Understanding gene-environment interactions in a mouse model of Crohn's disease

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Crohn's disease and ulcerative colitis are autoimmune chronic inflammatory disorders of the intestine that are known collectively as inflammatory bowel diseases (IBDs). The incidence of IBDs has stabilised over recent years in regions of high prevalence, such as the USA and northern Europe; however, the incidence is on the increase in regions of low incidence, such as Asia and southern Europe (Loftus, 2004). Studies of identical twins have shown around 50% concordance for Crohn's disease, indicating that both genetic and environmental factors influence susceptibility to IBD (Halfvarsen et al., 2003). In addition, it is widely accepted that infection by some bacterial strains can increase the likelihood of developing IBD. However, the aetiology of these debilitating conditions remains to be fully elucidated. A recent report provides the first example of a viral infection that causes altered phenotypic responses in mice carrying a mutation in a Crohn's disease susceptibility gene (Cadwell et al., 2010). The results of this study demonstrate that modulation of complex interactions between disease-susceptibility alleles, commensal bacteria and viral infection can influence disease pathology.

Approximately 30 susceptibility loci for Crohn's disease have been identified to date, some of which are associated with genes that control autophagy (Barrett et al., 2008). For example, there is a strong association between the incidence of Crohn's disease and the presence of polymorphisms in autophagy-related gene 16-like 1 (*ATG16L1*) (Hampe et al., 2007). Autophagy is an important cellular process by which double-membrane-bound vesicles containing cellular components or pathogens are transported to lysosomes for degradation or recycling. Although it is thought that errors in this process can lead to inefficient bacterial killing and defective antigen presentation, it is not clear which downstream functions of the autophagy pathway in which ATG16L1 is involved contribute to the pathogenesis of Crohn's disease. Defects in the ATG16L1 protein might result in reduced production of antimicrobial peptides by small-intestinal Paneth cells (specialised antimicrobial cells located at the crypt base of the small-intestinal epithelium) and increased production of secreted proinflammatory cytokines by macrophages (Cadwell et al., 2008; Saitoh et al., 2008). These abnormalities might result in an altered intestinal microbiome and, in turn, might lead to increased susceptibility to the initiation of intestinal inflammation. The *ATG16L1* polymorphism is present in around 50% of individuals in the European population; however, it confers only an ~twofold increase in Crohn's disease susceptibility (Hampe et al., 2007; Prescott et al., 2007), indicating that gene-environment interactions are probably required for disease initiation and/or progression.

Cadwell et al. previously established mouse colonies with hypomorphic Atg16L1 protein expression (Atg16L1^{HM}), which leads to aberrant disorganised granule packaging and a reduction of granule number in Paneth cells, as well as defective autophagy in thymocytes. The aberrations in Paneth cell phenotype that were observed in Atg16L1^{HM} mice that were raised in conventional animal house conditions were similar to those observed in patients with active Crohn's disease that also carry a disease-associated *ATG16L1* variant (Cadwell et al., 2008). In a follow-up study, the authors showed that Atg16L1^{HM} mice that had been re-derived by embryo transfer from conventional animal house facilities to enhanced specific-pathogen-free conditions had Paneth cells that were indistinguishable from those of wild-type littermate control mice. This finding suggested that bacterial or viral infection was the source of the abnormal Paneth-cell phenotype in the Atg16L1^{HM} mice housed in conventional conditions. Subsequent experiments identified that infection with persistent murine norovirus (MNV) caused abnormal Paneth cell morphology in the context of the hypomorphic Atg16L1 protein. By contrast, a non-persistent strain of MNV did not induce the same morphological changes in Paneth cells (Cadwell et al., 2010).

The pathological basis of IBD is incompletely understood, but it is generally considered to be an exaggerated inflammatory response to intestinal lumenal contents, triggered by defective intestinal barrier function. Dextran sulphate sodium (DSS) is administered orally to induce colitis in mice: it causes a breakdown of intestinal barrier function by an unknown mechanism, causing exposure of microbial-derived antigens to the immune system. The acute effects of DSS are believed to mainly involve the innate immune system, which is similarly activated in both ulcerative colitis and Crohn's disease. Characteristics of acute DSS colitis include weight loss, bloody diarrhoea, ulcerations, granulocyte infiltration and decreased epithelial cell proliferation (Okayasu et al., 1990). In the recent study by Cadwell et al., DSS was administered to Atg16L1^{HM} mice to determine whether aberrant intestinal pathology was observed as a result of the interaction between compromised barrier function, commensal bacteria and the Crohn's disease susceptibility allele *ATG16L1* (Cadwell et al., 2010). Atg16L1^{HM} mice did not display altered susceptibility to DSS-induced colitis compared with wild-type mice under normal conditions. However, when the mice were infected with persistent MNV prior to the onset of DSS-induced colitis, Atg16L1^{HM} mice, but not wild-type mice, showed more severe pathology and several more characteristics of human Crohn's disease.

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Additional characteristics observed in Atg16L1^{HM} mice included increased inflammation in the muscularis and associated mesenteric fat and blood vessels, increases in lymphoid aggregates, subserosal fibrosis, hypertrophy of the muscularis propria and proximal epithelial hyperplasia. Although pathology of the small intestine is not normally observed in DSS-induced colitis, Atg16L1^{HM} mice that were persistently infected with MNV before receiving DSS showed atrophy of the small intestine and blunted villi, which are characteristic features of human Crohn's disease, coeliac disease and norovirus-induced gastroenteritis. Mice infected with a non-persistent strain of MNV prior to receiving DSS, and mice that received DSS and a persistent strain of MNV simultaneously, did not show these features. These findings demonstrate that the timing of infection can determine the consequence of the interplay between a specific virus and a Crohn's disease susceptibility gene.

Immune responses that are mediated by lamina propria mononuclear cells and are caused by inappropriate reactions to commensal bacteria result in overproduction of proinflammatory cytokines such as tumour necrosis factor-α (TNFα) and interferon-γ (IFNγ). These cytokines play a substantial role in the pathogenesis of Crohn's disease, and anti-TNFα therapy is often used to treat patients with this disease. Blocking TNFα and IFNγ in persistently infected DSS-treated Atg16L1^{HM} mice dramatically reduced Crohn's-disease-like pathology, whereas treatment with blocking antibodies to these cytokines in uninfected DSS-treated Atg16L1^{HM} mice had no effect (Cadwell et al., 2010). These inflammatory cytokines are therefore necessary for the virus–susceptibility-gene effect on pathology in the DSS-induced colitis model. The effects of commensal bacteria were also assessed: antibiotic treatment (which eliminates the intestinal microbiota) prevented the abnormal pathologies observed in persistently MNV-infected DSS-treated Atg16L1^{HM} mice. Therefore, commensal bacteria are also required for the virus–susceptibility-gene effect in this model of Crohn's disease.

Cadwell et al. previously described a role in Crohn's disease for the autophagy pathway specifically in Paneth cells. More specifically, autophagy-deficient Paneth cells were shown to have elevated expression of genes associated with inflammatory responses (Cadwell et al., 2009). From the current study (Cadwell et al., 2010), it is not clear whether Paneth cell abnormalities contribute to the aberrant pathology observed in persistently MNV-infected Atg16L1^{HM} mice with DSS-induced colitis. MNV was not detected in Paneth cells, suggesting that direct infection of Paneth cells does not cause the observed pathologies. To examine this issue directly, it would be informative to cross the Atg16L1^{HM} mouse strain with Gfi1-deficient mice, which lack Paneth cells (Shroyer et al., 2005), and to determine whether the resulting strain was protected from Crohn's-disease-like symptoms when infected by a persistent strain of MNV and exposed to DSS. Follow-up studies that examine the effect of persistent MNV infection in mice that are hypomorphic for single or multiple autophagy-related susceptibility alleles, such as *NOD2*, and studies that involve modulation of colonic bacteria in the presence of persistent MNV infection in mouse strains carrying genes that are associated with differential Crohn's disease susceptibility in humans, should help to further elucidate the aetiology of Crohn's disease.

These findings in mice provide an example of how genetic and environmental factors that do not cause overt pathology on their own can interact to induce pathology resembling that observed in human disease. Although the results of Cadwell et al. (Cadwell et al., 2010) demonstrate a virus–susceptibility-gene interaction in the context of Crohn's disease, they also support the intriguing possibility that many human diseases could be influenced by an interaction between host genes and a single viral infection. In addition, this study demonstrates why individuals might display extensively varied responses to treatment for a pathologically defined disease, and highlights the need for monitoring past and current viral infections in future investigations that assess the association between genetic variation and disease susceptibility.

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