Published in final edited form as:
Expert Rev Clin Immunol. 2014 July ; 10(7): 875–884. doi:10.1586/1744666X.2014.913481.

Evidence based feeding strategies before and after the development of necrotizing enterocolitis

Misty Good[1], **Chhinder P. Sodhi**[2], and **David J. Hackam**[2]

[1]Division of Newborn Medicine, Department of Pediatrics, Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine

[2]Division of Pediatric Surgery, Department of Surgery, Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine

Abstract

Necrotizing enterocolitis (NEC) is a devastating disease of premature infants and is associated with significant morbidity and mortality. While the pathogenesis of NEC remains incompletely understood, it is well established that the risk of disease is increased by the administration of infant formula and decreased by the administration of breast milk. This review will focus on the mechanisms by which breast milk may serve to protect against NEC, and will review the evidence regarding various feeding strategies that may be utilized before and after an episode of NEC.

Keywords

Necrotizing enterocolitis; breast milk; innate immunity; toll-like receptor 4; amniotic fluid; prematurity; growth factors

Introduction

Necrotizing enterocolitis (NEC) is the most frequent and lethal gastrointestinal disorder affecting preterm infants [1,2], and is characterized by intestinal barrier disruption leading to intestinal necrosis, multi-system organ failure and death. NEC affects 7–12% of preterm infants weighing less than 1500 grams, and the frequency of disease appears to be either stable or rising in several studies [1–3]. The typical patient who develops NEC is a premature infant who displays a rapid progression from mild feeding intolerance to systemic sepsis, and up to 30% of infants will die from this disease [3,4]. In its early stages, NEC is difficult to diagnose, as the initial presentation includes temperature instability, apnea, bradycardia, lethargy, and mild feeding intolerance, which are symptoms that are shared with many other septic processes[5]. There is no effective cure for NEC, and the overall survival rate has not changed in the past 30 years [3]. The current treatment regimen for

Address correspondence and reprint requests to: David J. Hackam, MD, PhD, Division of Pediatric Surgery, Children's Hospital of Pittsburgh of UPMC, One Children's Hospital Drive, 4401 Penn Avenue, Pittsburgh, PA 15224, Tel 412 692 8449 Fax 412 692 3466, david.hackam@chp.edu.

Financial Disclosures
Dr. Hackam receives a research grant from Abbott Nutrition; Dr. Good receives funding from the Gerber Foundation.

infants with NEC includes cardiorespiratory support, nasogastric decompression, broad-spectrum antibiotics and cessation of enteral feedings [4,6,7]. Surgical intervention - which involves the removal of necrotic intestine or peritoneal drainage - is required in up to 50% of the cases [3], and is indicated when NEC fails to improve with medical management or in the setting of pneumoperitoneum [6,8]. Despite extensive clinical experience in the management of NEC, there is no agreement regarding how these infants should be fed either before or after an episode of the disease, although it seems intuitive that the manner of feeding may influence disease recurrence [9,10]. The objective of this article is to review the evidence pertaining to various feeding strategies that may be employed in premature infants so as to gain a greater understanding of how to optimally feed a premature infant with the goal of reducing either the development or the recurrence of NEC. To do so, we will first review the evidence in favor of the benefits of breast milk versus formula, will then highlight the various feeding strategies of premature infants before and after the development of NEC, and will then discuss novel and emerging approaches to feeding the premature infant so as to reduce the likelihood of NEC development in the first place.

Benefits of breast milk over infant formulas in the protection against the development of NEC

There is general consensus in the field that the administration of breast milk is the most effective strategy for protecting against the development of NEC [11–13]. The protective effects of breast milk may be achieved when the milk is obtained either from the infant's own mother or from a donor source, further underscoring its importance as a protective agent [14–16]. A Cochrane review in 2008 detailed the effects of formula feeding versus donor breast milk in preterm or low birth weight infants [16]. Eight studies were evaluated for effects on the primary outcomes of growth and development with secondary outcomes of death in the newborn period, the development of NEC, feeding intolerance, time to full enteral feedings and incidence of invasive infection [14,17–24]. These studies included a total of 1017 infants and most were less than 32 weeks gestation and less than 1800 grams. Most of the trials excluded infants who were small for gestational age, had congenital anomalies and gastrointestinal or neurological abnormalities. On meta-analysis, they concluded that administration of formula increases short term growth rates, but is associated with a higher risk of developing NEC versus donor breast milk, with a relative risk of 2.5% [16]. An additional Cochrane review evaluated several randomized controlled trials comparing human breast milk to formula feeding in preterm or low birth weight infants [24]. This analysis concluded that no data exists from randomized controlled trials to determine whether feeding premature infants with formula versus maternal breast milk impacts growth and development [24]. However, one of the excluded studies performed by Lucas et al found that NEC was 6–10 times more common in premature infants who were formula fed than those who were exclusively fed breast milk [11]. Furthermore, in premature infants who were greater than 30 weeks gestation, NEC was 20 times more common in formula fed infants versus breast fed infants [11], again confirming the protective role of breast milk in patients at risk for the development of NEC. More recently, a multicenter, blinded, randomized controlled trial was performed by Cristofalo et al comparing extremely preterm infants fed an exclusive human milk diet of pasteurized donor human milk with a human

milk based fortifier or bovine based preterm formula in infants whose mothers did not intend to provide breast milk to their infant [25]. The infants in each group were fed by initiating trophic feeds at 10–20 mL/kg/day within 1–4 days after birth as tolerated for up to 5 days. Subsequent feeds were advanced by a rate of 10–20 mL/kg/day at the discretion of the clinical teams based on the feeding guidelines of the study [25]. This study detected a significant decrease in the duration of parenteral nutrition with the infants that were fed the human milk diet. Additionally, these authors discovered that the incidence of surgical NEC was significantly decreased in the human milk diet group versus the bovine-based preterm formula, and further concluded that the number of patients needed to be fed an exclusive human milk diet to prevent one case of surgical NEC was 6 infants [25]. Taken together, these studies point to a clear protective role for breast milk in premature infants to prevent the development of NEC.

It is important to note that in a study by Meinzen-Derr et al, the protective effects of breast milk were found to be dependent on the amount of breast milk administered [13]. In 1272 subjects, these authors determined that for every 100 mL/kg cumulative increase in human milk an infant received in the first 14 days of life, the risk of NEC or death was decreased by 13% [13]. Schanler et al and Furman et al previously reported lower NEC rates in very low birth weight infants who received greater than 50 mL/kg/day of fortified human milk versus premature infant formula [26,27]. Taken together, these findings strongly argue in favor of the protective effects of breast milk in reducing the incidence of NEC and that the effects may be dose dependent. We will next highlight the potential components of breast milk that may confer this protection.

Components of breast milk that could potentially mediate the protective effects against the development of NEC

Various components of breast milk have been implicated in its protective role against the development of NEC. These include nitrites/nitrates, L-arginine, glutamine, human milk oligosaccharides, lactoferrin and growth factors, and will be reviewed in detail below.

Nitrate/Nitrite

Nitric oxide (NO) is produced by a variety of cells including macrophages and the intestinal epithelium, but when derived from the endothelium is known to be a potent vasodilator, and is an essential regulator of blood flow to tissues. Dietary nitrate and nitrite may be converted to NO within the gastrointestinal tract, which can then regulate intestinal blood flow [28–31]. Sodium nitrate is actively concentrated in saliva and reduced by bacteria to sodium nitrite in the oral cavity of humans, which enhances oral nitric oxide production [32]. In neonates, the bacterial conversion of nitrate to nitrite in the mouth is reduced compared to older children [33], and thus, the only available sources of sodium nitrite in infants are from their diet. Our group recently demonstrated that compared to infant formula, human and mouse breast milk were enriched in sodium nitrate, which is a precursor for the enteral generation of nitric oxide and nitrite [34]. Further, we determined that the supplementation of formula with sodium nitrate/nitrite restored intestinal perfusion, decreased the amount of pro-inflammatory cytokine expression within the intestine and reduced experimental NEC

severity in a murine NEC model. Our experimental NEC model consists of providing gavage formula feedings and intermittent doses of hypoxia to neonatal mouse pups. The use of an experimental animal model of NEC has its limitations as reviewed by Sodhi et al [35], although this study clearly showed that sodium nitrate or nitrite may be protective in the pathogenesis of NEC due to its vasodilatory effects on the intestine [34]. Another important study performed by Jones and colleagues recently showed that the levels of nitrate and nitrite change in the handling and storage of breast milk. Specifically that when the breast milk was freeze-thawed, the concentrations of nitrite in the breast milk produced by mothers of preterm and term infants was reduced [28]. Additionally, this group determined that the sodium nitrite concentrations in the milk of mothers of preterm infants were significantly less than the milk of mothers with term infants. These findings raise the possibility that sodium nitrate or nitrite are important components of breast milk that may exert a protective effect in NEC through the enhancement of intestinal blood flow, and thus the amelioration of intestinal necrosis.

L-Arginine

Since nitric oxide can regulate intestinal blood flow, but cannot be administered directly to the gastrointestinal tract, dietary supplementation with agents that may release nitric oxide may offer an appropriate method to achieve this protective effect. One such supplemental option could be L-arginine, an amino acid that synthesizes nitric oxide and is found to be deficient in premature infants [36]. Amin et al evaluated the effects of L-arginine supplementation in premature infants (<32 weeks and <1250 grams body weight) during the first 28 days of life on the incidence of NEC [37], and determined in a randomized, double blind, placebo controlled study that L-arginine reduced the incidence of all stages of NEC severity [37]. However, one of the limitations of their study was that early stage NEC was included as an outcome, which led to a NEC incidence in their study of 27%, well above the incidence that is typically observed in other neonatal centers. Further, when patients with early stage NEC were excluded, the protective effects of L-Arginine were no longer significant (P=0.77) due to the sample size of the study [37]. In a related study, Polycarpou et al performed a randomized, double blind pilot study assessing enteral L-Arginine supplementation on the incidence of NEC [38] and found that L-arginine did reduce the incidence of the most severe stage of NEC (2.5% in the L-arginine group vs. 18.6% in the control group) [38]. Additional studies that include more patients are required before recommendations regarding the role of L-arginine as a protective agent against NEC development can be made.

L-Glutamine

L-Glutamine is an amino acid that is present in breast milk, and which can stimulate intestinal cell proliferation by providing metabolic fuel to intestinal epithelial cells [39][40]. A nutritional deficiency of glutamine has been proposed to be a risk factor for necrotizing enterocolitis. Becker et al reported that plasma glutamine levels were decreased for at least 10 days prior to the onset of NEC in humans [41]. To address whether glutamine supplementation can attenuate NEC severity, Poindexter et al performed a multicenter, randomized controlled trial of 721 infants, which demonstrated no benefit of glutamine supplementation in reducing death, late-onset sepsis or NEC [40]. A more recent study by

Pawlik et al evaluated the frequency of feeding intolerance, NEC, intestinal perforation, sepsis and death between enteral administration of a glutamine and amino acid solution [42]. This clinical study of 106 very low birth weight infants demonstrated a significantly lower risk of feeding intolerance, lower incidence of NEC, intestinal perforation sepsis and death in the glutamine solution group versus the control groups [42]. However, a recent Cochrane meta-analysis evaluated the incidence of NEC with infants treated with enteral or parenteral glutamine and found no statistically significant difference between the ten studies assessed for NEC as a secondary outcome [43]. Although the available data do not provide concrete evidence that administration of glutamine prevents NEC, more studies are needed to determine the potential beneficial intestinal effects of glutamine.

Human Milk Oligosaccharides

Human milk oligosaccharides (HMOs) are non-digestible carbohydrates, which are a major component of breast milk and are thought to be in part responsible for its protective effects against the development of NEC [44]. HMOs are thought to act by influencing the microbial flora within the gastrointestinal tract [45], modulating immune cell activity [46,47], reducing the infiltration and activation of neutrophils *in vitro* [44], and providing a substrate for the proliferation of the health-promoting bacteria *Lactobacillus* and *Bifidobacteria* [48]. Jantscher-Krenn et al investigated a rat model of NEC and demonstrated that HMOs improved survival and attenuated experimental NEC severity [49]. To date there have been nearly 200 HMOs identified, but, the specific HMO that was found to mediate protection against NEC in these studies was disialyllacto-N-tetraose (DSNLT) and they further discovered that sialic acid is required for the protection [49]. This study is of importance nor only as a novel means of NEC prevention, but also by identying this HMO as a biomarker that could identify which infants may be at greater risk of NEC development, based upon the concentration of DSNLT present in a mother's breast milk. Further preclinical studies are required to determine the precise mechanism which mediates the protection. In addition, clinical studies in premature neonates are needed to test the efficacy of disialyllacto-N-tetraose and its role - if any - in the prevention of NEC.

Lactoferrin

Lactoferrin is a glycoprotein that is present in breast milk and has been implicated in the beneficial effects of breast milk for NEC via its reported antimicrobial properties [50]. Lactoferrin has also been shown to attenuate lipopolysaccharide-mediated pro-inflammatory cytokine release from monocytic cells [51], and stimulate enterocyte proliferation [52], which is important in maintaining the integrity of the intestinal mucosa, an important factor in NEC pathogenesis. In an experimental rat model of invasive E. coli infection, human lactoferrin was found to be synergistically protective against infection along with *Lactobacillus rhamnosus* GG [53]. Manzoni et al evaluated the role of bovine lactoferrin alone or in combination with *Lactobacillus rhamnosus* GG in the prevention of sepsis with NEC as a secondary outcome measure in a prospective, randomized multi-center double-blinded study of very low birth weight infants [54]. NEC was found to occur less frequently in the bovine lactoferrin group plus *Lactobacillus rhamnosus* GG (0/151 infants [0%]) versus the control group (10/168 infants [6%]), but not with bovine lactoferrin alone (3/153 [1.9%]) compared to the control group [54]. These findings are significant because they

demonstrate the protective effect of *Lactobacillus rhamnosus* GG delivered in combination with lactoferrin on NEC and raise the possibility that synergistic treatments may be a preventative option in NEC. Given that lactoferrin has been found in breast milk from a variety of species [55], these findings suggest that further studies evaluating the role of this protein may provide important mechanistic insights into the protective effects of breast milk against NEC.

Growth Factors

Breast milk is known to be enriched in various growth factors, which together are known to promote intestinal mucosal health via effects on intestinal epithelial migration, proliferation and maturation [56–59]. In particular, epidermal growth factor (EGF) is critical for intestinal development and is found in breast milk [60–62]. Dvorak and colleagues have demonstrated that EGF attenuates the severity of experimental NEC in rats [63], protects against intestinal barrier failure, normalizes expression of tight junction proteins in the intestine [62] and inhibits enterocyte apoptosis commonly seen in NEC [64]. Another growth factor that has been studied in an experimental model of NEC is heparin-binding epidermal growth factor-like growth factor (HB-EGF). Besner and colleagues have demonstrated that HB-EGF protects against experimental NEC by promoting enterocyte migration and proliferation [58]. Additionally, their group has shown that HB-EGF increases intestinal microvascular blood flow in experimental NEC [65]. Dvorak and colleagues compared the efficacy of treatment with EGF and/or HB-EGF on the prevention of experimental NEC and found that although, both agents demonstrated protection at various doses, EGF protected against NEC at more physiological doses than HB-EGF [66]. Given the fact that breast milk is rich in EGF and other growth factors, these studies illustrate the importance of evaluating these agents in greater detail.

Evidence based feeding protocols for the prevention of NEC

While the evidence is convincing that breast milk compared to formula feeding reduces the incidence of NEC in preterm infants, many mothers of premature infants are unable to produce adequate amounts of breast milk for their child, illustrating the need for formula based feeding preparations that may limit the propensity for NEC to develop in the first place. There are several such formulations available that have been designed to mimic the composition of human milk so that they meet the estimated caloric needs and nutrient requirements that enhance the growth and development of premature infants. Despite the availability of such preparations, the precise protocols by which these should be used to feed premature infants without causing harm remains incompletely understood, and a topic of great interest in the field. Several studies have evaluated the importance of the timing of introducing feeds, their rate of advancement or strategies of formula fortification in order to prevent NEC. We will next review the evidence supporting optimal delivery strategies, and will focus on the rate of delivery, and the rate of advancement of infant feeds.

i. What is the optimal rate of delivery of enteral feeds?

There is not a consensus in the literature regarding the rate of delivery of feeds to premature infants so as to reduce the likelihood of NEC development. One strategy of delivery is that

of providing minimal enteral nutrition or trophic feedings, which consists of 15–20 mL/kg/day of breast milk or formula every 2–3 hours during the first week of life. In a recent Cochran Review, Morgan et al concluded that there was insufficient evidence demonstrating that early trophic feedings compared to enteral fasting improves feeding intolerance or prevents NEC [67]. A study by McClure et al reported that infants receiving minimal enteral nutrition group did have a significantly lower incidence of culture-confirmed sepsis (0.5 versus 1.2 in the control group) [68]. A cohort study by Sallakh-Niknezhad et al sought to evaluate the benefits of “early” (within 48 hours of life) versus “late” (after 72 hours) feeding in premature infants weighing less than 1500 grams [69]. A total of 125 infants received enteral feedings while 45 infants were in the late feeding group and both groups followed a similar enteral feeding protocol which consisted of starting with 1–2 mL/kg every 4–6 hours with advancement of 1–2 mL/kg/day. This study determined that there was decreased time to gain weight, decreased duration of parenteral nutrition and decreased hospital stay in the “early” fed group compared to the “late” feeding group [69]. In a randomized, controlled trial of 141 preterm infants who were either fed 20 mL/kg/day for the first 10 days of life (“minimal feeding group”) or initiated at 20 mL/kg/day and advanced daily by 20 mL/kg/day (“advancing group”), Berseth and colleagues determined that the “advancing group” had a higher incidence of NEC (7 infants versus 1 infant in the “minimal feeding group”) [70]. Although this study determined that the minimal feeding group had prolonged use of parenteral nutrition and central line placement in comparison to the “advancing group”, the authors concluded that the slow rate of advancement of feeds should be considered safe in premature infants [70].

ii. How quickly should feeds be advanced in premature infants?

In addition to the timing of initiation of enteral feedings, the rate with which to advance the feedings has been a topic of some debate, and several studies have evaluated whether slow versus rapid advancement of feedings could contribute to the development of NEC. A recent Cochrane Review evaluated the effects of advancing enteral feeds slowly on the incidence of NEC in very low birth weight infants [71]. There were five studies included in the meta-analysis and each began interval bolus feedings within the first five days of life (n=588 infants, <32 weeks gestation; <1500 grams) [71]. Feedings in all of the studies were advanced between 15–20 mL/kg/day (slow) versus 30–35 mL/kg/day (fast). The authors determined that there was not a statistically significant difference in the effect of slow versus fast feedings on the development of NEC or on mortality [71]. Each of the trials did report that in the slow advancement group, the infants took a statistically significant longer time to regain birth weight [72–76], but there was no effect on secondary outcome measures of feeding intolerance [74–76] or the incidence of invasive infection [75,76]. A recent retrospective study by Maas and colleagues evaluated premature infants who were born at less than 32 weeks gestational age and under 1500 grams and determined that there was no significant difference in the incidence of NEC seen in the accelerated feeding advancement group (3.3% accelerated versus 2.7% in the slower group), but they acknowledge that their study was underpowered to detect small differences between the two groups [77]. Adequately powered randomized controlled trials are needed to provide evidence-based approaches on the rate of advancement of enteral feeding in order to prevent NEC.

iii. Effects of the addition of various nutritive supplements on the development of NEC

Various nutritive supplements have been developed with the goal of enhancing infant growth without also increasing the likelihood of developing NEC. Stephens et al demonstrated the importance of early protein and energy intake within the first week of life on neurodevelopmental outcomes [78], and discovered that for every 1 gram/kg/day increase in protein intake during the first week of life, there was a significant increase in performance on scores of mental performance (specifically, the Bayley Mental Development Index) [78]. In order to meet the increased energy and protein requirements of premature infants, various types of fortifiers have been added to the breast milk or formula. Kuschel et al reviewed the effects of fortified human milk on weight gain, length and head growth, bone metabolism and neurodevelopmental outcomes and found that there was increased short term but not long term growth in the fortified group, and found no significant difference in bone mineralization or long term neurodevelopmental outcomes advantage [79]. A recent study comparing the effects of two human milk fortifiers on clinical outcomes in premature infants by Theone et al found that the use of acidified liquid human milk fortifier versus powdered human milk fortifier led to poor growth [80], and also determined that the incidence of NEC was significantly increased in the acidified liquid human milk fortifier group versus the powdered group (13% versus 0%, $p = 0.03$) [80]. Additional studies are required in order to more accurately assess the role of various additives on the development of NEC.

iv. Probiotics and NEC

The administration of probiotics i.e. “live bacteria with potential benefits to human health” has become a popular and controversial area of study in the prevention of NEC[81,82]. There is a large and growing body of literature regarding the health benefits of probiotics in preventing diseases including NEC [83,84], and recent meta-analyses of several randomized controlled trials have demonstrated that probiotics can reduce the incidence of NEC [83–90]. Concerns exist regarding which probiotic to use, and in what combination [86,91,92]. Further safety and efficacy studies are needed to determine the precise probiotic(s) that demonstrate protection against NEC, as well as to alleviate ongoing concerns regarding the potential infective risk of providing live bacteria to premature infants.

v. Blood transfusions and their association with NEC

Packed red blood cell transfusions are administered frequently to premature infants as part of routine care in the treatment of anemia. Recent reports have raised questions regarding the potential risks of this routine practice, and in particular, have suggested an association between blood transfusions and the subsequent development of NEC. This association was reported by Mally et al, who identified that in 908 neonatal admissions, 17 of those infants developed NEC and 6 out of the 17 infants were associated with transfusions (35%) [93]. The transfusion associated necrotizing enterocolitis group developed signs of NEC within 22 ± 5 hours of the PRBC transfusion, were more likely to be on full oral feedings (100% vs. 9% in the non-TANEC) and three of six patients died within 48 hours of the diagnosis of NEC [93]. A meta-analysis was performed by Mohamed et al comparing ten retrospective case-control studies evaluating whether neonates who developed NEC were exposed to PRBCs 48hrs prior to the onset of NEC [94]. One of the studies included in the meta-

analysis reported that 56% of the cases of NEC occurred within 48hrs of transfusion with PRBCs versus 20% in the non-transfusion associated group, and after their practice was to withhold feeds feeds during a transfusion, the incidence of NEC decreased from 5.3% to 1.3% [95]. While such a causal relationship between blood transfusion and NEC development may exist, additional studies are required in order for such an association to be established with certainty.

Optimal strategies for administration of feeds after surviving an initial episode of NEC

While the above strategies provide insights into how to optimally feed a premature infant so as to reduce the likelihood of NEC developing, the fact remains that 10–15% of premature infants in most neonatal intensive care units will develop NEC. A variety of studies indicate that there are no established guidelines regarding the optimal timing for re-introduction of enteral feeds after an episode of NEC, which obviously must be carefully administered so as to prevent a recurrence. A period of 7–10 days of fasting has been recommended for patients with NEC in whom the disease resolves without surgery, while a period of 14 days has been recommended in cases of NEC that require surgical resection [3]. The incidence of recurrent NEC is approximately 4–6% [96,97] and in a study by Stringer et al, 16 infants who developed recurrent NEC in a 10 year period had a median gestational age of 32 weeks, a median birth weight of 1260 grams and developed recurrent NEC after a median interval of 37 days form the initial episode [97]. These authors found no association between the recurrent NEC episode and the original management of disease (surgical or medical), the type or timing of enteral feeds or the anatomic site of the initial disease [97]. A more recent review by Thyoka et al of 212 infants found that the risk of NEC recurrence was 10% at their center [98], and determined that infants with recurrent NEC had similar characteristics such as gestational age, admission weight, gender, presence of a stricture, need for surgery and mortality rates than those infants with a single episode of NEC, although patients with recurrent NEC were found to be more likely to be dependent on parenteral nutrition for a longer period of time [98]. Although NEC recurrence is a rare entity, clinicians still remain cautious about when to restart feedings, and there is little data to guide such decision-making. Bohnhorst et al evaluated the effects of feeding after 3 consecutive days without evidence of portal venous gas (a sign of diseased bowel), and found no increase in the incidence of NEC when compared to infants that were not fed for 14 days [99]. A major limitation of the study was the small number of patients that were examined. Despite this limitation, this study suggested that the practice of prolonged fasting in infants with a diagnosis of NEC may not be necessary. More studies are clearly needed to determine the safest length of time of fasting and how to introduce enteral feeds in the post-NEC period.

Novel approaches to feeding premature infants so as to reduce the likelihood of NEC development

Given that NEC development is so closely linked to the administration of enteral formula, there is clearly a great need to understand the pathogenesis of NEC from a molecular viewpoint so as to design novel formulas with the capability of preventing NEC from

developing. Our laboratory and others have shown that the development of NEC develops in part as a result of exaggerated signaling through the receptor for gram negative endotoxin, namely toll-like receptor 4 (TLR4), which is present on the surface of the intestinal epithelium, and that this signaling occurs in response to colonization of the intestine within the neonatal intensive care unit [100–106]. Further, we and others have shown that that intestinal TLR4 expression was significantly increased in mice and humans with NEC compared to controls [101] and that TLR4 activation within the intestinal epithelium leads to an increase in enterocyte apoptosis [107] and impaired proliferation [108]. Further, we have demonstrated that mice lacking enterocyte TLR4 are protected from NEC development, highlighting the importance of TLR4 in NEC pathogenesis [101,109]. Moreover, the more premature the infant is, the higher the level of TLR4 expression that is observed within the intestinal epithelium, perhaps explaining the predisposition that the premature infant has to NEC development [110]. These laboratory findings suggest that dietary or pharmacologic strategies designed to inhibit TLR4 may prevent NEC from developing in the first place. In this regard, we have used an *in silico* strategy to identify small molecule inhibitors to TLR4 that could be used to supplement nutritive formulas in premature infants. Our lead compound, C34, is a 2-acetamidopyranoside (MW 389) with the formula C17H27NO9, which inhibited TLR4 in enterocytes and macrophages *in vitro*, reduced systemic inflammation in mouse models of endotoxemia, and reduced the incidence of experimental NEC and in human tissue *ex vivo* obtained from infants with NEC [111]. These findings raise the possibility that supplementation of existing infant formulas with C34 or its analogues may offer novel preventative approaches to NEC.

An additional and very novel future approach to the prevention of NEC may involve the use of a synthetic amniotic fluid. It is known that during development, the fetus swallows amniotic fluid, which bathes the fetal intestine, and since the premature infant lacks amniotic fluid exposure as a consequence of early birth, we sought to determine whether amniotic fluid supplementation could prevent NEC development in experimental models. In support of this possibility, we found that enteral administration of amniotic fluid to mice prevented the development of experimental NEC, and did so through inhibition of the bacterial receptor toll-like receptor 4 [112]. Furthermore, we identified that the specific component within amniotic fluid that was largely responsible for the inhibition of TLR4 signaling was epidermal growth factor (EGF) as demonstrated by the fact that mice lacking the EGF receptor were not protected from NEC development after enteral administration of amniotic fluid [112]. The protective effects of amniotic fluid were also confirmed in a piglet model of NEC [113], as well as in the rat model of experimental NEC [114], where hepatocyte growth factor was found to mediate the protective effects of amniotic fluid. Taken together, these findings raise the exciting possibility that amniotic fluid – or a nutritive formulation with similar properties – may one day be used to prevent the development of NEC in premature infants.

Future directions in the management of infants at risk for the development of NEC

The above review indicates that nutritional considerations may have significant impact on the development of NEC in the premature infant, and their overall outcomes. Further prospective randomized clinical trials are needed in order to more accurately define the optimal approach to how and when to initiate and advance feeds in premature infants at risk for NEC. Furthermore, it is tempting to speculate that strategies designed to optimize infant formulations using a variety of molecular components - including those presently found within breast milk that - may reduce the incidence or severity of NEC. This is an extremely exciting area of research, and one that offers the possibility of helping a large number of infants, so as to reduce the burden of this devastating disease.

Expert Commentary

A wide variety of feeding practices exist on how to feed the premature infant in the hopes of preventing necrotizing enterocolitis. There have been several meta-analysis reviewing the timing of administration and rate of advancement of enteral feedings in the premature infant as reviewed above, but there is no consensus on the precise feeding strategy to prevent this disease. The exclusive use of human breast milk is recommended for all premature infants and is associated with a significant decrease in the incidence of NEC [11–13]. By determining the specific ingredients in breast milk that are protective against NEC, it is our hope that this devastating disease will one day be preventable.

Five-year View

Necrotizing enterocolitis is a deadly disease affecting the intestine of the premature infant, but the exact pathogenesis remains incompletely understood. As novel preventative approaches are identified, strategies to limit the disease may be better elucidated.

Acknowledgments

Grant support: DJH is supported by R01GM078238 and R01DK083752 from the National Institutes of Health and a research grant from Abbott Nutrition; MG is supported by MG is supported by 5K12HD052892-05 from the National Institutes of Health, The Children’s Hospital of Pittsburgh of the UPMC Health System and The Gerber Foundation.

References

Papers of special note are highlighted below as:

*of interest

**of considerable interest

1. Henry MC, Moss RL. Necrotizing enterocolitis. Annual review of medicine. 2009; 60:111–124.
2. Caplan MS, Jilling T. New concepts in necrotizing enterocolitis. Current opinion in pediatrics. 2001; 13(2):111–115. [PubMed: 11317050]
3. Neu J, Walker WA. Necrotizing enterocolitis. The New England journal of medicine. 2011; 364(3): 255–264. [PubMed: 21247316]

4. Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet. 2006; 368(9543):1271–1283. [PubMed: 17027734]

5. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: pathogenesis, classification, and spectrum of illness. Current problems in pediatrics. 1987; 17(4):213–288. [PubMed: 3556038]

6. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Annals of surgery. 1978; 187(1):1–7. [PubMed: 413500]

7. Neu J. Neonatal necrotizing enterocolitis: an update. Acta paediatrica. 2005; 94(449):100–105. [PubMed: 16214774]

8. Lee JS, Polin RA. Treatment and prevention of necrotizing enterocolitis. Seminars in neonatology: SN. 2003; 8(6):449–459. [PubMed: 15001117]

9. Frantz ID 3rd, L'Heureux P, Engel RR, Hunt CE. Necrotizing enterocolitis. The Journal of pediatrics. 1975; 86(2):259–263. [PubMed: 803385]

10. Vollman JH, Smith WL, Tsang RC. Necrotizing enterocolitis with recurrent hepatic portal venous gas. The Journal of pediatrics. 1976; 88(3):486–487. [PubMed: 1245964]

11*. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. Lancet. 1990; 336(8730): 1519–1523. Describes the strong relationship of the protective effect of breast milk on NEC. [PubMed: 1979363]

12. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatric research. 2007; 61(1):2–8. [PubMed: 17211132]

13**. Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. Journal of perinatology: official journal of the California Perinatal Association. 2009; 29(1):57–62. Demonstrated that the protective effects of breast milk on NEC are dose-dependent. [PubMed: 18716628]

14. Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. Pediatrics. 2005; 116(2):400–406. [PubMed: 16061595]

15. McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Archives of disease in childhood. Fetal and neonatal edition. 2003; 88(1):F11–14. [PubMed: 12496220]

16**. Quigley MA, Henderson G, Anthony MY, McGuire W. Formula milk versus donor breast milk for feeding preterm or low birth weight infants. Cochrane database of systematic reviews. 2007; (4):CD002971. Meta-analysis demonstrating the strong relationship of formula feeding and increased incidence of NEC. [PubMed: 17943776]

17. Davies DP. Adequacy of expressed breast milk for early growth of preterm infants. Archives of disease in childhood. 1977; 52(4):296–301. [PubMed: 558739]

18. Gross SJ. Growth and biochemical response of preterm infants fed human milk or modified infant formula. The New England journal of medicine. 1983; 308(5):237–241. [PubMed: 6848932]

19. Lucas A, Gore SM, Cole TJ, et al. Multicentre trial on feeding low birthweight infants: effects of diet on early growth. Archives of disease in childhood. 1984; 59(8):722–730. [PubMed: 6476868]

20. Lucas A, Morley R, Cole TJ, et al. Early diet in preterm babies and developmental status in infancy. Archives of disease in childhood. 1989; 64(11):1570–1578. [PubMed: 2690739]

21. Raiha NC, Heinonen K, Rassin DK, Gaull GE. Milk protein quantity and quality in low-birthweight infants: I. Metabolic responses and effects on growth. Pediatrics. 1976; 57(5):659–684. [PubMed: 7767]

22. Schultz K, Soltesz G, Mestyan J. The metabolic consequences of human milk and formula feeding in premature infants. Acta paediatrica Scandinavica. 1980; 69(5):647–652. [PubMed: 7234386]

23. Tyson JE, Lasky RE, Mize CE, et al. Growth, metabolic response, and development in very-low-birth-weight infants fed banked human milk or enriched formula. I. Neonatal findings. The Journal of pediatrics. 1983; 103(1):95–104. [PubMed: 6864403]

24. Henderson G, Anthony MY, McGuire W. Formula milk versus maternal breast milk for feeding preterm or low birth weight infants. Cochrane database of systematic reviews. 2007; (4):CD002972. [PubMed: 17943777]

25. Cristofalo EA, Schanler RJ, Blanco CL, et al. Randomized Trial of Exclusive Human Milk versus Preterm Formula Diets in Extremely Premature Infants. The Journal of pediatrics. 2013; 163(6): 1592–1595. e1591. [PubMed: 23968744]
26. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics. 1999; 103(6 Pt 1):1150–1157. [PubMed: 10353922]
27. Furman L, Taylor G, Minich N, Hack M. The effect of maternal milk on neonatal morbidity of very low-birth-weight infants. Archives of pediatrics & adolescent medicine. 2003; 157(1):66–71. [PubMed: 12517197]
28. Jones JA, Ninnis JR, Hopper AO, et al. Nitrite and Nitrate Concentrations and Metabolism in Breast Milk, Infant Formula, and Parenteral Nutrition. JPEN. Journal of parenteral and enteral nutrition. 2013
29. Petersson J, Phillipson M, Jansson EA, Patzak A, Lundberg JO, Holm L. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. American journal of physiology. Gastrointestinal and liver physiology. 2007; 292(3):G718–724. [PubMed: 17082222]
30. Nankervis CA, Giannone PJ, Reber KM. The neonatal intestinal vasculature: contributing factors to necrotizing enterocolitis. Seminars in perinatology. 2008; 32(2):83–91. [PubMed: 18346531]
31. Nankervis CA, Reber KM, Nowicki PT. Age-dependent changes in the postnatal intestinal microcirculation. Microcirculation. 2001; 8(6):377–387. [PubMed: 11781811]
32. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. Nature medicine. 1995; 1(6):546–551.
33. Kanady JA, Aruni AW, Ninnis JR, et al. Nitrate reductase activity of bacteria in saliva of term and preterm infants. Nitric oxide: biology and chemistry/official journal of the Nitric Oxide Society. 2012; 27(4):193–200. [PubMed: 22842223]
34. Yazji, I.; Sodhi, CP.; Lee, EK., et al. Endothelial TLR4 activation impairs intestinal microcirculatory perfusion in necrotizing enterocolitis via eNOS-NO-nitrite signaling. Proceedings of the National Academy of Sciences of the United States of America; 2013.
35. Sodhi C, Richardson W, Gribar S, Hackam DJ. The development of animal models for the study of necrotizing enterocolitis. Disease models & mechanisms. 2008; 1(2–3):94–98. [PubMed: 19048070]
36. Polycarpou E, Zachaki S, Tsolia M, et al. Enteral L-arginine supplementation for prevention of necrotizing enterocolitis in very low birth weight neonates: a double-blind randomized pilot study of efficacy and safety. JPEN. Journal of parenteral and enteral nutrition. 2013; 37(5):617–622. [PubMed: 23329787]
37. Amin HJ, Zamora SA, McMillan DD, et al. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. The Journal of pediatrics. 2002; 140(4):425–431. [PubMed: 12006956]
38. Polycarpou E, Zachaki S, Tsolia M, et al. Enteral L-arginine Supplementation for Prevention of Necrotizing Enterocolitis in Very Low Birth Weight Neonates: A Double-Blind Randomized Pilot Study of Efficacy and Safety. JPEN. Journal of parenteral and enteral nutrition. 2013
39. Rhoads JM, Argenzio RA, Chen W, et al. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. The American journal of physiology. 1997; 272(5 Pt 1):G943–953. [PubMed: 9176200]
40. Poindexter BB, Ehrenkranz RA, Stoll BJ, et al. Parenteral glutamine supplementation does not reduce the risk of mortality or late-onset sepsis in extremely low birth weight infants. Pediatrics. 2004; 113(5):1209–1215. [PubMed: 15121931]
41. Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. The Journal of pediatrics. 2000; 137(6):785–793. [PubMed: 11113834]
42. Pawlik D, Lauterbach R, Hurkala J, Radziszewska R. The effects of enteral administration of glutamine enriched solution in very low birth weight infants on reducing the symptoms of feeding intolerance. A prospective, randomized pilot study. Medycyna wieku rozwojowego. 2012; 16(3): 205–211. [PubMed: 23378398]

43. Moe-Byrne T, Wagner JV, McGuire W. Glutamine supplementation to prevent morbidity and mortality in preterm infants. Cochrane database of systematic reviews. 2012; 3:CD001457. [PubMed: 22419279]

44. Newburg DS. Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. Journal of animal science. 2009; 87(13 Suppl):26–34. [PubMed: 19028867]

45. Ruiz-Palacios GM, Cervantes LE, Ramos P, Chavez-Munguia B, Newburg DS. Campylobacter jejuni binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. The Journal of biological chemistry. 2003; 278(16):14112–14120. [PubMed: 12562767]

46. Newburg DS, Ruiz-Palacios GM, Morrow AL. Human milk glycans protect infants against enteric pathogens. Annual review of nutrition. 2005; 25:37–58.

47. Bode L. Human milk oligosaccharides: prebiotics and beyond. Nutrition reviews. 2009; 67 (Suppl 2):S183–191. [PubMed: 19906222]

48. Weng M, Walker WA. The role of gut microbiota in programming the immune phenotype. Journal of developmental origins of health and disease. 2013; 4(3)

49**. Jantscher-Krenn E, Zherebtsov M, Nissan C, et al. The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. Gut. 2012; 61(10): 1417–1425. Authors demonstrate that the specific human milk oligosaccharide disialyllacto-N-tetraose prevents experimental NEC. [PubMed: 22138535]

50. Valenti P, Antonini G. Lactoferrin: an important host defence against microbial and viral attack. Cellular and molecular life sciences: CMLS. 2005; 62(22):2576–2587. [PubMed: 16261253]

51. Haversen L, Ohlsson BG, Hahn-Zoric M, Hanson LA, Mattsby-Baltzer I. Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-kappa B. Cellular immunology. 2002; 220(2):83–95. [PubMed: 12657243]

52. Buccigrossi V, de Marco G, Bruzzese E, et al. Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. Pediatric research. 2007; 61(4):410–414. [PubMed: 17515863]

53. Sherman MP, Bennett SH, Hwang FF, Yu C. Neonatal small bowel epithelia: enhancing anti-bacterial defense with lactoferrin and Lactobacillus GG. Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine. 2004; 17(3):285–289.

54**. 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: the journal of the American Medical Association. 2009; 302(13):1421–1428. Compared bovine lactoferrin plus *Lactobacillus rhamnosus* GG versus lactoferrin alone and found a decreased incidence of NEC in the lactoferrin and *Lactobacillus rhamnosus* GG group. [PubMed: 19809023]

55. Magnuson JS, Henry JF, Yip TT, Hutchens TW. Structural homology of human, bovine, and porcine milk lactoferrins: evidence for shared antigenic determinants. Pediatric research. 1990; 28(2):176–181. [PubMed: 1697671]

56. Dvorak B. Milk epidermal growth factor and gut protection. The Journal of pediatrics. 2010; 156(2 Suppl):S31–35. [PubMed: 20105663]

57. Nair RR, Warner BB, Warner BW. Role of epidermal growth factor and other growth factors in the prevention of necrotizing enterocolitis. Seminars in perinatology. 2008; 32(2):107–113. [PubMed: 18346534]

58. Feng J, Besner GE. Heparin-binding epidermal growth factor-like growth factor promotes enterocyte migration and proliferation in neonatal rats with necrotizing enterocolitis. Journal of pediatric surgery. 2007; 42(1):214–220. [PubMed: 17208569]

59. Sheng G, Bernabe KQ, Guo J, Warner BW. Epidermal growth factor receptor-mediated proliferation of enterocytes requires p21waf1/cip1 expression. Gastroenterology. 2006; 131(1): 153–164. [PubMed: 16831599]

60. Pollack PF, Goda T, Colony PC, et al. Effects of enterally fed epidermal growth factor on the small and large intestine of the suckling rat. Regulatory peptides. 1987; 17(3):121–132. [PubMed: 3495822]

61. Dvorak B, Williams CS, McWilliam DL, et al. Milk-borne epidermal growth factor modulates intestinal transforming growth factor-alpha levels in neonatal rats. Pediatric research. 2000; 47(2): 194–200. [PubMed: 10674346]

62. Clark JA, Doelle SM, Halpern MD, et al. Intestinal barrier failure during experimental necrotizing enterocolitis: protective effect of EGF treatment. American journal of physiology. Gastrointestinal and liver physiology. 2006; 291(5):G938–949. [PubMed: 16798726]

63. Dvorak B, Halpern MD, Holubec H, et al. Epidermal growth factor reduces the development of necrotizing enterocolitis in a neonatal rat model. American journal of physiology. Gastrointestinal and liver physiology. 2002; 282(1):G156–164. [PubMed: 11751169]

64. Clark JA, Lane RH, Maclennan NK, et al. Epidermal growth factor reduces intestinal apoptosis in an experimental model of necrotizing enterocolitis. American journal of physiology. Gastrointestinal and liver physiology. 2005; 288(4):G755–762. [PubMed: 15528252]

65. Yu X, Radulescu A, Zorko N, Besner GE. Heparin-binding EGF-like growth factor increases intestinal microvascular blood flow in necrotizing enterocolitis. Gastroenterology. 2009; 137(1): 221–230. [PubMed: 19361505]

66**. Dvorak B, Khailova L, Clark JA, et al. Comparison of epidermal growth factor and heparin-binding epidermal growth factor-like growth factor for prevention of experimental necrotizing enterocolitis. Journal of pediatric gastroenterology and nutrition. 2008; 47(1):11–18. Provides side by side comparison of two potential therapeutics for NEC and determines efficacy of each. [PubMed: 18607263]

67. Morgan J, Bombell S, McGuire W. Early trophic feeding versus enteral fasting for very preterm or very low birth weight infants. Cochrane database of systematic reviews. 2013; 3:CD000504. [PubMed: 23543508]

68. McClure RJ, Newell SJ. Randomised controlled study of clinical outcome following trophic feeding. Archives of disease in childhood. Fetal and neonatal edition. 2000; 82(1):F29–33. [PubMed: 10634838]

69. Sallakh-Niknezhad A, Bashar-Hashemi F, Satarzadeh N, Ghojazadeh M, Sahnazarli G. Early versus Late Trophic Feeding in Very Low Birth Weight Preterm Infants. Iranian journal of pediatrics. 2012; 22(2):171–176. [PubMed: 23056882]

70. Berseth CL, Bisquera JA, Paje VU. Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics. 2003; 111(3): 529–534. [PubMed: 12612232]

71. Morgan J, Young L, McGuire W. Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane database of systematic reviews. 2013; 3:CD001241. [PubMed: 23543511]

72. Rayyis SF, Ambalavanan N, Wright L, Carlo WA. Randomized trial of “slow” versus “fast” feed advancements on the incidence of necrotizing enterocolitis in very low birth weight infants. The Journal of pediatrics. 1999; 134(3):293–297. [PubMed: 10064664]

73. Caple J, Armentrout D, Huseby V, et al. Randomized, controlled trial of slow versus rapid feeding volume advancement in preterm infants. Pediatrics. 2004; 114(6):1597–1600. [PubMed: 15574620]

74. Salhotra A, Ramji S. Slow versus fast enteral feed advancement in very low birth weight infants: a randomized control trial. Indian pediatrics. 2004; 41(5):435–441. [PubMed: 15181294]

75. Krishnamurthy S, Gupta P, Debnath S, Gomber S. Slow versus rapid enteral feeding advancement in preterm newborn infants 1000–1499 g: a randomized controlled trial. Acta paediatrica. 2010; 99(1):42–46. [PubMed: 20002013]

76. Karagol BS, Zenciroglu A, Okumus N, Polin RA. Randomized controlled trial of slow vs rapid enteral feeding advancements on the clinical outcomes of preterm infants with birth weight 750–1250 g. JPEN. Journal of parenteral and enteral nutrition. 2013; 37(2):223–228. [PubMed: 22664861]

77. Maas C, Mitt S, Full A, et al. A historic cohort study on accelerated advancement of enteral feeding volumes in very premature infants. Neonatology. 2013; 103(1):67–73. [PubMed: 23095283]

78. Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics. 2009; 123(5):1337–1343. [PubMed: 19403500]

79. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane database of systematic reviews. 2004; (1):CD000343. [PubMed: 14973953]

80. Thoene M, Hanson C, Lyden E, Dugick L, Ruybal L, Anderson-Berry A. Comparison of the effect of two human milk fortifiers on clinical outcomes in premature infants. Nutrients. 2014; 6(1):261–275. [PubMed: 24394538]

81. FAO/WHO. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada: 2002. Guidelines for the Evaluation of Probiotics in Food.

82. Indian Council of Medical Research Task F, Co-ordinating Unit I, Co-ordinating Unit DBT. ICMR-DBT guidelines for evaluation of probiotics in food. The Indian journal of medical research. 2011; 134:22–25. [PubMed: 21808130]

83. 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):1–4. [PubMed: 15629973]

84. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biology of the neonate. 2002; 82(2):103–108. [PubMed: 12169832]

85. Li D, Rosito G, Slagle T. Probiotics for the prevention of necrotizing enterocolitis in neonates: an 8-year retrospective cohort study. Journal of clinical pharmacy and therapeutics. 2013

86. Bin-Nun A, Bromiker R, Wilschanski M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. The Journal of pediatrics. 2005; 147(2):192–196. [PubMed: 16126048]

87. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane database of systematic reviews. 2011; (3):CD005496. [PubMed: 21412889]

88. Wang Q, Dong J, Zhu Y. Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. Journal of pediatric surgery. 2012; 47(1):241–248. [PubMed: 22244424]

89. 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. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2006; 42(12):1735–1742. [PubMed: 16705580]

90. Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. The American journal of clinical nutrition. 2011; 93(1):81–86. [PubMed: 20980486]

91. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane database of systematic reviews. 2008; (1):CD005496. [PubMed: 18254081]

92. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet. 2007; 369(9573):1614–1620. [PubMed: 17499603]

93. Mally P, Golombek SG, Mishra R, et al. Association of necrotizing enterocolitis with elective packed red blood cell transfusions in stable, growing, premature neonates. American journal of perinatology. 2006; 23(8):451–458. [PubMed: 17009195]

94. Mohamed A, Shah PS. Transfusion associated necrotizing enterocolitis: a meta-analysis of observational data. Pediatrics. 2012; 129(3):529–540. [PubMed: 22351894]

95. El-Dib M, Narang S, Lee E, Massaro AN, Aly H. Red blood cell transfusion, feeding and necrotizing enterocolitis in preterm infants. Journal of perinatology: official journal of the California Perinatal Association. 2011; 31(3):183–187. [PubMed: 21252964]

96. Kastenberg ZJ, Sylvester KG. The surgical management of necrotizing enterocolitis. Clinics in perinatology. 2013; 40(1):135–148. [PubMed: 23415269]

97. Stringer MD, Brereton RJ, Drake DP, Kiely EM, Capps SN, Spitz L. Recurrent necrotizing enterocolitis. Journal of pediatric surgery. 1993; 28(8):979–981. [PubMed: 8229602]

98. Thyoka M, Eaton S, Hall NJ, et al. Advanced necrotizing enterocolitis part 2: recurrence of necrotizing enterocolitis. European journal of pediatric surgery: official journal of Austrian Association of Pediatric Surgery … [et al] = Zeitschrift fur Kinderchirurgie. 2012; 22(1):13–16.

99**. Bohnhorst B, Muller S, Dordelmann M, Peter CS, Petersen C, Poets CF. Early feeding after necrotizing enterocolitis in preterm infants. The Journal of pediatrics. 2003; 143(4):484–487. Demonstrates that early reintroduction of feeding after an episode of NEC does not increase the incidence of recurrent NEC. [PubMed: 14571225]

100. Grave GD, Nelson SA, Walker WA, et al. New therapies and preventive approaches for necrotizing enterocolitis: report of a research planning workshop. Pediatric research. 2007; 62(4): 510–514. [PubMed: 17667844]

101. Leaphart CL, Cavallo J, Gribar SC, et al. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. Journal of immunology. 2007; 179(7):4808–4820.

102. Jilling T, Simon D, Lu J, et al. The roles of bacteria and TLR4 in rat and murine models of necrotizing enterocolitis. Journal of immunology. 2006; 177(5):3273–3282.

103. Stoll BJ, Nahmias AJ, Wickliffe C, Brann AW Jr, Dowell VR Jr, Whaley DN. Bacterial toxin and neonatal necrotizing enterocolitis. The Journal of pediatrics. 1980; 96(1):114–115. [PubMed: 7350290]

104. 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(3):e17776. [PubMed: 21445298]

105. Wolfs TG, Derikx JP, Hodin CM, et al. Localization of the lipopolysaccharide recognition complex in the human healthy and inflamed premature and adult gut. Inflammatory bowel diseases. 2010; 16(1):68–75. [PubMed: 20014022]

106. Chan KL, Wong KF, Luk JM. Role of LPS/CD14/TLR4-mediated inflammation in necrotizing enterocolitis: pathogenesis and therapeutic implications. World journal of gastroenterology: WJG. 2009; 15(38):4745–4752. [PubMed: 19824106]

107. Richardson WM, Sodhi CP, Russo A, et al. Nucleotide-binding oligomerization domain-2 inhibits toll-like receptor-4 signaling in the intestinal epithelium. Gastroenterology. 2010; 139(3):904–917. 917 e901–906. [PubMed: 20580721]

108. Sodhi CP, Shi XH, Richardson WM, et al. Toll-like receptor-4 inhibits enterocyte proliferation via impaired beta-catenin signaling in necrotizing enterocolitis. Gastroenterology. 2010; 138(1): 185–196. [PubMed: 19786028]

109. Sodhi CP, Neal MD, Siggers R, et al. Intestinal epithelial Toll-like receptor 4 regulates goblet cell development and is required for necrotizing enterocolitis in mice. Gastroenterology. 2012; 143(3):708–718. e701–705. [PubMed: 22796522]

110. Gribar SC, Sodhi CP, Richardson WM, et al. Reciprocal expression and signaling of TLR4 and TLR9 in the pathogenesis and treatment of necrotizing enterocolitis. Journal of immunology. 2009; 182(1):636–646.

111. Neal MD, Jia H, Eyer B, et al. Discovery and validation of a new class of small molecule Toll-like receptor 4 (TLR4) inhibitors. PloS one. 2013; 8(6):e65779. [PubMed: 23776545]

112. Good M, Siggers RH, Sodhi CP, et al. Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. Proceedings of the National Academy of Sciences. 2012; 109(28):11330–11335.

113. Siggers J, Ostergaard MV, Siggers RH, et al. Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates. American journal of physiology. Gastrointestinal and liver physiology. 2013; 304(10):G864–875. [PubMed: 23518680]

114. Jain SK, Baggerman EW, Mohankumar K, et al. Amniotic Fluid-borne Hepatocyte Growth Factor Protects Rat Pups against Experimental Necrotizing Enterocolitis. American journal of physiology. Gastrointestinal and liver physiology. 2014

Key Issues

- Necrotizing enterocolitis (NEC) is a devastating disease of premature infants and feeding practices have been suggested to be a modifiable risk factor.
- Breast milk is the most effective protective agent for NEC.
- Various components of breast milk are thought to mediate protection against NEC and include nitrate/nitrite, lactoferrin, human milk oligosaccharides (HMOs) and growth factors.
- Further investigation is needed to address the safest way to feed a premature infant after an episode of NEC to prevent recurrence.
- Toll-like receptor 4 (TLR4) signaling is important in the pathogenesis of NEC and further identification of TLR4 inhibitors should be investigated for their role in the prevention of NEC.