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The road to Crohn's disease:

A stress pathway directs the antimicrobial response to an enteric pathogen

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Crohn's disease, one of the two main forms of inflammatory bowel disease (IBD), is characterized in most patients by inflammation of the terminal ileum of the small intestine—a site of intense microbe–host engagement. Impairment in the conjoined action of autophagy (a cellular recycling pathway) and the endoplasmic reticulum (ER) stress response (called the unfolded protein response, UPR) within Paneth cells, specialized small intestinal epithelial cells (IECs), can instigate ileal inflammation (1). On page 1047 of this issue, Bel *et al.* (2) extend this notion by characterizing a mechanism of release of the antimicrobial peptide lysozyme via secretory autophagy from Paneth cells that might contribute to our understanding of how gene variants that affect both the UPR and autophagy predispose to Crohn's disease.

Bel *et al.* show that ER stress induced by infection with the intestinal bacterial pathogen *Salmonella enterica* serovar Typhimurium disrupts the secretory pathway in Paneth cells and hence prevents lysozyme secretion. Instead, this ER stress engages secretory autophagy to release lysozyme into the intestinal lumen. The authors demonstrate that during *S.* Typhimurium infection, relieving the attendant ER stress by treating mice with the small-molecule chaperone tauroursode-oxycholic acid abrogates the induction of secretory autophagy and lysozyme secretion from Paneth cells. This resulted in impaired antibacterial responses and increased *S.* Typhimurium burden. Notably, noninvasive *Bacteroides thetaiotaomicron* or invasion-deficient *S.* Typhimurium did not cause ER stress or Golgi breakdown, and therefore did not reroute lysozyme secretion via secretory autophagy. This pathway is impaired in *Atg16l1*^{T300A} (autophagy-related 16-like 1) mice, which express the major Crohn's disease risk variant (T300A). The authors further show that dendritic cells (a type of immune cell) respond to *S.* Typhimurium via Toll-like receptor (TLR) signaling, which triggers the activation of another type of immune cell, innate lymphoid cell type 3 (ILC3). These cells then produce interleukin-22 (IL-22), which acts on Paneth cells to control secretory autophagy of lysozyme (see the figure).

Abnormal Paneth cell lysozyme distribution is a notable, shared feature of impaired function of nucleotide-binding oligomerization domain-containing 2 (NOD2) and ATG16L1 (3, 4). Variants in the corresponding genes stand out among variants in hundreds of other genetic loci that are associated with IBD risk owing to their large effect size. Such lysozyme distribution abnormalities may reflect abnormal responses to pathogenic bacteria and

potentially commensal bacteria (which thrive in a symbiotic manner in the gut and do not cause infection). The abnormal distribution of lysozyme granules in Paneth cells from *ATG16L1*^{T300A} carriers in the absence of invasive bacterial infection (3) may in part also be a downstream consequence of ER stress. Consistent with this possibility, Crohn's disease patients with homozygous *ATG16L1*^{T300A} (5), or mice with IEC-specific deletion of *Atg16l1* (*Atg16l1*^{IEC}) (1), express markers of ER stress. Pathological immune responses that are inappropriately directed against commensal bacteria are thought to be a major driver of inflammation in Crohn's disease, and the microbiota exhibits compositional alterations ("dysbiosis") compared to healthy individuals (6). Although it is not clear whether dysbiosis is the cause or consequence of ileal inflammation in human Crohn's disease, it could be that defective secretory autophagy caused by *ATG16L1*-T300A may predispose to dysbiosis.

Bel *et al.* report that *S. Typhimurium* activates the PKR-like endoplasmic reticulum kinase (PERK) branch of the UPR to trigger secretory autophagy of lysozyme from Paneth cells (2). This involves PERK-mediated phosphorylation of eukaryotic translation initiation factor 2 subunit α (eIF2 α) and, consequently, induction of C/EBP-homologous protein (CHOP) (2). This pathway is identical to the mechanism that triggers canonical autophagy in Paneth cells upon deletion of the gene encoding the UPR transcription factor, X box-binding protein-1 (XBP1) (1). Although ER stress causes a mild form of enteritis in *Xbp1*^{IEC} mice (7), loss of PERK-induced autophagy in *Atg16l1*;*Xbp1*^{IEC} mice results in a transmural, discontinuous ileitis reminiscent of human Crohn's disease (1). Notably, in *Xbp1*^{IEC} mice, CHOP signaling induces the expression of the NKG2D ligand UL16-binding protein 1 (ULBP1), which causes an increase in NKG2D-expressing ILC1s and natural killer immune cells in the intraepithelial compartment (8). This is interesting because Bel *et al.* found that *S. Typhimurium*-induced IL-22 secretion from ILC3s is required for triggering lysozyme release via secretory autophagy in Paneth cells with ER stress (2). This raises the possibility that ER stress in IECs serves as a means to sense the presence of pathogens, creating a bidirectional system of communication that involves different subsets of immune cells that are ultimately involved in pathogen control. This may be a primitive function of ER stress in the IEC as shown by the critical role played by IRE1 α , the most evolutionarily conserved of the three branches of the UPR, in allowing the roundworm, *Caenorhabditis elegans*, to resist infection by *Pseudomonas aeruginosa* (9).

IRE1 α is an ER transmembrane receptor that forms homodimers upon ER stress, increasing to large clusters when ER stress persists. This is associated with a change from triggering a resolving UPR to one associated with inflammation (10). During persistent ER stress, a selective *ATG16L1*-dependent autophagy process is required to remove IRE1 α clusters and to terminate its signaling (11). Indeed, in patients with *ATG16L1*^{T300A} and in *Atg16l1*^{IEC} mice, IRE1 α accumulates in Paneth cells (11). In a related pathway, NOD2 signaling triggers I κ B kinase α (IKK α) activation, which directly phosphorylates and stabilizes *ATG16L1* (12). In the absence of IKK α , removal of IRE1 α clusters is disrupted (12). With increasing age, *Atg16l1*^{IEC} mice develop Crohn's disease-like ileitis (11) similar to the ileitis in young *Atg16l1*;*Xbp1*^{IEC} mice (1), and both types of ileitis are driven by accumulation and hyperactivation of IRE1 α (1, 11).

Whether NOD2 and immunity-related GTPase family M (IRGM), another autophagy pathway genetic risk element for Crohn's disease (13), contribute to secretory autophagy and/or the ileitis-inducing pathway described above will be interesting to explore. Notably, in myeloid cells infected with *Brucella abortus*, NOD2 is engaged downstream of IRE1 α activation and mediates ER stress-induced inflammation (14). NOD2 further relays signals from the commensal microbiota to leucine-rich repeat kinase 2 (LRRK2, encoded by another gene that is associated with Crohn's disease) to sort lysozyme into the secretory pathway, preventing its lysosomal degradation (4). Consequently, impaired function of NOD2, LRRK2, and IRGM all manifest with phenotypic abnormalities in the Paneth cell secretory compartment (4, 15). It is this remarkable convergence of Paneth cell phenotypes resulting from the impairment of important genes associated with risk of developing Crohn's disease that renders the underlying mechanisms of relevance for understanding Crohn's disease pathogenesis.

The majority of the genetic defects associated with Paneth cell dysfunction are not associated with spontaneous disease in genetically deficient mice. This highlights the critical role of the environment in triggering disease in genetically susceptible individuals. Only these yet-to-be defined environmental factors can provide the critical insights needed to explain the global increase in incidence and prevalence of Crohn's disease observed over recent decades. It is interesting in this regard that exposures to numerous types of enteropathogens represent one of the greatest environmental risk factors for the development of Crohn's disease. The study of Bel *et al.* may therefore provide an interesting link between these and other epidemiologic observations and the genetic landscape of Crohn's disease.

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Figure 1. Autophagy bypasses a secretory block in Paneth cells

Infection with the intestinal pathogen *Salmonella enterica* serovar Typhimurium blocks conventional secretion of the antimicrobial peptide lysozyme. To overcome this, Paneth cells engage the ER stress response to trigger secretory autophagy.