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Commentary

Pathogenesis and biomarkers for necrotizing enterocolitis: Getting any closer?

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Every year, fifteen million infants are born preterm (<37 weeks of gestation) and one million die worldwide [1]. Many survivors display both short- and long-term morbidities, partly explained by the premature exposure of their immature organ systems to the exogenous environment, oral milk feeding and bacteria [1]. Among the feeding- and bacteria-related complications, necrotizing enterocolitis (NEC) is the most serious disease, occurring in 5–10% of premature babies with a mortality of 20–30% [2]. Despite numerous investigations on the predisposing factors for NEC, the etiology and pathogenesis of NEC are poorly understood. It remains important to look for highly specific and sensitive biomarkers for early NEC diagnosis and treatments.

Multiple investigations have applied both conventional and -omics approaches to search for biomarkers [3,4], but no reliable biomarkers from biological samples have been obtained, although some results are promising. Recently, Weitkamp et al. detected a significant reduction of lamina propria Treg density in the ileum of NEC infants [5], suggesting impaired intestinal Treg function and excessive inflammatory responses during NEC progression. Further, NEC gut tissues also display increased activation of TLR4 signaling [6] and formation of neutrophil extracellular traps (NETs) with release of antimicrobial neutrophil components, including calprotectin [7]. As a result, fecal calprotectin has been suggested as a potential biomarker for NEC diagnosis [7]. However, both feces and urine are less than ideal biological samples for early NEC diagnosis as they are not always available from the infants when needed during suspected NEC.

In the article of EBioMedicine [8], Ma et al. reported elevation of blood $CCR9^+CD4^+$ T cells as well as $CCR9^+$ IL-17 producing Treg in human patients and mice with NEC. Of note, this particular T cell subpopulation possessed the markers of Treg but their immune suppressive activity was severely impaired, as tested *in vitro*. The authors also verified in experimental mice following NEC induction that the levels of $CCR9^+$ IL-17-producing Treg in the gut and blood were positively and negatively correlated, respectively, with histological NEC severity. This suggests that circulating $CCR9^+$ IL-17 producing Treg can be used as a biomarker for NEC diagnosis and NEC severity because these cells are likely polarized from conventional Treg and homed to the gut during NEC progression with the infiltration levels depending on the degree of gut inflammation. Adaptive T cell responses are important mechanisms in gut inflammation, especially in chronic gut diseases. In order to exert these responses, the homing of $CD4^+$ T cells from the circulation to the intestinal mucosa is key, and one of the homing mechanisms is mediated *via* the chemokine receptor CCR9. This mechanism has previously been found to be involved in inflammatory bowel diseases in adults [9] but this is the first report from the neonatal gut inflammatory condition, NEC.

The authors showed that the polarization of $CCR9^+$ Treg into $CCR9^+$ IL-17 producing Treg was regulated by IL-6 activity. Blocking IL-6 signaling by an IL-6 receptor antibody inhibited this polarization *in vitro*, and decreased NEC severity and infiltration of IL-17 producing Treg in mice. The authors therefore proposed that IL-6 receptor antibody can be used to treat NEC. The conversion of Treg into IL-17-producing Treg can be obtained *in vitro* by stimulation of conventional Treg with several cytokines, including IL-6, IL-2, IL-1β and IL-21 [10]. In the current paper, the authors applied the co-stimulation with IL-6 and IL-1β at 10–20 ng/mL to convert Treg into IL-17 producing Treg cells. These *in vitro* cytokine levels were much greater than those in the blood of NEC patients (10–200 pg/mL). The polarization of T cell subsets depends on specific conditions of the microenvironment but it cannot be excluded that the formation of IL-17 producing Treg *in vivo* in the blood and gut may be mediated by (many) more factors than IL-6 alone. Despite the effects of blocking IL-6 signaling on decreased NEC severity and infiltration of IL-17 producing Treg in mice, it remains elusive whether this particular T cell sub-population in the blood can also be ameliorated by IL-6 receptor antibody. In addition, it is unknown whether the high correlation detected between IL-17 producing Treg levels in the gut and NEC severity was only indirect, as the *in vivo* pathological role of this cell population was not examined. It will be interesting in the future to investigate this question and other potential therapeutic interventions to reduce the gut infiltration with IL-17 producing Treg cells, *e.g.* attenuation of gut chemokines targeting CCR9 receptor (CCL25).

In summary, the study by Ma et al. has added another important layer into the understanding of NEC pathogenesis and potential diagnosis and treatment for NEC. The exciting results from this study may pave the way for future investigations on new disease blood biomarkers as well as therapeutic approaches with inhibition of gut chemokines or IL-6 signaling. NEC remains an enigmatic and multifactorial disease

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that is difficult to predict and treat. The pathways of NEC progression may also vary according to gestational age at birth, postnatal age, feeding regimen and bacterial exposure – and not the least among different mammals. Hence, it is important to confirm and substantiate the present findings also in other animal models of NEC and in various subtypes of NEC in infants.

Declaration of Competing Interest

None.

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