commentary

α-Galactosylceramide: a potent immunomodulator produced by gut microbes[1]

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Intestinal bacteria have coevolved with humans to respond to and regulate metabolism in a species-specific manner. This commensalism, in turn, influences local and systemic energy homeostasis and immune regulation. Although recent advances in high-throughput technologies have enabled researchers to connect these unique genetic and metabolic microbial signatures with human health and disease, gaps remain in our understanding of the specific mechanisms by which intestinal bacteria impact complex human biology.

In this issue of *Journal of Lipid Research*, von Gerichten et al. establish that a potent immunomodulator of microbial origin, α-galactosylceramide, is present in the murine large intestine and contributes to immune regulation. The source is likely Bacteroides, a genus of the phylum Bacteroidetes, a bacterial phylum that developed early in evolutionary history and appears soon after birth in the intestinal tract of human infants (1). An α-galactosylceramide was previously discovered to be produced by the species *Bacteroides fragilis* (2) and to be a natural ligand for CD1d-mediated iNKT cell activation. Using their previously published method of chromatography-based LC-MS2 to separate glucosyl- and galactosylceramides and their stereoisomers (3), von Gerichten et al. isolated α-galactosylceramide from mouse intestine (which they term αGalCerMLI), established its large-intestinal origin (not finding it in duodenum nor jejunum), and concluded that it was of bacterial origin, because it was absent in germ-free NMRI mice. Using multiple mass spectrometric techniques (e.g., HILIC-MS2, LC-MS2, RP18-LC-MS2, and high resolution FTICR-MS2), they elucidated the structure to be αGalCer (d18:0;βh16:0) (i.e., with a β-hydroxylated FA and an α-glycosidic sugar), which differed from the *B. fragilis* αGalCer by two carbons in the sphingoid base and acyl chain.

The authors also elegantly show that, similar to αGalCer from *B. fragilis*, αGalCerMLI activates iNKT cells and IL-2 cytokine release in a dendritic and iNKT cell coculture in vitro system. Moreover, they demonstrate that iNKT activation by αGalCerMLI is CD1 dependent as evidenced by the release of IFN-γ in splenocyte-derived primary iNKT cells of wild-type mice but not those of $CD1d^{-/-}$ mice.

But do any other factors affect the levels of this immunomodulatory lipid? Indeed they do. The authors also demonstrated that mice subjected to high-fat, high-sugar diet feeding, DSS-induced colitis, or influenza infection have significantly reduced αGalCerMLI levels relative to controls. While one can only speculate at this point that these reductions in αGalCerMLI are due to reduced Bacteroides content [as seen in patients with inflammatory bowel disease (4), the clinical correlate of DSS colitis]; it is clear that αGalCerMLI levels are influenced by diet, colonic inflammation, and influenza infection and could be important regulators of T-cell immunity.

The results by von Gerichten et al. draw attention to the intimate, interwoven relationship among nutrients, α-galactosylceramide-producing gut microbiota, and inflammation. Their work substantiates prior lines of evidence demonstrating a role of Bacteroidetes in human nutrition and metabolism. For example, both obese humans (5) and healthy volunteers who consume a plant-based, carbohydrate-rich diet (6) have a lower abundance of Bacteroidetes compared with other phyla. That these healthy volunteers additionally consumed a low-fat diet suggests that the high-fat, high-sugar diet αGalCerMLI-reducing effects in the murine intestine are predominantly driven by the high carbohydrate load. The implications of these observations suggest that in addition to their known ability to ferment carbohydrates, αGal-producing bacteria such as Bacteroidetes have the capacity to sense intraintestinal carbohydrate load. This ability may be important for harvesting energy for the host and/or other intestinal bacteria in low carbohydrate settings.

Perhaps most provocative is the authors' highlighted inverse relationship between systemic influenza viral infection and αGalCerMLI content. Their findings are supportive of Yildiz et al. (7), who established a short-term, small intestine dysbiotic response to transient influenza A infection in mice that resulted largely from depletion of Bacteroidetes and Firmicutes phyla. Although the specific mechanism of this observation is unknown and exposes the complex relationship between viral infection and gut

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bacteria immunity (8), it is tempting to speculate that the response of gut α-galactosylceramide-producing bacteria both to systemic viral infection and inflammation-inducing high carbohydrate loads (9) is an attempt by these bacteria to escape immune cell recognition. This paradigm has been observed previously in *Bacteroides fragilis* organisms as they can alter their surface polysaccharides to avoid surveillance by local immune cells (10).

Because of the uniqueness of this lipid molecule, it is expected that this new frontier in gut microbiome research will lend itself to therapeutic application. One can envision that modulating gut microbial-derived immunogenic lipids like α-galactosylceramide may impact both energy homeostasis and immunity. For example, strategies that modulate αGalCerMLI content without worsening inflammatory responses may be relevant for obesity therapies. Similarly, as loss of gut microbial diversity has been linked with influenza vaccine success (11) (albeit B-cell mediated), modulation of αGalCerMLI could potentially improve response to viral vaccinations.

In summary, von Gerichten et al. have very convincingly placed αGalCer at the forefront of gut microbiome research aiming to establish concrete connections between microbiota diversity, energy homeostasis, and immunity.

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