Probiotics, Prebiotics, and Synbiotics for the Prevention of Necrotizing Enterocolitis\textsuperscript{1,2}

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Abstract

Necrotizing enterocolitis (NEC) is a devastating intestinal disease in preterm infants characterized by barrier disruption, intestinal microbial dysbiosis, and persistent inflammation of the colon, which results in high mortality rates. Current strategies used to manage this disease are not sufficient, although the use of human breast milk reduces the risk of NEC. Mother’s milk is regarded as a fundamental nutritional source for neonates, but pasteurization of donor breast milk affects the composition of bioactive compounds. Current research is evaluating the benefits and potential pitfalls of adding probiotics and prebiotics to pasteurized milk so as to improve the functionality of the milk and thereby reduce the burden of illness caused by NEC. Probiotics (live microorganisms that confer health to the host) and prebiotics (nondigestible oligosaccharides that stimulate the growth of healthy bacteria) are functional foods known to mediate immune responses and modulate microbial populations in the gut. Clinical research shows strain- and compound-specific responses when probiotics or prebiotics are administered in conjunction with donor breast milk for the prevention of NEC. Despite ongoing controversy surrounding optimal treatment strategies, randomized controlled studies are now investigating the use of synbiotics to reduce the incidence and severity of NEC. Synbiotics, a combination of probiotics and prebiotics, have been proposed to enhance beneficial health effects in the intestinal tract more than either agent administered alone. This review considers the implications of using probiotic-, prebiotic-, and synbiotic-supplemented breast milk as a strategy to prevent NEC and issues that could be encountered with the preparations. Adv Nutr 2016;7:928–37.

Keywords: synbiotics, probiotics, prebiotics, functional foods, necrotizing enterocolitis, inflammation, breast milk, human milk oligosaccharides, neonatology, premature

Introduction

The human body contains a complex and still far from completely understood array of trillions of microbes, including bacteria, viruses, phages, Archeae, and fungi (1). A healthy microbiome consists predominantly of commensal bacteria, which play an important role in providing the balance between health and disease (2). Alterations in this microbial homeostasis can lead to the onset of specific disorders. For instance, the development of diseases such as inflammatory bowel diseases (IBDs\textsuperscript{8}) (3), allergy (4), cancer (5), diabetes and obesity (6), and necrotizing enterocolitis (NEC) (7) are all considered to be associated with gut dysbiosis and perturbations in the composition and function of the intestinal microflora.

More recently, investigators have searched for ways to modulate the human bacterial flora so as to either prevent or treat human disease. Some studies have shown that the use of functional foods can modulate the composition and function of the gut microbiome as a way to alleviate symptoms and signs of IBD, irritable bowel syndrome (8), and colic in infants (9) and to prevent NEC in premature newborns (10).

NEC is a leading cause of morbidity and mortality in very-low-birth-weight (VLBW) premature infants (11). The estimated cost of health care for these infants is between $500 million and $1 billion/y in the United States (12). Common symptoms include gastric retention of enteral feedings, abdominal distension, and blood per rectum (12). This devastating and debilitating disease causes mucosal inflammation, intestinal epithelial cell death, sepsis, transmural
perforations resulting in the contents of the intestine leaking out into the peritoneal cavity, and eventually multiple organ failure (13).

The American Society for Parenteral and Enteral Nutrition clinical practice guidelines outline many questions surrounding preventive and treatment strategies for NEC (14). Randomized controlled studies showed that the mother’s own breast milk markedly improved health and survival in this vulnerable population of premature infants (15, 16). Although enteral administration of human breast milk, either as the mother’s own or by the use of donor milk banks, is a widely used prevention strategy in many parts of the world (17), probiotics, prebiotics, and more recently, synbiotics are also being evaluated for their potential use as supplements in either donor breast milk or formula to reduce the incidence of NEC (18). The purpose of this review is to consider critically the implications of using a symbiotic preparation as a supplement to enhance gut function and promote health in infants at risk of developing NEC.

Current Status of Knowledge

NEC

NEC is a life-threatening disease of unknown etiology in premature infants, which is characterized by excessive intestinal mucosal inflammation and epithelial cell death (12). The incidence of definitive NEC (defined as Bell stages II and III) (19) is ~7% among VLBW premature infants (20) but varies in prevalence over time and in various parts of the world. Regional differences in the occurrence of disease within countries have raised the consideration of environmental and infectious triggers as potential etiologic or exacerbating factors in the pathobiology of NEC (12). The mortality rate is high and has not decreased in the past decade despite remarkable advances in most other aspects of perinatal care (11). Surgical intervention rates remain as high as 50% (21).

The higher risk of developing NEC in premature newborns is considered to be associated with the immaturity of the developing host immune system (22), defects in intestinal epithelial barrier function, gut dysmotility, and impaired regulation of the microvascular circulation (12, 13). Because gut microbes can potentially affect each of these functions (23, 24), there is now increasing interest in considering both the composition and the function of the developing gut microbiota as another factor potentially related to the risk of premature neonates developing NEC.

Interestingly, antibiotic treatment has been associated with decreased microbial diversity and an increased risk of infants developing NEC (25). Prospective studies that analyzed stool samples before the onset of NEC suggest that the disease is associated with reduced microbial diversity that could accentuate the impact of single dominant species of microbes. Such dysbiosis is promoted by the widespread use of antibiotics in the neonatal intensive care unit setting (26, 27). The results of meta-analyses of prospective randomized clinical trials related to the role of probiotics in preventing NEC also support the importance of the gut microbiota in the pathogenesis of disease (28). Recent studies support the importance of the gut microbiome and suggest that maintaining environmental homeostasis (microbial, immune balance) could prove beneficial for preventing NEC in preterm infants (29).

Management strategies

To date, the medical management of NEC consists primarily of supportive care, bowel rest, withdrawal of enteral feedings, administration of broad-spectrum antibiotics, and surgery to remove severely affected areas of intestine (12).

Breast milk. Randomized controlled trials have shown that the enteral administration of breast milk markedly improves the health and survival of premature infants (30), although the specific mechanisms by which breast milk elicits such beneficial effects remain largely unknown. Human breast milk contains Bifidobacteria and lactic-acid producing Lactobacillus species (31), bacteria that can dampen host proinflammatory immune responses, enhance gut epithelial barrier integrity, and promote the fermentation of dietary carbohydrates. In addition, human breast milk is rich with nutrients such as carbohydrates, proteins, and fats that are considered the optimal source of nutrition for newborns ≤6 mo of age (32).

In addition to fulfilling nutritional demands, breast milk also provides immunologic factors that compensate for the immature immune system of the newborn. This is especially important to the preterm infant (33, 34). For example, at birth, preterm infants do not have fully mature antibody-producing plasma cells, which results in a temporary deficiency in secretory IgA (sIgA) required to protect intestinal mucosa from luminal microorganisms. Breast milk also stimulates beneficial microbes (Bifidobacteria and Lactobacilli) in the gut lumen to trigger sIgA production (35).

Mother’s own milk contains an abundance of bioactive growth factors, such as lactoferrin, epidermal growth factor (EGF), and TGF-α and TGF-β. These growth factors promote the functional maturation of the intestinal epithelium, regulate inflammatory responses, and enhance tissue healing in damaged cells (32). It is currently thought that intestinal barrier disruption, a cardinal feature triggering NEC, is a toll-like receptor (TLR) 4–dependent phenomenon (36, 37). Humans with NEC and rodent models of NEC both show elevated TLR4 expression that triggers enterocyte apoptosis and mucosal inflammation (38). EGF is highly abundant in both amniotic fluid as well as in breast milk; it inhibits TLR-4 signaling and prevents the development of NEC in animal models (39–41).

In addition to promoting the developing mucosal immune system in newborns, breast milk has an important role in establishing the newborn gut microbiota (42). Full-term breastfed neonates are colonized with a number of bacterial species, including Bifidobacteria, Lactobacilli, Bacteroides, Proteobacteria, and Streptococci (43), all of which contribute to gut function. VLBW preterm infants possess a greater number of pathogenic microbes, such as Escherichia coli and Clostridium difficile, despite being fed breast milk (27).
There are many sugars present in human milk, the most abundant components being lactose and oligosaccharides (44). The gut microbial composition is influenced by human-milk oligosaccharides (HMOs), which are an abundant and diverse family of glycans. Unlike lactose, HMOs traverse the gastrointestinal tract unaffected by stomach acidity, intestinal absorption, and enzymatic hydrolysis (45). Once reaching the distal ileum and colon, specific Bifidobacteria species such as *Bifidobacterium longum* subsp. *infantis*, utilize HMOs as a main carbon source in exclusively breastfed infants (46). To degrade HMOs, specific HMO-utilization genes, such as those expressing cleavage enzymes or transporters, are transcriptionally activated in bacteria to confer a growth advantage in the mixed microbial community present in the distal small bowel and large intestine (47). These effects are essential for microbiome development at birth because HMOs are the first prebiotic substrates ingested by neonates. Differences in exposure during early life likely contribute to distinct “pioneer” microbial communities, which may have long-term consequences on promoting health and affecting the development of chronic noncommunicable diseases later in life (48). For instance, the growth of *Bifidobacteria* can compete with harmful pathogens and generate metabolites such as SCFAs (butyrate, acetate, propionate), which promote intestinal epithelial barrier function (49). Moreover, HMOs structurally mimic the carbohydrate-binding motifs of certain enteric pathogens, such as enteropathogenic *E. coli* and *Candida albicans*, to prevent microbial adhesion to the luminal surface of enterocytes (50). The effects of HMOs are multifactorial, including direct effects on epithelial cells and indirect effects on the immune system and microbial composition (45), which could prove beneficial in alleviating NEC. It is interesting to note that, although HMOs are intrinsic to mother’s milk, the composition is not universal. One study that investigated the constituents of milk samples from 52 mothers at 38 wk of gestation showed that, although there were a number of conserved metabolites between subjects, there was great variability in the types of oligosaccharides present (44).

The consumption of breast milk by preterm infants reduces the occurrence of NEC, as shown in both prospective randomized controlled trials and in observational studies (15, 51). In VLBW infants (those with a birth weight <1 kg), each 100-mg/kg increase in breast-milk intake incrementally reduces both the incidence of NEC and associated mortality (15).

The mother’s own milk is an ideal source of neonatal nutrition, but in cases of premature birth, mothers frequently do not have sufficient amounts of breast milk for their infant. Thus, donated pasteurized milk can be used as an alternative. Pasteurization is intended to eliminate the transfer of harmful bacterial species. In addition to inactivating harmful bacterial components, however, this process also depletes beneficial bacteria (*Bacteroidetes*, *Lactobacillus*), affects immunologic factors, and reduces the number of IgG antibodies (52, 53), thereby altering the overall composition of breast milk (54). A recent preliminary study that investigated the effects of unpasteurized compared with pasteurized milk in 323 preterm infants found no significant differences between the groups for late-onset sepsis (15.9% compared with 15.1%; *P* = 0.49) but noted a trend toward decreases in the occurrence of NEC (2.4% compared with 4.4%; *P* = 0.25) in infants administered pasteurized milk (55). Supplementation and fortification of pasteurized breast milk with specific nutrients, such as vitamin D, proteins, and calcium, increased weight accretion in premature infants (53, 56) but not the frequency of NEC (57).

The unique and complex microbiological composition of unpasteurized human breast milk along with the abundance of HMOs makes for a blend of components that should provide additional beneficial nutritional and immunologic factors for neonates. Human breast milk contains a combination of probiotics (in the form of *Bifidobacteria* and *Lactobacillus*) and prebiotics (HMOs), which, if ingested in sufficient amounts, have the ability to affect the composition of the gut microbial community and impact the development of the mucosal immune system. Attempts are now underway to mimic some of the health properties of unpasteurized human breast milk with the addition of probiotics and prebiotics.

**Probiotics.** Probiotics are defined as live microorganisms that, when administered in adequate numbers, confer health benefits to the host (58). Underlying mechanisms of action of probiotics appear to be dose-dependent, species-specific, and multifactorial in nature (59). Probiotics modulate both innate (mucus and antimicrobial peptide and defensin production) and adaptive immune responses, including the production of sIgA and anti-inflammatory cytokines such as IL-10 and TGF-β; modulate interepithelial cell tight junction proteins [e.g., zona occludins 1 (ZO-1) and the Claudins]; increase the number of beneficial microbes within the gut; and competitively exclude pathogenic bacteria (59). It has been postulated that each of these positive attributes are potentially beneficial in preventing NEC.

Studies undertaken in preterm newborns also reported increased gut motility, reduced feeding intolerance (60), and enhanced degradation of intact protein antigens with the administration of various probiotic strains (61). Animal models that described the beneficial effects of probiotics on the enteric nervous system (62) and intestinal motility (63) corroborate findings in humans.

A retrospective study that looked at a cohort of VLBW neonates with NEC (≤1000 g) showed that infants administered *Lactobacillus reuteri* DSM 17938 had a lower incidence of NEC (64). In addition, multiple meta-analyses of prospective, randomized, placebo-controlled trials analyzed the impact of probiotics on reducing the prevalence of NEC in premature infants (65–67). Overall, probiotics are reported to significantly reduce the incidence of NEC (5.7% in infants receiving placebo compared with 2.4% in those administered probiotics; number-needed-to-treat = 30) and mortality (6.9% in placebo-treated compared with 4.54% in probiotic-treated groups; number-needed-to-treat = 42). A more recent meta-analysis of observational
multicenter studies also reported that preterm infants administered either single or multistain probiotic preparations had a lower incidence of NEC and sepsis (10). Interestingly, Baucells et al. (68) carried out a systematic review of randomized controlled trials that looked at the effect of various probiotic compounds and mixtures on the incidence of NEC. The authors found that a combination of *L. acidophilus* and *Bifidobacterium bifidum* was more beneficial in reducing the incidence of disease than either probiotic alone or a multistain combination (*B. infantis*, *Streptococcus thermophilius*, *Bifidobacterium lactis*, *B. bifidum*, *B. longum*, and *L. acidophilus*). These findings emphasize the importance of understanding the variability in probiotic functionality related to the particular strain(s) used.

There is ongoing debate as to whether there is sufficient evidence to recommend probiotics to premature infants on the basis of these meta-analyses. The Cochrane collaboration has argued that there already is evidence to warrant a change in clinical practice (28, 69), whereas others have raised concerns about the methodologic rigor of many of the published trials and the appropriateness of combining studies of various strains in a meta-analyses (70). In this context, the results of 2 recent large, prospective, randomized clinical trials that described completely different outcomes have been very interesting. The highly awaited Probiotics in Preterm Infants Study trial evaluated the potential preventive effect of the probiotic bacterium *Bifidobacterium breve* BBG-001 on NEC and sepsis (71). This high-quality, multicenter, randomized placebo-controlled trial included 1315 premature infants born between gestational weeks 23 and 30 who were cared for in neonatal intensive care units in the southeast of England. The study product was manufactured and regulated as a pharmaceutical drug, contrary to most other probiotics on the market, and was granted Clinical Trial Authorization from the Medicines and Healthcare Products Regulatory Agency in the United Kingdom. Given the size of the trial, it had sufficient power to detect relevant effects on both NEC and sepsis. Importantly, in contrast to most of the previous trials on the probiotic prevention of NEC, this study was also double-blinded. The primary outcome of this study provided negative results: in the probiotic group, 61 infants (9%) developed NEC compared with 66 (10%) in the placebo group (adjusted risk ratio: 0.93; 95% CI: 0.68, 1.27). Seventy-three (11%) infants in the probiotics group developed sepsis, compared with 77 (12%) in the placebo group (adjusted risk ratio: 0.97; 95% CI: 0.73, 1.29). These findings highlight the fact that only probiotic strains that have been proven to be effective in clinical trials should be used in clinical practice. The results also emphasize the fact that general recommendations for a specific treatment should be based on findings of randomized controlled trials with a high level of quality. Meta-analyses with considerable heterogeneity of included trials are simply not sufficient.

By contrast, the ProPrem trial was an adequately powered, double-blinded, placebo-controlled trial replicating a previous effective NEC prevention study in VLBW infants (72). This study was carried out in 10 centers in Australia and New Zealand with a low baseline incidence of NEC due to a high rate of using breast-milk feedings. The ProPrem study, which enrolled 1099 premature infants, describes a significant reduction in NEC—from 4.4% to 2.0%—but no effect on mortality (4.9% compared with 5.1%). The findings of the ProPrem trial replicate the positive results of a previous study (73) that used a combination of 3 probiotic strains: *B. infantis* DSM 96579, *Bifidobacterium animalis* subspecies *lactis* DSM 15954, and *S. thermophillus* DSM 15957. It should be noted, however, that in 2014, the manufacturer recalled the product (ABC Dophilus; Solgar, Inc.) used in the ProPrem trial, because the CDC reported contamination with the fungus *Rhizopus oryzae*, which had caused a lethal infection in premature infants treated with the product (74). This tragic event highlights that in addition to well-executed randomized trials proving efficacy, there is a pressing need for the development of probiotics as drugs that fulfill pharmaceutical requirements and regulations.

As highlighted in a recent commentary (75), there is ongoing controversy about the use of probiotics to prevent NEC in premature infants. Contrasting findings may well be attributed to variability in the probiotic strains and doses of strains tested, as well as diversity in their underlying mechanisms of action. It is known that the gut microbiome varies greatly from person to person in the types of genes expressed, although the roles played in modulating health and disease are yet to be elucidated (76). Genetic and functional characterization of differences between probiotic strains could well provide a better understanding of their use to prevent or treat gastrointestinal diseases, including NEC (77). There is an emerging general consensus for the need to more carefully decipher probiotic strain–specific underlying mechanisms of action in relation to the setting of specific disease states, particularly when combination products are used.

**Prebiotics.** Prebiotics are nondigestible food ingredients that stimulate the growth and/or activities of gut microbes while having the potential to produce beneficial metabolic and health benefits to the host (78). Although prebiotics are derived from digestible fibers, not all fibers are prebiotics. Prebiotics cause changes in gut microbial communities and stimulate the growth of beneficial gut microbes. Once prebiotics are fermented by *Bifidobacteria* and lactic acid–producing bacteria, SCFAs such as butyrate, acetate, and propionate are produced. SCFAs are then utilized as energy sources by host epithelial cells, modulate intestinal immunity, enhance barrier function, and inhibit adhesion of pathogenic bacteria (79).

Examples of commonly used prebiotics include galacto-oligosaccharides (GOSs) found in human breast milk and fructo-oligosaccharides (FOSs) present in a variety of food-stuffs including asparagus, leeks, and onions (78). Unlike HMOs, most commercial preparations of prebiotics are structurally homogenous; for instance, FOSs consist of repeating monomers of fructose with a terminal glucose,
compared with 5 different monosaccharides in HMOs. Despite these differences, prebiotics are functionally similar to HMOs with respect to their ability to modulate the growth of subpopulations of gut microbial communities. For instance, similar to HMOs, GOSs stimulate the selective growth of Bifidobacteria and lactic acid–producing bacteria. Prebiotic combinations, such as oligofructose/FOSs (1:1, 4 or 8 g/L) and GOSs/FOSs (9:1 at 8 g/L) stimulate gut microbiota profiles more comparable to those found in breastfed infants than in infants fed formula without prebiotics (80, 81). Similarly, prebiotic supplementation with either GOSs or FOSs increases the numbers of Bifidobacteria present in stools of preterm infants (82–85). However, such bifidogenic properties are not generalizable to all prebiotics: experiments that used a mixture of inulin and maltodextrin showed relatively poor Bifidobacteria growth (86). In addition to modulating the composition of the gut microbiome, current studies indicate that these prebiotic oligosaccharides can inhibit enteropathogen adhesion to host surfaces (87) and directly influence epithelial cell anti-inflammatory cytokine gene expression (79). Prebiotics can also directly promote intestinal cell barrier integrity by activating TLR–2 (88), enhancing tight junction claudin-3 assembly (89), and directly altering epithelial cytokine expression (90). These effects were thought to be due to the activation of pattern recognition receptors (91); however, evidence from animal studies is limited and further studies in this area are needed.

Despite microbial compositional changes induced by supplementation with prebiotics, evidence for the use of prebiotics to prevent NEC is not compelling. For instance, a meta-analysis concluded that, although prebiotic oligosaccharides are safe and do stimulate the growth of beneficial microbes, there is no reduction in the incidence of NEC and sepsis (85). Similarly, a recent multicenter study reported that inulin used as a prebiotic does not decrease NEC incidence in premature VLBW infants compared with placebo (92). By contrast, another study described a reduction in the incidence of NEC in VLBW newborns fed breast milk supplemented with a mixture of short-chain and long-chain FOSs (93).

Although changes in the composition of the gut microbiome are seen in animal models of NEC, prebiotic (GOS) supplementation did not protect pups against developing intestinal injury (94). Emerging issues arising from the published literature include a lack of consistency in the selection of specific prebiotics used in various studies and a lack of understanding of their specific underlying mechanism(s) of action. Analogous to the strain-specific effect of probiotics, it seems that the effect of prebiotics may well depend on their specific molecular structure. In a murine model, a specific isomer of disialyllacto-N-tetraose, a naturally occurring HMO, was identified to be protective against NEC, whereas GOSs, currently added to infant formula to mimic the properties of HMOs, had no effect (94).

**FIGURE 1** Search methodology using PubMed, MEDLINE, and CONSORT databases. Keywords necrotizing/necrotising enterocolitis (NEC) and synbiotics and probiotics and prebiotics were used for published articles on the use of synbiotics in the management of NEC. NEC, necrotizing enterocolitis.
Although there are a number of studies that reported no adverse effects when using prebiotics, some studies reported that prebiotics in high doses may elicit unwanted side effects such as bloating, increased flatulence, abdominal pain, increased bowel movements, and altered stool consistency (95). Despite these potential dose-related side effects, benefits to using manufactured prebiotics relate to issues of ease of long-term storage, clarity of dose, and absence of any viable microorganisms in the preparations.

**Synbiotics.** Synbiotics, a term first introduced by Kolida and Gibson (96), are “mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare.” Synbiotics are believed to act synergistically to increase overall gut health by offering more benefits than the use of either a probiotic or prebiotic agent alone. Such an integrated product is thought to allow for increased function of exogenously administered bacteria (probiotics) as well as providing substrate for endogenous commensal bacteria. Synbiotics have been studied in the settings of allergy, cancer, liver disease, pancreatitis, IBD, and irritable bowel syndrome and in surgical patients. Variable clinical outcomes reported indicate that synbiotics may well behave differently under various pathological conditions (97). A disadvantage to using synbiotics is that it is difficult to predict the selectivity and specificity of each of the components and what the resulting mechanisms of action will be.

A comprehensive review of the literature was undertaken by using the databases PubMed, MEDLINE, and CONSORT with the use of the key words necrotizing/necrotising enterocolitis (NEC) and synbiotics and probiotics and prebiotics. Additional relevant research articles were selected from the bibliographies of published articles. The resulting literature reviewed came from articles related to synbiotic use in the management of NEC (Figure 1). The search yielded 21 related articles, with 3 of the publications describing the use of synbiotics for the treatment of NEC in randomized clinical trials and 2 in animal-related publications (Table 1) (18, 30, 92, 98, 99).

To date, there are few studies investigating the effects of synbiotics in the setting of NEC. To elucidate some of the underlying mechanisms of action, various techniques have been used to precipitate a comparable disease state in rodents. One such approach uses hyperoxia/hypoxia to cause formula-induced inflammation in rats pups, similar to that seen in NEC (98). In this study, formula supplemented with a synbiotic mixture of *Saccharomyces boulardii*, with 90% FOSs and 10% GOSs, downregulated TLR2 to a greater extent than either the probiotic or prebiotic alone. In addition, the synbiotic preparation preferentially downregulated IFN-γ, whereas IFN-β was downregulated by the probiotic and IL-6 downregulated by the prebiotics. Interestingly, there was no improvement in body weight in the treatment groups compared with the untreated controls. Another study used postnatal malnutrition to stimulate NEC-like responses in rats (99). The same probiotic, prebiotic, and synbiotic formulations produced similar results with respect to weight gain, whereas variations in the degree of oxidative stress, histopathology, and NO signaling and biosynthesis were evident between treatment groups. Both of these studies showed the variability, complexity, and unpredictability of host intestinal responses to synbiotics. These studies show that, although probiotics and prebiotics stimulate specific responses, the synbiotic mixture does not necessarily

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<th>Study (ref)</th>
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<td>Nandhini et al. (18)</td>
<td><em>Lactobacillus acidophilus</em> (700 million), <em>Bifidobacterium longum</em> (400 million), <em>Bifidobacterium infantis</em> (300 million), <em>Lactobacillus rhamnosus</em> (400 million), <em>Lactobacillus plantarum</em> (300 million), <em>Lactobacillus casei</em> (300 million), <em>Lactobacillus bulgaricus</em> (300 million), <em>Bifidobacterium breve</em> (300 million), FOSs (100 mg)</td>
<td>RCT, synbiotic provided with mother’s milk</td>
<td>No significant change in incidence of NEC, no change in severity, no change in sepsis, no change in mortality</td>
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<td>Sreenivasa et al. (30)</td>
<td><em>L. acidophilus</em> (300 million), <em>B. longum</em> (150 million), <em>Bifidobacterium bifidum</em> (150 million), <em>Streptococcus thermophiles</em> (150 mg), FOSs (100 mg)</td>
<td>RCT, synbiotic provided enterally with breast milk</td>
<td>Reduced incidence and severity of NEC</td>
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<td>Dilli et al. (92)</td>
<td><em>Bifidobacterium lactis</em> (500 billion), inulin (30 mg)</td>
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<td>Decreased incidence of NEC, decreased rates of sepsis, reduced mortality</td>
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<td>D’Souza et al. (98)</td>
<td><em>Saccharomyces boulardii</em> lyo [Florastor kids (Biocodex, Inc.), 5 mg/mL], 90% FOSs and 10% GOSs</td>
<td>Hypoxic/hyperoxic-induced NEC in rats, synbiotic provided in formula</td>
<td>Weight loss; decreased TLR, TNFα, EGF</td>
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<td>D’Souza et al. (99)</td>
<td><em>S. boulardii</em> lyo (Florastor kids, 5 mg/mL), 90% FOSs and 10% GOSs</td>
<td>Postnatal malnutrition-induced NEC in rats, synbiotic provided in formula</td>
<td>Weight loss; no hemorrhages, inflammation, or necrosis; modulation of oxidative stress responses</td>
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1 EGF, epidermal growth factor; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; NEC, necrotizing enterocolitis; RCT, randomized controlled trial; ref, reference; TLR, toll-like receptor.
cause a synergistic effect and potentially can stimulate diverse responses in the host.

A blinded placebo-controlled study, which used a combination of the probiotic *B. lactis* and the prebiotic inulin, described a lower incidence of NEC and reduced mortality in both the probiotic and synbiotic study arms, but with no beneficial effects observed in infants administered prebiotic alone (92). It should be emphasized that, in this study, the synbiotics did not have any additive effect compared with the use of probiotics alone. Another prospective randomized controlled study reported that infants provided with a synbiotic preparation containing *L. acidophilus*, *B. longum*, *B. bifidum*, *S. thermophiles*, and FOSs had a lower incidence and reduced severity of NEC (30). A randomized controlled trial showed that synbiotics differentially affected the incidence of NEC and the severity of symptoms in patients who developed the disease (18). This randomized controlled study indicated that preterm infants weighing >1000 g administered a synbiotic preparation containing 8 probiotic strains (mixture of various strains of *Lactobacillus* and *Bifidobacterium*), in combination with FOSs, did not show significant improvements in the severity of NEC, sepsis, or mortality compared with the untreated cohort (18).

None of the studies that evaluated the efficacy of synbiotics in premature infants as a strategy to prevent NEC have investigated potential underlying mechanisms of action. Despite the complexity and cost of clinical trials, to add to our current knowledge base and to direct future dietary interventions the study of underlying potential mechanisms of

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**FIGURE 2** Potential interactions to consider when formulating synbiotic preparations.

**FIGURE 3** Schematic representation of proposed direct and indirect effects of probiotics (A), prebiotics (B), and synbiotics (C) that promote beneficial effects on the host, which include bacterial colonization, epithelial barrier integrity, intracellular signaling, innate and adaptive immunity, and metabolic processes in the intestinal tract.
action should be included as an integral part of all future clinical trials in this field.

Conclusions

The complexity of defining the most appropriate probiotic, prebiotic, or synbiotic combination for use in various clinical settings is becoming increasingly evident. Probiotics exert strain-specific mechanisms of action (100). Prebiotics also exert effects on the host in a structure-specific manner (79). Varying doses of probiotics and prebiotics, and the complementary or synergistic effects of compounds contained in a synbiotic preparation (96), are each important considerations to take into account when formulating an intervention strategy.

Potentiation of individual responses is frequently considered when justifying the use of synbiotic preparations (Figure 2). Although it is presumed that a combination of 2 individual bioactive agents may prove better that either one alone, antagonistic effects should also be considered (Figure 3). Although it is often thought that there is little or no interaction between the probiotic and the probiotic contained in a synbiotic preparation, a recent study showed, with the use of epithelial cells grown in tissue culture, that prebiotics have a direct effect on the adherence ability of certain probiotic strains while having no impact on other strains (101). An in-depth analysis of potential prebiotic-probiotic interactions should be conducted by using relevant and replicable in vitro model systems and in animal models of human disease.

To date, there appears to be insufficient evidence to warrant recommending the use of synbiotics in conjunction with pasteurized breast milk for the prevention of NEC outside of the setting of a registered randomized clinical trial. Advances in the field are likely to be driven by studies that include hypothesis-driven research focused on unraveling the underlying mechanisms of action of the ingredients contained in the synbiotic preparation under evaluation. Probiotics and prebiotics on their own each have complex and diverse mechanisms of action. Trying to formulate a synthetic compound with the biological and structural complexities of human breast milk has proven to be challenging. Combining both prebiotics and probiotics into a synbiotic preparation is a first rough approximation to mimic the composition of HMOs and probiotics contained in human breast milk. Factors that must be taken into account when investigating synbiotics include the potential for synergistic responses, antagonistic reactions, additive effects, and either the enhancement or masking of adverse effects. In addition to future studies that assess the efficacy of synbiotics and potential mechanisms of action of individual components, documenting the safety of the combined preparations will be essential before considering the more widespread use of such formulations in premature infants as a management strategy to prevent NEC.

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All authors read and approved the final manuscript.

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