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INSIGHTS

IgA—about the unexpected

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In this issue of *JEM*, Nakajima et al. (https://doi.org/10.1084/jem.20180427) demonstrate that glycan-dependent, epitope-independent IgA coating of intestinal bacteria alters bacterial gene expression and metabolism. This conferred coated bacteria with fitness within the mucus niche and contributed to intestinal homeostasis through cross-phylum interactions.

IgA may be considered rather a maverick among antibody isotypes. Intestinal IgA is positioned exactly at the heart of host–microbial mutualism but risks its integrity in the harshest of protease-rich environments. It also (partly) departs from the traditional idea of immunoglobulin function: that the antibody-binding fragments (Fab) composed of one domain each of the heavy and light chains provide complementarity to bind the antigenic epitope, and the constant Fc region of each isotype heavy chain contains the chemical signals that tell the immune system what to do next. In the paper by Nakajima et al., glycosyl side chains direct binding to bacterial cell walls, causing alterations in bacterial metabolism and the ability of IgA-bound taxa to seed beneficial communities of microbes in the outer mucus layer of the large intestine (Nakajima et al., 2018). The emblematic monoclonal studied in their work was highly glycosylated and specific for OVA, which could be used to saturate the (Fab) "antigen" binding sites without affecting bacterial binding.

Both polymeric secretory IgA and its secretory component (SC), the residual polypeptide from the polymeric immunoglobulin receptor (pIgR), which remains covalently bound once the secreted IgA has been freed by pIgR proteolysis after epithelial transcytosis, are highly glycosylated (Mathias and Corthesy, 2011). These carbohydrate chains have been shown to interact with pathogenic bacteria either as adhesion competitors or by anchoring SIgA to the mucus layer to exert a functional protective effect (Schroten et al., 1998; Royle et al., 2003). Glycosylation has also been shown to allow binding to nonpathogenic bacteria by SIgA monoclonals specific for *Shigella*, *Salmonella*, and respiratory syncytial virus, and by isolated SCs (Mathias and Corthesy, 2011). The glycosyl chains are also protective against SIgA proteolysis in the harsh intestinal environment, including by bacterial pathogens that secrete specific IgA proteases as a pathogenicity mechanism.

This noncanonical maverick behavior of IgA must be taken in context. IgA has well-described canonical functions of high affinity binding allowing neutralization of toxins and viruses, and there is evidence that bacterial binding to nonpathogens is mediated through the Fab as well as through glycosyl interactions (Mathias and Corthesy, 2011). The importance of canonical (Fab) and noncanonical (glycosyl) binding of microbial molecules by SIgA has so far been unclear for a number of the ways in which the antibody isotype functions. These include limiting exposure of mucosal and systemic tissues to taxa of the intestinal microbiota (Macpherson and Uhr, 2004), delaying the induction of IgA in neonates through the protective effect of IgA in the maternal milk (Kramer and Cebra, 1995), and shaping the ultimate composition of the microbiota as neonates are colonized by successive waves of microbial taxa before reasonable stability of the microbiota is achieved (Rogier et al., 2014).

The mammalian microbiota is usually presented in terms of its estimated composition derived from metagenomic sequencing data or from the proportions of phylum- or genus-specific polymorphisms in the sampled consortia. All learning is an approximation of the truth: despite considerable oro-anal flow along the intestine, taxonomy of one site in the intestine gives only limited information about the different compositions of microbial taxa in other intestinal niches. Equally, knowledge of the genomes of different microbial taxa allows inference of possible overall microbial metabolic function in a particular consortium, but does not necessarily tell us whether those genome-encoded functions are being used or not. The paper by Nakajima et al. (2018) links noncanonical glycosyl-mediated IgA binding to bacteria with its consequences on bacterial gene expression in the outer mucus layer of the colon and stabilization of a healthy microbial consortium.

Insights from Macpherson and Ganal-Vonarburg.

The outer mucus layer of the colon contains rather dense consortia of intestinal microbes, in contrast to the tightly arranged mucin polymers of the inner mucus layer, which are largely bacteria-free. Previous gnotobiotic studies that compared bacterial gene expression and metabolite compositions in the outer mucus layer with the lumen of the colon have shown that despite their proximity, these are two rather distinct niches (Li et al., 2015).

Recently, the Mazmanian group engineered mutants of *Bacteroides fragilis*, a human commensal, with an altered surface capsule, which were better targets for IgA binding. This rendered the bacteria more competitive and stable within the gut and enhanced attachment to intestinal epithelial cells shown in vitro. In vivo, the coating with IgA seemed to promote location of *B. fragilis*

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within the mucus niche. This was confirmed with other bacterial strains using IgA-deficient mice (Donaldson et al., 2018).

The observation that IgA binding to commensal bacteria can shift the bacterial gene expression to promote survival and homeostasis within the intestine is now consolidated in the paper by Nakajima et al. (2018). The authors generated mice whose intestinal IgA was predominantly directed against OVA by adoptively transferring OT-II (OVA specific) $CD4^+$ T cells into T cell–deficient hosts and exposing them to OVA in the drinking water. The resulting OVA-specific antibodies coated intestinal bacteria but could still bind OVA through free Fab sites. After generating hybridomas from the small intestinal lamina propria cells of these mice, a highly glycosylated OVA-specific IgA clone was chosen for further experiments. This IgA bound different microbial taxa, but most efficiently coated *Bacteroides thetaiotaomicron* through its LPS: the binding was not blocked by OVA, indicating that it did not depend on classical Fab epitope interactions.

To see how the 7-6IgA hybridoma would function in vivo, a "backpack" experiment was performed. 7-6IgA or control hybridoma cells were transplanted onto $Rag1^{-/-}$ mice, so the recipients expressed a single monoclonal (7-6 or control) Ig. When these animals were colonized with *B. theta* after treatment with antibiotics, Nakajima et al. (2018) showed that although 7-6IgA does not bind directly to MAFF, it induced the expression of a group of polysaccharide utilization transcripts in *B. theta*, collectively named Mucus-Associated Functional Factors (MAFFs).

MAFFs are shown to be generally important in host–microbial mutualism. (1) They contribute to in vivo fitness. Other commensal Bacteroidales in both mouse and humans have orthologous genes that are up-regulated in bacteria inhabiting the mucus niche. Deletion of two *B. theta* MAFF genes (Δ*maffC* and Δ*maffD*) caused growth retardation in minimal medium in vitro and reduced fitness in mouse competition assays in vivo, including smaller size than the wild-type strain. Induction of MAFF genes seemed to enhance the ability of *B. theta* to use dietary carbohydrates. (2) MAFFs determine the phenotype of *B. theta* in the presence of other taxa. The larger size of *B. theta* wild-type compared with the Δ*maff* strain is lost in germ-free mice, even when the wild-type strain was small, indicating that the size effect of the MAFF gene function in vivo depends on the presence of a diverse microbiota, possibly through cross-feeding. (3) MAFF function can influence the composition of other taxa in the microbiota and the resilience of the host to inflammatory challenges. Colonization of antibiotic-treated specific pathogen-free mice with the wild-type *B. theta* strain compared with the Δ*maff* double mutant increased the abundance of Clostridiales and the gene expression profile of Firmicutes, resulting in protection from chemically induced colitis with higher short-chain fatty acid production. The beneficial effects on microbiota composition appear to be enhanced in the 7-6IgA backpack model. Hence, the data of Nakajima et al. (2018) highlight the role of epitope-independent, glycan-mediated binding of IgA to bacteria followed by induction of MAFF genes. This promotes bacterial fitness within the outer mucus layer and interphylum interactions among commensal bacteria, improving intestinal homeostasis.

Gordon and colleagues have previously shown beneficial effects of monoclonal IgA in a similar backpack model using *B. theta* monocolonized $Rag^{-/-}$ mice carrying a hybridoma for a specific polysaccharide capsular epitope. Here, the loss of binding was highly specific according to the particular capsular polysaccharide gene locus that was disrupted in a transposon screen, and an acid hydrolysis–sensitive epitope could be extracted for binding studies. Specific IgA binding to this *B. theta* epitope limited innate immune responses in the host intestine and protected the bacterium from oxidative stress (Peterson et al., 2007). The group has subsequently reported another IgA monoclonal against the LPS O-Ag polysaccharide that can also modulate bacterial gene expression in vivo (Peterson et al., 2015).

These experiments start to dissect the multidimensional interplay between IgA binding to intestinal microbes, bacterial metabolism, innate responses of the mucosal immune system, niche occupancy within the outer mucus layer, and interactions with other commensals. They also illustrate that nonconventional binding and amplification of natural specificities (Bunker et al., 2017) may be sufficient for IgA function. Despite the elegance of these experiments, they are likely facets of a much bigger picture. Just as alternative behavior of mavericks is often successful in business, perhaps reducing IgA canonical binding constraints is part of its secret of success and can be exploited therapeutically, such as modulation of the microbiota to protect premature infants from necrotizing enterocolitis.

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