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Evidence and Experience in Neonatal Medicine

Necrotising Enterocolitis: The State of the Science

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ABSTRACT

Necrotizing enterocolitis is the most common gastrointestinal emergency of the neonate, affecting 5-10% of infants, yet the pathogenesis remains unclear. Widely accepted risk factors include prematurity, enteral feeds, bacterial colonization and mucosal injury. How these or other yet identified factors come together to create the classic clinical and pathologic features is the subject of much research. The activation of the cytokine cascade, in part by bacterial ligands, appears to play a key role in mucosal injury. Two mediators that may also contribute are platelet activating factor and intestinal toll-like receptors. Short chain fatty acids, the products of bacterial fermentation of carbohydrates, have been thought to cause mucosal injury. Overgrowth of pathogenic bacteria in the face of a decreased commensal population may play a key role. A current focus of clinical research involves probiotics, enterally fed forms of commsenal bacteria. This may set the stage for a healthier intestinal ecosystem and possibly, decreased risk of NEC. **[Indian J Pediatr 2007; 74 (1) : 67-72]** *E-mail: Ian.Holzman@MSSM.EDU*

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Necrotizing Enterocolitis (NEC) is the most common gastrointestinal emergency of the neonate, yet the true etiology remains unclear despite years of research. Many theories have attempted to elucidate the true pathogenesis over the last 40 years without success. These theories and subsequent research efforts have centered on what are felt to be the most important risk factors: prematurity, enteral feeds, mucosal injury and the presence of bacteria. Historically, clinical research has focused particularly on feeding practices – when, with what and how fast to increase them. Attempts to identify a consistent infectious etiology have failed, yet strict infectious control parameters can decrease the incidence. With the advances of molecular biology, the identification of a few factors holds promise, including platelet activating factor (PAF), intestinal toll-like receptors (TLR), short chain fatty acids (SCFAs), and the activation of the cytokine induced inflammatory cascade. They appear to play a pivotal role in not only mucosal injury but the maintenance of an intact mucosal barrier. One area of clinical research is focused on probiotics and their impact on the normal commensal flora. This article will review the most current theories on the pathogenesis of NEC, all which revolve around the most widely accepted risk factors.

Prematurity

Prematurity remains the most consistent factor, although term babies can develop NEC. Approximately 5-10% of infants with a birth weight less than 1500 grams will develop NEC and the incidence increases with decreasing gestational age.[1, 2] In the 1960s Santulli described some of the first cases of NEC in premature infants with low apgar scores and hyaline membrane disease. These infants were dependent upon ventilatory support, had umbilical catheters in place, and developed the hallmark signs of NEC after the initiation of enteral feeds.[3] These patients provided the basis for the concept that in order for NEC to occur, three things needed to be present – bacteria, ischemia, and substrate (enteral feeds). Neonatologists in the modern NICU find themselves with far fewer asphyxiated preterm infants, more limited use of umbilical catheters, shorter time on ventilators, and judicious feeding.

As a result, the focus has shifted to the role of the immature mucosal barrier coupled with an impaired immune response to intestinal pathogens. While secretory IgA (sIgA) doesn't participate in the inflammatory cascade its deficiency may facilitate bacterial translocation.[4] It is known that sIgA plays an important role in mucosal defense by binding with antigens and impairing their absorption by enterocytes. A second suggested immune system interaction is the possibility that aberrant T lymphocyte activity may fail to recognize a breakdown in the mucosal barrier in a premature infant.[4]

Feeding

Enteral feeds have almost always been administered when NEC occurs, although symptoms usually develop

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weeks after the introduction of feeds, but often not far after achieving full enteral feeds. Many times infants develop symptoms following recent volume advancement or after reinitiating feeds. Human milk reduces the incidence but doesn't prevent it entirely. Barlow published a study in 1974 citing the importance of human milk in decreasing the risk of NEC and suggesting the role of protective factors such as sIgA mentioned above.[5] Others have speculated on the effects of osmolality in damaging the intestine but published data have failed to support this. While many clinicians have written on the topic of "safe" feeding advancement, the available studies are conflicting. Most authors agree that 20 ml/Kg/day is a safe advancement rate.[6] Many NICUs have a policy of attempting "minimal enteral nutrition" or trophic feeds for a period of time before advancing feeds in an attempt to prime the intestine.

Infectious Agents

It is clear that infectious agents play a role in the clinical appearance of NEC. No single pathogen has been identified – gram negative bacteria are the most common, followed by gram positive bacteria, but yeast and even viruses have been implicated. Cases of NEC are usually sporadic, but the many reports of clusters suggest colonization with particularly virulent strains may be important. Only 1/3 of infants will have a positive blood culture, but bacteremia is seen more often with advanced disease. Pneumatosis intestinalis, the hallmark radiologic finding of NEC, is the intraluminal and portal system presence of hydrogen gas produced by the fermentation of carbohydrates by bacteria.

The gastrointestinal tract of the newborn infant is sterile but colonized within 12-24 hours – first with maternal vaginal flora, followed by that of the external environment. The normal commensal flora – *Lactobacilli* and *Bifidobacterium* are found weeks later. Colonization is affected in part by feeding practices. Feeding with human milk is associated with earlier colonization by anerobic commensals. The therapeutic strategies used in the NICU – broad spectrum antibiotics and delayed initiation of enteral feeds contributes to the fact that the VLBW infant often has a delayed onset and aberrant pattern of colonization.[7] This may favor the presence of more pathogenic strains over the usual commsensal flora.

Ischemia

Ischemia has long been thought to play a role in NEC. It was thought that ischemia was the inciting event, with interrupted mucosal integrity and bacterial translocation the end result. The hallmark histological findings of mucosal edema, ulceration, inflammation and coagulation necrosis support the accepted belief that ischemia plays a role at some point. Historical risk factors such as perinatal asphyxia, presence of umbilical lines, polycythemia, hypotension, or the use of indomethacin supported the role of ischemia. However, epidemiologic studies have failed to confirm an association between NEC and most of these risk factors.

Current research efforts are focused on the role of intrinsic vascular regulation. The intestinal circulation of the newborn has a low vascular resistance which facilitates a high rate of blood flow. This regulation occurs by the balancing of elements that favor dilatation or constriction of the vasculature. Endothelin-1 is a peptide that promotes vasoconstriction. Nitric oxide (NO) is a free radical produced by an intact endothelium that favors vasodilatation. NO production occurs by way of an enzyme- endothelial nitric oxide synthetase or eNOS - expressed at low levels during fetal life and increased postnatally. At baseline the balance favors an excess of NO and thus a vasodilated intestinal microcirculation. The principal sites of resistance are the arterioles within the submucosal plexus.[8] Events that alter this balance would shift the equilibrium towards increased vascular resistance and thus a limitation of blood flow and oxygen delivery.[8] Ischemia-reperfusion injury following perinatal asphyxia, or the prolonged state of low flow perfusion in growth retarded fetuses are two examples that are a focus of considerable research interest. Animal models support their role in the dysregulation of intestinal vascular resistance, primarily by disrupting NO production.[8] However, whether the implicated role of ischemia is the inciting cause for NEC or the end result of an initial inflammatory disruption of the mucosal barrier remains unknown.

Inflammatory Mediators

Current evidence points to the role of activated inflammatory mediators and an inadequate anti-inflammatory response in the breakdown of mucosal integrity, which may represent the final common pathway in NEC. In clinical studies, patients with NEC often have elevated inflammatory mediators and attempts have been made to correlate the presence and concentration of these factors with disease presentation.[9]

Intestinal epithelial cells produce many of the cytokines that are implicated as mediators of intestinal inflammation and injury. Cytokines are soluble molecules that bind cell receptors and play a role in both humoral and cell-mediated immune responses. IL-6 induces secretion of acute phase proteins, enhances T cell proliferation and antibody production by B cells. IL-8 recruits inflammatory cells. The synthesis of TNFα is generated in part by microbial products and other cytokines. TNFα recruits inflammatory cells, but is also involved in cytotoxicity and programmed cell death.

Platelet Activating Factor (PAF) is one of the mediators most intensely studied. PAF is an endogenous phospholipid inflammatory mediator that is produced by inflammatory cells, endothelial cells, platelets and bacteria. There are PAF receptors on most cells. The

receptor gene is expressed in many organs, but evidence exists that the greatest receptor expression is found in the ileum- the most common site of involvement in NEC.[10] Activation of the PAF receptor induces the production of additional molecules such as TNFα, IL-6, and IL-8. In addition, it activates pathways triggering apoptosis in intestinal epithelial cells.[10] In various experimental models, PAF causes capillary leak, myocardial dysfunction, renal dysfunction, neutropenia, thrombocytopenia, and hypotension.[10] Conversion by PAF-acetylhydrolase renders it inactive. Human neonates have low / absent circulating PAF-acetylhydrolase, and human milk contains significant quantities.

Animal models have attempted to reproduce the clinical and pathologic features based on accepted risk factors. Barlow created the first model in the 1970s of newborn rats stressed with asphyxia, colonized with enteric bacteria by nasogastric tubes, and subsequently fed. It was this animal model that first highlighted the importance of breast milk in preventing NEC.[5] More recently, investigators have used a similar animal model to illustrate the importance of endogenous mediators such as PAF in the pathogenesis of NEC. They induced bowel necrosis by infusion of PAF, TNFα or lipopolysaccharide (LPS). PAF infusion caused focal necrosis, but affected the entire small bowel when given in large quantities. LPS acted as a priming agent for PAF, as small doses were synergistic with low doses of PAF. LPS-induced intestinal injury was blocked by pretreatment with a PAF antagonist.[11]

Toll-Like Receptors

Toll-like receptors (TLRs) on the cell surface act as sensors of microbial infection and play a role in the initiation of the inflammatory and immune defense response. Both commensal and pathogenic bacteria secrete molecules that serve as ligands to TLRs, such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA). LPS has been shown to act specifically on TLR4, and LTA on TLR2. TLRs may play a role in the preservation of intestinal epithelial integrity.

Rakoff-Nahoum and colleagues studied the interaction of TLRs and commsensal organsisms in a series of experiments with mice.[12] They found that activation of TLRs by the commsensal flora played a fundamental role not only in the preservation of intestinal epithelial integrity but also in protection from injury. Animals deficient in TLR signaling were more vulnerable to induced intestinal injury as compared with controls, resulting in a near 100% mortality as opposed to 100% survival. In addition, these animals produced low levels of factors integral in cellular protection and repair before and after injury. An interesting component of these series of experiments was the role of commensals in protection from injury. Wild type mice depleted of commensals by an antibiotic regimen had similar rates of morbidity / mortality following induced intestinal injury as did the mice deficient in TLR signaling, and these mice had low concentrations of the factors that play a key role in cellular protection and repair. The authors concluded that TLRs are crucial in maintaining intestinal epithelial homeostasis particularly following injury, and that commensal flora induce the production of protective factors following epithelial injury.

However, activation of TLRs does result in cytokine activation and, potentially, a considerable inflammatory response. Therefore, especially in a neonate lacking commensal flora, TLRs may play a role in the activation of a pathologic inflammatory response and subsequent injury. Colonization with organisms that secrete LPS or LTA, known ligands for TLRs, occurs early and prior to that of anaerobic commensals. Caplan *et al.* proposed a potential role of TLRs in activation of the pathologic inflammatory response, as opposed to protection from injury.[10] In a newborn mice model of NEC, asphyxiated animals fed with formula were found to have up-regulated TLR4 expression. In addition, PAF given to the animals induced TLR4 expression and TLR4 deficient mice had a decreased incidence of NEC. The authors concluded that activation of TLR4 by bacterial ligands and PAF may be critical in the initiation of events that culminate in NEC.

Short Chain Fatty Acids and Bacterial Overgrowth

It is well accepted that the presence of bacteria usually is a prerequisite for NEC to develop, yet the actual role of bacteria is undefined. Do they incite the injury or do they gain access to the cells below the epithelial lining only after injury occurs by another mechanism? SCFAs, mainly acetic acid, propionic acid and butyric acid, are the products of bacterial fermentation of carbohydrates in the intestinal lumen. In newborn infants, normal intestinal bacterial colonization is established once enteral feeding is achieved. In the anaerobic environment of the colon, bacteria rapidly ferment carbohydrates to gases (hydrogen, carbon dioxide, and in some cases, methane) and SCFAs.[13] This process of bacterial fermentation of undigested carbohydrates plays an important role in normal intestinal biology such as water and salt absorption in the colon, energy salvation, and colonic mucosal maturation.[13] In the premature infant who has a relative lactase deficiency, lactose ingested in the form of milk may be fermented into SCFAs and subsequently absorbed. In some premature infants, an abnormal state of SCFA over-production may arise during periods of significant carbohydrate malabsorption and / or bacterial overgrowth. This may exceed the buffering and absorptive capacity of the colon and lead to an increased concentration of SCFAs in the colon.[13] Levels of SCFAs in the distal ileum may also increase by way of reflux across the ileo-cecal valve or due to local bacterial overgrowth. Lin *et al* have demonstrated that intraluminal

administration of SCFAs can induce concentration and maturation-dependent intestinal mucosal injury in newborn rats with pathology similar to that seen in NEC.[14, 15] Intraluminal administration of lactic acid, the fermentation product of lactic acid-producing probiotics, does not induce identifiable intestinal mucosal injury. Their findings suggest that overproduction / accumulation of SCFAs, but not lactic acid, in the proximal colon and / or distal ileum may play a key role in the pathogenesis of NEC.

A recently published study by Sangild *et al* used a novel design in an animal model to illustrate how formula feeding and bacterial colonization could induce NEC without a prior asphyxial insult.[16] In a series of experiments, they documented the spontaneous development on NEC in formula and colostrum fed premature pigs. Animals fed formula had higher rates of NEC (53% *vs* 5% in the colostrum group), associated with villous atrophy even in those that appeared clinically healthy. Animals fed colostrum had higher levels of brush border enzymes. Animals raised in a sterile environment and subsequently fed lacked villous atrophy, and formula fed animals were found to have high rates of *Clostridium* colonization. After formula fed animals were passively immunized with plasma containing high levels of titers against toxins to *C.perfringens* and *E.coli*, they failed to develop clinical or histopathologic signs of NEC. This study implicates the interaction of bacteria and substrate in the pathogenesis of NEC.

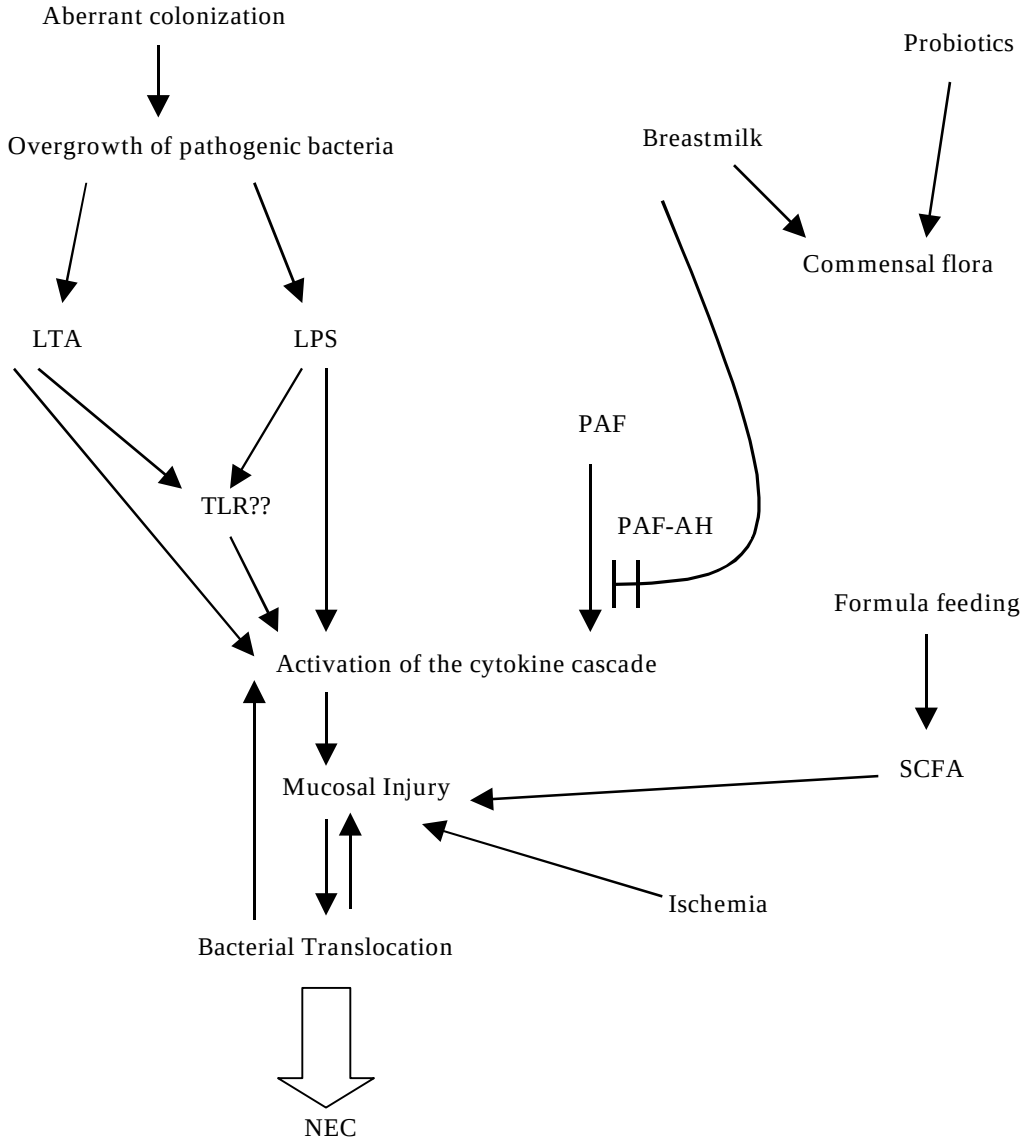

Fig 1. Flow diagram of proposed factors involved in the pathogenesis of necrotizing enterocolitis. LTA - Lipoteichoic acid; LPS – Lipopolysaccharide; TLR – Toll-like receptor; PAF – Platelet activating factor; PAF-AH – Platelet activating factor acetyl-hydrolase; SCFA – Short chained fatty acids; NEC – Necrotising Enterocolitis.

TABLE. Probiotics: Recent Clinical Trials

Author	Study Method	Subjects	Probiotics	Primary outcome	Finding
Dani Italy, 2002	Prospective RCT 12 NICUs	N = 585 total <33wks, <1500g	**Dicoflor** *Lactobacillus GG*	UTI Bacterial sepsis NEC	Not significant
Bin Nun Israel, 2005	Prospective RCT 1 NICU	N = 145 total <1500g	**ABC Dophilus** Bifidobacteria infantis Streptococcus thermophilus *Bifidobacteria bifidus*	NEC and / or Death	Significant: Cases and severity of NEC Not Significant: NEC associated mortality
Lin **Taiwan, 2005**	Prospective RCT 1 NICU	N = 367 total **<1500g**	**Infloran** *Lactobacillus acidophilus* *Bifidobacterium infantis*	NEC or Death	Significant: Reduction in incidence and severity of NEC

Probiotics

It has been increasingly recognized that normal healthy gut microflora play a vital role in human health and perform important metabolic functions that support the digestive system. Probiotics are living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition. Most probiotics are lactic acid-producing bacteria. This is a large group of bacteria so named because they produce lactic acid as an end product of fermentation. Probiotics currently being investigated in clinical practice are enterally fed forms of these normal commensals that fail to translocate or cause mucosal injury and in fact may protect against mucosal injury. Among them, *Bifidobacterium* and *Lactobacillus* are some of the most common probiotics found in the intestine of healthy newborn infants

Administration of some common probiotics has been shown to reduce the incidence of NEC in experimental animal models as well as in clinical trials. Using an animal model of NEC, Caplan et al demonstrated that probiotics may play a role in the prevention of NEC.[17] Rats were inoculated with *B. infantis*, *E.coli*, or saline control and exposed to an NEC protocol (formula feeding + asphyxia). They demonstrated colonization with *B. infantis* in the intestine and stool within 48 hours. Levels of the inflammatory mediators, plasma endotoxin and phospholipase A2, were lower in the *B. infantis* group. In addition, *B. infantis* treated animals had a significant reduction in the incidence of NEC compared with control and E.coli treated animals.

The use of probiotics in the clinical setting has been a source of great interest, and a few published reports support their use. Three recent prospective randomized trials have sought to illustrate the beneficial impact of enterally fed probiotics in the prevention of NEC (Table). In two of the studies, in which the primary outcome was death or significant NEC, a statistically significant reduction in the incidence and / or severity of NEC occurred in the probiotic fed group. Both of these centers had a fairly high (15 and 23%) pre-study incidence of NEC.[18, 19] The third study, which examined probiotic use in the prevention of urinary tract infections, bacterial sepsis and NEC, found no statistically significant impact.[20]

In summary, the typical patient with NEC is at its simplest a premature infant who has been fed. What sets the stage for this potentially devastating illness remains one of the great mysteries of neonatology. Once the mucosal barrier is disrupted bacterial invasion into the wall of the intestine could occur. Bacterial translocation may propagate further injury by the additional activation of inflammatory mediators. The end result, ischemia, apoptosis, and concomitant mucosal disruption leads to pneumatosis and the characteristic lesions of coagulation necrosis. Inflammatory mediators such as TNFα and IL-8 are well known to induce shock and the systemic inflammatory response syndrome (SIRS). If this vicious cycle continues, there is a high likelihood of intestinal necrosis and perforation. Using probiotics to produce a healthier intestinal ecosystem in premature infants may have a potential role in the prevention of NEC, an elusive goal for decades. No longer must we sacrifice early enteral nutritional support (an approach that can lead to fewer central lines infections and less hepatotoxicity from parenteral nutrition) to our concerns about eventual bowel necrosis. While the story will continue, the ending is now at least in sight!

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