IgA Targets the Troublemakers

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Resident microbes within the gastrointestinal tract are known to influence the progression of inflammatory bowel diseases (IBD); however, the identity of the bacteria that exacerbate inflammation remains unknown. In the most recent issue of Cell, Palm et al. (2014) utilize the host immune response as a way to identify colitogenic bacteria.

IBD such as Crohn’s disease (CD) and ulcerative colitis (UC) have become prominent problems over the last several decades within western civilizations. The etiology of these diseases has remained enigmatic but is thought to be the result of a complex interplay between genetic susceptibility, environmental stimuli, and inappropriate immune responses. The environmental component is thought to involve bacteria as IBD patients have often been found to harbor altered intestinal microbial communities (microbiota) and symptoms are often ameliorated with antibiotic treatment (Manichanh et al., 2012). Supporting this, animal models of IBD rely on the presence of commensal bacteria, as disease cannot be induced in germ-free settings. Decades of research have failed to identify individual pathogens that can induce IBD alone, suggesting that compositional changes within the microbiota might influence disease. However, it has been difficult to identify which members of the microbiota are colitogenic, as most studies have relied on correlation analysis of the microbiota in healthy versus sick patients. Given the large amount of interpersonal variation in resident bacterial communities between individuals and many genetic susceptibility loci that may feed back to influence community composition, it is likely that some bacteria may cause problems only in a few individuals while being perfectly benign in others. So how can one identify which organisms are causing the problem in any given person? In the most recent issue of Cell, Flavell and colleagues let the host immune system point the way (Palm et al., 2014).

Humans dedicate a lot of energy to making over 2 g of Immunoglobulin A (IgA) a day, making it the most abundant antibody in the human body. A majority of IgA is found within the gastrointestinal tract where it functions in multiple ways to protect the intestinal tissue from invasion and destruction by commensal and pathogenic bacteria (Mantis et al., 2011). It accomplishes this task by neutralizing bacterial toxins and sculpting epitope expression of the microbiota to prevent motility or immunogenicity (Cullender et al., 2013; Peterson et al., 2007). Thus, the production of IgA is a key mechanism to directly influence the function and structure of the microbiota and maintain intestinal health.

Based on the importance of IgA, the authors posited that IgA might be more reactive against disease-driving bacteria. To test this, the authors utilize magnetic bead purification and flow cytometry to separate IgA-coated from noncoated bacteria followed by 16 s rRNA gene sequencing to identify members of the microbiota directly targeted by the host immune system. IgA within the gut can be generated in two ways, one depending on interactions with T cells (T cell-dependent, or TD) (Caselli et al., 2012) while the other relies on production of the cytokines BAFF and APRIL by dendritic cells and is independent of T cell direction (TDI) (Figure 1) (Bemark et al., 2012). TDI interactions are thought to mainly produce low-affinity oligoclonal antibody while TD-generated antibodies have greater affinity and higher specificity for a given antigen. Thus, TD-generated antibodies should not have off-target effects and provide more effective immunity against problem-causing microbes. Traditional approaches to assay antibody production, such as ELISA, require a lower affinity for binding when compared to flow cytometry, which requires $10^9$ l/mol; thus the FACS-based IgA approach (IgA-SEQ) used by Flavell and colleagues enriches for the high-affinity antibody against the microbiota that has likely been generated by TD interactions (Slack et al., 2012). Supporting this, high levels of IgA coating of most members of the microbiota are lost in animals that lack T cells. Using this approach, the authors demonstrate that the microbiota found within the IgA+ fraction in mice is distinct from the non-IgA-coated bacteria. In particular, the IgA+ fraction was enriched for unclassified but abundant Bacteroidales group S24-7, segmented filamentous bacteria (SFB), Lactobacillus, and an unclassified genus of Erysipelotrichaceae, indicating that IgA targets specific members of the microbiota.

To test whether IgA coating could identify colitogenic bacterial members, these authors utilized an animal that lacked expression of the inflammasome adaptor protein, ASC. They had previously demonstrated that the development of colitis within this model depends on the presence of Prevotellaceae species and can be transferred to wild-type specific pathogen-free (SPF) mice that were cohoused with ASC−/− animals (termed SPF31/32) (Elinav et al., 2011). Employing IgA-SEQ to either SPF or SPF31/32 mice revealed that while IgA still targeted SFB most significantly in both SPF and SPF31/32 mice, an unclassified genus from Prevotellaceae was most abundantly coated with IgA in SPF31/32 animals. The consistent, high-level targeting of SFBs, which are intimately associated with the epithelium, may suggest that proximity is a major determinant of IgA induction (Caselli et al., 2012). Since Prevotellaceae were previously identified to be colitogenic in this model, these data suggest that IgA targeting by the host might identify members of the microbiota that elicit disease.
Can this assay be used to identify colitogenic bacteria in humans suffering from IBD? To examine this, the authors performed IgA-SEQ on 27 people with CD, 8 with UC, and 20 healthy controls. As previously reported, the percentage of bacteria coated with IgA was significantly increased in people with CD or UC. As expected, there is a high amount of bacterial diversity between individuals; however, there were 35 species of bacteria that were found to be abundantly coated in individuals with IBD. Several species were found in both healthy and IBD patients, but were only highly coated by IgA in IBD patients. Additionally, the authors identify specific taxa that appear to be more highly coated specifically in UC or CD patients.

Many studies on microbial communities have stopped at identifying potentially colitogenic members with such 16S rRNA gene surveys; however, these authors took the impressive approach of creating culture collections from IBD patients and testing these bacterial strains in vivo mouse models. Members of the microbiota from 11 IBD patients were isolated and grown within the laboratory to create personalized culture collections. Representative bacterial members were rationally chosen to form an IgA+ and IgA- consortia and used to colonize germ-free mice. Importantly, colonization of animals with either group of bacteria did not elicit intestinal disease, indicating that these bacteria were not colitogenic on their own. However, upon challenge with the DSS model of colitis, mice colonized with the IgA+ consortia exhibited more severe intestinal disease and bleeding while animals colonized with the IgA- bacteria showed minimal inflammation, indicating that the species present within the IgA+ consortia exacerbated disease. All but two members of the cultured human microbiota were able to stably colonize mice, highlighting the value of germ-free model systems to test the functionality of bacterial members isolated from human patients.

In agreement with the idea that close association with the host induces IgA binding, members from the IgA+ consortia were more invasive and found more prominently within the mucus, a region normally thought to be depleted of bacteria in healthy intestines. A recent paper from Ley and colleagues used this same technique to identify that IgA-specific responses toward flagellin cause a downregulation of flagella within the microbiota to decrease motility. Thus, a major function of IgA might be to prevent bacterial expression of genes that are potentially harmful to the host.

Given that there is not one single gene polymorphism or one single microbe that is linked to IBD, it is likely that future treatment options for this disease will need to be more personalized. Further validating this need, the authors used whole-genome sequencing of two isolates of B. fragilis that were differentially coated with IgA to show that although these organisms are taxonomically related, they were genetically distinct. More importantly, colonization of germ-free mice with highly coated IgA+ B. fragilis elicited worsened disease when compared to animals colonized with a B. fragilis strain that was IgA-, highlighting the limitations of using 16S rRNA gene sequencing surveys alone that generally cannot differentiate bacterial strain level differences.

These exciting findings suggest a mechanism to identify bacterial organisms that worsen the development of intestinal disease, allowing specific organisms to be therapeutically targeted. One
Tethering Viral Restriction to Signal Transduction

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Tetherin serves as an innate sensor of viral infection in addition to its role in inhibiting virus release from infected cells. In this issue, Galão et al. (2014) provide important insights into the mechanism of virus-induced signal transduction by tetherin.

Tetherin (BST-2 or CD317) is rapidly upregulated on the cell surface by type I interferons, where it prevents the detachment of virus particles from infected cells. This activity reflects the unique topology of tetherin, which includes a short cytoplasmic tail followed by an N-terminal transmembrane domain, an extracellular coiled-coil domain, and a C-terminal glycosyl-phosphatidylinositol anchor (reviewed in Neil [2013]). These structural features allow opposite ends of tetherin dimers to partition between viral and cellular membranes during viral budding, thereby linking nascent virions to the plasma membrane (Neil, 2013).

Although first identified as an HIV-1 restriction factor, tetherin is now understood to have broad activity against diverse families of enveloped viruses, many of which have in turn evolved countermeasures to tetherin (Neil, 2013). Among the primate lentiviruses, at least three different viral gene products have acquired the ability to counteract tetherin. Whereas Nef is used by the majority of simian immunodeficiency viruses (SIVs) to antagonize the tetherin proteins of their nonhuman primate hosts, HIV-1 Vpu and HIV-2 Env have acquired the ability to counteract human tetherin due to the absence of a five amino acid sequence in the cytoplasmic tail of human tetherin that confers susceptibility to Nef (Neil, 2013). While these observations imply that tetherin has potent antiviral activity, the immunological mechanisms contributing to the antiviral effects of tetherin are not fully understood.

Earlier reports by Galão et al. and others demonstrated a role for tetherin in signal transduction (Cocka and Bates, 2012; Galão et al., 2012; Tokarev et al., 2013). Under conditions of protein overexpression or virion-induced clustering, tetherin activates NFκB by a pathway involving the recruitment of the mitogen-activated protein kinase TAK1. NFκB activation was found to be dependent on sequences in the extracellular domain of tetherin that participate in virion retention, and on a dual-tyrosine motif in the cytoplasmic tail (Y9DYSR) previously implicated in the constitutive cycling of tetherin between the plasma membrane and the trans-Golgi network (Galão et al., 2012). However, signaling is separable from the role of this dual-tyrosine motif in tetherin internalization. Indeed,