Necrotizing enterocolitis (NEC) is a common and devastating disease of premature infants. Immaturity of the innate immune system of the gut is central to the pathogenesis of NEC. Recent studies suggest a key role for Paneth cells in this disease. Addressing basic questions on the development and function of immature Paneth cells may shed light on the puzzling pathophysiology of NEC. Current animal models of NEC are limited in their capacity to answer these questions.

Paneth cells are highly specialized secretory cells located at the base of the crypts of Lieberkühn in the small intestine that play a central role in intestinal innate immunity. Paneth cell granules contain high concentrations of antimicrobial peptides and various immune mediators that are released into the intestinal lumen to shape the intestinal microbiota and protect the intestinal epithelium and its stem cells against pathogens. In the human fetus, Paneth cells first appear in the first trimester, mature by the current age of viability (22–24 weeks gestation), and increase in numbers by term gestation. The role of Paneth cells in utero is unknown given that there are few, if any, bacteria in the fetal gut. Microbial colonization of the intestine of the premature infant begins at birth, and the dynamics differ significantly from that of the term infant due to antibiotic exposure, environmental factors including those due to prolonged hospitalization, and immaturity of virtually every aspect of neonatal intestinal immunity (including Paneth cell function).

The distribution of Paneth cells along the healthy intestine reflects the differing physiology of the small intestine and the colon. Small bowel function requires villi with enterocytes, goblet cells and enteroendocrine cells to perform the primary roles of digestion and absorption of nutrients. Here, the mucus layer is relatively thin and non-homogeneous. Paneth cells likely fortify this vulnerable mucosal surface with its rapid turnover, keeping microbes at bay. In the colon, however, there are no villi; the crypts are lined with epithelial cells, most notably goblet cells that produce a thick layer of mucus to shield the mucosa from exposure to the high numbers of bacteria. Paneth cells are generally absent, but metaplastic Paneth cells are sometimes observed in the colon, especially in diseases of dysbiosis (aberrations in the composition of the intestinal microbiota) such as inflammatory bowel disease.

Necrotizing enterocolitis (NEC) is a common and devastating disease of premature infants that appears to result from a combination of immaturity of intestinal defenses, enteral feeding and dysbiosis. Careful studies of the potential role of Paneth cells in NEC over the past 15 y have yielded unclear and sometimes seemingly contradictory results. Early studies comparing premature infants with NEC to control infants with intestinal atresia demonstrated an apparent decrease in lysozyme-staining Paneth cells in one study, and an apparent increase in Paneth cell numbers and human defensin 5 at the mRNA level, but not at the protein level, in another study. Subsequently, investigators have shown a decrease in lysozyme-staining Paneth cells in premature infants with NEC compared with premature infants with spontaneous intestinal perforation (a clinically distinct entity usually
occurring in the first week of life in premature infants and often associated with the administration of indomethacin and/or corticosteroids. Lysosome-staining Paneth cells were decreased, or not significantly different, in premature infants with NEC compared with preterm infants with atresia or other non-inflammatory intestinal disease. Following surgery for either NEC or atresia, Paneth cell numbers appear to increase and colonic Paneth cell metaplasia is common.

These observations generate many questions: is the increase in mRNA expression of Paneth cell antimicrobial peptides in premature infants and rat pups an unsuccessful attempt by the immature innate immune system to respond to NEC? Are the differences between mRNA expression and protein expression due to an increase in secretion, or alternatively a defect in granule-formation that predisposes the premature infant to NEC? Is the apparent decrease in Paneth cell numbers in NEC an artifact of Paneth cell detection related to secretion/degranulation, or truly a reflection of decreased cell populations? One of the major challenges of using human tissues to determine NEC pathogenesis is the highly variable quality of tissue that can be obtained at surgery due to varying degrees of necrosis. Paneth cell identification usually depends on either histologic identification based on location at the base of the crypts and presence of plump secretory granules, or on immunohistochemical identification of Paneth cell products such as lysozyme. Neither of these approaches may be adequate to identify immature or degranulated Paneth cells, as are likely present in premature and stressed neonates. To address the above questions, paneth cell markers that are not linked to the secretory process or electron microscopy to trace the production, packaging, and secretion of Paneth cell products may be helpful.

An alternative hypothesis is that the differentiation program or steady-state numbers of Paneth cells is disrupted, i.e., that dysfunction of Paneth cells may be an early event that predisposes the premature infant to NEC. If so, this predisposition may be due either to inability of the Paneth cells to effectively shape the intestinal microbiota or inability of the Paneth cell to respond to invasive mucosal pathogens. Animal models are a logical approach to addressing these hypotheses. Table 1 presents a summary of the early animal models of NEC. In Table 2 the most commonly used current models are presented, all of which have histologic

### Table 1. Early animal models of NEC

| Animal | Age at intervention | Intervention | Gross pathology | Microscopic Pathology |
|--------|---------------------|--------------|-----------------|-----------------------|
| Mouse | 6–10 weeks | PAF | Mild: focal congestion | Villous necrosis |
| Rat (loop of colon) | 6–8 weeks | Infusion of low pH fatty acids +/- increased intraluminal pressure | Pallor, edema | Crypt necrosis, inflammatory cell infiltrate, focal hemorrhage, mucosal ulceration |
| Piglet (term) | 12–48 h | Hypoxia + hyperthermia | Villous necrosis, inflammatory cell infiltrate, mucosal ulceration |
| Quail | 13 d | Lactose + C. butyricum | Hemosprage, pneumoniosis | Congestion, inflammatory infiltrate, mucosal hyperplasia, hemorrhagic ulcerations, focal necrosis |

### Table 2. Current animal models of NEC

| Animal | Age at intervention | Intervention | Gross pathology | Microscopic pathology |
|--------|---------------------|--------------|-----------------|-----------------------|
| Mouse | < 12 h | E. faecalis + hyperthermia + formula feeding | Transmural coagulative necrosis with villous sloughing |
| Mouse | 10–12 d | PAF + LPS | Mild: separation of submucosa | Severe: transmural necrosis |
| Mouse | 14–16 d | Dithizone + K. pneumonia | Mucosal edema, loss of villi, intramural air, transmural necrosis |
| Rat (preterm) | 2 h | Hypoxia + hyperthermia + formula feeding | Mild: submucosal edema | Severe: transmural necrosis, loss of villi |
| Piglet (preterm) | Birth | Total parenteral nutrition + enteral formula feeding | Hyperemia, edema, hemorrhage, pneumoniosis, necrosis | Mild: hyperemia with stunted villi | Severe: transmural necrosis |

PAF, platelet activating factor; LPS, lipopolysaccharide.
findings that are common in human NEC. Mouse models are appealing because of the tremendous possibilities for genetic manipulation, however the extremely small size of newborn mouse pups presents a significant challenge. Mice and rats do not have intestinal crypts at birth; these cells resulting in an increase in Paneth cell numbers, however the Paneth cells in infected pigs is debated, but clearly the piglet model is not ideal to assess the role of Paneth cells in NEC. In the traditional rat model wherein NEC is triggered in four-day-old rat pups by formula feeding, hypoxia and hypothermia, expression of several Paneth cell antimicrobial molecules (including lysozyme, secretory phospholipase A2 and two peptides from the RegIII family of lectins) is markedly increased at the mRNA level, but changes were not obvious at the protein level with current methodologies.

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