Immune system implicated in prematurity complication

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Despite advances in neonatal care, necrotizing enterocolitis (NEC) – the most common gastrointestinal emergency in premature infants – continues to be a deadly disease.

"We haven't made a lot of progress in identifying babies early who may be at risk for NEC, preventing it or treating it," said Jörn-Hendrik Weitkamp, M.D., a neonatologist and assistant professor of Pediatrics at Monroe Carell Jr. Children's Hospital at Vanderbilt.

Now, Weitkamp and his colleagues have discovered that disruptions in immune system regulation — not previously considered to be important in NEC pathophysiology — may play a role in the disease. The findings, reported in the journal *Gut*, suggest a new target for therapeutic interventions for NEC.

NEC is an inflammatory disease that kills intestinal tissue. Premature babies, and particularly those who have early formula feedings (breast milk has protective properties), are at increased risk for developing NEC.

About 40 percent of babies with NEC require surgery to remove dead bowel tissue — and half of these babies do not survive. NEC survivors suffer long-term complications including bowel-related problems and impairments in motor and cognitive function.

Weitkamp wanted to explore whether differences in immune system

regulation — particularly in the "adaptive" immune response mediated by B cells and T cells — might play a role in NEC. This type of cellular immunity was not considered important in the early neonatal period because it takes time to develop, and because it was not found in mouse models, Weitkamp said. But he knew from other studies that immune responses in newborn mice are not identical to immune responses in newborn humans.

Weitkamp and his colleagues turned to stored human intestinal tissue samples that had been surgically removed from preterm babies diagnosed with NEC or other intestinal diseases. They found high levels of T cells called T regulatory (Treg) cells in the intestine of premature infants, which was surprising given the lack of these cells in the gut of newborn mice. Treg cells suppress the immune response and are critical for keeping the immune system in balance, preventing harmful inflammation.

Because of limitations related to Treg cell detection in stored tissue samples, the investigators collected fresh intestinal tissue samples from babies having surgery for NEC and for non-NEC problems, during or immediately after surgery.

Using multiple cellular "markers" and flow cytometry to identify the Treg cells, they confirmed that premature babies have an abundance of Treg cells in the intestines. Babies with NEC had about 60 percent fewer Treg cells than babies with non-NEC problems.

The investigators also detected increased expression of inflammatory cytokines (immune system signaling molecules) — particularly those that suppress Treg cell development — in the NEC tissue samples. And in assays of cell function, they found that the Treg cells suppressed cytokine production by and proliferation of T cells.

Follow-up studies on tissue samples from infants who had a second surgery suggest that the reduction in Treg cells is not because of an immune system defect in the babies.

"We believe that the T regulatory cells we've identified are in fact functional Treg cells and that they are down-regulated at the time of NEC," Weitkamp said. "Our studies challenge the dogma that cellular immunity is not important in the immediate neonatal period or in the pathophysiology of NEC."

Now, Weitkamp and his colleagues including neonatology fellow Joann Romano-Keeler, M.D., are studying the intestinal microbiome — the collection of microbes colonizing the gut — in the surgically removed samples.

"We know that the microbiome and the immune system are important in shaping each other," Weitkamp said.

"We need to understand exactly how that works and which components typically found in human milk — such as certain vitamins, prebiotics and probiotics — are important for healthy microbiome and healthy immune system development."

Such components, he said, might become the basis for interventions to prevent the development of NEC.

Vanderbilt's collaborative environment and the support of colleagues in Pediatric Surgery, Pathology and Neonatology made the collection of fresh surgical tissue samples possible, Weitkamp said.

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