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Models of the pathogenesis of necrotizing enterocolitis

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Neonatal necrotizing enterocolitis is a disease of unknown origin.[1, 2] The initiating events in the pathogenesis of NEC have not been established. Nonetheless, epidemiologic observations emphasize the potential roles of infection, enteric feeding, and local vascular compromise of the gastrointestinal tract in the pathogenesis of this disease.

Necrotizing enterocolitis is the most common serious acquired gastrointestinal tract problem in neonatal intensive care units. It has a significant mortality rate and delayed chronic morbidity caused by short bowel syndrome.[3-5] Manifestation is by systemic signs and symptoms suggestive of neonatal sepsis and shock. The gastrointestinal manifestations include abdominal tenderness and distension, gastrointestinal hemorrhage, emesis, ileus (gastric residuals), intestinal perforation, and peritonitis.[1-5]

The diagnosis is suggested when an infant has the signs and symptoms described above and is confirmed by the radiologic signs of pneumatosis intestinalis or portal venous gas, or both. The appearance of the intestine reveals hemorrhagic or coagulation necrosis with visible gas-filled cysts along the subserosal and submucosal layers.[1, 2, 5] These cysts correspond to the pneumatosis intestinalis observed on the abdominal radiograph. The microscopic appearance of the intestinal tissue demonstrates mucosal edema, hemorrhage, coagulation necrosis, and mucosal ulceration covered by a pseudomembranous exudate.

EPIDEMIOLOGY

Necrotizing enterocolitis is seen in premature infants; 90% of cases are in infants born at less than 36 weeks of gestation.[2, 5-9] The disease usually develops during the first 2 weeks after birth. The more immature the infant is at birth, the later is the onset of NEC. It has been proposed that immaturity of the gastrointestinal tract predisposes to NEC.[9] This immaturity may relate to mucosal integrity or permeability,[10] and also to the regulation of gastrointestinal blood flow,[10, 11] motility,[12] or enzyme function.[13]

Additional observations have emphasized the role of various perinatal risk factors. Because many of these factors produce hypoxia and ischemia of the gastrointestinal tract, vascular compromise has been proposed as a theoretic event in the pathogenesis. It is now thought that many of the proposed risk factors for NEC are coincidental findings. New studies confirm that prematurity is the primary risk factor for NEC.

NEC	Necrotizing enterocolitis
PAF	Platelet activating factor
TNF	Tumor necrosis factor

Other data have indicated that milk feeding is an etiologic factor. Another factor may be oral feeding, 95% of affected patients having been fed by mouth before the onset of disease. In addition, the occurrence of NEC in epidemics has suggested that NEC is due to an infectious agent.

HYPOXIC-ISCHEMIC INJURY

Mesenteric blood flow in infants may decline in the presence of polycythemia, extreme hypoxia, and severe abdominal distension.[10, 11, 14-16] The result of these individual events is increased mesenteric vascular resistance. When severe hypoxia produces mesenteric acidemia, mesenteric vascular resistance increases and may result in a reduction of intestinal oxygen extraction.[10, 11] The first effects of hy-

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poxic intestinal injury are seen in the mucosa. Mucosal injury may produce malabsorption by inhibition of active transport.[14, 17] Hypoxic injury may also result in mucosal necrosis with ulceration and tissue sloughing.

Many of the fetal or neonatal hypoxic risk factors for NEC could induce the dive reflex. Events such as chronic placental insufficiency with increased fetal vascular resistance (as determined by fetal Doppler ultrasound examination), acute fetal asphyxia, exposure to cocaine, respiratory distress syndrome, shock, polycythemia, the use of intravascular catheters, and exchange transfusions could potentially produce fetal or neonatal hypoxia and mesenteric circulatory insufficiency.[18, 19]

After an acute hypoxic-ischemic event, reperfusion-induced tissue damage can produce ongoing injury to the intestinal mucosa. The damage may include cytotoxic vascular endothelial cell damage with further ischemia and the cytotoxic effects on cells of oxygen-free radicals.[20, 21]

Although experimental data suggest that ischemic or postischemic events can produce injury resembling NEC, there is little information to indicate that hypoxia and ischemia in human infants are consistent predisposing factors. Many of the risk factors associated with ischemia are common to all premature infants. It is therefore probable that the identification of these factors in a patient is coincidental and not causal. When patients with NEC are compared with normal, weight-matched control subjects, many of these predisposing variables are found with equal incidence in both groups.[2]

ENTERAL ALIMENTATION

In more than 90% of infants with NEC, the disease develops after enteric feeding starts. It was once thought that immunoprotective factors in fresh human milk (e.g., macrophages, IgA) could prevent NEC.[1] Despite interesting animal experiments, there is little evidence to suggest that human milk influences the incidence of this disease.

Oral administration of IgA to premature infants has lowered the incidence of NEC.[22] It is not understood why this IgA preparation effectively reduced the incidence of NEC when fresh human milk did not. Treatment with IgA may have achieved higher gut levels of specific antibodies against potential pathogens. Alternatively, immunoglogulins may have other protective roles. NEC has been associated with some forms of milk protein allergy; exogenous immunoglobulins may block the allergic-immune response.[1]

The volume or rate of increase of milk feedings may be a contributor to the development of NEC. Some studies have suggested that giving nothing by mouth for 1 or 2 weeks may lower the incidence of NEC.[23] However, new evidence suggests that early enteral feeding does not necessarily predispose infants to a higher incidence of NEC.[24-29]

Excessive milk volume and rapid increases of volume have been suggested as a risk factor for NEC.[2, 27, 30] Large volumes may produce stress on an already injured intestinal mucosa. Overfeeding and distension may interfere with the ability to increase mesenteric blood flow after a meal and thus may produce local intestinal hypoxemia. In addition, overfeeding may cause malabsorption of lactose, which is common if the premature infant is overfed. The colonic bacteria ferment lactose to short-chain organic acids and hydrogen gas; the latter is present in large quantities in the lumen and in the gas-filled cysts noted as pneumatosis intestinalis.[31] The large amount of hydrogen produced by the colonic bacteria results in intestinal distension. Gaseous distension may increase luminal tension; as the intraluminal pressure increases, the perfusion to the mucosa becomes compromised and may produce vascular insufficiency.

The relationship between enteral feeding and the etiology of NEC has not been well defined. Nonetheless, feeding practices must take into consideration the vulnerability of the premature infant's intestine. Formula increments of approximately 10 to 20 ml/kg/day have been safe and are often recommended.[27, 28] Greater daily feeding increments (35 to 60 ml/kg/day) have been associated with an increased incidence of NEC.[27, 30]

INFECTIOUS DISEASE

Circumstantial data indicate that NEC may be associated with an infectious agent. The disease occurs in epidemics[1, 32]; the spectrum ranges from benign hemorrhagic colitis to fulminant lethal enterocolitis with intestinal perforation. Epidemics have been associated with single pathogens such as *Escherichia coli, Klebsiella pneumoniae, Salmonella* species, *Staphylococcus epidermidis, Clostridium butyricum,* coronavirus, enterovirus, and rotavirus.[1, 2, 4, 6] The suspicion of a transmissible agent has been suggested by the observation that intensive care personnel may be sick at the same time as the infants during these episodes of NEC.[32, 33, 34] During most epidemics, including those in which there were illnesses in the neonatal intensive care staff, the investigators have not been able to identify a responsible microbiologic agent.

The difficulty of isolating specific microorganisms has created the thought that NEC may be due to a bacterial toxin.[1, 2] NEC has similarities to many types of enterotoxemia in newborn mammals and to that seen in older human patients with pigbel. The epidemiologic and pathologic features of pigbel are similar to those of NEC. Before the un-

derstanding that *Clostridium perfringens* type C toxin causes pigbel, one theory for the pathogenesis of this disease was that ischemia was responsible.

The blood culture is positive in approximately one third of patients with NEC.[1, 35] The bacteria associated with NEC reflect the colonic flora; they are not necessarily the responsible agents in the pathogenesis of NEC. After mucosal injury, the intestinal protective mechanisms are disrupted, permitting the colonic bacteria to enter the circulation. The bacteria recovered in blood cultures in patients with NEC probably reflect the current colonic colonization flora of the low birth weight infant. Thus, between 1950 and 1970, the common pathogens identified in cultures of blood specimens from patients with NEC were gram-negative enteric bacilli; more recently gram-negative bacteria have been noted with a frequency equal to that of *S. epidermidis*.

INFLAMMATORY MEDIATORS

One mechanism that may cause intestinal injury may be the spontaneous endotoxemia noted with enteral feeding in premature infants.[36, 37] Release of presumably enteric bacteria-derived endotoxin has been noted with feeding difficulties (intolerances) and possibly NEC. It has not been ascertained whether this endotoxemia is a result of increased toxin production, decreased catabolism, or increased mucosal permeability.

Endotoxemia in premature infants may produce signs and symptoms of septic shock. The response noted during gram-negative bacterial septic shock includes thermal instability, disseminated intravascular coagulation, increased vascular permeability, metabolic acidosis, hypotension, and decreased left ventricular function.[38, 39]

Many of the effects of endotoxemia are mediated by cytokines. Tumor necrosis factor is produced by endotoxin-stimulated macrophages. Injection of TNF into the circulation results in adverse cardiopulmonary changes similar to those in septic shock, including hypotension, fever, hemorrhage, and increased vascular permeability.[40, 41] Antibody against TNF protects mice from lethal doses of endotoxin.[40-43] Endotoxin causes a sevenfold elevation of plasma TNF concentration in adults. Bloody diarrhea is also produced by intravenous administration of TNF.[40] The intestinal histopathologic changes after injection of TNF into animals resemble the morphologic features of NEC in human beings. The initial interpretation of TNF-induced intestinal necrosis was that this lesion appeared similar to mesenteric ischemia. These studies suggest an important role for endotoxin-stimulated TNF production with subsequent intestinal injury resembling ischemia, NEC, or both.

Other studies have suggested that TNF-induced bowel necrosis in experimental models may also be mediated by platelet activating factor.[44] The release of TNF causes local intestinal PAF production. Endotoxin's adverse effects are additive to the TNF stimulation of PAF release. Additional evidence that PAF may mediate some of the intestinal necrosis after TNF injection is the observation that PAF antagonists block the effects of TNF. PAF increases the production of local intestinal leukotriene D_4, resulting in vasoconstriction of local arteries.[45]

The adverse effects of inflammatory mediators could also produce the hematologic and physiologic profiles seen in NEC: neuropenia, thrombocytopenia (with or without disseminated intravascular coagulation), metabolic acidosis, increased vascular permeability, and hypotension. The hematologic findings in NEC suggest that local inflammatory mediators such as TNF or PAF may play a role in the local intestinal consumption of neutrophils and platelets.

POTENTIAL MULTIFACTORIAL EVENTS: ONE COMMON FINAL PATHWAY

Many adverse events for the intestinal mucosa as a result of infectious agents, inflammatory mediators, circulatory instability, and excessive feeding may be responsible for the initial mucosal injury of NEC. Mucosal injury may produce malabsorption of lactose and excessive intestinal gas production. The mucosal injury may also cause an ileus and thus intestinal stasis. Disruption of the mucosa may cause increased permeability of inflammatory mediators, endotoxin, bacteria, or hydrogen gas.

Ileus, stasis, and gas production may be associated with endotoxemia and distension. Increased intraluminal pressure with or without inflammatory mediator activation may result in serious vascular compromise. The final result of these multiple events could be the "ischemic looking" hemorrhagic necrosis identified as NEC. This final common pathway may explain NEC noted in an epidemic caused by a microbiologic agent or endemic cases of NEC after polycythemia, exchange transfusion, or excessive volumes of oral feedings.

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