Autophagy, Microbial Sensing, Endoplasmic Reticulum Stress, and Epithelial Function in Inflammatory Bowel Disease

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Increasing evidence has emerged that supports an important intersection between 3 fundamental cell biologic pathways in the pathogenesis of inflammatory bowel disease. These include the intersection between autophagy, as revealed by the original identification of *ATG16L1* and *IRGM* as major genetic risk factors for Crohn's disease, and intracellular bacterial sensing, as shown by the importance of NOD2 in autophagy induction upon bacterial entry into the cell. A pathway closely linked to autophagy and innate immunity is the unfolded protein response, initiated by endoplasmic reticulum stress due to the accumulation of misfolded proteins, which is genetically related to ulcerative colitis and Crohn's disease (*XBP1* and *ORMDL3*). Hypomorphic ATG16L1, NOD2, and X box binding protein-1 possess the common attribute of profoundly affecting Paneth cells, specialized epithelial cells at the bottom of intestinal crypts involved in antimicrobial function. Together with their functional juxtaposition in the environmentally exposed intestinal epithelial cell, their remarkable functional convergence on Paneth cells and their behavior in response to environmental factors, including microbes, these 3 pathways are of increasing importance to understanding the pathogenesis of inflammatory bowel disease. Moreover, in conjunction with studies that model deficient nuclear factor-κB function, these studies suggest a central role for altered intestinal epithelial cell function as one of the earliest events in the development of inflammatory bowel disease.

Keywords: Inflammatory Bowel Disease; Crohn's Disease; Ulcerative Colitis; Pathogenesis; Autophagy; Endoplasmic Reticulum Stress; Unfolded Protein Response; Innate Immunity; Genetics.

The inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) have long been considered to arise from an inappropriate immune response directed toward the commensal microbiota in a genetically susceptible host.[1,2] Based on twin experiments, the genetic contribution in CD has been estimated at 50%, and in UC at approximately 20%, with the remainder ascribed to environmental factors; most notably smoking and enteropathogens.[1,3,4] These interrelated genetic and environmental factors, which are associated with risk for developing IBD, likely share the common feature that they beneficially or adversely affect the functional relationships between the commensal microbiota, intestinal epithelium, and gut-associated lymphoid tissues.[1] Although much remains to be learned about the relevant environmental factors that affect these relationships, significant progress has been made in understanding the immunobiology of IBD and, more recently, its genetic underpinnings. The latter has largely been facilitated by genome-wide association studies (GWAS),[5,6] with the identification of >90 genetic loci associated with CD and UC,[7–10] but also linkage and candidate gene

Abbreviations used in this paper: Agr2, anterior gradient gene 2; AIEC, enteroadherent-invasive *Escherichia coli*; Atg, autophagy; CD, Crohn's disease; CHOP, C/EBP homologous protein; DC, dendritic cell; eIF2α, eukaryotic initiation factor 2α; grp78, glucose-regulated protein 78; GWAS, genome-wide association studies; HM, hypomorphic; IBD, inflammatory bowel disease; IL, interleukin; JNK, c-Jun N-terminal kinase; MDP, muramyl dipeptide; MNV, murine norovirus; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; NLR, Nod-like receptor; PERK, pancreatic ER kinase; S1P, Site-1 protease; ssRNA, single-stranded RNA; Th17, T helper 17; TLR, Toll-like receptor; TNF, tumor necrosis factor; UC, ulcerative colitis; UPR, unfolded protein response; XBP1, X box binding protein-1; XBP1s, spliced X box binding protein-1.

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studies that have allowed, in certain circumstances, for the identification of rare, functionally important variants, including those associated with early-onset IBD.[11–14] Despite this dramatic expansion in the number of genetic loci linked to IBD, in CD, a single gene, *NOD2* (followed by *IL23R* and *ATG16L1*), is associated with by far the largest, albeit limited, fraction of genetic heritability, with the remainder of genes imposing only limited degrees of risk.[15] However, the most recent meta-analysis of CD GWAS data has shown that 71 of the common genetic loci discovered so far may account for only 23% of CD's heritability; although this could be an underestimate.[15] Despite this, it is increasingly clear that the identified genetic risk factors appear to be operating within functionally interacting pathways and, as such, likely amplify their individual importance relative to disease pathogenesis. One group of potentially interacting pathways that are rapidly coming to light and have received a significant amount of attention because of the implications that they have for understanding relevant environmental factors is that associated with autophagy, the unfolded protein response (UPR), and intracellular bacterial sensing. This review will summarize the evidence for this in light of recent observations in each of these 3 areas.

Autophagy

The ability of GWAS to generate knowledge about the genetic underpinning of IBD in a hypothesis-free unbiased way was exemplified by the discovery of genes associated with a fundamental biological pathway that had not previously been considered in the context of IBD, namely autophagy.[16,17] Initially, a coding variant (T300A) in *ATG16L1* was identified in a genome-wide assessment of ~20,000 protein coding variants (nonsynonymous single nucleotide polymorphisms)[16] and was later independently identified in subsequent GWAS studies.[6,17] Further support for an involvement of autophagy in IBD came from association of *IRGM*,[6,18,19] another gene that had previously been linked to autophagy induction, and *LRRK2*.[8]

Macroautophagy (autophagy) refers to a conserved biological process that has evolved as a physiological response to the removal of damaged organelles and micro-organisms (selective) or cellular starvation (nonselective).[20,21] Other types of autophagy, such as chaperone and mitochondria-associated autophagy, are not yet linked to IBD but are worthy of mention (for review see references 22 and 23). Autophagy designates the engulfment of cellular macromolecular contents via a double-layered membrane (autophagosome) and its subsequent fusion with lysosomes (lysophagosome) and, consequently, lysosomal degradation.[20] This catabolic pathway allows for recycling of proteins and macromolecular components into their basic constituents and the availability of the latter for anabolic processes. It is therefore not surprising

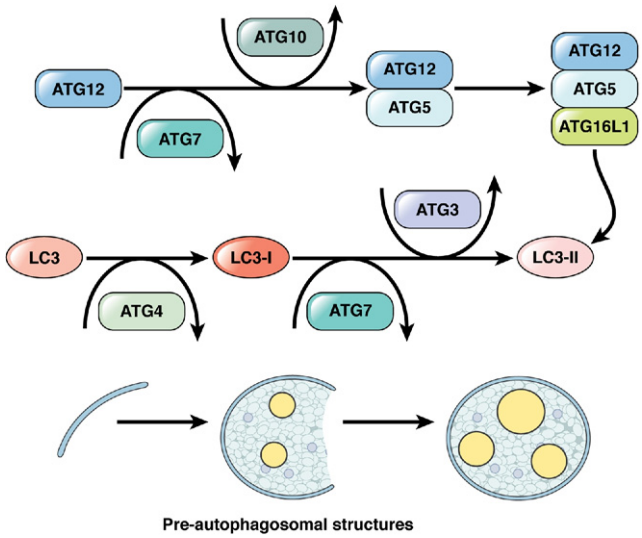

Figure 1. Molecules involved in autophagy induction.

that autophagy plays a fundamental role in embryogenesis, immune function, and tissue remodeling and, consequently, a wide variety of disease processes, including, neurodegeneration, cancer, infection, and inflammation among others.[21]

Several distinct steps that have largely been elucidated in yeast model systems are involved in the autophagy process.[20] These involve induction, cargo recognition, vesicle formation, autophagosome-vacuole fusion, and, finally, breakdown of the autophagic contents and the release of their degradation products into the cytosol (Figure 1).[20] All cells exhibit a certain low level of basal autophagy, which can be induced under various conditions (ie, induced autophagy). The latter mechanism allows cells to efficiently adapt to various intracellular and extracellular stressors. The serine/threonine protein kinase target of rapamycin plays a central role as an inhibitor of autophagy, and several signaling pathways that regulate autophagy induction mechanistically converge on mammalian target of rapamycin (mTOR).[20,24] Upon release of the inhibitory activity provided by mTOR, autophagy is generated by autophagy (Atg) proteins that function in a cascade of modular interactions. The most nascent complex includes Beclin 1, Atg1 (ULK1 and ULK2 in mammals), Atg13, and Atg17 (FIP2000 in mammals)[20] that motivate the recruitment of Atg8 (LC3-I) to the isolation membrane via a membrane anchoring, phosphatidylethanolamine modification of Atg8 (LC3-II). Identification of LC3-II recruitment to the isolation membrane is a major means to quantify autophagy (Figure 2). Other proteins involved in the formation of the isolation membrane that initially surrounds the organelles or organisms destined for degradation include Atg 2, 3, 9, and 16. Interestingly, the observation that sirolimus, an mTOR inhibitor, was clinically beneficial in a case report of a CD patient with