Pathol*. 2007;137(Suppl 1): S27–S31.
35. Otte JM, Podolsky DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol*. 2004;286:G613–G626.
36. Abreu MT. The Ying and Yang of bacterial signaling in necrotizing enterocolitis. *Gastroenterology*. 2010;138:39–43.
37. Liew FY, Xu D, Brint EK, et al. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol*. 2005;5:446–458.
38. Dai D, Nanthakumar NN, Newburg DS, et al. Role of oligosaccharides and glycoconjugates in intestinal host defense. *J Pediatr Gastroenterol Nutr*. 2000;30:S23–S33.
39. Chu SH, Walker WA. Developmental changes in the activities of sialyl- and fucosyltransferases in rat small intestine. *Biochem Biophys Acta*. 1986;883:496–500.
40. Snyder JD, Walker WA. Structure and function of intestinal mucin: developmental aspects. *Int Arch Allergy Appl Immunol*. 1987;82:351–356.
41. Udall JN Jr.. Gastrointestinal host defense and necrotizing enterocolitis. *J Pediatr*. 1990;117:S33–S43.
42. Eibl MM, Wolf HM, Furnkranz H, et al. Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. *N Engl J Med*. 1988;319:1–7.
43. Roberts SA, Freed DL. Neonatal IgA secretion enhanced by breast feeding. *Lancet*. 1977;2:1131.
44. Tan XD, Hsueh W, Chang H, et al. Characterization of a putative receptor for intestinal trefoil factor in rat small intestine: identification by in situ binding and ligand blotting. *Biochem Biophys Res Commun*. 1997;237:673–677.
45. Sands BE, Podolsky DK. The trefoil peptide family. *Annu Rev Physiol*. 1996;58:253–273.
46. Lin J, Holzman IR, Jiang P, et al. Expression of intestinal trefoil factor in developing rat intestine. *Biol Neonate*. 1999;76: 92–97.
47. Ouellette AJ. Paneth cells and innate immunity in the crypt microenvironment. *Gastroenterology*. 1997;113:1779–1784.
48. Salzman NH, Polin RA, Harris MC, et al. Enteric defensin expression in necrotizing enterocolitis. *Pediatr Res*. 1998; 44:20–26.
49. Claud EC, Lu L, Anton PM, et al. Developmentally regulated IkappaB expression in intestinal epithelium an susceptibility to flagellin-induced inflammation. *Proc Natl Acad Sci USA*. 2004;101:7404–7408.
50. Nanthakumar NN, Fusunyan RD, Sanderson I, et al. Inflammation in the developing human intestine: a possible pathophysiologic contribution to necrotizing enterocolitis. *Proc Natl Acad Sci USA*. 2000;97:6043–6048.
51. Fusunyan RD, Nanthakumar NN, Baldeon ME, et al. Evidence for an innate immune response in the immature intestine: toll-like receptors on fetal enterocytes. *Pediatr Res*. 2001;49:589–593.
52. Claud EC, Zhang X, Petrof EO, et al. Developmentally regulated tumor necrosis factor-alpha induced nuclear factor-kappaB activation in intestinal epithelium. *Am J Physiol Gastrointest Liver Physiol*. 2007;292:G1411–G1419.

53. Nanthakumar N, Meng D, Goldstein AM, et al. The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. *PLoS One*. 2011;6:e17776.
54. Barclay A, Stenson B, Simpson J, et al. Probiotics for necrotizing enterocolitis: a systematic review. *J Pediatr Gastroenterol Nutr*. 2007;45:569–576.
55. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev*. 2008;(1):CD005496.
56. Alfaleh K, Anabrees J, Bassler D, et al. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev*. 2011;(3):CD005496.
57. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *FASEB J*. 2001;15:1398–1403.
58. Morowitz MJ, Poroyko V, Caplan M, et al. Redefining the role of intestinal microbes in the pathogenesis of necrotizing enterocolitis. *Pediatrics*. 2010;125:777–785.
59. Wang Y, Hoenig JD, Malin KJ, et al. 16s rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. *ISME J*. 2009;3:944–954.
60. Mshvildadze M, Neu J, Shuster J, et al. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr*. 2010;156:20–25.
61. Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol*. 2010;7:503–514.
62. Gibbs K, Lin J, Hozman IR. Necrotizing enterocolitis: the state of the science. *Indian J Pediatr*. 2007;74:67–72.
63. Foligne B, Nutten S, Grangette C, et al. Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. *World J Gastrenterol*. 2007;13:236–243.
64. He F, Morita H, Hashimoto H, et al. Intestinal Bifidobacterium species induce varying cytokine production. *J Allergy Clin Immunol*. 2002;109:1035–1036.
65. Khailova L, Dvorak K, Arganbright KM, et al. Bifidobacterium bifidum improves intestinal integrity in a rat model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol*. 2009;297: G940–G949.
66. Bin-Nun A, Bromiker R, Wilschanski M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr*. 2005;147:192–196.
67. Dani C, Biadaioli R, Bertini G, et al. Probioics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants: a prospective double-blind study. *Biol Neonate*. 2002;82:103–108.
68. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. *Int J Infect Dis*. 1999;3:197–202.
69. Kitajima H, Sumida Y, Tanaka R, et al. Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed*. 1997; 76:F101–F107.
70. Lin HC, Su BH, Chen AC, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics*. 2005;115:1–4.
71. Deshpande G, Rao S, Patole S, et al. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics*. 2010;125:921–930.
72. Costalos C, Skouteri V, Gounaris A, et al. Enteral feeding of premature infants with Saccharomyces boulardii. *Early Human Development*. 2003;74:89–96.
73. Manzoni P, Mostert M, Leonessa ML, et al. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. *Clin Infect Dis*. 2006;42:1735–1742.
74. Mohan R, Koebnick C, Schildt J, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *J Clin Microbiol*. 2006;44:4025–4031.
75. Stratiki Z, Costalos C, Sevastiadou S, et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. *Early Hum Dev*. 2007;83:575–579.
76. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics*. 2008;122:693–700.
77. Samanta M, Sarkar M, Ghosh P, et al. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *J Trop Pediatr*. 2009;55:128–131.
78. Rouge C, Piloquet H, Butel MJ, et al. Oral supplementation with probiotics in very low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr*. 2009;89:1828–1835.
79. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. *JAMA*. 2009;302: 1421–1428.
80. Sari FN, Dizdar EA, Oguz S, et al. Oral probiotics: Lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *European Journal of Clinical Nutrition*. 2011;65:434–439.
81. Reuman PD, Duckworth DH, Smith KL, et al. Lack of effect of Lactobacillus on gastrointestinal bacterial colonization in premature infants. *Pediatr Infect Dis*. 1986;5:663–668.
82. Braga TD, Alves Pontes da Silva GA, Cabral de Lia PI, et al. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birht-weight preterm infants: a double-blind, randomized, controlled trial. *Am J Clin Nutr*. 2011;93:81–86.
83. Thompson C, McCarter YS, Krause PJ, et al. Lactobacillus acidophilus sepsis in a neonate. *J Perinatol*. 2001;21:258–260.
84. Broughton RA, Gruber WC, Haffar AA, et al. Neonatal meningitis due to lactobacillus. *Pediatr Infect Dis*. 1983;2:382–384.
85. Perapoch J, Planes AM, Querol A, et al. Fungemia with Saccharomyces cerevisiae in two newborns, only one of whom had been treated with ultra-levure. *Eur J Clin Microbiol Infect Dis*. 2000;19:468–470.
86. Ohishi A, Takahashi S, Ito Y, et al. Bifidobacterium septicemia associated with postoperative probiotic therapy in a neonate with omphalocele. *J Pediatr*. 2010;156:679–681.
87. Land MH, Rouster-Stevens K, Woods CR, et al. Lactobacillus sepsis associated with probiotic therapy. *Pediatrics*. 2005; 115:178–181.