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Role of interleukin-10 in the pathogenesis of necrotizing enterocolitis

Claudia N. Emami, M.D., M.P.H.[a], Nikunj Chokshi, M.D.[a], Jin Wang, M.S.[a], Catherine Hunter, M.D.[a], Yigit Guner, M.D.[b], Kerstin Goth, B.A.[a], Larry Wang, M.D.[a], Anatoly Grishin, Ph.D.[a], Henri R. Ford, M.D., M.H.A., F.A.C.S.[a,*]

[a]Department of Surgery, University of Southern California Keck School of Medicine, Children's Hospital Los Angeles, 4650 Sunset Blvd., Penthouse, MS 72, Los Angeles, CA 90027, USA; [b]Department of Surgery, University of California Davis School of Medicine, Los Angeles, CA, USA

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Abstract

BACKGROUND: Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in premature neonates. The pathogenesis of NEC is characterized by an intestinal epithelial injury caused by perinatal insults, leading to the activation of the mucosal innate immune system and exacerbation of the epithelial barrier damage. Cytokines play an important role in mucosal immunity. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that has been shown to play a role in epithelial integrity and modulation of the mucosal immune system. We hypothesized that IL-10 may protect against the development of experimental NEC by blunting the inflammatory response in the intestine.

METHODS: Wild-type and IL-10 -/- mice underwent a NEC-inducing regimen of formula feeding in combination with hypoxia and hypothermia (FF+HH). Integrity of the gut barrier was assessed through measurement of epithelial apoptosis, tight junction disruption, and inducible nitric oxide synthase. A total of 5 μg of exogenous IL-10 was administered intraperitoneally to IL-10-/-mouse pups before the initiation of FF+HH to test dependence of gene knockout phenotype on IL-10.

RESULTS: IL-10 -/- FF+HH showed more severe morphologic and histologic changes compared with controls as evidenced by increased epithelial apoptosis, decreased junctional adhesion molecule-1 localization, and increased intestinal inducible nitric oxide synthase expression. Administration of exogenous IL-10 alleviated the mucosal injury.

CONCLUSIONS: We conclude that IL-10 plays a protective role in the pathogenesis of NEC by attenuating the degree of intestinal inflammation.

Necrotizing enterocolitis (NEC) is the most devastating gastrointestinal disease affecting newborn infants. Although more than 90% of the infants affected by NEC are premature, some cases have been reported in full-term infants.[1] The incidence of NEC continues to increase because the number of premature births has increased during the last decade and a half.[2] Furthermore, recent advances in neonatal care have improved the survival of premature infants markedly. NEC is associated with a high mortality rate, which ranges from 10% to 50% according to different reports. However, in the group of infants with severe NEC, which is characterized by pan-intestinal involvement, the mortality rate approaches 100%.[3,4] Although the pathogen-

* Corresponding author. Tel.: +1-323-361-2111; fax: +1-323-361-8678.
E-mail address: hford@chla.usc.edu
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esis of NEC remains elusive, the principal initiating events are believed to involve gut ischemia, formula feeding, and intestinal colonization with opportunistic pathogens. These perinatal insults compromise the integrity of the immature gut barrier, leading to bacterial translocation and activation of innate immune responses.[5] NEC has been associated with increased levels of proinflammatory and anti-inflammatory cytokines in the inflamed intestine.[6] Although proinflammatory cytokines tend to promote gut barrier failure, evidence suggests that anti-inflammatory cytokines such as interleukin-10 (IL-10) are likely to play a protective role by dampening the inflammatory responses. Indeed, IL-10–deficient mice develop intestinal inflammation as evidenced by the presence of colitis.[7] By contrast, intraperitoneal injection of IL-10 in a mouse model of intestinal ischemia/reperfusion reduces local and systemic inflammation.[8]

In this study, we examined the role of IL-10 in the pathogenesis of experimental NEC. We show that levels of IL-10 increase in the serum of animals subjected to the NEC-inducing regimen of formula feeding, hypoxia, and hypothermia (FF+HH). IL-10 deficiency exacerbates the degree of intestinal inflammation in response to FF+HH. Administration of exogenous IL-10 before the initiation of FF+HH decreases the severity of NEC. These results show that IL-10 plays a protective role in the pathogenesis of NEC.

Methods

Animals

The Institutional Animal Care and Use Committee approved all animal experiments in this study. Timedated, pregnant Sprague–Dawley rats were purchased from Charles River Laboratories (Hollister, CA) and delivered at term. Newborn rat pups were randomly separated and subjected to the NEC-inducing regimen of FF+HH as previously described, or left to breastfeed with their mother.[9] Wild-type (WT) C57Bl/J and congenic IL-$10^{-/-}$ mice were purchased from Jackson Laboratories (Sacramento, CA) and bred at the Children's Hospital Los Angeles Animal Research Facility. Newborn mice were delivered by cesarean section of timed-pregnant mice at gestational day 18. NEC was induced using FF+HH as previously described; control animals were left to breastfeed with their mothers.[6] The newborn mice in the experimental group were kept at 35°C and 70% relative humidity; they were gavage-fed every 3 hours with formula (2× Esbilac Milk Replacer for Puppies; PetAg, Hampshire, IL; 20 μL on day 1 and 30 μL thereafter) through a 24-gauge polyurethane catheter (Luther Medical Products, Tustin, CA) inserted in the esophagus. The mouse model of NEC differs from the rat model with regard to feeding frequency (once every 3 h in mice vs 3 times/d in rats); amount of formula per feeding (20–30 μL in mice vs 200 μL in rats); administration of hypoxia (1 min at 0% O_2 in mice vs 10 min at 5% O_2 in rats); and the addition of hypothermia (10 min at 8°C).[6] Therefore, this latter regimen is referred to as formula feeding plus hypoxia and hypothermia (FF+HH). Animals displaying symptoms of NEC (abdominal distension, rectal bleeding, pneumatosis intestinalis) were promptly euthanized. All surviving animals were euthanized on day 3. NEC was diagnosed by microscopic examination of H&E–stained sections of terminal ileum. NEC grade (0–4, with increments of .5) was determined by a pathologist blinded to the groups, based on the extent of epithelial sloughing, submucosal edema, neutrophil infiltration, and derangement of intestinal villus architecture.[10]

Cytokine measurement

Blood samples from the pups were obtained via cardiac puncture and sent out to our collaborating laboratories at the University of Pittsburgh for analysis using the Luminex assay (Luminex, Austin, TX).

Immunofluorescence microscopy

Paraffin sections of small intestine were stained with anti–JAM-A antibody (Zymed, San Francisco, CA) or anti-inducible nitric oxide synthase (iNOS) antibody (Transduction Laboratories, San Diego, CA), followed by appropriate fluor-labeled secondary antibodies (Jackson Immunoresearch, West Grove, PA), as recommended by the manufacturers. Sections were stained for apoptosis using the ApopTag Red In Situ Apoptosis Detection Kit (Chemicon, Billerca, MA). Fluorescent images were obtained using the Olympus BX51 microscope equipped with a color camera and Picture Frame software (Olympus, Center Valley, PA). For comparisons, samples were processed in parallel on the same slide, and images were acquired and adjusted identically.

Results

Experimental NEC is associated with increased levels of IL-10

We previously showed that newborn rats subjected to formula feeding combined with hypoxia develop epithelial injury resembling NEC (Fig. 1A).[10] To elucidate the pattern of inflammatory cytokine expression in experimental NEC, we performed a Luminex analysis of the serum from neonatal rats after breastfeeding (BF) or FF+HH. Levels of IL-10 increased significantly after the second day of FF+HH compared with BF controls (Fig. 1B). These results indicate that experimental NEC is associated with increased levels of IL-10.