What's LPS Got to Do with It? A Role for Gut LPS Variants in Driving Autoimmune and Allergic Disease

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http://dx.doi.org/10.1016/j.chom.2016.04.025

The bacterial communities that live in and on our bodies have a profound influence on our health. In a new paper in *Cell*, Vatanen et al. (2016) report that the composition of the early-life gut microbiome, particularly those species producing lipopolysaccharide, influences the onset of autoimmune and allergic disease.

The increasing incidence of autoimmune and allergic disease in developed nations is a public health issue that has puzzled researchers for several decades. Genetic loci that contribute to disease susceptibility have been identified, but genetic variation alone cannot fully explain the rapidly rising number of patients. The hygiene hypothesis has been a popular explanation for the emergence of these so-called diseases of Western society (Strachan, 1989). Originally, this hypothesis suggested that increased vaccination and improved sanitation led to reduced exposure to infectious diseases, depriving the immune system of immunoregulatory stimuli required to prevent inappropriate hyperreactivity to innocuous self or environmental antigens (Strachan, 1989). More recent iterations of the hygiene hypothesis have been extended to include a role for the commensal microbiota in the maintenance of health, as these microbes are increasingly understood to play a critical role in regulating a large variety of host functions. It is now clear that the composition of these carefully selected co-evolved microbial communities has been disrupted by factors common to our modern environment and lifestyles. How this altered 21st century microbiota is contributing to the increasing prevalence of disease is the subject of intensive ongoing research.

As reported recently in *Cell*, Vatanen et al. (2016) have identified a previously unappreciated role for LPS variants in the gut microbiota in driving disease. Intrigued by the observation that the incidence of allergy and autoimmunity is markedly increased in a population of Finnish infants compared to their genetically similar counterparts in Russian Karelia, the authors examined the microbial composition of fecal samples collected from these two groups (along with a group from Estonia, where disease incidence is transitioning from Russian to Finnish levels) throughout the first 3 years of life. They found that Russian children had a bacterial microbiota dominated by *Bifidobacterium*, while infants from Finland and Estonia had increased abundance of *Bacteroides*, particularly during the first year of life. Finnish and Estonian infants also had increased concentrations of food allergen-specific and anti-insulin antibodies in their serum, early indicators of disrupted immune homeostasis.

Since *Bacteroides* and *Bifidobacterium* are the two major taxa that metabolize human milk oligosaccharides, the authors hypothesized that these genera play functionally similar roles but that the overrepresentation of *Bacteroides* in the Finnish and Estonian children had a differential effect on their immune system function. At the metagenome level, analysis of gene ontology categories revealed further distinctions between the Russian and Finnish microbiomes; lipid A and LPS biosynthesis genes were both strongly enriched in the Finnish cohort. *Bacteroides* strains are known to make lipid A variants that are structurally distinct (tetra- or penta-acylated) from canonical, hexa-acylated *E. coli* lipid A. Indeed, *Bacteroides dorei*, the dominant strain in the Finnish children, produced lipid A moieties with four or five acyl chains. When *Bacteroides* LPS was used to stimulate immune cells from either human peripheral blood or mouse bone marrow, its ability to induce cytokine production was severely impaired compared to LPS from *E. coli* or other bacteria. *Bacteroides* LPS also inhibited cytokine production when coadministered with *E. coli* LPS and was unable to induce endotoxin tolerance, as measured by inflammatory cytokine production, upon repeated exposure. This suggested that *Bacteroides* LPS may be less effective in providing early life signals required to maintain mucosal homeostasis (and prevent inflammation) than other forms of LPS (Figure 1). To test this idea, the authors examined the ability of different LPS variants to protect against type 1 diabetes (T1D) in the NOD mouse model. *E. coli* LPS delayed disease onset and severity, while *B. dorei* LPS administration had no effect. The authors concluded that, due to increased *Bacteroides* abundance, the children in Finland and Estonia have altered signaling via the LPS receptor Toll-like receptor (TLR) 4, and may, as a result, lack critical signals early in life that could prevent the onset of autoimmunity at a later time.

The idea that LPS exposure is important to educate the immune system and prevent allergic and autoimmune disease has a long history. Shortly after Strachan proposed the hygiene hypothesis in 1989, many studies noted differences in LPS (endotoxin) levels in different housing environments and associations between LPS in house dust and the incidence of asthma. For example, children raised on farms had reduced levels of allergy and asthma, and their homes were found to have an increased abundance of LPS in the dust, bedding, and mattresses (Braun-Fahrlander et al.,

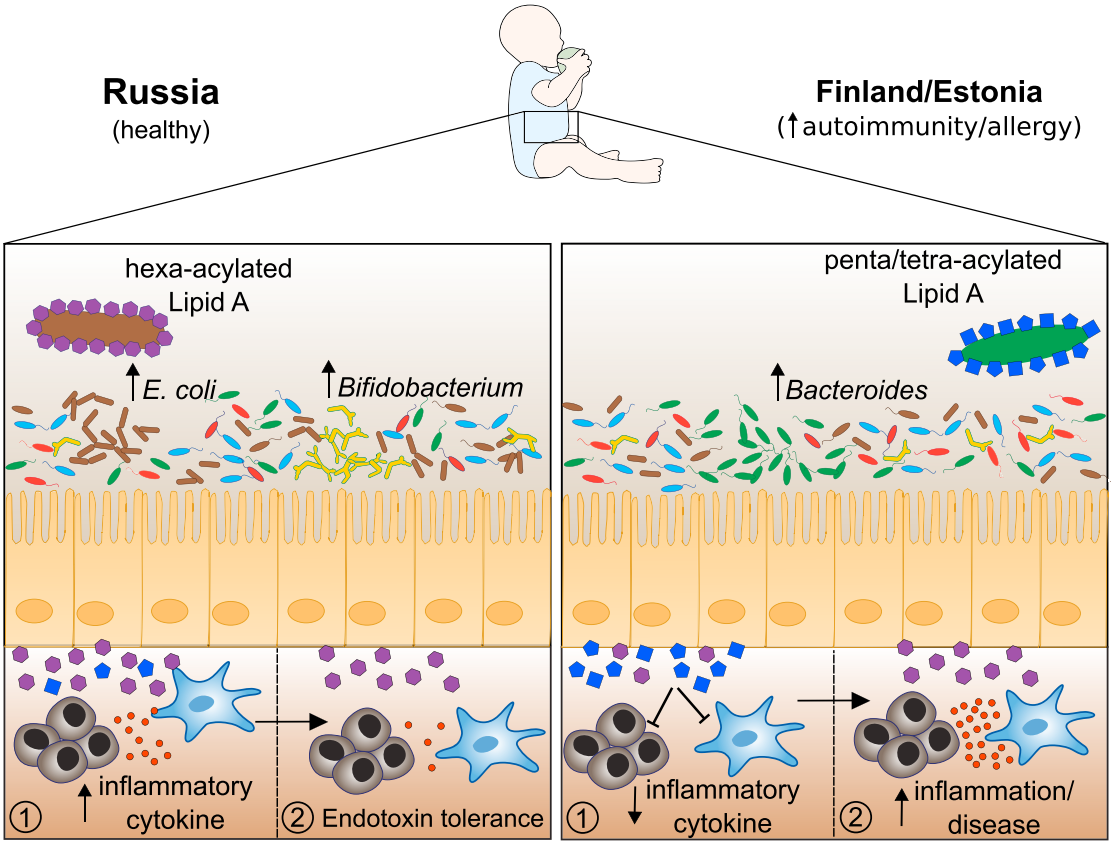

Figure 1. Early Life Exposure to *E. coli* Lipid A Induces Endotoxin Tolerance to Prevent Autoimmune and Allergic Disease
Children born in Russian Karelia have increased representation of *E. coli* in their microbiota, leading to greater exposure to inflammatory, hexa-acylated lipid A (1). Upon subsequent stimulation with lipid A, there is a reduced cytokine response, indicative of endotoxin tolerance (2). In Finnish and Estonian children, however, there is higher abundance of *Bacteroides*, with inhibitory penta- or tetra-acylated lipid A (1). Exposure to *Bacteroides* lipid A does not induce endotoxin tolerance, leading to exacerbated inflammatory responses later in life (2).

2002; von Mutius and Vercelli, 2010). These studies suggested that exposure to LPS (possibly through inhalation) could have an asthma-protective effect. Early animal model work showed that the increased susceptibility of C3H/HeJ mice to sensitization to food allergens was related to their inability to signal via TLR4 (Bashir et al., 2004). Neonatal administration of a cocktail of broad-spectrum antibiotics induced allergic responses to food in TLR4-sufficient mice similar to those seen in their TLR4-deficient counterparts, identifying intestinal bacteria as the source of the TLR4 ligand (Bashir et al., 2004). Several mouse model studies showed that NOD mice, which are prone to T1D, are protected from disease by oral or intraperitoneal administration of LPS, as the authors of the new study confirm (Vatanen et al., 2016).

The combination of metadata rich longitudinal sample collection, 16S gene profiling, and whole-genome shotgun (WGS) metagenomics performed in the present study has proved to be an effective hypothesis generating and screening platform for exploring possible links between early life microbiome composition and autoimmune risk in these cohorts. High-throughput 16S sequencing provides a cost-effective approach to characterize the community structure and taxonomic differences at higher levels. In combination with WGS metagenomics, the microbiome can be analyzed down to the species and strain level and supplemented with functional information, providing insight into "who is doing what," as reflected in the present study. By using relevant metadata for population stratification, this metagenomic analysis allowed the authors to establish biologically relevant comparisons and generate testable hypotheses. This screening platform approach could readily be applied to other diseases where dysbiosis may play an important role.

A limitation of this study is that its findings are, of necessity, correlative. The authors cannot establish a causal relationship between lipid A variants and the prevalence of autoimmunity in the populations studied. They also cannot explain what factors (presumably environmental) have driven compositional changes in the microbiome in the geographic locations studied. The Russian and Finnish study populations may also be exposed to environmental variables that function independent from the microbiota. Moreover, as the authors acknowledge, LPS signaling is likely only part of the story. For example, a recent report showed that *Veillonella*, one of the major lipid A producers in the Finnish children, is reduced during the first year of life in a cohort of asthmatic children in Canada (Arrieta et al., 2015). Administration of *Veillonella* as part of a four-species consortium was protective in a mouse model of asthma, suggesting that additional

factors can predispose to allergic diseases. Indeed, what remains unclear from the literature is how signaling through other TLRs early in life affects the development of autoimmune and allergic disease. LPS is not the only TLR ligand that can vary widely among different bacterial species. Flagellin, the ligand for TLR5, is also expressed in highly variable forms, but it is less clear how activation of this pathway influences the health and homeostasis of the host. Crohn's disease patients have increased serum antibody directed against the flagellin expressed by particular bacterial taxa (CBir) (Lodes et al., 2004; Targan et al., 2005), suggesting there may be differential stimulatory capacities among these TLR ligands as well. Other studies have demonstrated that stimulation of TLR2 by a polysaccharide derived from a *Bacteroides* strain, *B. fragilis*, protects against an experimentally induced colitis (Round et al., 2011; Round and Mazmanian, 2010). This brings up another interesting factor to consider in light of the current study. Although certain members of the microbiota, like *Bacteroides*, may inhibit TLR4 signaling, it is also possible that they provide beneficial cues through other receptors, or that they may be broadly immunosuppressive. It seems likely that a balance of multiple bacteria-derived signals is required for the maintenance of mucosal homeostasis, particularly in the gut.

Future studies delving into other innate immune pathways may provide a more complete picture of the signaling events required to establish healthy mucosal homeostasis early in life. Since metabolic pathways like glycolysis and protein catabolism are enriched in the Russian infant microbiotas, it would be interesting to also consider protective roles for bacterial metabolites. What becomes clear from the analysis performed in this paper is that the field is moving toward a systems biology approach, where the integration of data coming from different sources (DNA, RNA, proteins, metabolites) from both the host and microbes is needed to be able to understand the features that lead to the onset of immune disorders. With improved screening measures and increasing cohort size, there is greater opportunity to identify early life variations and develop novel, microbiome-modulating therapeutics to prevent disease later in life.

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