Necrotizing enterocolitis (NEC) is an inflammatory intestinal disorder that disproportionately affects premature infants and lacks in effective therapeutics. Mihi and colleagues demonstrated that the cytokine interleukin-22 (IL-22) promotes intestinal epithelial regeneration and reduces disease severity in an experimental model of NEC.

The pathogenesis of the NEC remains unclear. In preterm infants, a combination of an underdeveloped intestinal tract, alterations in the composition of the microbiota that trigger intestinal injury, and an exaggerated inflammatory response are all thought to contribute to disease development. Accordingly, breastfeeding, which promotes the stabilization of the microbiota and fluctuations in the microbiota composition and localization of the microbiota, is associated with decreased risk of NEC.

Extensive research has placed the cytokine interleukin-22 (IL-22) as a key regulator of intestinal homeostasis. IL-22 stimulates epithelial cell regeneration after intestinal injury and promotes the expression of antimicrobial factors that aid in the clearance of enteric pathogens. Preclinical studies in adults have highlighted the therapeutic role of IL-22 in inflammatory bowel disorders such as ulcerative colitis. However, the role of IL-22 in NEC has not been studied. Given that the disturbance of the intestinal barrier and fluctuations in the microbiota play important roles in the pathology of NEC, Mihi and colleagues tested the hypothesis that IL-22 can alleviate the disease severity of NEC.

To model the intestinal injury seen in human NEC, Mihi and colleagues separated mouse pups from their mothers at 4 days of age and fed them with human formula, which studies have linked with increased risk of NEC development in premature infants. To recapitulate the inflammatory responses seen in human NEC, they supplemented formula with lipopolysaccharide, a bacterial cell wall component that triggers inflammation, and an enteric bacterium isolated from a human infant diagnosed with NEC. To mimic the decreased intestinal perfusion seen clinically in infants with NEC, they subjected pups to hypoxia treatment twice daily. Analysis of intestinal function and disease severity was performed when pups reached day 7 of age, 3 days after experimental NEC induction.

The authors found that administration of recombinant IL-22 (rIL-22) to mice subjected to NEC decreased disease severity and reduced mucosal damage compared to PBS-treated control animals. Treatment with rIL-22 also limited the expression of IL-1β and CXCL2, pro-inflammatory factors that increase during human disease. To determine the underlying mechanism by which IL-22 conferred protection against NEC, the authors considered several hypotheses. In adults, IL-22 promotes the expression of Reg3γ, a potent antimicrobial peptide that reinforces intestinal homeostasis by regulating the composition and localization of the microbiota. Surprisingly, though rIL-22 treatment induced upregulation of Reg3γ by neonatal intestinal epithelial cells, neither exogenous administration nor neutralization of Reg3γ during NEC affected disease severity. IL-22 also induced expression of other antimicrobial factors during NEC, which could limit the expansion of inflammatory taxa. However, 16S-based sequencing did not reveal significant IL-22-mediated alterations in microbiota composition during NEC. While it is formally possible that cytokine treatment induced changes in microbial gene expression or metabolite production, these results suggest that IL-22 primarily functions via other mechanisms to limit the severity of experimental NEC.

IL-22 reinforces the mucosal barrier of adult mice by promoting the regeneration of intestinal epithelial cells. This was also true in the context of experimental NEC. The authors observed an expansion of proliferating intestinal transit amplifying cells following rIL-22 administration. Additionally, rIL-22 protected the integrity of the tight junctions in the intestinal epithelial cells as visualized by anti-occludin staining. Intestinal epithelial cells express high levels of the IL-22 receptor. Induction of NEC to pups lacking the IL-22 receptor on intestinal epithelial cells (IL-22ra1fl/fl × Villin-Cre) abrogated the protective effects of rIL-22 treatment.

Thus, through a systematic evaluation of the microbiota composition, antimicrobial peptide production, and intestinal barrier function, the authors concluded...
that IL-22 ameliorates NEC, at least in part, by enhancing the regeneration of intestinal epithelial cells and promoting barrier integrity.

A notable aspect of this study is the detailed characterization of the IL-22 response during early life. The authors confirmed previous observations that intestinal IL-22 levels are extremely low in neonates and showed that expression of IL-22 remained low even during the potent inflammatory response induced by experimental NEC. This finding may be attributed to the fact that the cells known to produce IL-22 (ILC3 and CD4+ T cells) are in a naive or undifferentiated state early in life. Surprisingly, despite the lack of production in early life, treatment with IL-22 could elicit potent responses both in neonatal mice and human enteroids cultured from a premature infant with NEC. It will be interesting to unravel the mechanisms underlying the IL-22 response. What causes the dramatic upregulation of IL-22 during the weaning transition in mice, and is this phenomenon also observed in healthy human infants? If IL-22 production is increased in full-term infants, the immaturity of the gut and the inability to mount an IL-22 response upon intestinal insult in premature infants could explain their heightened risk of developing NEC. Additionally, it remains unclear why IL-22 is not expressed in early life. While detrimental in the context of prematurity and NEC, perhaps the lack of IL-22 promotes host-microbiota interactions, thereby facilitating appropriate immune education in the developing neonate.

In light of the paucity of treatments available for NEC, this study shows exciting preclinical data for the potential of IL-22 in alleviating disease severity.

REFERENCES

1. Mihi, B., Gong, Q., Nolan, L.S., Gaie, S.E., Goree, M., Hu, E., Lanik, W.E., Rimer, J.M., Liu, V., Parks, O.B., Lewis, A.N., et al. (2021). Interleukin-22 signaling attenuates necrotizing enterocolitis by promoting epithelial cell regeneration. Cell Rep. Med. 2, this issue, 100320–1–100320–12.

2. Niño, D.F., Sodhi, C.P., and Hackam, D.J. (2016). Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat. Rev. Gastroenterol. Hepatol. 13, 590–600.

3. Fehr, K., Moossavi, S., Sbihi, H., Boutin, R.C.T., Bode, L., Robertson, B., Yonemitsu, C., Field, C.J., Becker, A.B., Manchane, P.J., et al. (2020). Breastmilk Feeding Practices Are Associated with the Co-Occurrence of Bacteria in Mothers’ Milk and the Infant Gut: the CHILD Cohort Study. Cell Host Microbe 28, 285–297.e4.

4. Thai, J.D., and Gregory, K.E. (2020). Bioactive Factors in Human Breast Milk Attenuate Intestinal Inflammation during Early Life. Nutrients 12, E581.

5. Keir, M., Yi, Y., Lu, T., and Ghiardi, N. (2020). The role of IL-22 in intestinal health and disease. J. Exp. Med. 217, e20192195.

6. Sugimoto, K., Ogawa, A., Mizoguchi, E., Shimomura, Y., Andoh, A., Bhan, A.K., Blumberg, R.S., Xavier, R.J., and Mizoguchi, A. (2008). IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J. Clin. Invest. 118, 534–544.

7. Cristofalo, E.A., Schanler, R.J., Blanco, C.L., Sullivan, S., Trawoeger, R., Kiecht-Kohlendorfer, U., Dudell, G., Rechtman, D.J., Lee, M.L., Lucas, A., and Abrams, S. (2013). Randomized trial of exclusive human milk versus preterm

Figure 1. Pathophysiology and preclinical model of NEC
(A) Risk factors for NEC include premature birth, perturbed microbial composition, formula feeding, and exaggerated inflammatory responses.
(B) Pups were separated from mothers 4 days after birth and fed six times a day with formula supplemented with lipopolysaccharide (LPS) and a bacterium isolated from a human infant. Pups were also subjected to hypoxia daily and administered PBS or rIL-22 via intraperitoneal injection daily. Intestinal samples were examined 3 days later.
8. Maheshwari, A., Schelonka, R.L., Dimmitt, R.A., Carlo, W.A., Munoz-Hernandez, B., Das, A., McDonald, S.A., Thorsen, P., Skogstrand, K., Hougaard, D.M., and Higgins, R.D.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2014). Cytokines associated with necrotizing enterocolitis in extremely-low-birth-weight infants. Pediatr. Res. 76, 100–108.

9. Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., de Sauvage, F.J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14, 282–289.

10. Torow, N., Yu, K., Hassani, K., Freitag, J., Schulz, O., Basic, M., Brennecke, A., Sparwasser, T., Wagner, N., Bleich, A., et al. (2015). Active suppression of intestinal CD4(+) TCRß(+) T-lymphocyte maturation during the postnatal period. Nat. Commun. 6, 7725.