

# ADVANCES IN IBD

Current Developments in the Treatment of Inflammatory Bowel Diseases

Section Editor: Stephen B. Hanauer, MD

## Investigating the Role of Autophagy in the Pathophysiology of IBD

Scott E. Plevy, MD  
University of North Carolina at Chapel Hill  
Chapel Hill, NC

**G&H** First, can you explain the difference between innate and adaptive immunity?

**SP** The cells involved in the innate immune response are neutrophils, macrophages, and dendritic cells. These are the frontline soldiers when the body is invaded by a microbial pathogen. These cells, by virtue of the cytokines they elaborate and their ability to process and eradicate microbes intracellularly, present surface antigens that then activate adaptive immunity.

The adaptive immune response, made up of T cells and B cells, is considered the hallmark of the mammalian immune system. T cells and B cells are the only cells in the body that can re-arrange DNA to create different receptors and therefore recognize any antigen that the body can encounter. These cells are what allow us to develop immunologic memory and immunity to infection through previous exposure.

Unlike adaptive immunity, the mechanisms of innate immunity are evolutionarily conserved. When we discuss microbial recognition and different forms of cell death, we see homologous processes and homologous proteins in lower organisms such as *Drosophila*, and even plants and yeast.

**G&H** How does the process of cell death fit into immune pathways and the pathogenesis of inflammatory bowel disease?

**SP** Clinicians are aware of a form of cell death called apoptosis, or programmed cell death. Apoptosis can be

characterized as fratricide: when cells undergo apoptotic death, it is initiated by a signal delivered from another cell, indicating that the target cell has served its purpose and it is now time to die. Apoptosis is important in several ways to the pathogenesis of inflammatory bowel disease (IBD). First, apoptosis is physiologic cell death. It is how cells are supposed to die. When the gut mucosa encounters an infection, proliferation of T cells and B cells is triggered to help eradicate the infectious organism. However, once these T cells and B cells have performed their function, they die through apoptosis. Defects in T-cell apoptosis in the gut have been described in patients with IBD, leading to the notion that these T cells inappropriately persist, and can perpetuate inflammation.

Another link that has been described between IBD and apoptosis is through the ability of antitumor necrosis factor monoclonal antibodies to induce apoptosis in inflammatory cells, which may correlate with efficacy in patients with IBD.

A second form of cell death is necrosis. Necrosis can be described as homicide: a destructive, inappropriate form of cell death. Necrosis occurs in the setting of physiologic stress, inflammation, and infection. In IBD, when intestinal cells are dying a necrotic death, these cells release endogenous proteins that have potent inflammatory effects. Clinicians (unknowingly) are familiar with quantitative measurements of cell necrosis in the gut because some of the proteins released from necrotic cells are markers of disease activity. The necrosis marker most recognized in clinical practice is fecal calprotectin.

Autophagy is a third form of cell death, which can be thought of as suicide. The term autophagy literally translates as “self-eating.” Autophagy occurs as a host response to metabolic stresses, such as nutrient deprivation and hypoxia. In order to survive, stressed cells can enter a state of semidormancy that allows them to survive until nutrients are again available. Initially, the cell will shut off protein synthesis. At this point, characteristic autophagocytic membranes begin digesting the cell’s machinery of protein synthesis. This is a way of re-utilizing proteins so that the cell can survive. The cell next eats the cytoplasm, saving the mitochondria to the very end. The mitochondria are the cell’s batteries. Once they have been consumed, the cell can no longer survive.

### G&H How has the process of autophagy been linked to IBD pathogenesis?

**SP** Several years ago, genetic researchers uncovered variations in two genes in the autophagy pathway, *ATG16L1* and *IRGM*, that confer Crohn’s disease susceptibility. There are several hypotheses regarding the function of these genes in Crohn’s pathogenesis, but much more work needs to be done. The discovery of autophagy genes illustrates the importance of the genetic approach to understanding disease pathogenesis. No one implicated autophagy in Crohn’s disease before these two genetic markers were described.

At one level, autophagy could be involved in settings of stress and starvation in the gut. Cells that have defective autophagocytic pathways may not survive as well and may die inappropriately via necrosis, thereby releasing inflammatory compounds that contribute to IBD.

Autophagy is also involved in the digestion of intracellular proteins and the process of antigen presentation, which is a link between innate and adaptive immunity. Through autophagocytic processes, foreign and self-antigens are expressed on the surface of innate immune cells, where they are recognized by T cells. Thus, defective autophagy may result in inappropriate activation of adaptive immune responses as well.

However, autophagy is also involved in the recognition and eradication of microbes by the innate immune system. Additionally, autophagy has been demonstrated in the intestinal epithelium. In the setting of autophagy defects, there may be insufficient recognition and killing of intracellular bacteria that normally reside in the gut. The characteristic autophagic double membrane, which engulfs self-proteins, also engulfs bacteria. There are particular types of bacteria that survive inside of cells where the host may be particularly reliant on autophagy for their elimination. Early work has suggested that the role

of autophagy in IBD may be related to the eradication of enteric bacteria.

### G&H How can these theories of the role of autophagy in IBD be related to new therapies or prognostic indicators?

**SP** We are at a very early stage of research, and I would urge caution at this juncture before accepting an emerging dogma that autophagy in IBD is solely related to antimicrobial responses in the gut. We need to establish better basic understanding of autophagic processes in IBD before translating this knowledge into therapeutic targets.

With regard to genetic markers, at this point we have identified approximately 40 IBD susceptibility genes, some of which are unique to Crohn’s disease. The unique Crohn’s susceptibility genes, such as *NOD2* and the autophagy genes, highlight the importance of innate immunity and host-microbial interactions. Other IBD susceptibility genes, like the interleukin (IL)-23 receptor, are associated with both Crohn’s disease and ulcerative colitis. Interestingly, the same genetic polymorphisms are linked to other chronic inflammatory diseases like psoriasis and the spondyloarthropathies. Ultimately, some genetic variants are master regulators of inflammation (like the IL-23 receptor), whereas others will determine organ-specific inflammation, and still others will determine specific phenotypes within a disease.

Data have been reported showing that the autophagy genetic variations are weakly associated with ileal fibrostenotic and perforating disease (as opposed to inflammatory colonic Crohn’s disease). However, similar phenotypic associations with the *NOD2* gene are more robust. Ultimately, combinations of genetic variants will be required to better predict disease phenotype and progression.

### G&H What are the next steps in researching autophagy genetic variations as they relate to IBD?

**SP** There are several important pathways forward. One will be through the development of reductionist systems that demonstrate how these genetic polymorphisms impact upon the physiologic roles of autophagy. However, there are differences in these genes between mice and humans, so it may be difficult to model these genetic variants in animals. In the laboratory, we ultimately need to study cells from people with Crohn’s disease genotyped for autophagy variants to best understand function.

At a clinically relevant level, we need to genotype large populations of Crohn’s disease patients for autophagy variants and determine whether genetics define a particular disease phenotype and/or predict disease severity, progres-

sion, and outcomes. It would be useful and interesting to know if autophagy variants correlate with serologic and gut immune responses. Ultimately, to advance the field, genetic polymorphisms, serologic markers, and measurable immune responses need to be integrated into clinical trials to determine whether patient subpopulations respond to specific therapeutic interventions.

### Suggested Reading

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