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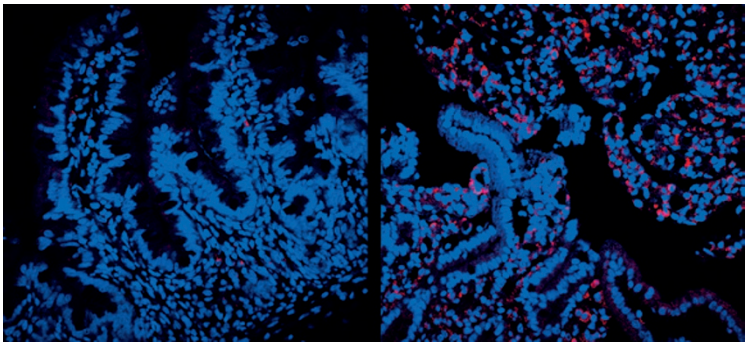
PAEDIATRICS

T cells in necrotizing enterocolitis

Toll-like receptor 4 (TLR4) via STAT3 signalling, has been shown to be important in the development of necrotizing enterocolitis (NEC), an inflammatory disease affecting premature infants. TLR4 signalling in intestinal epithelial cells was shown to increase the migration of T cells to intestinal tissue and skew the population of these cells in favour of proinflammatory type 17 T helper (T_H17) cells over anti-inflammatory regulatory T (T_{REG}) cells, leading to necrosis of intestinal tissue in mice. Restoration of the balance towards T_{REG} cells was achieved with enteral retinoic acid treatment and reduced the severity of disease in mice with NEC.

"This work stemmed from our desire to understand and to treat NEC, a devastating disease of premature infants that is associated with extremely high mortality, and for which there is no specific treatment. It has been known for many years that NEC is an inflammatory process that involves the intestines, but the specific cells that mediate this inflammation have received very little attention," says corresponding author David Hackam (John Hopkins University, Baltimore, USA).

The polarization of intestinal T cells into T_H17 cells and resultant decrease in the numbers of T_{REG} cells was confirmed in intestinal samples from both humans and mice with NEC. To see whether T cells were required for the development of NEC the authors attempted to induce NEC in *Rag*$^{-/-}$ mice, which have no functional T cells. These mice were protected from developing NEC, but transferring T cells from wild-type mice into *Rag*$^{-/-}$ mice restored susceptibility to induction of NEC.



Normal human intestine (left), human intestine with necrotizing enterocolitis (right), stained for T cells (red). Image courtesy of D. Hackam.

Previous work from this group has shown that TLR4 is involved in NEC pathogenesis and so the authors investigated whether TLR4 signalling was also responsible for the influx of T cells to the intestines and if it affects the composition of the T-cell pool.

Selective deletion or over-expression of *Tlr4* in mouse intestinal epithelial cells revealed a decrease or increase, respectively, in the migration of T cells compared with wild-type mice. This recruitment of T cells was found to be mediated via cognate chemokine ligand 25. In addition, mice without *Tlr4* expression had less of the T_H17 marker RAR-related orphan receptor C and more Foxp3$^+$ T_{REG} cells compared with wild-type mice with NEC. The opposite senario occurred in mice over-expressing *Tlr4*.

IL-17 receptor A (IL-17RA) expression was found to be upregulated in human and mouse NEC tissue samples. Blockade of the IL-17RA with intraperitoneal injection of an anti-IL-17 receptor antibody reduced the severity of NEC injury in mice. The integrity of intestinal epithelial tight junctions improved, enterocyte proliferation increased and apoptosis of crypt cells decreased. Crucially, IL-17RA is induced by TLR4 and so the authors believe IL-17RA–IL-17 signalling to be an important mechanism by which TLR4 causes disease.

Given the role of T cells in this model of NEC, the authors then aimed to modulate the population of these cells from T_H17 to T_{REG} cells to decrease disease severity. All-trans retinoic acid has been used in other contexts to effect this change in T-cell phenotypes and oral administration of the compound to mice with NEC substantially reduced disease severity. A decrease in IL-17 expression was observed, however, TLR4 expression was unaffected.

Results from this study have informed the desire to set up clinical trials investigating vitamin D supplementation for the treatment of NEC, as it has previously been shown to suppress the expression of T_H17 cytokines. The authors also plan to examine vaccine approaches to prevent T-cell migration to the intestines.

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ORIGINAL ARTICLE Egan, C. E. *et al.* Toll-like receptor 4–mediated lymphocyte influx induces neonatal necrotizing enterocolitis. *J. Clin. Invest.* http://dx.doi.org/10.1172/JCI83356