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Paneth cells and necrotizing enterocolitis

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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.

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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.[1] 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.[2] Here, the mucus layer is relatively thin and non-homogeneous.[3] Paneth cells likely fortify this vulnerable mucosal surface with its rapid turnover, keeping microbes at bay.[1] 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.[3] 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.[4]

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.[5] 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,[6] and an apparent increase in Paneth cell numbers and human defensin 5 at the mRNA level, but not at the protein level, in another.[7] Subsequently, investigators have shown a decrease in lysozyme-staining Paneth cells[8] and total Paneth cells (by histology)[9] in premature infants with NEC compared with premature infants with spontaneous intestinal perforation (a clinically distinct entity usually

Table 1. Early animal models of NEC

Animal	Age at intervention	Intervention	Gross pathology	Microscopic Pathology
Mouse[18]	6–10 weeks	PAF	Mild: focal congestion Severe: necrosis	Villous necrosis
Rat[19]	Young adult	PAF + LPS (intra-aortic)	Hemorrhagic necrosis of segments of jejunum and ileum	Mild: focal necrosis, often confined to the tips of villi Severe: transmural necrosis, complete loss of villi
Rabbit (loop of colon)[20]	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)[21]	12–48 h	Hypoxia + hypothermia		Villous necrosis, inflammatory cell infiltrate, mucosal ulceration
Quail[22]	13 d	Lactose + *C. butyricum*	Hemorrhage, pneumatosis	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[23]	< 12 h	*E. faecalis* + hypoxia + hypothermia + formula feeding		Transmural coagulative necrosis with villous sloughing
Mouse[24]	10–12 d	PAF + LPS		Mild: separation of submucosa Severe: transmural injury
Mouse[9]	14–16 d	Dithizone + *K. pneumonia*		Mucosal edema, loss of villi, intramural air, transmural necrosis
Rat (preterm)[25]	2 h	Hypoxia + Hypothermia + formula feeding		Mild: submucosal edema Severe: transmural necrosis, loss of villi
Piglet (preterm)[26]	Birth	Total parenteral nutrition + enteral formula feeding	Hyperemia, edema, hemorrhage, pneumatosis, necrosis	Mild: hyperemia with stunted villi Severe: transmural necrosis

PAF, platelet activating factor; LPS, lipopolysaccharide.

occurring in the first week of life in premature infants and often associated with the administration of indomethacin and/or corticosteroids). Lysozyme-staining Paneth cells were decreased,[8] or not significantly different,[10] 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.[10]

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

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 develop in the few days after birth. The mouse develops morphologically distinct Paneth cells at about the seventh day of life[11] and the rat at about day 11–14.[12,13] Consequently, models requiring younger pups will be unable to address the questions posed above. Pigs develop intestinal crypts prenatally, similar in timing to the human fetus, however pigs normally do not develop Paneth cells.[14] The presence of 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 Reg3γ family of lectins) is markedly increased at the mRNA level, but changes were not obvious at the protein level with current methodologies.[15]

In adult mice, oral inoculation with *Salmonella typhimurium* causes accelerated proliferation of intestinal progenitor cells resulting in an increase in Paneth cell numbers, however the Paneth cells had fewer secretory granules per Paneth cell consistent with either degranulation or immaturity of the newly generated cells.[16] It is unclear whether Paneth cells in 14–21-d-old mice are able to respond similarly to infection or dysbiosis. Differences in Paneth cell function and intestinal microbiota composition in mice of differing background strain have also been described.[17]

The recent report of a novel mouse model of NEC involving a "two-hit" mechanism that attempts to mimic the major risk factors for NEC (immaturity of intestinal defenses and abnormal colonization of the intestinal tract) in 14–16-d-old mice by first ablating Paneth cells and then exposing the injured intestinal mucosa to oral *Klebsiella pneumonia*[9] underscores the potential importance of Paneth cells in this disease. Interestingly the same insult in five-day-old mice, which do not yet have Paneth cells, does not result in NEC-like disease. Unfortunately, implicit in this model is the inability to make conclusions about changes in expression, packaging, and secretion of Paneth cell products in NEC (given the intentional damage to the Paneth cells).

Further investigations of the responsiveness of immature Paneth cells to insults including ability to package and release Paneth cell products and ability to alter the intestinal microbiota (e.g., mouse intestinal loop models with stimulators of Paneth cell degranulation such as pilocarpine or pathogens like *K. pneumoniae*) may shed light on these questions, and in turn, on the complex and puzzling pathophysiology of NEC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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