Molecules and Cells

Journal Club

Let Them Flourish for the First Weeks and Suffer Less

Bifidobacteria carrying beneficial genes utilizing human milk oligosaccharides (HMOs) help develop a healthy immune system in breastfed babies.

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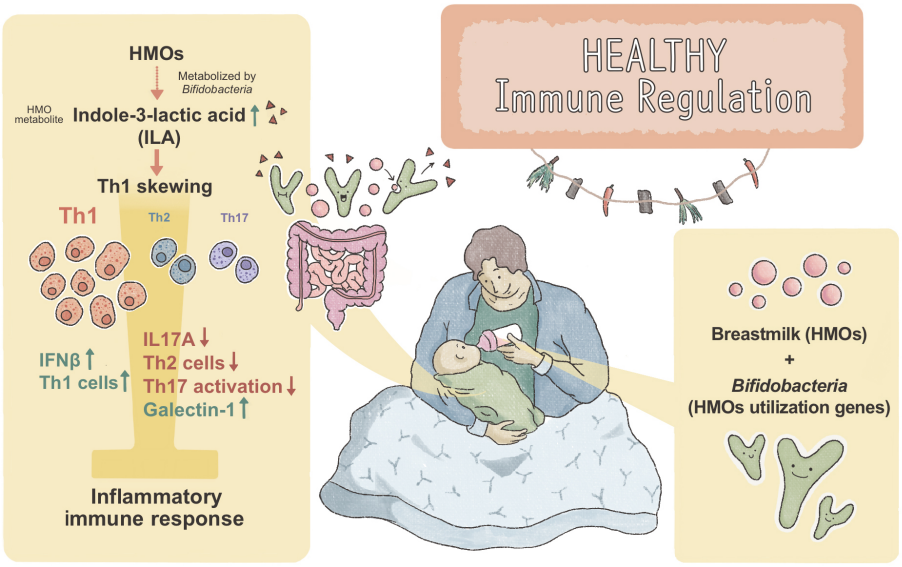

During the first three months of human life, intestinal immune systems are transiently activated, responding to colonizing gut microbiome, which eventually establishes healthy symbiosis with breastfed babies. Specific bacteria, such as *Bifidobacteria*, digest HMOs by HMO-utilization genes and produce beneficial metabolites, such as ILA. For example, in the presence of ILA, galactin-1 is upregulated and limits T cell activation in favor of Th1 state over Th2 and Th17, dampening intestinal inflammatory response. The systemic actions of beneficial symbiotic gut microbes and their HMO metabolites on the developing immune system for the first several weeks after birth potentially affect human health in the long term, such as developing immune-mediated diseases.

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"Sam-chil-il," the three-seven day in Korean tradition, means the 21st day after birth. When a baby is born, a house gate is decorated with "Gheum-Jool," a rice straw string skewed with wooden charcoal sticks that boot off evil spirits, signals a new arrival, and bans visitors to avoid potentially harmful contacts. On every 7th day for the first three weeks, offering seaweed soup to "Samsin," a goddess of child-bearing, is performed. After the final worship, "Gheum-Jool" is removed and visitors are allowed to meet and greet the newborn and her mother, welcoming the new arrival to the world. The next big event is "Baek-il," the 100th-day celebration. Parents share rice cakes with 100 people, wishing their child a long life of 100 years. While modern-day extricating newborn care reduces infant mortality for the first three months, mounting scientific evidence indicates that what babies experience and develop for the first three months is crucial for their well-being immediately and in their entire life.

Human breast milk contains numerous human milk oligosaccharides (HMOs) that play a vital role in babies' healthy growth (Andreas et al., 2015). Such complex, energetically expensive sugars are digested by babies even if they lack necessary glucosidases; however, their gut microbes are adapted to metabolizing HMOs, such as *Bifidobacterium*; thus, establishing a key link in coevolution between humans and gut microbes (Sela and Mills, 2010). Babies who lose *Bifidobacterium* tend to suffer enteric inflammation and immune-mediated disorders, such as pathogen infections, asthma, allergies, and other auto-immune diseases, but underlying molecular and cellular mechanisms are largely elusive (Arrieta et al., 2018; Henrick et al., 2019; Rhoads et al., 2018; Ryu et al., 2019; Vatanen et al., 2016). Henrick et al. (2021) showed that simple feeding of *Bifidobacteria* help in effective digestion of HMOs for the first three months and can relieve intestinal inflammation in breastfed babies by promoting proper immunoregulation. The study was conducted using a leading-edge system immunology approach with precision technology that makes it possible to process the baby's blood samples as little as 100 μl. The phenomenal cohort study with multi-omics analyses is enlightening, showing why the first three months of life bear the most desirable milestone in humans.

To monitor immunological events, Henrick et al. (2021) collected blood samples from newborns in Sweden, profiled 64 blood immune cell populations, and quantified 355 unique plasma proteins. Within the first three weeks, the same duration as Sam-chil-il, there was an initial innate immune response, such as expanding monocytes and a transient surge in interferon γ (IFNγ) and other indicators of active immune systems. There was also a gradual increase in the frequency of memory regulatory T cells (Tregs). Additionally, right after Sam-chil-il, the number of γδT cells, most abundant in the gut mucosa, robustly increased. These responses indicate transient innate and adaptive immune activities and key regulatory mechanisms during the first several weeks of human life.

They also observed an expansion of a mucosal-specific subset of memory $CD4^+$ T cells (Yi et al., 2019). In addition, analysis of enriched blood transcriptional modules revealed that the mucosal-specific T cells recognize antigens in babies' intestine after birth, circulate and expand in the bloodstream, and interact with natural killer cells and monocytes in the newborn intestinal immune system, indicating active immune responses in babies' intestine that early.

Metagenomics analysis of the newborns' stool waters indicates that gut bacterial composition was highly variable at birth but gradually converged later. Especially, Bifidobacteriaceae is a major microbe family that increased in abundance, reaching the first peak near the 8th week after birth. Additionally, breastfed babies not treated with antibiotics held expanded Bifidobacteriaceae, composed of multiple species, including *Bifidobacterium longum* and *B. bifidum*, the most frequently detected ones.

There were dramatic differences in immune system states between babies holding abundant Bifidobacteriaceae and those who failed. Babies with abundant Bifidobacteriaceae frequently had more anti-inflammatory monocytes, a highly suppressive Treg subset, and elevated Treg-associated cytokines, including IL-27. In contrast, babies lacking Bifidobacteriaceae had many neutrophils, basophils, plasmablasts, memory $CD8^+$ T cells (indicators of immune activation), and elevated levels of critical mediators of intestinal inflammation, such as tumor necrosis factor α (TNFα), IL-17A, and Th2 cytokines. Additionally, analysis of cell–cell interaction using Spearman correlation matrices confirmed the inverse correlation between memory Tregs and activated $CD8^+$ T cells and proinflammatory monocytes. Therefore, the lack of Bifidobacteriaceae for the first weeks may result in systemic immune dysregulation, leading to systemic and intestinal inflammation.

Henrick et al. (2021) observed that some HMO-utilization genes (key ecological determinants of fitness for *Bifidobacteria*) are commonly detected in the babies' fecal metagenomes, despite low abundance (Duar et al., 2020a; 2020b). Interestingly, however, the abundance of HMO-utilization genes was negatively correlated with many proinflammatory plasma proteins. In contrast, it was positively correlated with IL-27 cytokine, limiting Th2- and Th17-type responses favoring Th1 (Yoshida and Hunter, 2015). Therefore, HMO-utilization genes expressed by the whole gut microbiome, including *Bifidobacteria*, correlate with decreased systemic inflammation and reduced Th2- and Th17-type responses.

A peculiar point in the Swedish cohort is that there is no bacteria isolate expressing all HMO-utilization genes, likely responsible for the low abundance of HMO-utilization genes. To assess the beneficial effects of HMO-utilization genes, they fed breastfed babies in California with *B. longum* subsp. *infantis* EVC001, which expresses all HMO-utilization genes. These babies increased their level of anti-inflammatory IFNβ (suppressing inflammatory responses), which was not previously recognized. However, whether IFNβ induction directly affects T cells remains unknown.

To understand the direct effects of bifidobacterial metabolites and enteric cytokines on T cells, Henrick et al. (2021) isolated naïve $CD4^+$ T cells from human adults and treated them with stool water collected from EVC001-supplemented babies. Stool waters from EVC001-supplemented babies induced the Th1-like state, whereas those from control babies induced the Th2-like state. A tryptophan metabolite,

indole-3-lactic acid (ILA), is the most over-represented in EVC001-fed babies out of 564 significantly different biochemicals in assessing fecal metabolites. Thus, Naïve $CD4^+$ T cells were treated with ILA alone without any stool water, and their polarized cell types and transcripts were analyzed using a multi-omics approach (Jung et al., 2020). Galactin-1, a negative regulator of T cell activation, was highly upregulated in polarizing T cells. Galectin-1 induces IL-27, acting through IFNβ-dependent reprogramming of tissue macrophages, which is essential in resolving inflammation (Yaseen et al., 2020). Therefore, HMO-metabolizing bacteria may induce anti-inflammatory and tolerogenic responses by elevating IL-27, IFNβ, and ILA-mediated upregulation of galectin-1 that acts on $CD4^+$ T cells, which expresses AhR, an ILA receptor (Uhlen et al., 2019).

The study by Henrick et al. (2021) provides solid evidence of molecular and cellular mechanisms that helps explain why babies colonized early in life with *Bifidobacteria* are less likely to develop immune-mediated diseases (Arrieta et al., 2018; Vatanen et al., 2016). It also explains how the symbiosis of human and beneficial gut bacteria is established and the tolerance induction to the gut microbiota (Schaupp et al., 2020; Stefan et al., 2020; Sundblad et al., 2018). The study reveals a human-specific pre-weaning expansion of microbiota in breastfed babies that is different from mice, in which weaning triggers microbiota expansion (Al Nabhani et al., 2019; Knoop et al., 2017; Ryu et al., 2019).

Immediately after birth, the sterile baby gut becomes colonized by various microbes from the surroundings and mother. Many factors influence the intestinal microbiota composition and its establishment: vaginal birth or C-section; breastmilk or formula; antibiotics or herbal medicine, or just mom's touch (Kostandy and Ludington-Hoe, 2019; Lee et al., 2019). Choices are made depending on clinical, traditional, personal reasons, and availability, and whatever outcomes continue to be managed. Nevertheless, there is good news that *B. infantis* EVC001 is a commercially available probiotic. Therefore, it may be wise to observe Korean three-seven days. If applicable, taking full 12 weeks of or even longer maternity leave will greatly help make the jubilee Baek-il celebration in grace and peace.

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CONFLICT OF INTEREST

The author has no potential conflicts of interest to disclose.

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