Inflammatory Bowel Disease, Stress, and the Endoplasmic Reticulum

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The causes of Crohn's disease and ulcerative colitis are poorly understood, despite having been defined many decades ago on the basis of their clinical manifestations. Experiments involving transgenic mice and genomewide association studies involving patients have independently provided glimpses into the complexity of the genetic causes of these disorders. A recent study described by Kaser and colleagues combines experimental and genetic approaches to build a compelling case implicating the unfolded-protein response as a culprit in inflammatory bowel disease.

Stress in the endoplasmic reticulum is caused by the accumulation of unfolded nascent proteins in the lumen under adverse cellular conditions. This process activates several protective signal-transduction pathways, collectively termed the unfolded-protein response. This response regulates the expression of genes that function within the secretory pathway or affect the metabolism of proteins and lipids. Thus, it remodels the secretory machinery and resets cellular physiology to “blow off steam” in the endoplasmic reticulum.

The unfolded-protein response is initiated by transmembrane receptors (Fig. 1). These proteins sense the status of protein folding in the endoplasmic reticulum and then transmit signals through their cytoplasmic tails. One such stress sensor is inositol-requiring enzyme 1 (IRE1), a resident transmembrane protein in the endoplasmic reticulum. Its rather exotic cytoplasmic tail contains a protein kinase domain as well as endoribonuclease activity. IRE1 undergoes oligomerization when unfolded proteins accumulate, triggering its autophosphorylation. This in turn activates its unusual effector function: the precise endonucleolytic cleavage of messenger RNA (mRNA) that encodes a transcription factor called X-box binding protein 1 (XBP1). IRE1 cuts the precursor XBP1 mRNA twice, removing an internal fragment and thus inducing a frame shift. This activity has a dramatic effect on the encoded XBP1 protein. Whereas the protein that is encoded by the precursor mRNA is labile and represses target genes of the unfolded-protein response, the spliced XBP1 mRNA encodes a stable and potent activator of the same target genes.

Lee and colleagues previously used gene-knockout techniques to show that Xbp1 is essential for cells equipped with an extensive secretory endoplasmic reticulum, such as plasma cells and epithelial cells in the pancreas and salivary gland. Kaser et al. teamed up with these authors, prompted by a report that genetic deletion of an endoplasmic reticulum stress sensor exacerbated experimental colitis in mice, to generate a mouse with a conditional Xbp1 allele that could be excised uniquely in intestinal epithelial cells. One would predict that such cells would have increased stress in the endoplasmic reticulum, and this prediction was borne out. Moreover, the Xbp1-mutant small-intestinal epithelium had extensive inflammation, whereas secretory-cell lineages were either absent (Paneth cells) or significantly reduced in number (goblet cells). No spontaneous colitis was observed, yet the animals had an increased susceptibility to experimental colitis.

The authors then turned to human inflammatory bowel disease and documented increased levels of the spliced (“activated”) XBP1 mRNA in both healthy and inflamed tissues, indicative of ongoing stress in the endoplasmic reticulum in these patients. The combined observations implied that components of the pathway of the unfolded-protein response, such as XBP1, may contribute to a genetic susceptibility to inflammatory bowel disease. Three previous genomewide association studies suggested linkage between inflammatory bowel disease and a locus on chromosome 22q12, home of the XBP1 gene. Kaser et al. carried out three association studies, using genetic markers that span the 22q12 region and samples from patients with Crohn's disease or ulcerative colitis, in addition to samples from unaffected control individuals.
subjects. The investigators thereby identified both protective and risk haplotypes at the XBP1 locus.

They next applied “deep sequencing” (i.e., the resequencing of essential elements of the XBP1 gene) of samples from several hundred patients with Crohn’s disease or ulcerative colitis and from control subjects. They detected multiple novel polymorphisms, some of which were very rare, that affected the coding sequence of the XBP1 gene and occurred only in patients with inflammatory bowel disease. These polymorphisms were too rare to allow statistical analysis. Yet, when two of the XBP1 sequence variants were tested functionally, they turned out to decrease XBP1 function in cell-culture assays.

Previous genomewide association studies in patients with inflammatory bowel disease have unveiled more than a dozen risk genes and loci. Some of these genes point to disturbances of innate and adaptive immunity (NOD2 and IL23R, respectively), of autophagy (ATG16L1 and IRGM), and of the epithelial barrier function (DLG5). The study by Kaser et al. provides genetic evidence to implicate another biologic phenomenon in the cause of inflammatory bowel disease: stress in the endoplasmic reticulum. Experimental confirmation of this hypothesis may come from studies in mice that are engineered to express changes in the rare XBP1 gene found in some patients with inflammatory bowel disease. The unfolded-protein response is a highly conserved and well-characterized signaling pathway. If indeed stress in the endoplasmic reticulum contributes to inflammatory bowel disease, it is anticipated that ongoing linkage studies will turn up additional components of the pathway.

Dr. Clevers reports having an equity interest in Agamyxis. No other potential conflict of interest relevant to this article was reported.

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Figure 1. Cell Stress and Inflammatory Bowel Disease.
The stress receptor inositol-requiring enzyme 1 (IRE1) forms multimers in the membrane of the endoplasmic reticulum in stressed cells. Autophosphorylation by and of its cytosolic kinase domain unmasks dormant endoribonuclease activity. IRE1-mediated sequence-specific cleavage of X-box binding protein 1 (XBP1) messenger RNA (mRNA) excises a small RNA fragment. The two mRNA fragments are religated, which leads to a frame shift in the coding sequence. Spliced XBP1 mRNA encodes XBP1, a potent transcriptional activator of target genes of the unfolded-protein response, whereas unspliced XBP1 mRNA encodes a transcriptional inhibitor. Kaser et al.1 recently generated a mouse model of inducible inflammatory bowel disease by preventing this splicing reaction and thereby inactivating the unfolded-protein response. This finding gives rise to the hypotheses that the integrity of the intestinal epithelium depends on the unfolded-protein response and that such a compromised response renders persons susceptible to induced inflammatory bowel disease. N denotes endonuclease, and P autophosphorylation.

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