Milk and bugs educate infant immune systems

Spreckels, Johanne E.; Zhernakova, Alexandra

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Spotlight

Milk and bugs educate infant immune systems

Johanne E. Spreckels[1,2] and Alexandra Zhernakova[1,3,*]
[1]Department of Genetics, University Medical Center Groningen, Groningen, the Netherlands
[2]Twitter: @spreckelsje
[3]Twitter: @SashaZhernakova
*Correspondence: a.zhernakova@umcg.nl
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Immune-system maturation starts early in life, but studies investigating immune-system education in human infants remain scarce. In a recent issue of *Cell*, Henrick et al. study early gut microbiota and immune-system development in two infant cohorts. The authors describe that *Bifidobacteria* can use milk sugars to produce immunoregulatory compounds that induce immune tolerance and reduce intestinal inflammation.

The early-life gut microbiota is thought to shape the maturation of the human immune system, potentially leading to long-lasting effects on an individual's susceptibility to infections and immune-mediated diseases, like allergies and diabetes (Al Nabhani and Eberl, 2020; Vatanen et al., 2016). However, to date, most studies investigating immune development in early life have relied on animal models (Al Nabhani and Eberl, 2020), while data from human studies remains scarce. In a recent issue of *Cell*, Henrick et al. (2021) apply advanced technologies to study how early microbial colonizers of the human gut affect immune-system maturation. In human infant cohorts from Sweden and the United States, the authors observe that early colonization of the gut by breast-milk-consuming *Bifidobacteria* was associated with a more immunoregulatory phenotype, whereas limited *Bifidobacterial* colonization was associated with immune activation and intestinal inflammation (Figure 1).

The authors first describe the development of the gut microbiome and immune system during the first months of life in a cohort of 208 newborns from Sweden. Extensive immune-cell and plasma-protein profiling data from 858 longitudinally collected blood samples show transient innate immune activation soon after birth, followed by production of cytokines and an expansion of immune cells linked to adaptive immune responses. These immune-system changes were paralleled by gut microbial colonization in the weeks and months after birth. Shotgun metagenomic sequencing data from 347 fecal samples collected from 157 of the 208 infants show that the human gut microbiome was highly variable at birth but converged with increasing infant age and that the gut abundances of members of the Bacteroidaceae and Bifidobacteriaceae families increased over time—two findings that are in concordance with previous studies on the development of early infant gut microbial communities (Bäckhed et al., 2015; Yassour et al., 2016). Previously, *Bifidobacteria* were reported to have immunomodulatory functions and reduced enteric inflammation in preterm and breastfed-term infants (Nguyen et al., 2021; Wu et al., 2016). In the current study, the expansion of Bifidobacteriaceae varied between infants and the authors focus on studying the effect of differences in early *Bifidobacterial* colonization on the developing immune system. Consistent with the earlier findings, a low abundance of Bifidobacteriaceae in the gut was associated with intestinal inflammation and activation of innate and adaptive immune responses that were characterized by expanded populations of innate and adaptive immune cells and increased concentrations of pro-inflammatory cytokines (including tumor necrosis factor α, interleukin [IL]-1α, IL-13, and IL-17A) in plasma samples. In contrast, a high abundance of Bifidobacteriaceae was associated with an anti-inflammatory, immunoregulatory phenotype characterized by, among other factors, regulatory T cells and elevated plasma concentrations of the immunoregulatory cytokines IL-10 and IL-27.

Most of the infants in the cohort were breastfed beyond 3 months of age. When drinking their mother's milk, breastfed infants receive human milk oligosaccharides (HMOs), sugars with prebiotic properties that bacteria in the gut can use as a nutrient source. HMOs, which are one of the major components of breast milk, thus likely affect the infant gut microbial community and, concomitantly, immune-system maturation in early infancy. Indeed, Henrick et al. (2021) show that the capacity of gut bacteria to metabolize HMOs was correlated with plasma concentrations of immune-related proteins, specifically lower levels of pro-inflammatory cytokines and higher levels of the more immunoregulatory cytokine IL-27.

To further investigate how gut bacteria that can utilize HMOs affect immune regulation, the authors performed an intervention study in 60 American infants. All were exclusively breastfed and randomized to receive either no supplementation (n = 31) or supplementation with *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) EVC001 (n = 29), a *Bifidobacterium* strain carrying a complete set of genes for metabolizing HMOs. Supplementation was given from 7 to 24 days postpartum. For 20 infants of each group, shotgun metagenomic sequencing data and fecal cytokine concentrations were available at the age of 6 days, before the intervention started, and at 60 days, after the intervention stopped. In the combined analysis, the authors observed that the infant fecal abundances of the potentially pathogenic gut colonizers Clostridiaceae, Enterobacteriaceae, and Staphylococcaceae positively correlated with the fecal concentrations of pro-inflammatory cytokines that drive T helper 2 (Th2) and Th17 immune responses, whereas a higher fecal Bifidobacteriaceae abundance was negatively correlated with

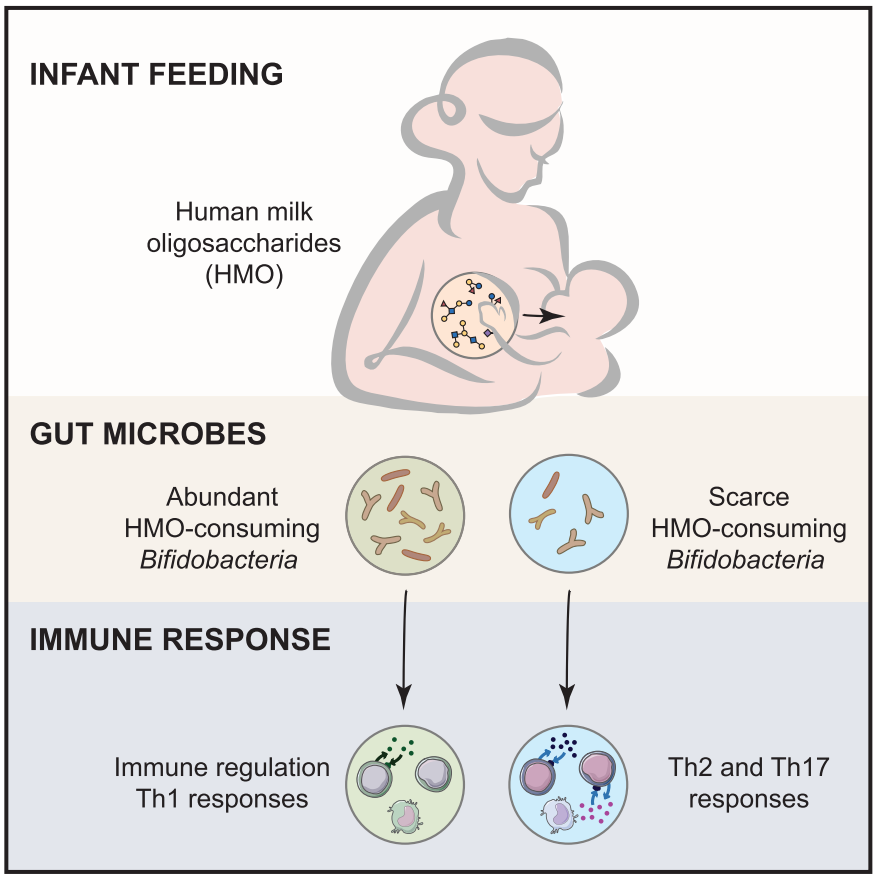

Figure 1. Human milk oligosaccharide-utilizing bacteria shape the infant immune system
Human milk oligosaccharide (HMO)-metabolizing gut bacteria like *Bifidobacteria* produce components that train the early infant immune system toward a more regulatory phenotype and Th1-like immune responses. Presence of only few HMO consumers in infant guts associates with increased immune activation and Th2 and Th17 immune responses.

the concentrations of these cytokines. Accordingly, pro-inflammatory Th2 and Th17 responses were reduced in the *B. infantis* EVC001 infant group after supplementation as compared to the control group without intervention. To further elucidate these findings, the authors performed *in vitro* T cell polarization assays using fecal waters from infants with and without the *B. infantis* EVC001 supplement followed by targeted transcriptomics and proteomics profiling. Here, they observed that naive Th0 cells polarized in the presence of fecal water from infants of the *B. infantis* group showed decreased expression of the Th17-associated *IL23R* gene and increased expression of Th1-associated genes compared to Th0 cells polarized in fecal water from controls. The T cell polarization assays were also used to explore which molecules drive Th cell polarization, with the tryptophan derivative indole-3-lactic acid identified as playing a key role. Indole-3-lactic acid is a bacterial metabolite that is produced in large quantities by *B. infantis* when HMOs are available as a substrate (Ehrlich et al., 2020). The indole-3-lactic acid upregulated the expression of galectin-1, a known inducer of immune tolerance (Sundblad et al., 2018), thereby mediating the immune system regulation observed in *B. infantis* EVC001-supplemented infants. In sum, data from both the birth and intervention cohorts show that the combination of HMOs from breast milk and the presence of HMO-metabolizing bacteria like *B. infantis* EVC001 in the infant gut can modulate immune functions in early infancy.

Henrick et al. (2021) provide unique insights into the interaction between early gut microbiome development and maturation of the human immune system. Their extensive analysis of the developing gut microbial communities, immune cells, and proteins in several hundred longitudinally collected blood and fecal samples from more than 200 newborns is exceptional, especially considering the difficulties associated with obtaining sufficient sample material for the different analyses from infants that early in life. In addition to their thorough characterization of the parallel development of the gut microbiota and immune system, the authors extend their study with data from an intervention cohort and *in vitro* cell-culture assays to describe how *Bifidobacteria* can utilize sugars from breast milk to produce immune-modulating components that reduce intestinal inflammation and induce immune tolerance in the first months of life.

As with any good study, the one by Henrick et al. (2021) raises further research questions. One yet-unexplored question is what effects the different HMOs in breast milk have on the developing gut microbiome and immune system. The HMO profile of breast milk depends on the mother's genetics, with genetic variance in the *FUT2* and *FUT3* genes determining the abundance of specific HMOs in breast milk (Lefebvre et al., 2020). Studying both the effect of maternal genetics on the interplay between early gut microbial colonization and immune-system development and which specific HMOs are utilized by gut bacteria to thrive and produce immune-modulatory compounds should help further our understanding of which factors aid early-life gut-microbiome development and immune maturation. Additionally, studies should also explore the role of infant formula, which rarely contains HMOs, on the development of the gut microbiome and immune system.

Another important question is whether changes in immune system education early in life will have the desired long-lasting effects on health outcomes, i.e., whether infants with more HMO metabolizers like *Bifidobacteria* colonizing their gut in the weeks following birth will have reduced incidences of immune-mediated diseases like allergies or type 1 diabetes. So far, most infant cohorts do not investigate the effects of early gut microbiota beyond the first few years of life. Longer follow-up studies in this and other large cohorts will be necessary to answer this

question. Additional (clinical) trials studying the immune-modulatory functions and therapeutic potential of the bacterial metabolite indole-3-lactic acid in young infants, as well as in individuals suffering from immune-mediated diseases, might help to develop new prevention and treatment strategies for many different immune-system-associated conditions.

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