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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Nerves Make the Bowel Happy, Even When the Enteric Nervous System Is Missing!

The bowel has its own "second brain" called the enteric nervous system with about as many nerve cells as the spinal cord and ~4× as many glia. These cells control most aspects of bowel function including motility, blood flow, epithelial cell biology, and immune cell activities.[1] In about 1:5000 infants, the enteric nervous system is absent from the end of the bowel. This life-threatening birth defect is called Hirschsprung disease. Bowel without neurons (aganglionic bowel) tonically contracts and lacks propagating contractions, preventing efficient passage of air and stool. Children with Hirschsprung disease also commonly develop bowel inflammation resulting in explosive diarrhea and a predisposition to bacterial translocation into the blood, a problem called Hirschsprung disease associated enterocolitis. To treat Hirschsprung disease, surgeons remove bowel that lacks enteric nervous system cells and reconnect "good" bowel to near the anal verge. This procedure is called pull-through surgery. Although pull-through surgery can be lifesaving, many children have problems after pull-through surgery including enterocolitis. Mechanisms underlying enterocolitis remain incompletely understood. In particular, it is not clear why some children have recurrent enterocolitis, and other children have no enterocolitis symptoms.

To learn more about enterocolitis, Keck et al[2] studied colons removed from 44 children during Hirschsprung disease pull-through surgery. As Meier-Ruge et al[3] observed in 1972, colon that lacks enteric nervous system often has abundant acetylcholinesterase staining, so acetylcholinesterase analysis is routinely used at many medical centers as part of Hirschsprung disease diagnosis. Acetylcholinesterase degrades the neurotransmitter acetylcholine, a signaling molecule for many types of enteric neurons and also for extrinsic parasympathetic nerve fibers that enter the bowel. Although it is well-known that the intensity of acetylcholinesterase staining varies from child to child with Hirschsprung disease, I am not aware of prior literature suggesting that the abundance of acetylcholinesterase staining in Hirschsprung bowel has any particular significance. Keck et al hypothesized that increased acetylcholinesterase might mean increased acetylcholine signaling and recognized that acetylcholine is a key component of the "cholinergic anti-inflammatory pathway" first described by Tracey's group.[4] Using the Swiss roll technique, they classified 31 children as "fiber-low" and 13 children as "fiber-high" on the basis of the intensity of small β3tubulin+ nerve fibers and the intensity of acetylcholinesterase antibody staining near mucosa of rectosigmoid colon.

Remarkably, fiber-high aganglionic rectosigmoid colon had fewer Th17+ lymphocytes on average than fiber-low rectosigmoid. The mean Th17/Treg ratio in fiber-high colon also trended lower compared with fiber-low colon ($P = .0699$). The balance between Th17 and Treg cells is thought to be a key factor controlling intestinal inflammation.[5] Th17 cells protect the intestinal mucosa but can increase intestinal inflammation via the production of proinflammatory cytokines. Treg cells produce anti-inflammatory cytokines. Th17 and Treg cells each differentiate from naive CD4+ T cells. For Th17 cells, interleukin (IL) 6, transforming growth factor (TGF)-β, IL21, and IL23 promote differentiation, maturation, and maintenance. In contrast, Treg cells are generated from naive T cells in the presence of TGF-β and IL2. Keck et al[2] found that macrophages isolated from fiber-low and fiber-high colon both had a gut imprinted M2 phenotype (CX3CR1, AREG, AhR, CSR1R, CD200R, CD209, TGF-β2, IL10), but more macrophages from the fiber-low colon were IL23+, and fiber-low macrophages had higher mRNA levels of the Th17-inducing cytokines IL23a (an IL23 subunit) and IL6 and of the IL17 chemokine CCL-20. Interestingly, CD64+ macrophages closely associated with nerve fibers in submucosa of fiber-high colons and were more likely to have a bipolar shape typical of anti-inflammatory (M2) macrophages instead of the rounded or stellate shape typical of proinflammatory (M1) macrophages. These observations make it plausible that extrinsic nerve fibers abundant in fiber-high colons could alter macrophage phenotypes, and that these macrophages in turn regulate the Th17 and Treg balance to impact bowel inflammation. Although the relevance of these findings might be questioned, within a year of pull-through surgery 9 of 42 children had postoperative enterocolitis. Seven of the children with enterocolitis were in the fiber-low group. In a second independent Hirschsprung disease cohort, 14 of 29 children had postoperative enterocolitis, with 12 of 14 in the fiber-low group. These clinical observations suggest that extrinsic innervation alters likelihood of developing post–pull-through enterocolitis in children with Hirschsprung disease, or that immune cells and dysbiosis impact innervation density. The data also suggest that fiber-low colon predicts increased likelihood of Hirschsprung disease associated enterocolitis after pull-through surgery, and that targeting IL17 or IL23 might help treat or prevent enterocolitis. Details about the neuroimmune interactions that underlie the observations of Keck et al[2] including the origin of extrinsic nerve fibers, reasons for differences in nerve fiber abundance, and relevant neurotransmitters still need to be investigated because this could lead to new targeted neuromodulatory therapy for Hirschsprung

disease associated enterocolitis. This remarkable study opens many new avenues for investigation and identified new meaning for acetylcholinesterase staining that has been performed on Hirschsprung disease colon biopsies for almost 50 years!

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