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Objectives

After completing this article, readers should be able to:

1. Describe the basic epidemiologic aspects of neonatal enterocolitis (NEC).
2. List the major risk factors that predispose neonates to NEC.
3. Delineate the current views on the mechanisms involved in the pathogenesis of NEC.
4. Discuss the various models used to study NEC.
5. List the major mediators believed to be involved in the pathogenesis of NEC.

Introduction

Despite many years of clinical observation and experimental investigation, the pathogenesis of neonatal enterocolitis (NEC) remains elusive. The multifactorial theory suggests that four key risk factors—prematurity, formula feeding, intestinal ischemia, and bacterial colonization—are important prerequisites to the initiation of intestinal injury in neonates (Figure). Current hypotheses suggest that these risk factors stimulate activation of the inflammatory cascade that ultimately results in the final common pathway of bowel necrosis that is the hallmark of neonatal NEC.

Prematurity

Prematurity is the most consistent and important risk factor associated with neonatal NEC. The disease occurs in 10% of babies born in the United States who weigh less than 1,500 g, but it is extraordinarily infrequent among term newborns and almost never is diagnosed in older infants or children. Furthermore, data clearly show that the more preterm the infant, the higher the risk of NEC. Nonetheless, the specific reason(s) for this particular predisposition are not well understood.

Evidence from animal and human studies have shown significant differences between term and preterm neonates in several aspects of intestinal development and function. The mucosal barrier matures throughout gestation and even remains deficient in the term neonate during the first few weeks of life. It is well known that several mucosal enzymes and gastrointestinal hormones are suppressed or deficient in preterm animals/humans. Many aspects of the intestinal host defense system, a complex and important cascade responsible for limiting the invasion of multiple pathogens, are deficient or dysfunctional in the preterm infant, including the secretory immunoglobulin A (IgA) response, neutrophil function, macrophage activation, cytokine production and function, and activity of intestinal defensins. Furthermore, evidence suggests that autoregulation of the microcirculation differs in newborns compared with older animals and that peristalsis, a key physiologic mechanism in the prevention of bacterial overgrowth, is dysfunctional in the preterm neonate. As will be discussed later, preterm infants hospitalized in the neonatal intensive care unit (NICU) develop marked differences in the pattern of intestinal bacterial colonization compared with term newborns, which may contribute to the initiation of NEC. Nonetheless, the specific factors responsible for the peculiar epidemiology of this disease affecting preterm infants remain unclear.

Formula Feeding

More than 90% of patients diagnosed with NEC have been fed enterally. Much debate and investigation over the past 30 years pertains to the role of enteral nutrition on the initiation

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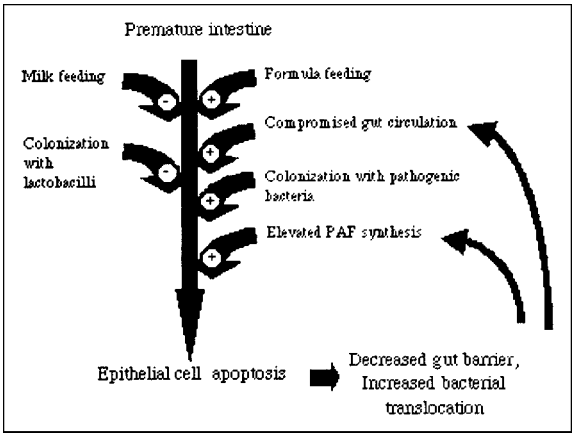

Figure. Schematic of NEC pathomechanism.

of bowel injury. Hypotheses have considered the importance of formula osmolality and strength; the rate of daily feeding volume advancement; the provision of nutrition by the nasogastric or nasojejunal route; cycling as bolus or continuous infusion of formula; and the differences between preterm formula, term formula, and human milk. Only breastfeeding clearly shows a beneficial role in reducing the incidence of NEC compared with alternative food sources.

A careful feeding regimen that was believed to reduce the risk of NEC markedly in preterm infants was described several years ago. Since that time, most neonatologists advance feeding volumes slowly and cautiously, although few studies have confirmed the benefit of this technique. A recent report has shown that careful feeding decreased the rate of NEC substantially in an NICU. Despite the use of historical controls and lack of randomization, this study highlighted the diversity with which preterm newborns are fed and the impact that such variety might have on their developing intestines. In a randomized, controlled, prospective trial, preterm infants weighing less than 1,500 g were randomized to receive either "slow" feeding advancement of 15 mL/kg per day or "fast" advancement at 35 mL/kg per day. The authors found no difference in the incidence of NEC between the groups (13% with the slow regimen versus 9% with the fast regimen), but the group randomized to fast advancement regained their birthweights more rapidly. These unexpected findings suggest that very low-birthweight infants may not require as much caution in their feeding regimen as previously suggested, but few neonatologists currently advance feedings rapidly in preterm infants.

In addition to feeding volume, several studies suggest that the food source and feeding approach affect the development of neonatal NEC. In a recent randomized, controlled trial of four feeding groups that included human milk and preterm formula and compared continuous nasogastric feeds, nasogastric bolus and early priming, and no early trophic feeding, no differences were documented in the incidence of NEC. Furthermore, additional morbidities were fewer with human milk, and the rate to acquisition of full feedings was faster with the bolus method. This study underscores that feeding approaches differ among NICUs and that clear standards of care for feeding preterm infants are lacking.

Although most neonatologists assume that human milk feedings reduce the incidence of NEC, only a few scientific reports address this clinical effect. More than 20 years ago, the effectiveness of human milk supplementation was examined in two retrospective analyses. In one study of 109 cases of NEC, the findings identified several cases fed fresh human milk exclusively and were unable to identify specific risk factors peculiar to any of the three feeding classes, which included human milk, milk and formula, and formula alone. In another study, many fewer cases of NEC were analyzed according to feeding type, and the authors concluded (based on soft data) that frozen human milk was beneficial to all infants, but that it did not protect fully against the disease in the highest risk, lowest birthweight groups. To assess better the beneficial effect of human milk, a multicenter, prospective, controlled trial was conducted that was randomized for some infants. Among the randomized patients, donor milk reduced the incidence of NEC compared with preterm formula, although inadequate power precluded statistical significance (1% versus 5% of confirmed cases). Nonetheless, in the nonrandomized groups, donor milk reduced the incidence of NEC in all birthweight subcategories. Despite the flaws in study design, these data suggest a beneficial role for human milk. Although physicians empirically assume that fresh human milk may provide greater benefit than frozen or pasteurized preparations, specific randomized trials have not confirmed this supposition.

Several potent bioactive factors in human milk, which are absent in preterm formula preparations, may play a role in modulating the inflammatory cascade and influencing the incidence of NEC. For example, human milk contains neonatal antigen-specific antibodies (IgA, IgM, and IgG), leukocytes, enzymes, lactoferrin, growth factors, hormones, oligosaccharides, polyunsaturated fatty acids, nucleotides, and specific glycoproteins. All of these compounds have been postulated to alter the mucosal environment, thereby reducing the risk for neonatal NEC. Despite the enormous work pursuing these impor-

tant human milk components, their specific importance on the intestinal milieu requires additional investigation.

Intestinal Ischemia

It is theorized that compromised intestinal blood flow in the fetus and neonate contributes to the pathophysiology of neonatal NEC. Multiple experimental models have been designed to evaluate the importance of intestinal ischemia in the developing animal, and several observations from the human neonate have contributed to our understanding. It is generally understood that most term newborns who develop intestinal necrosis have associated conditions that compromise the intestinal circulation, including polycythemia/hyperviscosity, birth asphyxia, exchange transfusion, congenital heart disease, or intrauterine growth restriction with reversed end-diastolic flow in the umbilical artery. In these situations, the presentation and clinical progression of disease may be quite different from that which is observed in preterm neonates who have NEC.

Several clinical situations in the preterm infant that increase the risk of NEC are associated with compromised intestinal blood flow. For example, patients who have an active patent ductus arteriosus (PDA) associated with a large left-to-right shunt have decreased gut perfusion and appear to have an increased risk for NEC. In a provocative randomized trial evaluating the effect of prophylactic ductal ligation in the first day of life in preterm infants weighing less than 1,000 g, investigators in Alabama documented a significantly lower incidence of NEC in treated infants compared with standard management controls. Furthermore, patients treated with indomethacin to close the PDA have an increased risk for NEC, presumably due to the effect of the drug on the intestinal circulation. Significant controversy over the years has surrounded the importance of umbilical artery catheters on the incidence of NEC, and some neonatologists are reluctant to feed these patients enterally for fear of this disease. It is hypothesized that the presence of umbilical artery catheters reduces intestinal circulation or allows for emboli to traverse the superior mesenteric artery. Nonetheless, the data are not convincing that umbilical artery catheters alter the incidence of NEC. Although apnea and bradycardia spells are frequent in the preterm infant and may compromise the intestinal microcirculation, no convincing data suggest that these events modulate the pathophysiology of NEC. Finally, maternal cocaine abuse has been associated with an increased incidence of NEC in several retrospective cohort studies, and animal data suggest that intestinal blood flow is reduced with cocaine exposure.

Bacterial Colonization

Before birth, the fetus has a sterile intestinal environment, and cases of NEC have not been described in utero. It is presumed that intestinal stenosis or atresia results from the onset of severe ischemia prior to birth. It follows that bacterial colonization is a prerequisite for the initiation of inflammatory bowel necrosis and the clinical presentation of NEC. Following delivery, intestines are colonized rapidly with bacteria, resulting in a large array of microorganisms that include the anaerobic bacteria bifidobacteria and lactobacilli among breastfed infants. Preterm infants hospitalized in an NICU who are not exposed early to human milk typically exhibit different intestinal microflora than healthy term infants. In a study of stool microflora from extremely low-birthweight infants, investigators found: 1) a paucity of bacterial species in most cases (fewer than three by the 10th day of life), 2) increased species diversity with human milk feeding, 3) reduced species number with antibiotic therapy, and 4) only 1 in 29 infants colonized by anaerobic bacteria (eg, bifidobacteria or lactobacilli). Although epidemics of NEC due to specific microorganisms have been described, most cases of NEC are isolated, and recent data suggest that specific bacterial species do not cause NEC. These findings imply that these high-risk infants are susceptible to overgrowth of specific pathogens that could initiate the inflammatory cascade that results in NEC.

Experimental Models for Assessing the Pathogenesis of NEC

Comparative analysis of data derived from healthy neonates and those affected by NEC has provided valuable information and served as a stepping stone for various in vivo animal experiments and in vitro model systems. Studies in the laboratory further contributed to the understanding of the pathogenesis of NEC and allowed the design of various preventive strategies.

Analysis of human samples typically extends to serum samples, stool samples, and surgical or pathologic specimens of the small intestine. Other, less typical parameters collected from other sources or via noninvasive imaging techniques include duodenal aspirate and Doppler or near-infrared imaging of abdominal circulation.

Analysis of these parameters uncovered a potential role for compromised mesenteric circulation, increased mucosal inflammation, and intestinal epithelial apoptosis as contributing mechanisms and identified platelet-activating factor (PAF) as a potential key molecule in the pathogenesis of NEC.

Mesenteric Blood Flow

Mesenteric circulation is extremely vulnerable to hemodynamic challenges. As is well known from the phenomenon of "diver's reflex," the mesenteric blood flow is very low on the priority list when perfusion is limited or oxygenation has to be redirected to vital organs. In the presence of compromised hemodynamics or compromised oxygenation in the systemic circulation, increased vascular resistance reduces blood flow in the mesenteric circulation. Consequently, several studies addressed the effects of various conditions affecting the systemic circulation or oxygenation on mesenteric blood flow, either by using Doppler ultrasonography or near-infrared spectroscopy. Apneic episodes, PDA, extracorporeal membrane oxygenation, umbilical arterial catheterization, and hypothermia during cardiac surgery have been shown to reduce splanchnic blood flow, and these changes correlate with compromised mucosal barrier function and a subsequently increased incidence of NEC, even in term neonates. The hemodynamics of the mesentery are even more at risk in preterm neonates. In one remarkable study, the critical limits of mesenteric circulation and oxygen delivery have been described on an isolated segment of human small intestine that was removed during transplantation. With perfusion rates greater than 30 mL/min per 100 g intestinal tissue, oxygen consumption was flow-independent; below these rates, oxygen consumption became flow-limited.

Findings from these clinical observations have been validated using various animal models, including rats, piglets, and rabbits. These experiments allowed even more detailed characterization of the mechanisms by which mesenteric blood flow is regulated and enabled researchers to test various preventive strategies to augment mesenteric blood flow or to prevent the consequences of reduced perfusion under conditions that predispose an infant to compromised splanchnic circulation. Among other mediators, epinephrine, cocaine, endothelin-1, tumor necrosis factor-alpha, and endotoxin have been shown to play a role in regulating pathologic mesenteric circulation, with the latter two acting through nitric oxide metabolism. An experimental preventive strategy was designed to ameliorate the consequences of reduced mesenteric blood flow during cardiac surgery by perfusing intestinal lumen with oxygenated fluorocarbons during hypoxic episodes.

Although reduced splanchnic blood flow certainly can act as a predisposing factor for NEC, it cannot be viewed as a sole cause of the disease. In a rat model of NEC, PAF antagonism prevented necrotic changes in the small intestine induced by lipopolysaccharide plus hypoxia challenge without significantly affecting circulatory changes. Furthermore, in piglets whose mesenteric blood flow can be precisely monitored with Doppler ultrasonography, PAF has been shown to cause only a transient effect on splanchnic circulation via a cardiotoxic effect. These findings suggest that the well-documented role of PAF in the pathogenesis of NEC probably is independent of its effect on mesenteric circulation.

PAF

PAF has emerged as a primary mediator in the pathogenesis of NEC. Gonzalez-Crussi and Hsueh have shown that intra-aortic injection of PAF results in experimental bowel necrosis that is similar to NEC. This model has been widely used to investigate the molecular and cellular events that lead to experimental bowel necrosis. Findings from human samples validated the role of PAF in NEC. Plasma levels of PAF-acetylhydrolase, the PAF-degrading enzyme, have been shown to be significantly lower in preterm neonates, and PAF levels in stool increase following the initiation of enteral feeding. A neonatal rat model of NEC employing common risk factors for NEC, such as hypoxic stress and formula feeding, also has been used to investigate the role of PAF in NEC. In this model, formula feeding and hypoxia stress synergistically increased the intestinal expression of phospholipase A2 and PAF receptor mRNA. Conversely, PAF receptor antagonists or PAF-acetylhydrolase mixed in the formula prevented the development of experimental NEC.

Because evidence indicating a central role for PAF in NEC was so strong, the various effects of PAF on epithelial cells had to be evaluated. Given that ion transport has been implicated in various forms of intestinal pathology, we investigated the regulation of ion transport properties of cultured intestinal epithelial cells by PAF. We mounted monolayers of HT29-CL19A cells (ie, colonic adenocarcinoma cells) in Ussing chambers and measured vectorial transepithelial ion transport. PAF stimulated secretory chloride transport across epithelial cell monolayers, but only when cells were exposed to PAF from the luminal side. The validity of this surprising observation was verified by the detection of PAF receptors in the apical plasma membrane. It is yet to be determined whether the stimulation of ion transport by PAF represents physiologic regulation or it is responsible for PAF-induced pathology.

Intestinal Mucosal Barrier and Apoptosis of Epithelial Cells

It has been postulated that one primary reason for the increased risk for NEC in preterm neonates is an unde-

veloped mucosal barrier, which may allow the translocation of bacteria and unprocessed food antigens into the lamina propria that, in turn, might activate inflammatory cells. The mucosal barrier consists of a single layer of epithelial cells that line the lumen of the intestine and are sealed together by tight junctions. Consequently, in addition to the inherently premature barrier, mucosal permeability may be augmented further by damage to the integrity of the epithelial layer. Epithelial cells turn over naturally by the removal of some cells via programmed cell death (apoptosis) and by replacement of the dying cells with proliferating cells in the crypts. Abundant apoptosis is a logical first step that might cause a breach in the mucosal barrier, thereby initiating a cascade of events consisting of bacterial translocation into the submucosa and the activation of an inflammatory cascade.

Ford and associates detected the presence of abundant apoptotic nuclei in the epithelial cells of bowel specimens derived from bowel resections of patients who had NEC. To investigate systematically the involvement of epithelial apoptosis in the pathogenesis of experimental NEC, we evaluated the presence of epithelial apoptosis in a neonatal rat model of NEC. Formula feeding and hypoxia stress caused an abundance of apoptosis in the intestinal epithelium, and apoptosis preceded the gross morphologic damage to the intestinal wall. Further studies need to evaluate whether a specific blockade of apoptosis can prevent NEC in this model and verify that apoptosis is an underlying cause of further damage. Nevertheless, existing data suggest that accelerated apoptosis might be an important early event in NEC pathogenesis and might set the stage for subsequent bacterial translocation and activation of the inflammatory cascade. The abundance of inflammatory mediators might further accelerate epithelial apoptosis, thus completing a vicious circle.

To evaluate a possible connection between altered PAF metabolism and aberrantly high epithelial apoptosis, we studied the regulation of apoptosis by PAF and other inflammatory mediators in tissue culture models of the developing intestine. PAF is a potent stimulator of apoptosis in cultured intestinal epithelial cells, and it promotes apoptosis in epithelial cells via the activation of caspase 8 and caspase 3. Accordingly, caspase inhibitors efficiently block PAF-induced apoptosis in epithelial cells. Based on these results, we are currently evaluating whether caspase inhibition can prevent experimental NEC in the neonatal rat model.

Bacterial Colonization

Inappropriate colonization of the intestine with enteropathogenic bacteria has been thought to play a role in NEC. Nonates fed human milk tend to colonize their intestine with bifidobacteria and lactobacilli (facultative anaerobes); formula-fed neonates develop an intestinal flora abundant with potentially pathogenic gram-negative bacteria. These distinct colonization profiles might affect susceptibility to NEC. Because enterococci and lactobacilli use lactose as their primary energy source, successful colonization with these gram-positive organisms exhausts the primary energy source that would allow the growth of pathogenic organisms. Furthermore, facultative anaerobes generate metabolic byproducts such as the short-chain fatty acids, acetate, propionate, and butyrate that promote differentiation of intestinal epithelial cells. These bacteria also are believed to interact directly with complex carbohydrates on apical surfaces of epithelial cells, initiating signal transduction and differentiation. In the absence of colonization with these beneficial gram-positive bacteria, various enteropathogenic gram-negative organisms may overgrow in the intestinal milieu. In an animal model, an NEC-like histology was achieved by filling rabbit ileal loops with enteropathogenic *Escherichia coli*. Release of endotoxins from these gram-negative organisms might contribute both to accelerated apoptosis and to compromised splanchnic hemodynamics. We have found that bacterial lipopolysaccharide is a potent activator of epithelial apoptosis, and others have found that it can severely reduce mesenteric blood flow.

These findings suggest that early colonization of the gut might have an important role in developing susceptibility to NEC. Accordingly, the supplementation of infant formula with probiotics might be a feasible prevention strategy to reduce the incidence of NEC in high-risk populations. In an experimental model of NEC in quails and in the aforementioned neonatal rat model of NEC, supplementation of formula with live bifidobacteria prevented the development of NEC. Using the neonatal rat model of NEC, we have performed a preliminary characterization of the mechanisms by which bifidobacteria prevented NEC. Circulating plasma endotoxin levels were 10-fold lower in formula-fed and asphyxia stressed animals that received bifidobacteria. Furthermore, bifidobacteria supplementation of formula prevented the increase of PLA2-II gene expression by formula feeding and asphyxia stressing in the neonatal intestine. These findings in the animal model offer encouragement for future clinical trials to determine whether bifidobacteria can prevent NEC in humans.

Summary

Although the precise mechanisms that are responsible for the development of NEC remain elusive, a basic understanding of the key risk factors and the major steps that lead to this disease is developing. Based on this initial evidence, several novel prevention strategies are being evaluated that might help to reduce the incidence of NEC.

Suggested Reading

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NeoReviews Quiz

1. Prematurity is the most consistent and important risk factor in the epidemiology of necrotizing enterocolitis (NEC). Of the following, the estimated incidence of NEC among very low-birthweight (<1,500 g) infants is *closest* to:

   A. 5%.
   B. 10%.
   C. 15%.
   D. 20%.
   E. 30%.

2. Enteral feeding is one of the prerequisites for initiating intestinal injury that results in NEC. Of the following, the factor *most* likely to be protective against NEC is:

   A. Continuous orogastric infusion.
   B. Early gut priming (trophic feedings).
   C. Exclusive human milk feeding.
   D. Low caloric density of milk.
   E. Slow advancement of feeding volume.

3. A compromised intestinal blood flow in the fetus and/or the neonate contributes to the pathogenesis of NEC. Of the following, the *most* convincing cause of intestinal ischemia is:

   A. Anemia of prematurity.
   B. Indomethacin treatment.
   C. Partial exchange transfusion.
   D. Recurrent apnea and bradycardia.
   E. Umbilical artery catheterization.

4. Intestinal epithelial apoptosis contributes to the pathogenesis of NEC. Of the following, the medication *most* likely to cause intestinal epithelial apoptosis is:

   A. Endothelin-1.
   B. Endotoxin.
   C. Epinephrine.
   D. Platelet-activating factor.
   E. Tumor necrosis factor.

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