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Commensal and probiotic bacteria may prevent NEC by maturing intestinal host defenses

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Abstract

Necrotizing enterocolitis (NEC) is a devastating disease of prematurity with significant morbidity and mortality. Immaturity of intestinal host defenses predisposes the premature infant gut to injury. An abnormal bacterial colonization pattern with a deficiency of commensal bacteria may lead to a further breakdown of these host defense mechanisms, predisposing the infant to NEC. The presence of probiotic and commensal bacteria within the gut has been shown to mature the intestinal defense system through a variety of mechanisms. We have shown that commensal and probiotic bacteria can promote intestinal host defenses by reducing apoptotic signaling, blocking inflammatory signaling, and maturing barrier function in immature intestinal epithelia. Future studies aimed at elucidating the mechanisms by which probiotic and commensal bacteria exert their effects will be critical to developing effective preventive therapies for NEC.
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1. Introduction

The extremely preterm neonate faces many challenges following early birth. Their fragile gastrointestinal (GI) systems are in a critical state of development and are forced to adapt quickly to extrauterine life. This complex organ provides a large interface with the external environment. When the GI system functions properly, it not only facilitates optimal nutrition, but also plays a unique role in host defense. A breakdown in this essential function poses a significant threat to the health of the premature infant. More specifically, immaturity of intestinal host defenses may predispose infants to necrotizing enterocolitis (NEC). Despite advances in the medical care of premature infants, NEC rates remain unchanged [1–3], and it continues to be one of the most devastating and unpredictable diseases of prematurity. Unfortunate patients who contract this disease are at high risk for adverse neurodevelopmental outcomes [4,5]. The etiology of NEC has not been fully elucidated, but it is likely multifactorial, involving immaturity of intestinal host defenses and abnormal bacterial colonization [6–9].

Colonization of the initially sterile intestinal ecosystem occurs postnatally as dietary and environmental changes occur. Complete colonization resembling that of an adult is reached by two years of age and, remarkably, contains up to 1×10^{14} colony-forming units (CFUs) [10]. Appropriate colonization with commensal bacteria is important for intestinal function and development [11–13] and may play a central role in the postnatal maturation of intestinal host defenses [14]. Neonates who are born prematurely have an abnormal intestinal microbial composition [13,15,16], which may predispose them to a failure of postnatal evolution of critical innate defenses and lead to NEC. As further evidence for the importance of commensal bacteria, preterm infants with altered intestinal flora due to prolonged antibiotic therapy are more likely to develop NEC [17,18]. Other factors that influence proper colonization include maternal exposure to antibiotics, mode of delivery, human breast milk feedings, and the hospital environment. Preterm infants are more likely to be delivered by cesarean section and experience delayed enteral feeding, which make them less likely to acquire commensal flora

Abbreviations: IFN, interferon; NF-κB, nuclear factor kappa B; IL, interleukin; TNF-α, tumor necrosis factor alpha; MIP, macrophage inflammatory protein; ROS, reactive oxygen species; LGG, *Lactobacillus rhamnosus* GG.

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perinatally from passage through the birth canal or from human milk feedings. This may lead to decreased colonization of beneficial probiotic bacteria, including species of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* [19,20]. The hospital environment, with its preponderance of pathogenic organisms [21], also negatively affects the intestinal colonization of beneficial commensal bacteria.

Studies have shown commensal bacteria regulate many intestinal defenses including barrier function, mucin and IgA secretion, inflammation, and homeostatic processes such as proliferation and apoptosis [22–26]. We believe that immature intestinal host defenses play a critical role in the pathogenesis of neonatal intestinal inflammatory diseases, including NEC, and commensal bacteria (or their products) can promote the maturation of these host defenses. Understanding this process is crucial to developing potential therapeutic (prebiotic, probiotic or postbiotic) interventions to better treat or prevent these devastating diseases. Specific areas of host defense that can be promoted by commensal and probiotic bacteria include intestinal epithelial cell proliferation and apoptosis, innate immune regulation, and epithelial barrier function.

2. Murine model of postnatal intestinal development

Epidemiologic studies indicate that incidence and postnatal age of NEC onset is inversely proportional to gestational age [9,27–30]. Thus, infants born earlier not only have a higher NEC incidence but also develop NEC at a later postnatal age, with a developmental window of susceptibility at 30-32 weeks [9,27–30]. Susceptibility to NEC is likely due to developmental immaturity in intestinal host defenses such as exaggerated apoptotic and inflammatory responses [7,31] and decreased barrier function. In order to better understand developmental differences in intestinal host defenses that may play a role in NEC, our laboratory has successfully modeled premature intestinal epithelia in 0-3 week old preweaned mice. Rodents are altricial species and thus their intestines are functionally immature at birth [32]. Premature human intestines and preweaned murine intestines are similar in that mucosal immunity and GALT are maturing postnatally [14,33]. The neonatal murine intestinal epithelial architecture and barrier function are relatively immature at birth compared to the neonatal human intestine and continues to mature over the first three weeks of life [14,34]. Rodent intestines in the 2nd week of life are thought to represent the maturity of early 3rd trimester human intestines, and thus, we and others have successfully used murine intestines in the 2nd week of life to model premature human intestines [14,32,35–48].

Using this murine model of postnatal intestinal development, we have characterized the ontogeny of key intestinal host defenses. Specifically, we have shown that intestinal epithelial apoptotic [49,50] and proinflammatory [51] responses peak at 2 weeks in the murine gut at a time when intestinal epithelial barrier function remains immature [52]. This developmental period of dysregulated intestinal host defenses may resemble the developmental period of peak susceptibility to NEC seen in premature infants. Thus the 2-week-old murine intestine may be an ideal model for the preterm human intestine. Further, we have successfully employed this novel murine model of postnatal intestinal development to investigate the mechanisms by which commensal and probiotic bacteria promote regulation and maturation of intestinal host defenses.

3. Commensal and probiotic bacteria regulate apoptotic responses in immature intestinal epithelia

Apoptosis, when regulated correctly, is generally regarded as a protective process for the host and results in a balance between cell proliferation and cell death. It allows damaged cells to be removed in response to injurious stimuli (microbial, hypoxic, or chemical) [23] without further impairment of the surrounding tissue, but problems may arise when apoptotic activity is excessive or aberrant. There is histopathologic evidence in humans that apoptosis plays a role in the early events of the development of NEC [53,54], and, in animal models of NEC, epithelial apoptosis precedes gross bowel necrosis [55]. We have shown that immature intestinal epithelial cells exhibit exaggerated responses to apoptotic stimuli [49–51]. Commensal bacteria have been shown to reduce the incidence of NEC [56]. Therefore, we investigated whether commensal *Escherichia coli* could regulate apoptotic signaling in the developing gut.

Commensal strains of *E. coli*, which are obtained from the maternal GI tract, populate the intestines of term newborns very early in life [11,12,57]. Mirpuri et al. showed that a previously known, anti-inflammatory [22,58] commensal strain of *E. coli* isolated from healthy human colon reduces epithelial apoptosis in a murine model of developing intestine [50]. Ontogeny studies demonstrated that immature murine intestinal epithelia were most susceptible to inducible apoptosis at 2 weeks of postnatal age. However, when we fed neonatal mice with *E. coli* prior to inducing apoptosis, we found that intestinal epithelia in both the small intestine and colon showed a 50% reduction in induced apoptosis. *E. coli* mitigated apoptotic responses via IFN-αA-dependent upregulation of the antiapoptotic protein, GBP-1.

We have shown that probiotic bacteria can also regulate intestinal epithelial apoptotic responses. Probiotics are defined as 'living micro-organisms, which upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition' [59]. These beneficial bacteria can foster a normal intestinal colonization pattern in addition to directly supporting optimal functioning of intestinal epithelial cells. Experimental NEC in animal models has shown a reduction in both the severity [60] and incidence [61,62] of NEC after receiving probiotics. There has been much enthusiasm surrounding similar results in human trials, with a reduced incidence of NEC following probiotic administration [56,63]. However,