Editorial

Anti-TNF Antibodies and Autophagy: A Hidden Nexus for a Successful Therapeutic Response?

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Crohn’s disease [CD] and ulcerative colitis [UC] are the two major forms of inflammatory bowel disease [IBD]. First-line therapies are based on neutralisation of the immune system by corticosteroids, or on blockage of pro-inflammatory cytokines such as tumour necrosis factor-α [TNF-α]. It is now becoming clear that these treatments required a personalized benefit versus risk assessment; therapy will be based on individual clinical patient profiles, determined through available biomarkers and tissue signatures. Therefore, it is necessary to improve our current understanding of IBD pathogenesis, with the ultimate goal of personalising therapeutic intervention.

IBDs are characterised by chronic intestinal inflammation and an excessive recruitment of leukocytes into the intestinal mucosa. A current hypothesis is that alterations of the gut microbiota have a pivotal role in the initiation and maintenance of inflammation, in genetically predisposed individuals.

The research for genetic determinants of disease onset and progression has recently culminated in the Immunochip project, which has identified more than 160 loci containing IBD susceptibility genes. The relevance of genome-wide association studies [GWAS] initially was confirmed by the identification of a nucleotide-binding oligomerization domain containing two [NOD2] variants, which remain the strongest determinants of susceptibility to CD, after more than one decade from its discovery. NOD2 is an intracellular sensor of bacterial infections, which drives the production of pro-inflammatory cytokines in macrophages and antimicrobial peptides such as α-defensin in Paneth cells, confirming the relevance of innate immune responses to gut microbiota and priming of adaptive immunity. Moreover, performing GWAS allowed uncovering novel disease-associated pathways, such as autophagy.

Autophagy was initially implicated in the pathogenesis of CD by the discovery of the Thr300Ala [T300A] variant in the autophagy related 16-like 1 [ATG16L1] gene in a non-synonymous single nucleotide polymorphism [SNP] association study. Soon afterward, the immunity-related GTPase family M [IRGM] gene variants were associated with an increased risk of developing both CD and UC, confirming the relevance of autophagy in the control of intestinal inflammation.

However, the mechanisms through which IRGM regulates autophagy were poorly understood, and only recently has been elucidated the involvement of IRGM in the recruitment of the autophagy machinery in order to actively conduct antimicrobial defense. In contrast, ATG16L1 activities have been deeply investigated in mice, healthy individuals, and patients with CD. Using Atg16L1-deficient and hypomorphic mice, it has been clarified that ATG16L1 is able to control both canonical and bacteria-induced autophagy, Paneth cell homeostasis, and IL-1β secretion; in support of this, changes in the morphology of Paneth cells were observed in CD patients homozygous for the risk allele of ATG16L1. However, studies focusing on T300A have shown conflicting results. Indeed, T300A variants are fully competent in the formation of autophagosomes, even if T300A-expressing cells were found to be defective in the capture of internalised Salmonella within autophagosomes.

Therefore, it is becoming evident that autophagy contributes to IBD pathogenesis through multiple mechanisms that are not mutually exclusive and rely on the cell-type specific control of antimicrobial activities.

Concurring with this, a recent study identified a novel role for the myotubularin-related protein 3 [MTMR3] in amplifying pattern recognition receptor [PRR]-induced cytokine secretion in human macrophages down-modulating phosphatidylinositol 3-phosphate [PtdIns3P] activation and autophagy levels. Similarly, the work of Levin and colleagues investigated the possibility that autophagy is involved in directing the transition of human macrophages into a regulatory phenotype mediated by anti-TNF antibodies. Macrophages were characterised from a mixed leukocyte reaction [MLR] after exposure to infliximab and positive isolation through CD14 beads. Only in the presence of anti-TNF antibodies did the macrophage population express high levels of the regulatory marker CD206 and of autophagy-related genes, in comparison with both classically IFN-γ induced M1 macrophages and IL-4 induced M2 macrophages. Of note, macrophages treated with infliximab are also prone to express high levels of LC3II, and analyses by confocal microscopy confirmed the occurrence of an increased number of autophagosomes. Furthermore, Levin and colleagues clarified that...
the effects elicited by anti-TNF treatment were dependent on the activity of the lysosomal enzyme cathepsin S, since the administration of an inhibitor was able to abrogate the induction of CD206+ macrophages.19

Taking together, these data clearly indicate that autophagy is increased in anti-TNF induced macrophages and that, on the other hand, autophagy is required to promptly induce regulatory macrophages.

Noteworthy, Levin A and colleagues had the opportunity to explore the contribution of ATG16L1 allele variant T300A in expanding regulatory macrophages. Indeed, MLR were generated from 1:1 cultures of peripheral blood mononuclear cells [PBMC] from healthy donors, genotyped for the ATG16L1 risk allele. Importantly, the number of CD206+ macrophages was directly proportional to the number of wild-type [WT] allele in cultures.

Even if the exact mechanism behind the effects mediated by the ATG16L1 risk allele was not investigated, this striking evidence suggested that an intact autophagy pathway is actually required for an optimal response to anti-TNF therapy, providing the rationale to prioritise this pathway as a new potential target for drug development.

Clinically available drugs that up-regulate autophagy are sirolimus and everolimus, two rapamycin analogues. Interestingly, two different case reports indicated a successful treatment of refractory CD patients with either everolimus16 or sirolimus.17 However, a double-blind randomised multicentre study has failed to demonstrate benefit when comparing everolimus with azathioprine or placebo in maintaining steroid-induced remission in active CD patients,18 suggesting that several issues remain to be addressed.

In particular, it is necessary to assess the efficacy, safety, and long-term outcomes of up-regulating autophagy, since it has been suggested that augmented autophagy might worsen the progression of established colorectal cancers, exacerbating the polarisation of M2 macrophages.19,20

In conclusion, the challenge will be now to identify those patients who are more likely to respond to anti-TNF treatment in combination with autophagy inducers, which could be most effective in the treatment of IBD.

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**References**


