A Microbe-Dependent Viral Key to Crohn’s Box

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Once Pandora unlocked her fateful box and liberated the evil forces within, it became impossible to put them back. Now, new work on Crohn’s disease suggests the existence of a viral “key” that irreversibly renders a genetically susceptible mouse prone to pathogenesis. Indeed, dangerous liaisons among host genotype, viral infection, intestinal injury, and trillions of gut microbes may in part determine which individuals progress to a full-blown disease state. These findings suggest that viral and bacterial triggers may serve as therapeutic targets for Crohn’s and prompt new hypotheses that relate inflammation, host immune status, microbial community structure, and human health.

MAKING SENSE OF GWAS

We are currently in a new era of biomedical research when genetic components that influence disease can be identified by genome-wide association studies (GWAS), but the need to uncover the mechanisms that link genetic polymorphisms to disease raises challenging scientific questions that call for rigorous experimentation. A recent report that examines one of the genes identified as a risk factor for Crohn’s disease has demonstrated how follow-up studies that focus on genes identified in GWAS can not only lead to insight into protein function but also pave the way to defining the environmental factors that drive disease pathogenesis (1).

When the human genome project was completed nearly 10 years ago (2), researchers predicted that a new era of personalized medicine based on genetic testing would follow. However, despite the identification of numerous genetic polymorphisms that can predispose individuals to Crohn’s disease, the environmental factors that can trigger this disease in a subset of individuals who are at risk remained largely ill-defined (3). The initial results from multiple international human microbiome projects, mostly driven by a reduction in the cost of high-throughput sequencing, have emphasized the need to take into account not only our human genome, but also the genomes within the trillions of microorganisms that are associated with each human being (the microbiota) (4, 5). The combination of human surveys and more controlled experimentation in animal models has implicated the microbiome in a variety of disorders, ranging from metabolic syndrome (6–14) to inflammatory bowel diseases (IBDs) (15, 16). IBDs form a spectrum of conditions with inflammation in the gut that ranges from ulcerative colitis, a neutrophilic inflammatory process limited to the colon, to Crohn’s disease, which occurs predominately in the distal small intestine and colon and consists of lesions that span the depth of the intestinal wall (transmural lesions) and often contain granulomatous inflammation.

A pioneering study recently published in the journal Cell suggests that the pathogenesis of Crohn’s disease may be even more complex than originally anticipated (1). The authors show that in a susceptible host, disease progression requires both a trigger (viral infection) and the presence of the gut microbiota (Fig. 1). These findings are consistent with those of earlier studies of mice and humans. Previous research in humans has implicated the autophagy gene ATG16L1 in Crohn’s, but carriers of the specified disease variant (~50% of Europeans) have a less than twofold increase in susceptibility to Crohn’s disease (17–19). ATG16L11039 mice—a model of Crohn’s disease in which expression of the mouse ATG16L1 gene is perturbed (hypomorphic)—show decreased protein production and a reduction in autophagy (20), changes intended to mimic the human ATG16L1 T300A polymorphism, which exhibits reduced but not abrogated function (21). These changes are accompanied by abnormal histology and gene expression in their Paneth cells—epithelial cells at the base of mucosal crypts in the ileum region of the small intestine of the gut that are involved in local immunity. These symptoms are similar to those seen in Crohn’s disease patients who are homozygous for the ATG16L1 disease variant.

In their new work, Cadwell et al. show that this phenotype can be triggered by exposure to a virus commonly found in mouse facilities, the murine norovirus (MNV) (1). When ATG16L11039 mice were re-derived in an enhanced-barrier facility, their gut symptoms were ameliorated. Furthermore, the Crohn’s-like phenotype was induced upon exposure to a specific strain of mouse norovirus (MNV CR6) but not after exposure to a transient infection of a closely related virus (MNV CW3).

Perhaps most strikingly, after the intestinal tissue was injured with a chemical assault—treatment with dextran sodium sulfate (DSS)—the ATG16L11039 mice infected with MNV CR6 displayed some of the hallmarks of Crohn’s disease, including an increased number of lymphoid aggregates (collections of lymphocytes in the epithelial layer) and increased inflammation in the muscularis, a layer of smooth muscle in the intestinal tract. This response was specific to MNV CR6 and not seen in mice infected with MNV CW3. The observed viral strain–dependent phenotype is interesting, because MNV CR6 is capable of persistent infection (22). These observations suggest that the level of inflammation, the amount of adaptive immune response required to clear the virus, and the collateral damage induced by the virus are all key factors in Crohn’s disease pathogenesis.

Together, these findings link host genotype and viral infection with a response to chemical challenge, resulting in Crohn’s-like symptoms, a virus–plus-susceptibility gene interaction. However, the story gets even more complicated, because this interaction was shown to depend not only on the host inflammatory cytokines TNF-α and interferon-γ but also on the gut microbiome. Treatment of MNV CR6–infected ATG16L11039 mice with broad-spectrum antibiotics resulted in protection against intestinal injury from DSS. These findings are consistent with other models of IBD that are clearly dependent on the presence of gut bacteria and can be produced in germ-free mice colonized with defined bacterial consortia in the absence of a viral trigger (23).
Fig. 1. Trigger points. Shown here is a proposed model for the viral trigger of colitis in ATG16L<sup>−/−</sup> and WT mice. (A) In the absence of viral infection, the intestinal crypts of ATG16L<sup>−/−</sup> mice are essentially the same as those of WT mice. The microbiome consists of mainly Firmicutes and Bacteroidetes, with Proteobacteria at low relative abundance. The mucus layer that lines the gut is intact, preventing bacterial translocation. (B) Both WT and ATG16L<sup>−/−</sup> mice experience a similar initial response to MNV Cr6 infection. Inflammation during acute infection may lead to changes in the microbiota, increased permeability of the epithelial barrier as a result of gaps in the mucus layer, and activation of innate effector cells, such as Paneth cells, neutrophils, and natural killer (NK) cells. The mucosal immune response to the virus also includes adaptive responses by B and T cells that presumably contribute to viral control. The coordinated immune response between the adaptive and innate immune systems must also neutralize and clear any microbes that penetrate the epithelial barrier during inflammation. (C) In WT mice, resolution of viral infection goes well. Defensins and antimicrobial peptides (including lysozyme-P) are secreted to re-sterilize the crypts, mucus production by goblet cells increases the mucus barrier, and T cell–dependent and –independent IgA production against offending bacteria lead to their exclusion and removal from the mucosal layer. (D) In ATG16L<sup>−/−</sup> mice, the resolution phase of the viral infection is abnormal and colitis is established. The mechanisms that lead to this pathology are still not defined, but some of the possibilities are described in the text. In this state, the mucus layer has many functional gaps, the bacterial population of the microbiome may have shifted to inflammation-resistant microbes (more Bacteroidetes and/or Proteobacteria and less Firmicutes), the Paneth cell population is inflamed, and granulomatous inflammation spreads in the mucosal layer. CTL, cytotoxic T lymphocyte; DC, dendritic cell; NK, natural killer cell; PC, plasma cell; T<sub>H1</sub>, T helper cell.
IMMUNE INTERPLAY

These studies suggest that the microbiota is a key component of colitis; in mouse models, colitis develops in the context of abnormal adaptive or innate immune responses that fail to prevent translocation across the epithelial layer and the presentation of gut bacteria to immune cells, or result in excess activation of the adaptive immune system. The influence of the innate immune response in regulating the composition of the gut microbiota has been observed in multiple animal models. A comparison between the microbiota in MyD88-deficient (MyD88KO) and Rag1-deficient mice—the former with impaired innate immune responses and the latter with no adaptive immune response—revealed that the lack of an innate immune response had a greater impact on the composition of the gut microbiota (8). MyD88KO mice fed a standard low-fat plant-polysaccharide diet had an increased abundance of Bacteroidetes and a decreased abundance of Firmicutes, two of the dominant bacterial phyla in the mouse gut, relative to the Rag1-deficient or wild-type (WT) mice (8). Similarly, a comparison of MyD88KO mice with heterozygous MyD88KO/+ mice, both generated in a non-obese diabetic (NOD) genetic background, revealed a relative decrease in the ratio of Firmicutes to Bacteroidetes in the mice that lacked an intact innate immune response (the MyD88KO mice), accompanied by an increased relative abundance of three bacterial families: Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae (13). In addition, α-defensins, groups of Paneth cell–derived antimicrobial peptides that provide defense against enteric pathogens, have been associated with changes in small intestinal microbial ecology. Mice that lack Mmp7, a gene that encodes a protein involved in processing mouse defensins, have a significantly higher abundance of Firmicutes and a lower amount of Bacteroidetes than do WT controls. Conversely, mice that express a human α-defensin gene (DEFA5) have decreased and increased relative abundances of Firmicutes and Bacteroidetes, respectively, relative to the WT animals (24). Together, these results suggest that changes in the innate immune response as a result of the ATG16L1 mutation could also alter the gut microbiota.

A fascinating observation from Cadwell et al. is that susceptibility to colitis induction can be switched from off to on; mice in a colitis-resistant state before infection with the virus become susceptible to injury-induced colitis after viral infection, and, once the colitis-sensitive state is induced, cannot go back to a colitis-resistant state. Multiple mechanisms could account for this pattern (Fig. 1).

One possibility is immunological memory: Once a peripheral tolerance to a gut microbial antigen is lost and memory T cells are created, it may not be possible for these T cells to become re-tolerized. Indeed, because of changes in sensitivity and migration patterns that are characteristic of memory T cells, they may no longer be indifferent to the presentation of their cognate antigen in the gut (25). A second possible mechanism is that an immune-compromised host such as the ATG16L1HM mouse could have a weakened gut barrier function that, once altered by the virus, may remain weakened, so that it cannot prevent penetration of the gut microbiota to stop inflammation and reestablish equilibrium. A third possibility is that viral infection changes the gut microbiota in a pathological way, giving rise to a colitis-propagating milieu. Inflammation may provide a selection pressure for a microbiota enriched in inflammation-resistant microbes that may also be pro-inflammatory, resulting in a positive feedback loop that can be stopped only by the use of broad-spectrum antibiotics to block this altered microbial community. Finally, it is possible that the phenotype observed in the ATG16L1HM mouse model is related to alterations in the host’s adaptive immune response. A reduction in the ATG16L1 gene product has been associated with increased antigen presentation (26), and immunoglobulin A (IgA) antibodies specifically directed toward symbiotic bacteria have been shown to mediate homeostasis in the gut (27). Alterations in the T cell–dependent antimicrobial IgA could result from ATG16L1-dependent perturbation of antigen presentation to T cells in the gut lamina propria, which could give rise to an increased inflammatory response in the Paneth cell population.

All of these diverse findings suggest that it is necessary to take into account multiple facets of the human microbiome when considering complex diseases such as Crohn’s. Polymorphisms in key susceptibility genes in our human genome, such as ATG16L1, may only serve to weaken the first link in the chain that protects the intestinal epithelia from a combination of viral infection, microbial stimulation of inflammation, and other dietary or xenobiotic factors. These mechanistic links suggest a new model for translational medicine, in which the root causes of Crohn’s disease are not targeted. Instead, the various triggers of disease could be identified and targeted therapeutically. These potential points of intervention could include specific viral strain vaccines, manipulations of the bacteria in the intestine through antibiotics or dietary modification, or preventative measures to block the host response to these challenges (Fig. 1).

One key need in moving forward will be to better define and characterize the human viriome. A small number of studies have begun to explore the immense diversity of DNA viruses that colonize the distal human gut (28, 29), as well as RNA viruses, which can be introduced through the diet (30). A recent analysis of the fecal viromes of healthy adult female monozygotic twins and their mothers at three time points over the course of a year revealed that each virome was unique to each individual regardless of their genetic relatedness, but remained remarkably stable over time (29). Although Cadwell et al. do not speculate that noroviruses are the trigger for human Crohn’s disease, it is important to note that Norwalk-like viruses are common and have been shown to account for 93% of nonbacterial diarrheal outbreaks in the United States (31). Although neglected as human pathogens unless found to be the cause of large disease outbreaks, it may be that these minor pathogens wreak serious havoc on the unfortunate few that are genetically susceptible. As Cadwell et al. have done with murine noroviruses, the isolation and characterization of viruses from healthy people and Crohn’s disease patients will help to determine whether specific viruses are associated with the human disease and, if so, to define the range of strains that can trigger abnormalities in susceptible human cell lines or mouse models.

Many important questions about the observed virus–susceptibility gene interaction remain. For example, are specific bacteria involved in the interaction, or is the phenotype dependent only on the cumulative load of microbes in the gut? What other viruses can or cannot trigger this phenotype? Is viral persistence the key component, or is there something unique about MNV CR6? What is the mechanism behind this interaction, and what mechanistic components represent weak links?
that could be targeted therapeutically? Despite all these provocative questions, one thing is certain: These investigations promise to shed new light on the complex interactions between the microbiome and our pathophysiology, with the hope of developing new avenues to prevent disease.

REFERENCES AND NOTES


32. Competing interests: The authors declare no competing interests.