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REVIEW

Pathogenesis of Necrotizing Enterocolitis

Modeling the Innate Immune Response

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Necrotizing enterocolitis (NEC) is a major cause of morbidity and mortality in premature infants. The pathophysiology is likely secondary to innate immune responses to intestinal microbiota by the premature infant's intestinal tract, leading to inflammation and injury. This review provides an updated summary of the components of the innate immune system involved in NEC pathogenesis. In addition, we evaluate the animal models that have been used to study NEC with regard to the involvement of innate immune factors and histopathological changes as compared to those seen in infants with NEC. Finally, we discuss new approaches to studying NEC, including mathematical models of intestinal injury and the use of humanized mice. *(Am J Pathol 2015, 185: 4—16; http://dx.doi.org/10.1016/j.ajpath.2014.08.028)*

Necrotizing enterocolitis (NEC) is a disorder characterized by intestinal necrosis in premature infants that results in significant morbidity and mortality.[1] Approximately 7% of infants with a birth weight between 500 and 1500 g develop NEC.[1] The pathogenesis is characterized by intestinal inflammation that can progress to systemic infection/inflammation, multiorgan failure, and death. The bowel is distended and hemorrhagic on gross inspection. On microscopic examination, signs of inflammation, mucosal edema, epithelial regeneration, bacterial overgrowth, submucosal gas bubbles, and ischemic transmural necrosis are seen (Figure 1, A—E).[2]

Currently the pathogenesis of NEC is believed to have multifactorial causes, including intestinal immaturity and microbial dysbiosis. Intestinal immaturity leads to a compromised intestinal epithelial barrier, an underdeveloped immune defense, and altered vascular development and tone. The compromised epithelial barrier and underdeveloped immune system, when exposed to luminal microbiota that have been shaped by formula feedings, antibiotic exposure, and Cesarean delivery, can lead to intestinal inflammation and sepsis. Despite therapeutic success in animal model systems, there are relatively few therapeutic strategies that have allowed for significantly improved outcomes in infants with NEC. Two hurdles that persist are our incomplete understanding of the developing immune system in premature infants and our inability to adequately replicate these complex factors in animal models.[3,4] This review summarizes the complex intestinal immune system in premature infants and details what is known about the involvement of innate immune factors in NEC, both in animal models and in human disease.

The Neonatal Intestinal Ecosystem

The neonatal intestinal ecosystem is extremely fragile. At birth, the newborn is exposed to the external environment for

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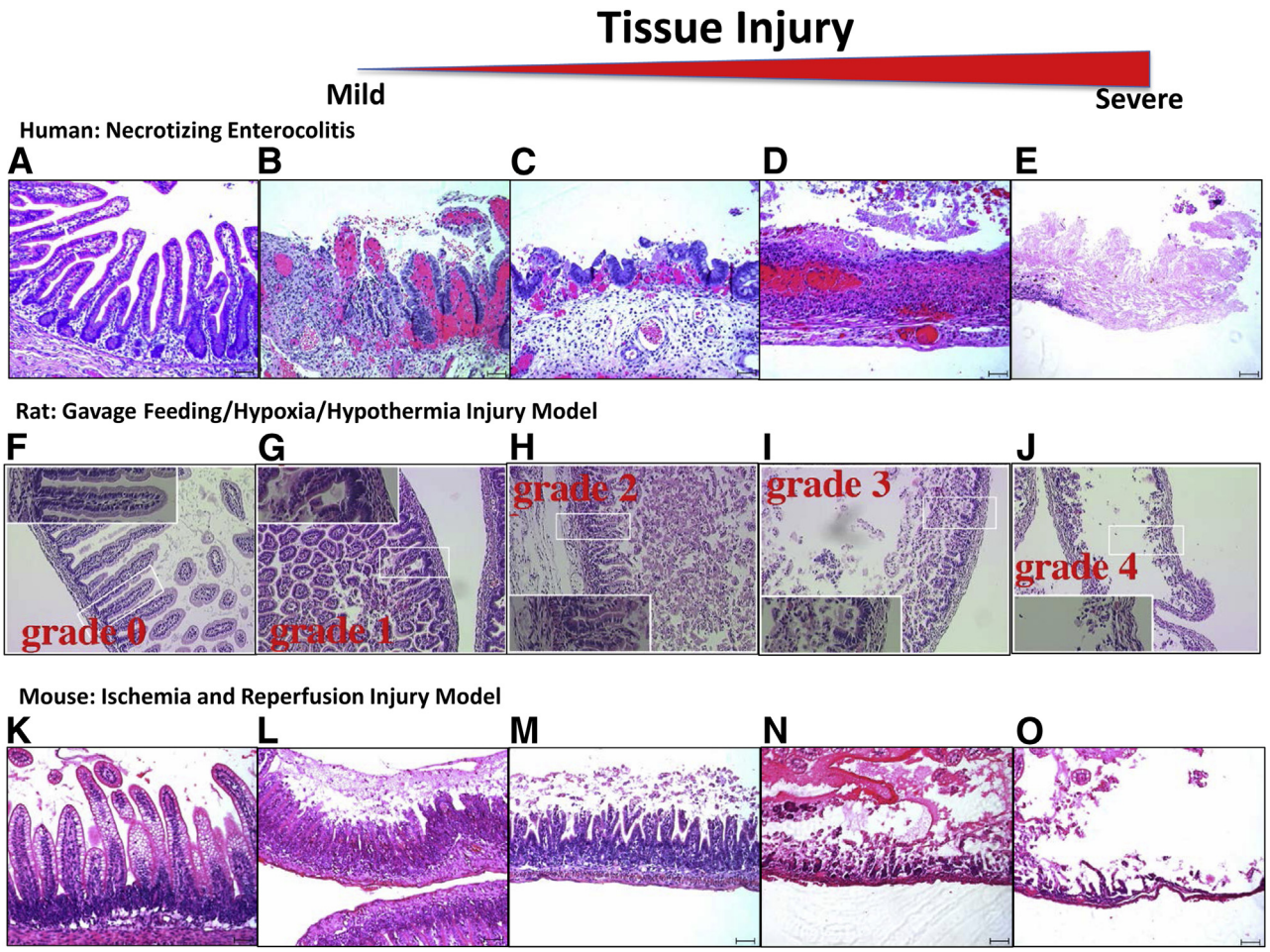

Figure 1 Examples of the various grades of morphological damage in hematoxylin and eosin–stained specimens. **A–E:** Representative samples of premature infants with necrotizing enterocolitis. **A:** Age-matched control from patient with jejunal atresia. **B:** Mild injury with hemorrhagic necrosis of mucosa and loss of villus tip architecture. **C:** Progressive injury with inflammatory infiltration of muscularis with complete villus destruction. **D:** Severe muscular and epithelial damage with complete loss of mucosa. **E:** Perforation with transmural necrosis with complete loss of epithelial and muscular architecture. **F–J:** Representative samples from intestinal injury secondary to gavage feeding in the setting of hypothermia and hypoxia in neonatal rats. **F:** Intact morphology, grade 0. **G:** Sloughing of villus tips, grade 1. **H:** Mid-villus necrosis, grade 2. **I:** Loss of villi, grade 3. **J:** Complete destruction of the mucosa, grade 4. **Insets** in **F–J** show higher magnified portions of the same sections, corresponding to the **boxed regions**. **K–O:** Representative images of tissue injury secondary to 60 minutes of intestinal ischemia and 90 minutes of reperfusion in 2-week-old mice. **K:** Sham-operated mice (no ischemia). **L:** Villus tip necrosis. **M:** Mid-villus necrosis. **N:** Loss of villus architecture. **O:** Complete loss of mucosal architecture. **F–J**, reprinted with permission from Nature Publishing Group.[28] Scale bars = 50 μm (**A–E**, **K–O**). Original magnification, ×20 (**A–O, main images,** and **F–J, insets**).

the first time, and the immune response must begin to distinguish between self and nonself. In particular, the recognition of food antigens and commensal microbiota must be distinguished from that of potential pathogens. This process is even more challenging in premature infants, as they are typically placed on broad-spectrum antibiotics that disrupt both the timing and the diversity of the initial bacterial colonization of the intestinal tract.[1] In addition, the immature epithelial barrier appears to be more sensitive to the detection of bacteria[5,6] and more susceptible to bacterial translocation,[7] allowing for both an unwarranted inflammatory response to commensals and the translocation of pathogens, both of which may contribute to intestinal damage.

The innate immune system is the first line of defense against infections. Innate immune cells respond in a nonspecific manner and do not confer long-lasting immunity to the host.[8] The major components are cells (including macrophages, neutrophils, dendritic cells (DCs), natural killer cells, B1 B cells, innate lymphoid cells, and γδ T cells) and anatomical barriers (such as the intestinal epithelium and the gastrointestinal mucus layer). In addition, the presence of commensal microbiota can serve as a part of the innate immune system by preventing the colonization by pathogenic bacteria (colonization resistance).

Mucosal Innate Immunity

Several major components of the human mucosal immune system are in place before birth. These components include IgM^+ B cells; γδ T cells found in the intraepithelial lymphocyte compartment, which are seen as early as 12 to 15 weeks of embryonic age; and Peyer's patches, which can