Characterizing the role of timing on the impact of interleukin-22 treatment during experimental necrotizing enterocolitis

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Introduction

- Necrotizing enterocolitis (NEC) is a severe intestinal disease that impacts ~7% of premature infants.[1]
- The etiology of NEC remains incompletely understood, but NEC results from uncontrolled inflammation leading to irreversible intestinal epithelial injury and intestinal necrosis (**Fig. 1**).
- One of the primary goals of the Good Lab is to identify factors that protect against intestinal injury in NEC.
- They previously demonstrated that the cytokine interleukin-22 (IL-22) attenuates intestinal inflammation, reduces intestinal injury, and improves epithelial barrier integrity during experimental NEC in mice.[2]

Pathophysiology of NEC

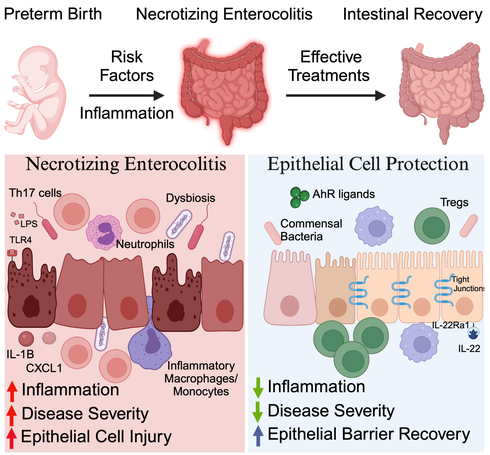

Figure 1. NEC Pathogenesis. NEC primarily impacts preterm infants and results from activation of the innate receptor Toll-like receptor 4 (TLR4) on intestinal epithelial cells by lipopolysaccharide (LPS) on the dysbiotic intestinal bacteria. This initiates an inflammatory cascade leading to epithelial injury and irreversible intestinal necrosis. Studies from the Good Lab found that treatment with IL-22 reduces inflammation, decreases disease severity, and promotes epithelial barrier recovery in mice.[2]

Methods

Study Design

Rationale: Our lab demonstrated that recombinant IL-22 (rIL-22) treatment decreased inflammation in experimental NEC when given preventatively.[2] The purpose of these studies was to determine the optimal timing of rIL-22 administration in our mouse model of experimental NEC (**Fig 2**).

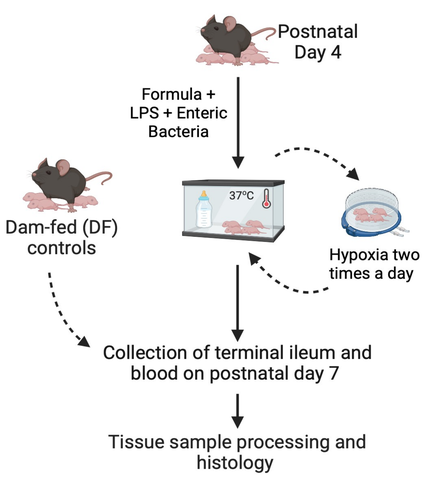

Figure 2. Mouse model of experimental NEC.[3] Intestinal inflammation can be induced in a murine NEC model. In this model, neonatal mice received oral gavage feeds of a formula containing LPS and dysbiotic enteric bacteria from a neonate who died from NEC. They were exposed to hypoxia twice a day. For these experiments, IL-22 was injected at 6, 12, and 24 h after initiation of the model. Intestinal samples were collected for qPCR and immunofluorescent staining.

Results

Early administration of rIL-22 downregulates inflammation and improves survival during experimental NEC in mice.

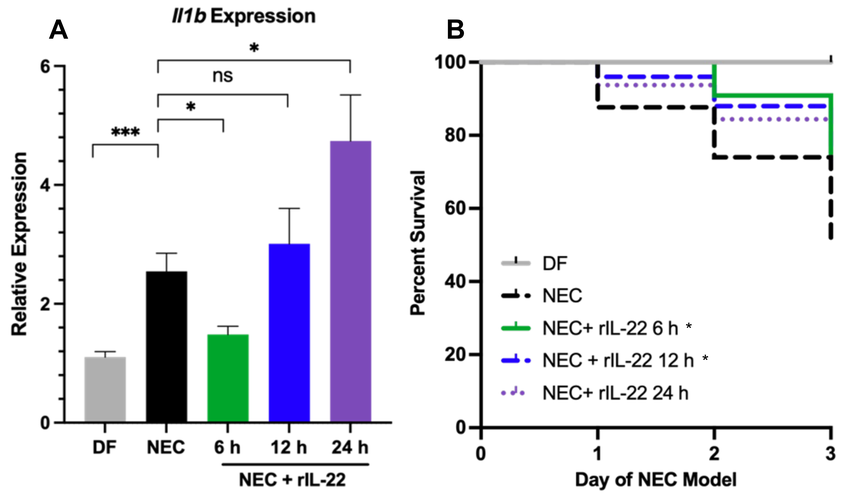

Figure 3. Treatment with rIL-22 at 6 h of experimental NEC decreases intestinal inflammation and improves survival, while treatment at 24 h worsens inflammation. (**A**) Gene expression in the ileum for dam fed (DF) controls or mice subjected to NEC that were untreated or received rIL-22 at 6, 12, or 24 h. Expression of *il1b* was significantly decreased at 6 h and increased at 24 h relative to no treatment. (**B**) rIL-22 significantly improved survival in NEC when given at 6 h or 12 h but not 24 h.

Immunofluorescent staining of the intestine from mice subjected to the mouse model of NEC.

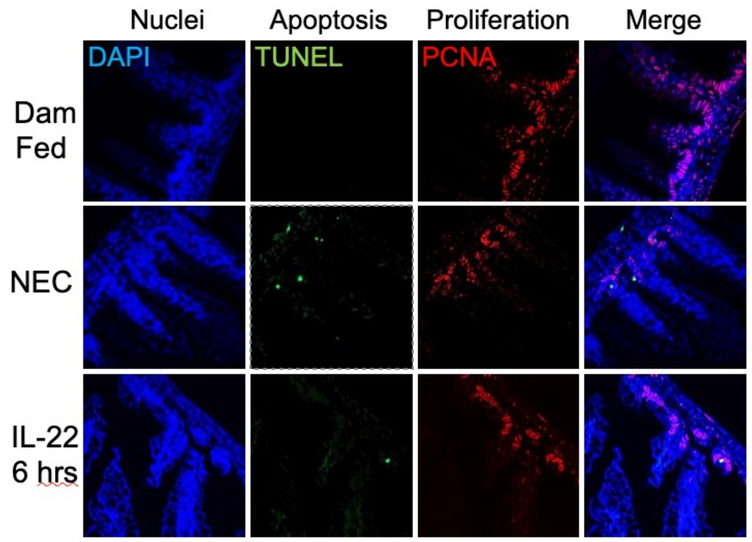

Figure 4. Intestinal epithelial cell apoptosis is increased and proliferation is decreased during experimental NEC, and improved by treatment with rIL-22. After NEC model completion, intestinal tissue was stained for nuclei (DAPI, blue), apoptosis (TUNEL, green), and proliferation (PCNA, red). Staining revealed increased epithelial cell apoptosis and decreased epithelial cell proliferation during NEC. Administration of rIL-22 at 6 h reduced apoptosis and enhanced proliferation in NEC.

Results

Quantification of the impact of rIL-22 treatment on epithelial cell apoptosis and proliferation in NEC.

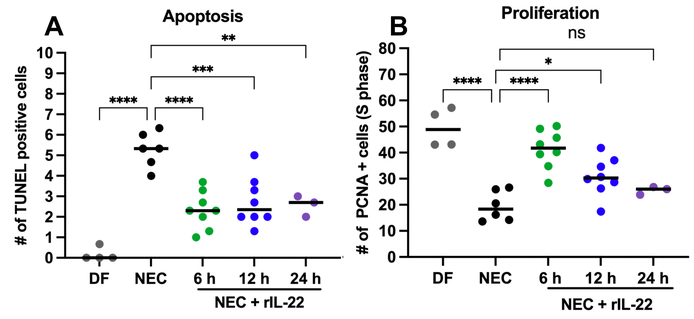

Figure 5. Treatment with rIL-22 prevented intestinal epithelial cell apoptosis and enhanced proliferation during NEC. **(A)** Compared to dam fed (DF) controls, the number of apoptotic cells (TUNEL staining) in the intestine of mice subjected to the NEC model was significantly increased. Enterocyte apoptosis was significantly decreased after treatment with rIL-22 at 6, 12, and 24 h of NEC. (**B**) Quantification of enterocyte proliferation (PCNA staining), revealed significantly decreased proliferation with NEC relative to DF controls and improvement in proliferation with treatment with rIL-22 at 6 and 12 h. Quantification was performed on stained intestinal sections, as in Figure 4.

Conclusions

- These studies support that rIL-22 is protective in our mouse model of NEC-like intestinal inflammation.
- rIL-22 provides the most benefit at 6 h after experimental NEC, but administration as late as 12 h may have some benefits, including improving survival, decreasing enterocyte apoptosis, and enhancing proliferation.
- Future studies will determine both the timing and dose of rIL-22 administration to optimize treatment outcomes for preterm neonates with NEC.

Acknowledgements

LCF is supported by a Thrasher Research Fund Early Career Award, UNC School of Medicine Physician Scientist Training Program Faculty Award, and UNC Children's Development Early Career Investigator Grant through the generous support of donors to UNC. MG is supported by National Institutes of Health (NIH) grants R01DK124614, R01DK118568, R01HD105301, R44HD110306, the Chan Zuckerberg Initiative Grant number 2022-316749, and the University of North Carolina at Chapel Hill Department of Pediatrics.

I would also like to thank Dr. Misty Good, Dr. Lauren Frazer, and all members of the Good Lab for all their work and contributions to this project.

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