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Review Article

Review Article

Inflammatory Bowel Disease Drugs: A Focus on Autophagy

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Abstract

Inflammatory bowel disease [IBD] is characterized by chronic inflammation of the gastrointestinal tract. Medications such as corticosteroids, thiopurines, immunomodulators and biologic agents are used to induce and maintain remission; however, response to these drugs is variable and can diminish over time. Defective autophagy has been strongly linked to IBD pathogenesis, with evidence showing that enhancing autophagy may be therapeutically beneficial by regulating inflammation and clearing intestinal pathogens. It is plausible that the therapeutic effects of some IBD drugs are mediated in part through modulation of the autophagy pathway, with studies investigating a wide range of diseases and cell types demonstrating autophagy pathway regulation by these agents. This review will highlight the current evidence, both *in vitro* and *in vivo*, for the modulation of autophagy by drugs routinely used in IBD. A clearer understanding of their mechanisms of action will be invaluable to utilize these drugs in a more targeted and personalized manner in this diverse and often complex group of patients.

Key Words: Autophagy; drugs; IBD; Crohn's disease

1. Introduction

The major inflammatory bowel diseases [IBD], Crohn's disease [CD] and ulcerative colitis [UC], are characterized by chronic inflammation of the gastrointestinal [GI] tract and affect up to 1 in 250 people in the UK.[1] A recent National Health Service [NHS] review estimated IBD treatment costs of £720 million per year,[1] with roughly a quarter of these costs directly attributed to drug treatments.[2] At present there is no cure for IBD, and medications are aimed at inducing and maintaining remission of disease by modifying inflammatory processes.[3] The efficacy of current drugs for the treatment of IBD continues to come under scrutiny, as response to treatment often diminishes over time, resulting in disease complications. A recent review of European cohorts estimates that 10–35% of CD patients required surgery within 1 year of diagnosis and up to 61% by 10 years.[4] Development of new drugs is a long and expensive process associated with high failure rates; therefore, making better use of drugs that have already been approved for clinical use is essential. The Crohn's and Colitis Foundation of America has recently highlighted this need for research into optimizing medical therapies,[5] with patient stratification and personalized medicine of key importance in this context.[6] In order to improve the efficacy of existing drugs, a more comprehensive characterization of their mechanism of action is required. Here we give an overview of IBD drugs that have been linked to the modulation of autophagy, a cellular process that has been implicated in CD pathogenesis, and summarize what is currently known regarding their mechanism of action.

2. Aetiology of IBD

The aetiopathogenesis of IBD remains poorly understood but is almost certainly multifactorial in nature, with genetic predisposition,

environmental triggers [such as smoking, antibiotics and diet] and a dysregulated immune response to intestinal microflora all contributing.[7] Genome-wide association studies [GWAS] have now identified multiple susceptibility loci for CD and confirmed the previously recognized association of nucleotide-binding oligomerization domain-containing protein 2 [*NOD2*], genes involved in T cell-dependent immunity and autophagy, including autophagy-related protein 16-1 [*ATG16L1*], immunity-related GTPase family M protein [*IRGM*] and leucine rich repeat kinase 2 [*LRRK2*].[8] Genetic association with the transcription factor x-box-binding protein 1 [*XBP1*], a key component of the endoplasmic reticulum [ER]-stress response, with both forms of IBD have also been identified and replicated.[9] These genetic studies have led to an increase in research linking autophagy dysregulation to CD pathogenesis.

2.1. Autophagy

Autophagy is an intracellular process that degrades excessive, damaged or aged proteins and organelles to maintain cellular homeostasis.[10] These homeostatic functions impact on many essential cellular processes including development and differentiation, survival, senescence and innate and adaptive immunity, with dysregulated autophagy linked to a multitude of diseases.[11] When macroautophagy [hereafter referred to as autophagy] is initiated, the isolation membrane, an expanding lipid bilayer, forms a double membrane vesicle [the autophagosome] around the cargo to be degraded [Figure 1]. The mature autophagosome then fuses with a lysosome to form an autophagolysosome, in which lysosomal enzymes degrade the inner membrane and cargo. The process of autophagy is controlled by the coordinated activity of ATG [autophagy-related] proteins. The further detailed and complex molecular machinery involved in biogenesis of the isolation membrane and autophagosome is beyond the scope of this focused review and has been discussed comprehensively elsewhere[12]; however, it is appropriate to highlight the role of ATG16L1 in this process. Two ubiquitin-like molecules, LC3 [microtubule-associated proteins 1A/1B light chain 3A]/ATG8 and ATG12 are involved in autophagosome biogenesis. LC3/ATG8 is conjugated to phosphatidylethanolamine [PE] to form lipidated LC3-II and is associated with autophagosome formation. ATG12 is conjugated to ATG5 and forms a complex with ATG16L1 [ATG16L1 complex]. The ATG16L1 complex is proposed to specify the site of LC3 lipidation for autophagosome formation [Figure 1][13].

2.2. Autophagy signalling pathways

Autophagy is active at a basal level in most cell types to maintain homeostasis, and this activity is modulated in response to a myriad of stresses and stimuli that include starvation, hypoxia, infection and ER stress.[14] Autophagy is largely regulated, but not exclusively, by the mTORC1 [mechanistic target of rapamycin complex 1] and Beclin1/B cell lymphoma 2 [Bcl-2] signalling pathways [Figure 2]. The mTORC1 pathway plays a central role in the inhibition of autophagy, for example blocking mTORC1 activity with the small macrolide antibiotic rapamycin stimulates induction of autophagy. Class I phosphatidylinositide 3-kinases [PI3K], Akt and Ras/Mek/Erk signalling pathways are involved in the activation of mTORC1 and subsequent inhibition of autophagy.[14] mTORC1 inhibits autophagy via phosphorylation of Unc-51 like autophagy activating kinase 1 [ULK1] and ATG13 to inhibit the ULK1-ATG13-FIP200 complex, which is important for initiation of autophagosome formation.[15] Conversely, AMP-activated protein kinase [AMPK] is involved in the inhibition of mTORC1 and stimulates autophagy via phosphorylation of ULK1 at sites distinct from mTORC1.[16] Activated ULK1 and AMPK subsequently phosphorylate Beclin1 for the induction of autophagy.[16,17] Beclin1 induces autophagy through the formation of the class III PI3K complex consisting of Vps34-Vps15-Beclin1.[18] Interaction of the class III PI3K complex with ATG14 is important for recruitment of autophagy proteins, including the ATG16L1 complex and LC3/ATG8, to the autophagosome membrane during early stages of the pathway [Figure 2].[12]

Beclin1 was originally identified as an interacting protein with Bcl-2 [19], an anti-apoptotic protein that inhibits autophagy when it is in complex with Beclin1.[20,21] In response to nutrient deprivation, c-Jun N-terminal kinase [JNK]-1-mediated phosphorylation of Bcl-2 occurs, causing the dissociation of the Beclin1-Bcl-2 complex and induction of autophagy.[22] However, during periods of prolonged nutrient deprivation, increased levels of Bcl-2 phosphorylation prevent Bcl-2 from binding to and inhibiting pro-apoptotic proteins including Bcl-2 associated X protein [BAX] and Bcl-2-antagonist/killer [Bak].[23,24] Therefore, Bcl-2 phosphorylation

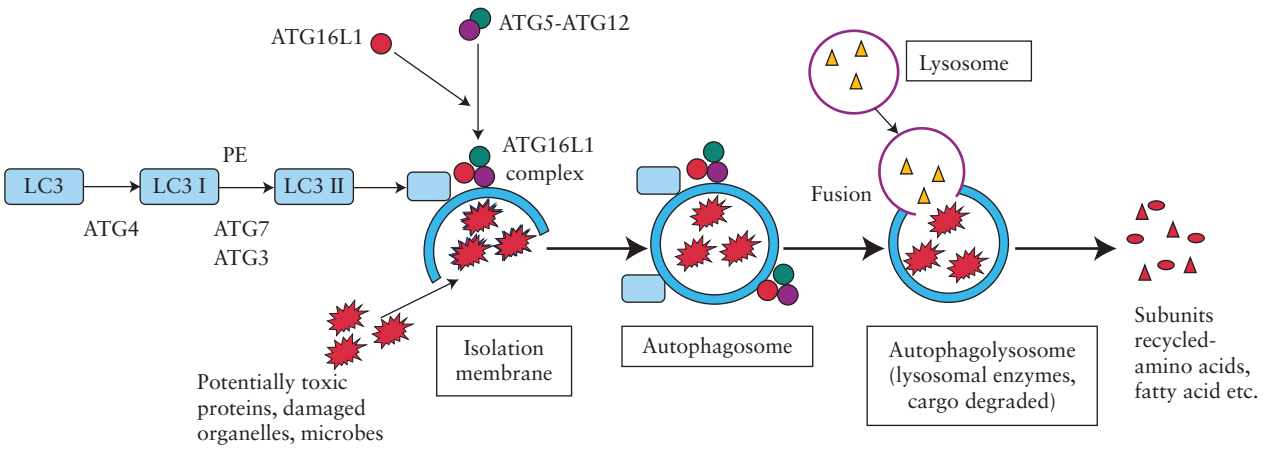

Figure 1. The autophagy pathway. During the initial stages of autophagy, the isolation membrane forms a double membrane vesicle [the autophagosome] around the cargo to be degraded. The mature autophagosome then fuses with a lysosome to form an autophagolysosome, in which cargo are degraded by lysosomal enzymes and subunits are recycled. Autophagy is controlled by the coordinated activity of ATG proteins. Two ubiquitin-like molecules, LC3 and ATG12, are involved in autophagosome biogenesis. LC3 is conjugated to PE to form lipidated LC3-II and is associated with the autophagosome outer membrane. ATG12 is conjugated to ATG5 and forms a complex with ATG16L1 [ATG16L1 complex]. The ATG16L1 complex is proposed to specify the site of LC3 lipidation for autophagosome formation.

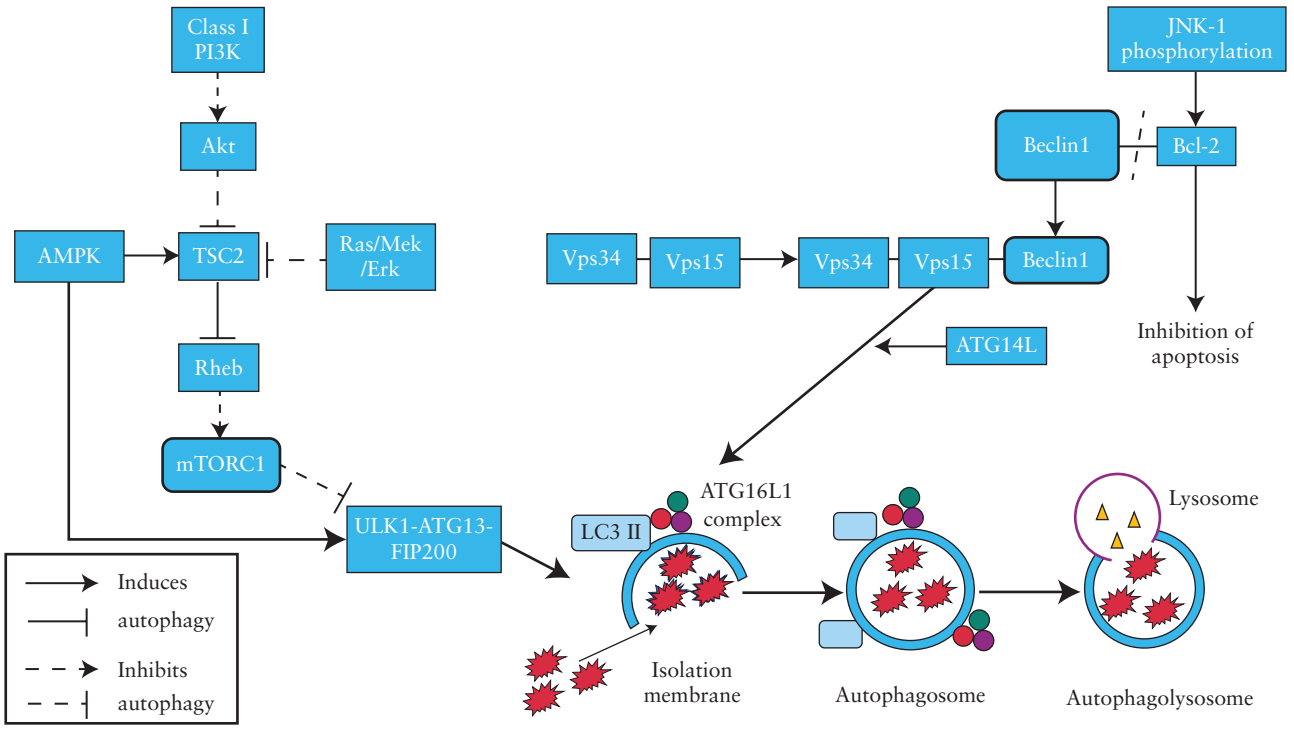

Figure 2. Autophagy regulation. The central pathways in autophagy regulation are mTORC1 and Beclin1/Bcl-2. class I PI3K, via Akt and Ras/Mek/Erk signalling pathways phosphorylate Tuberin [TSC2] to promote Rheb-dependent activation of mTORC1. When active, mTORC1 inhibits formation of the ULK1-ATG13-FIP200 complex, which is necessary for initiation of autophagy. Conversely, AMPK is involved in the inhibition of mTORC1 and stimulates autophagy via phosphorylation of ULK1 at sites distinct from mTORC1. Bcl-2 is dissociated from Beclin1 due to JNK-1-dependent phosphorylation of Bcl-2. Bcl-2 is then free to inhibit apoptosis through binding of BAX and Bak. Beclin1 is free to bind Vps34-Vps15 [the mammalian homologue of Vps15 is p150] to induce autophagy. The Vps34-Vps15-Beclin1 complex binds to ATG14L to induce further ATG protein recruitment and elongation of the isolation membrane in the initial stages of autophagy. Activated ULK1 and AMPK can also directly phosphorylate Beclin1 for the induction of autophagy [not shown].

can act as a switch between autophagy, a pro-survival response to cellular stress and apoptosis, a mechanism to limit damage to neighbouring cells under conditions of prolonged stress.[24] A rheostat model proposed by Pattingre *et al.*[20] suggests that when autophagy exceeds physiological levels, then autophagic-cell death can occur due to over-digestion of essential cellular components. The complex relationship between autophagy and apoptotic cell death has been reviewed elsewhere.[25]

3. Autophagy and Crohn's Disease

Xenophagy [a specific type of autophagy that degrades microorganisms] is central to the innate immune response. It can target and degrade intracellular pathogens, stimulate the production of host defence peptides and present antigens to initiate the adaptive immune response.[26] During infection, microbe-associated molecular patterns [MAMPs] are detected by a family of proteins called pattern recognition receptors [PRRs] located within host cells. PRRs involved in xenophagy include the Nod-like receptors [NLRs], Toll-like receptors [TLRs] and sequestosome 1/p62-like receptors [SLRs] [27].

The PRR *NOD2* was the first gene to be linked to CD susceptibility in 2001,[28–30] with the three most common CD-associated *NOD2* single nucleotide polymorphism [SNP] variants [R702W, G908R and L1007f/s] identified in roughly one-third of patients.[31] Furthermore, homozygous mutation of the *NOD2* gene increases the risk of developing CD 20- to 40-fold.[31,32] The NOD2 L1007f/s variant is unable to detect muramyl dipeptide [MDP], a component of bacterial cell walls, which results in deficient nuclear factor kappa-light-chain-enhancer of activated B cells [NFκB] signalling and host defence peptide secretion.[33] In 2007 the first autophagy gene, *ATG16L1*, was linked to CD susceptibility,[34] followed by the identification of variants in autophagy genes including *IRGM* and *LRRK2*.[8] An SNP identified in *ATG16L1*, that encodes for a single amino acid substitution [T300A],[34] has been modelled in hypomorphic mice.[35] These mice do not spontaneously develop intestinal inflammation but do show evidence of Paneth cell dysfunction that is similar to Paneth cells from patients homozygous for the T300A allele.[36] A recent functional study using a T300A knock-in mouse model has demonstrated that the T300A variant creates a caspase cleavage site, making ATG16L1 more susceptible to caspase-3-mediated degradation.[37]

The majority of functional studies have focused on NOD2 and ATG16L1, which are among the strongest risk factors in CD. These studies have reported decreased autophagy levels in a range of cell types derived from CD patients, and cells harbouring NOD2 L1007f/s or ATG16L1 T300A variants exhibit a number of disrupted functions linked to autophagy, including impaired autophagosome formation and degradation of cytoplasmic microorganisms, defective presentation of bacterial antigens to $CD4^+$ T cells and alterations in Paneth cell granule formation.[33,38–41] Importantly, in intestinal epithelial cells and dendritic cells [DCs] that harbour the NOD2 L1007f/s or ATG16L1 T300A variants, MDP-induced autophagy is diminished, leading to ineffective killing of pathogens such as *Salmonella typhimurium*, *Shigella flexneri* and Adherent Invasive *Escherichia coli* [AIEC].[33] It has been suggested this may be due to the inability of NOD2 L1007f/s to recruit ATG16L1 T300A protein and the autophagy machinery to

sites of bacterial entry at the cytoplasmic membrane.[42] The increased levels of pro-inflammatory cytokines observed in CD patients have also been linked to autophagy dysregulation. Loss of functional ATG16L1 protein results in increased pro-inflammatory IL-1β and IL-18 production in murine studies[39] and in human peripheral blood mononuclear cells.[40] It has been suggested that when bound to NOD2, ATG16L1 acts as a modulator of NOD2 activity, shifting the balance between autophagy and cytokine production; loss of functional ATG16L1 shifts NOD2 activity towards pro-inflammatory signalling [40]. Autophagy is required for presentation of antigens derived from degraded bacterial components to the adaptive immune system.[26] This is of particular importance as dysregulation of T-cell responses are a key feature of CD pathogenesis. DCs from CD patients expressing the NOD2 L1007f/s or ATG16L1 T300A variants have disrupted antigen sampling and processing[41] and are incapable of antigen presentation via major histocompatibility complex [MHC] II.[33]

Little is known about the function of IRGM and LRRK2 in CD. A deletion polymorphism immediately upstream of *IRGM* found in strong linkage disequilibrium with the most strongly CD-associated SNP, causes *IRGM* to segregate into CD risk variant [deletion] and protective variant [no deletion].[43] Subsequently it has been shown that a family of microRNAs [miRNAs], miR-196, that is overexpressed in the inflammatory intestinal epithelia of individuals with CD, downregulates the IRGM protective variant but not the risk-associated variant. Functionally, the loss of IRGM protective variant expression compromises autophagy and control of the intracellular replication of CD-associated AIEC.[44] Interestingly, a recent study has placed IRGM in a central role for the orchestration of core autophagy machinery in response to microbial infection.[44] It was shown that IRGM regulates the formation of a complex containing NOD2 and ATG16L1 that is necessary for the induction of xenophagy. The interaction of IRGM with NOD2 also stimulates phosphorylation cascades involving AMPK, ULK1 and Beclin1 that regulate autophagy initiation complexes.[44] LRRK2 expression is enriched in human immune cells and is increased in colonic biopsy specimens from patients with CD.[45] Functionally, LRRK2 can enhance NFκB-dependent transcription, whereas small interfering RNA [siRNA] knockdown of LRRK2 in RAW 264.7 macrophages interferes with reactive oxygen species production and bacterial killing.[45]

Common upstream signalling pathways regulate autophagy; however, its activation can have different functional outcomes that operate in a cell-type specific manner. Consistent with this conditional knockout mouse models of autophagy genes *ATG16l1* and *ATG5* are selectively important for the biology of the Paneth cell, with notable abnormalities observed in the granule exocytosis pathway.[36] *IRGM1*-deficient mice also exhibit abnormalities in Paneth cell location and granule morphology, accompanied with increased susceptibility to inflammation in the colon and ileum.[46] LRRK2 deficiency confers enhanced susceptibility to experimental colitis in mice; however, this was associated with enhanced nuclear localization of the transcription factor nuclear factor of activated T cells [NFAT1], important for regulating innate immune responses.[47] Specifically, it was found that there was aberrant activation of bone marrow-derived macrophages from the LRRK2 deficient mice following exposure to various stimulators of innate immunity. Clearly, a comprehensive understanding of the cell-specific nature of autophagy and autophagy-related proteins is essential for understanding its role in IBD.

3.1. ER stress and autophagy

ER stress results from unfolded and misfolded protein accumulation in the ER, with cells that naturally secrete large amounts of protein, such as Paneth cells, being more susceptible to ER stress.[48] The ability of highly secretory cells to respond to and resolve the ER stress depends on the unfolded protein response [UPR].[48] Genetic studies have identified several ER stress/UPR genes that are associated with IBD,[49] most notably *XBP1*, and there is evidence that ER stress levels are increased in the intestines of patients with IBD.[9] Autophagy activity is high in Paneth cells[50] and can act to counterbalance ER stress [51]; therefore ER stress is a significant risk when the UPR or autophagy is not functional. Consistent with this, targeted deletion of either *XBP1* or *ATG16L1* in intestinal epithelial cells is associated with severe spontaneous CD-like transmural ileitis if both genes are compromised.[50] Importantly, in Paneth cells of patients harbouring an *ATG16L1* T300A risk allele, the ER-stress markers 78 kDa glucose-regulated protein [GRP78] and phospho-eukaryotic initiation factor 2 α subunit [pEIF2α] were highly expressed.[52] This has led to suggestion that the ATG16L1 T300A variant may define a specific subtype of patients with CD, characterized by Paneth cell ER stress, which correlates with bacterial persistence and reduced antimicrobial functionality.[52] Interestingly, a recent study has demonstrated a direct link between NOD1/2 and ER stress-induced inflammation.[53] In mouse and human cells, the ER stress inducers thapsigargin and dithiothreitol trigger the production of the pro-inflammatory cytokine IL-6 in a NOD1/2-dependent manner. Furthermore, IL-6 production induced by the intracellular pathogen *Brucella abortus*, which also induces ER stress, was dependent upon NOD1/2-signalling. Therefore, it is significant that major risk factors for CD, ATG16L1 and NOD2, functionally intersect with ER stress and the UPR. The convergence between autophagy and ER stress provides new opportunity for the treatment of IBD. For example, modulation of the UPR in combination with autophagy inducers is a promising therapeutic strategy.

3.2. Current IBD drugs

The mechanism of action of current IBD drugs remains incompletely understood [Table 1]. However, progress has been made in recent years towards characterising their effects, with the modulation of immunoregulatory signalling pathways often linked directly or indirectly to the autophagy response [Table 2]. Importantly these heterogeneous studies have been conducted in a wide variety of disease settings and cell types; highlighting the need to explore the effect of these drugs on autophagy pathway activity in the context of IBD.

3.3. Corticosteroids

The first-line treatment for CD and UC is often corticosteroids. Corticosteroids downregulate pro-inflammatory cytokines including IL-1, IL-6 and tumor necrosis factor alpha (TNFα), by inhibiting the transcription of genes involved in their production and affecting the stability of messenger RNA [mRNA] to inhibit protein expression.[54] Furthermore, inflammatory signalling induced by NFκB is decreased due to interaction with corticosteroid receptors.[54] Although there is limited knowledge of the effect of corticosteroids on autophagy in IBD, there has been some progress in understanding their effect on autophagy in other disease settings.

The clinical response to corticosteroids in UC patients has been linked to mTORC1 [Figure 3]. In a transcriptomics study, it was observed that miRNA and mRNA profiles in the rectal mucosa of UC patients differed between responders and non-responders to corticosteroid treatment.[55] The mRNA with the most significant differential expression between groups was DNA damage-induced transcript 4 [DDIT4], an inhibitor of mTORC1 activity, which was upregulated in responders after 3 days of corticosteroid treatment.

Table 1. IBD drugs mechanism of action.

Drug class	Examples	Mechanism of action
Corticosteroids	Prednisolone, budesonide	• Downregulation of pro-inflammatory cytokines[54] • Interference with NFκB inflammatory signalling[54]
Aminosalicylates	Sulphasalazine, mesalazine	• Scavenging of damaging reactive oxygen species [ROS], upregulation of endogenous antioxidant systems, inhibition of leukocyte motility and leukotriene and platelet activation, interference with NFκB, TNFα, IL-1 and TGF-β, inhibition of nitric oxide formation, prevention of mitochondrial damage and colonic epithelial cell arrest in S-phase [9]
Thiopurines	Azathioprine, 6-mercaptopurine	• Inhibition of DNA, RNA and protein synthesis, causing results in immune suppression and cytotoxicity[75] • Induce T cell apoptosis through co-stimulation of CD28 due to the blockage of RAC1 activation of NFκB[76]
Immunomodulators	Methotrexate, cyclosporin and tacrolimus	• Methotrexate inhibits DNA and RNA synthesis in rapidly dividing cells[83] • Cyclosporin and tacrolimus alter IL-2 transcription causing reduced T-cell activity[83]
Biologics [Anti-TNF agents]	Infliximab, adalimumab	• Anti-TNFα antibodies neutralize TNFα to prevent pro-inflammatory functions

Table 2. Inflammatory bowel disease drugs linked to autophagy modulation.

Drug class	Evidence of autophagy modulation
Corticosteroids	• Corticosteroids upregulated mTORC1 inhibitors to induce autophagy in skeletal muscle *in vivo*, L6 myoblasts[57] and primary human lymphocytes[58] • Dexamethasone induced autophagy in T lymphocytes[62–64] • Inhibition of autophagy in human monocytes infected with *Aspergillus fumigatus*[65]
Aminosalicylates	• Sulphasalazine decreased autophagy via NFκB inhibition in an *in vivo* murine model of cachectic cancer[70] • Sulphasalazine induced autophagic cell death through inhibition of the Akt pathway and activation of the ERK pathway in an oral squamous cell carcinoma cell line[72]
Thiopurines	• Autophagy is activated in hepatocytes treated with thiopurines[73] • Increased autophagy in epithelial cells of animal colitis model due to rapid local bacterial conversion of thioguanine pro-drug to active metabolite[80]
Immunomodulators	• Cyclosporin cytotoxicity induced autophagy as a survival process in malignant glioma cells[83], primary cultured human renal tubular cells and *in vivo* with rat kidneys[84] and in kidney proximal tubule epithelial cells[85] • Cyclosporin induced autophagic-cell death in a rat pituitary cell line[86]. • Tacrolimus induced autophagy in mouse neuroblastoma and microglial cell lines and in the brains of tacrolimus-treated mice[90]
Biologics [Anti-TNF agents]	• Anti-TNF agents can induce reactivation of TB, at least partially due to decreased autophagy [97] • TNF stimulates autophagy in synovial fibroblasts from rheumatoid arthritis patients,[93] in skeletal muscle,[94] in atherosclerotic vascular smooth cells,[59] in trophoblastic cells[95] and in mouse macrophages[96]

Furthermore, three miRNAs that were differentially expressed in responders could potentially target DDIT4.

In the hippocampus of rats, it has also been shown that corticosterone treatment affects mTORC1 signalling pathways.[56] In this study, corticosterone upregulated the expression of DDIT4, as well as FK506-binding protein 51 [FKBP51], but downregulated DDIT3. DDIT4 and FKBP51 inhibit mTORC1 activity, whereas the pro-apoptotic transcription factor DDIT3 is itself regulated by mTORC1.[56] In agreement, Wang *et al.*[57] found that dexamethasone treatment of *in vivo* skeletal muscle and cultured L6 myoblasts increased DDIT4 expression and confirmed that DDIT4 downregulates mTORC1 activity. Another study, investigating the effects of dexamethasone treatment on T lymphocytes from healthy donors, found that there was a reduction in mTORC1 expression.[58] Taken together, these studies strongly suggest that the mTORC1 pathway and autophagy play an important role in the response to treatment with corticosteroids.

Corticosteroid treatment is often associated with secondary osteoporosis and several studies have investigated the effects of corticosteroids on osteocyte cell fate. It has been shown *in vitro* and *in vivo* that low doses of prednisolone and dexamethasone induce autophagy in osteocytes and this is associated with osteocyte viability.[59,60] However, higher doses of corticosteroids induce apoptosis, suggesting that autophagy may act as a protective mechanism against the cytotoxic effects of corticosteroids. [59] Autophagy is also activated in spinal cord injuries [SCL] along with apoptosis and necrosis; however, rats treated with methylprednisolone exhibited decreased autophagy post-SCL.[61] The effects of methylprednisolone on autophagy in this study may therefore be attributed to direct inhibition of autophagy or to a decrease in inflammation associated with injury, which indirectly reduces autophagy.

Corticosteroids are also used to treat lymphoid malignancies by blocking cell proliferation and inducing apoptosis in immature T cells. It has been shown that glucocorticoids induce autophagy in

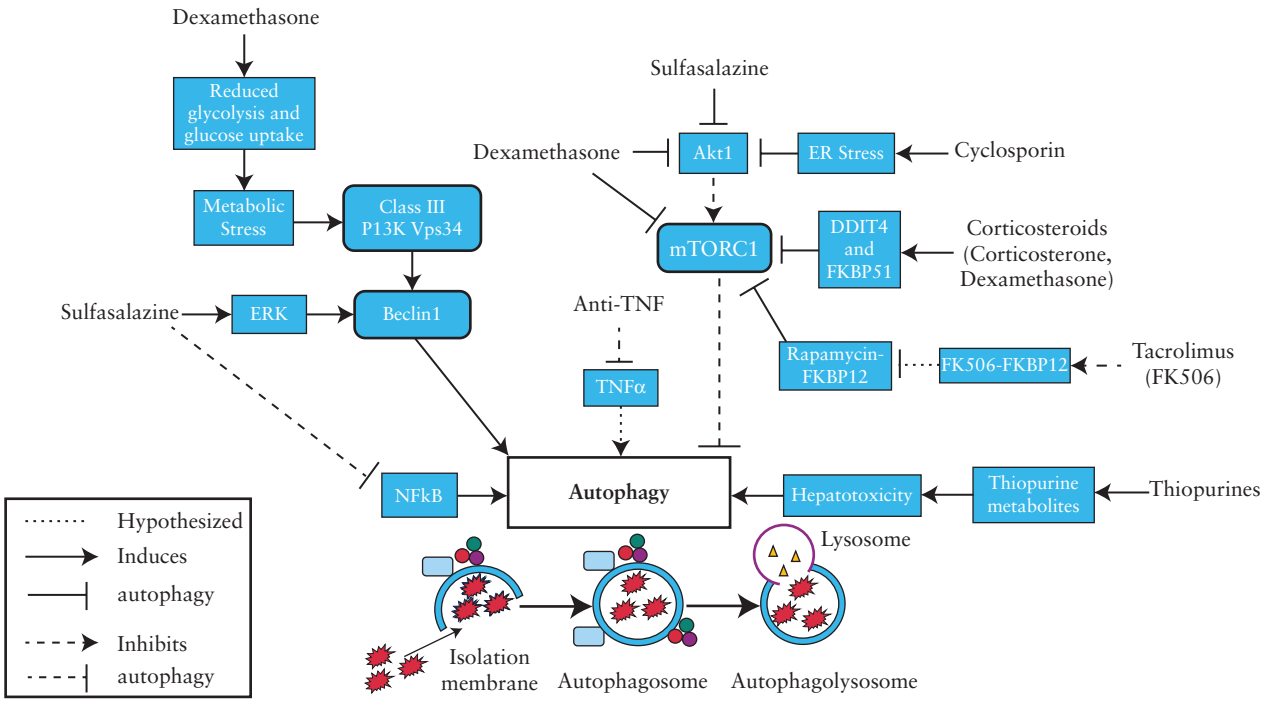

Figure 3. Current inflammatory bowel disease drugs modulation of autophagy pathways. Refer to the text and Table 2 for details.

immature T cell populations,[62] lymphoid cell lines[63] and primary leukaemia cells.[64] The dexamethasone-induced increase in autophagy was also associated with inhibition of mTORC1, possibly through regulation of the Src kinase Fyn.[62] Swerdlow *et al.*[63] suggested that a contributing factor to dexamethasone-induced autophagy could be metabolic stress caused by reduced glycolysis and glucose uptake in corticosteroid-treated lymphocytes [Figure 3]. Autophagy stimulation by glucocorticoids is relevant for treatment of lymphoid malignancies as it is intimately linked to the induction of apoptosis in T lymphocytes.[63,64] Corticosteroids are able to induce apoptosis in immature T lymphocytes, as these cells lack the inhibitor of apoptosis protein Bcl-2. When Bcl-2 was overexpressed in immature T lymphocytes, dexamethasone-induced apoptosis was shown to be inhibited.[63] Although Bcl-2 usually inhibits autophagy by binding to Beclin1 [Figure 2], it has been shown that overexpression of Bcl-2 in immature T lymphocytes can increase autophagy levels, presumably due to inhibition of apoptosis.[63] Furthermore, autophagy induction prolonged the survival of dexamethasone-treated cells, and autophagy inhibition decreased survival time.[63] In contrast, Laane *et al* [64] found that autophagy played a positive role in dexamethasone-induced apoptosis in lymphoid leukaemia cells. In this study, dexamethasone induced cell death through promyelocytic leukaemia [PML] protein-dependent dephosphorylation of the autophagy inhibitor Akt, stimulating the induction of autophagy [Figure 3].

Investigating fungal pathogen elimination in human monocytes demonstrated that corticosteroids could block autophagy protein recruitment to pathogen-containing phagosomes.[65] Detection of the fungal ligand β-glucan by Dectin-1 receptors triggered Syk kinase-dependent production of reactive oxygen species [ROS], which stimulate autophagy when cells are infected by *Aspergillus fumigatus*.[65] When autophagy was directly inhibited, or cells were treated with corticosteroids [*in vivo* and *ex vivo*], phagosome maturation [including fusion with the lysosome] and *A. fumigatus* killing were impaired.[65] This highlights the importance of autophagy as a defence mechanism against fungal infections, but contradicts studies suggesting that autophagy is induced by corticosteroid treatment. Whereas this study focused on the effects of corticosteroids on xenophagy with *A. fumigatus*, other studies investigating T lymphocytes focused on non-selective macroautophagy induced by cellular stress. The contrasting results could be due to differences between the types of immune cells investigated, the disease pathogenesis, the types of corticosteroids used or the different types of autophagy that were investigated, and serves to highlight the cell-type specific nature of autophagy and the need to investigate the effect of corticosteroids on cell types that are relevant to IBD.

3.4. Aminosalicylates

Aminosalicylates are effective as first-line drugs to induce and maintain remission in mild to moderate cases of UC.[66] Despite a lack of evidence for their efficacy in CD treatment, they are often prescribed as adjuvant therapy due to minimal side effects, low cost and chemo-preventative properties.[3,67] Sulphasalazine or salicylazosulphapyridine [SASP] was originally developed for rheumatoid arthritis and contains 5-aminosalicyclate [5-ASA] bound to sulphapyridine [68]. Sulphapyridine exhibits direct antimicrobial activity and treatments with sulphapyridine have been linked to alterations in faecal bacterial profiles.[69] Sulphapyridine has been associated with additional adverse effects,[3] leading to the development of other forms of aminosalicylates including mesalazine. These consist of only the active moiety of SASP and does not contain sulphapyridine; pro-drugs of mesalazine, for example balsalazide and olsalazine, are also in use.[68] The anti-inflammatory activities of 5-ASA include the scavenging of damaging ROS, upregulation of endogenous antioxidant systems, inhibition of leukocyte motility, leukotriene and platelet activation, interference with NFκB, TNFα, IL-1 and TGF-β, inhibition of nitric oxide formation, prevention of mitochondrial damage and colonic epithelial cell-cycle arrest in S-phase.[69] In theory, many of these activities could directly or indirectly affect autophagy

due to a reduction of cellular stress. One study, investigating sulphasalazine as an NFκB inhibitor in an *in vivo* murine model of cancer cachexia, reported a decrease in autophagy[70] [Figure 3]. This could be due to a direct effect of NFκB inhibition, as NFκB signalling regulates autophagy in a context-dependent manner,[71] or through one or more of the other pathways regulated by sulphasalazine. In addition, this response may be specific to the disease or to the muscle tissues being examined in murine models. In contrast, Han *et al.*[72] reported that sulphasalazine treatment in an oral squamous cell carcinoma [OSCC] cell line, HSC-4, induced autophagic cell death through inhibition of the Akt pathway and activation of the ERK pathway [Figure 3]. The seemingly opposing effects of sulphasalazine observed in these studies may be due to differences in dosage. Dosage is extremely difficult to compare between *in vitro* and *in vivo* studies; however, it is possible that the induction of autophagic cell death observed by Han *et al.*[72] may be representative of a concentration range that is cytotoxic.

3.5. Thiopurines

Thiopurines, including azathioprine, 6-mercaptopurine and 6-thioguanine, are immunosuppressant drugs used to treat IBD.[73] They have a relatively slow onset but can maintain remission in moderate to severe cases of CD and have also shown some effectiveness for the induction of remission.[3,74] The commonly used pro-drug azathioprine is converted to 6-mercaptopurine [6-MP] by glutathione in the intestinal wall. Through a multi-step enzymatic pathway, the drug is broken down to thiopurine metabolites, thioguanine nucleotides [TGN] and methylmercaptopurine nucleotides [MMPN]. These nucleotides act as purine antagonists causing the inhibition of DNA, RNA and protein synthesis, which results in immunosuppression and cytotoxicity.[75] Azathioprine can also generate 6-thioguanine GTP, which has been shown to induce T cell apoptosis through co-stimulation of the CD28 receptor due to blockage of Ras-related C3 botulinum toxin substrate [Rac1] activation of NFκB.[76] Erythrocyte concentrations of thiopurine metabolites are now carefully monitored in many centres, to maintain therapeutic levels and to assess adherence, as increases in blood concentration have been associated with hepatotoxicity.[77] Other severe adverse effects associated with thiopurines are pancreatitis and myelosuppression,[3] with 15–20% of patients treated with thiopurines having to discontinue treatment due to these side effects.[75]

Due to the severe adverse effects of thiopurines, a potential protective role for autophagy in hepatocytes has been investigated. Autophagy is activated in hepatocytes treated with thiopurines, possibly as a secondary response to the hepatotoxic effects of the drug [Figure 3]; however, it could also indicate that autophagy is directly modulated to balance immune responses in patients.[73,78] Despite the lack of understanding of the mechanism of action of thiopurines, it has been shown that autophagy has a protective role in hepatocytes during thiopurine therapy,[73] suggesting that a combination treatment of thiopurines with drugs that induce autophagy may reduce their adverse effects, enhancing their efficacy and safety.

A very recent study has correlated ATG16L1 genotype and response to thiopurines in two IBD cohorts and found that the ATG16L1 risk variant associates with response to thiopurine treatment specifically in patients with CD but not with UC.[79] Furthermore, a defect in the autophagosomal regulation of active Rac1, a member of the Rho family of GTPases linked to the regulation of diverse cellular functions including cytoskeletal rearrangement, underlies the association between ATG16L1 and CD through decreased myeloid cell migration.[79] As thiopurine can inhibit Rac1 activity, the authors suggest that ATG16L1 genotyping may be used to identify patients who would benefit from thiopurine treatment. In another new study, the rapid local bacterial conversion of thioguanine pro-drug to active metabolite was shown to augment autophagy in epithelial cells, resulting in increased intracellular bacterial killing and decreased intestinal inflammation and immune activation in spontaneous and induced animal colitis models.[80]

3.6. Methotrexate, cyclosporin and tacrolimus

Methotrexate, cyclosporin and tacrolimus are immunomodulatory drugs used mainly as second-line treatments to maintain remission in severe, steroid-refractory CD,[81] with more recent evidence suggesting a role for tacrolimus in UC.[82] Methotrexate inhibits DNA and RNA synthesis in rapidly dividing cells, and cyclosporin and tacrolimus alter IL-2 transcription causing reduced T cell activity.[83] Although some evidence suggests that cyclosporin and tacrolimus modulate autophagy as part of their mechanism of action, no link has been identified between methotrexate and autophagy modulation.

Cyclosporin, originally used to prevent organ transplant rejection, acts by blocking lymphocyte and other immune cell activation.[83] As this drug has very cytotoxic effects, several studies have shown that treatment with cyclosporin can induce autophagy in response to the toxicity either as a survival process or as part of a cell death mechanism.[83–86] Toxic levels of cyclosporin induced autophagy *in vivo* and *in vitro* in malignant glioma cells. [83] This was accompanied by mTORC1 inhibition and an ER stress response, with blockage of ER signalling decreasing accumulation of the autophagy marker LC3-II[83] [Figure 3]. Furthermore, when autophagy is inhibited by blocking ULK1, ATG5 or ATG7, cyclosporin-induced cell death was shown to increase.[83] These results suggest that autophagy is induced as a protective response to the cytotoxic effects of cyclosporin.

In a study of cyclosporin-induced nephrotoxicity, ER stress-dependent autophagy induction [Figure 3] has been demonstrated in primary cultured human renal tubular cells and *in vivo* within rat kidneys.[84] In addition, cyclosporin can cause chronic metabolic stress, which leads to autophagy induction in kidney proximal tubule epithelial cells.[85] In this study, autophagy-competent cells allow for metabolic adaptation to cyclosporin treatment, whereas autophagy deficiency resulted in cyclosporin-induced deterioration of the tricarboxylic acid [TCA] cycle and the overall energy status of the cell. In a rat pituitary cell line model, cyclosporin induced apoptosis and autophagic-cell death in a dose-dependent manner.[86] From these studies, it appears that autophagy is stimulated by cyclosporin only as a secondary response to the drug's cytotoxic effects.

The mechanism of action of tacrolimus, also known as FK506, is similar to that of cyclosporin as both drugs inhibit the protein phosphatase calcineurin to block T cell function and IL-2 transcription. FK506 inhibits calcineurin by forming a complex with the immunophilin FKBP12 [FK506 binding protein], which is involved in immunoregulation.[87] FKBP12 is also the direct target of rapamycin, an inhibitor of mTORC1.

A recent study by Ge *et al.*[88] investigating a novel activator of mTORC1, 3-benzyl-5-[[2-nitrophenoxy] methyl]–dihydrofuran-2[3H]-one [3BDO], demonstrated that 3BDO could activate mTORC1 by occupying the rapamycin-binding site in FKBP12.[89] This study suggested that FK506, through a mechanism involving the formation of an FK506-FKBP12 complex, has the potential to act as an mTORC1 activator and autophagy inhibitor [Figure 3]. In another study investigating the use of FK506 as a novel therapeutic for prion infections, FK506 was shown to induce autophagy in mouse neuroblastoma [N2a58] and mouse microglial [MG20] cell lines and in the brains of mice.[90] FK506 treatment significantly increased LC3-II, ATG5, ATG7 and autolysosome formation,

concomitant with decreased prion protein levels in cell cultures and increased survival of mice due to delayed accumulation of prion proteins.[90]

3.7. Biologic agents

Overproduction of pro-inflammatory cytokines and chemokines are a common feature associated with inflammatory diseases. Monoclonal antibodies that target and neutralise cytokines such as TNFα, IL-12, IL-23, IL-21, IL-22, IL-32 and IFN-γ, with a view to decreasing pro-inflammatory signaling, are used for the treatment of IBD.[91] These biologic agents are usually reserved for the treatment of refractory CD or steroid-dependent patients to induce and maintain remission.

The most commonly used biologic agent for IBD is the anti-TNFα antibody, infliximab. Other anti-TNFα treatments approved for treatment of IBD patients include adalimumab, golimumab for UC only, and certolizumab pegol, which is approved in the USA, Switzerland and Russia. Anti-TNFα biosimilars, which are cheaper versions of licensed biologic agents whose patents have now expired, have also recently been developed.[92]

TNFα plays a major role in modulating the inflammatory response, and while the effects of TNFα have been extensively studied in a variety of cell types, its mechanism of action in the gut remains unknown. One confirmed effect of TNFα is the modulation of autophagy, which has been observed in synovial fibroblasts from rheumatoid arthritis patients,[93] in skeletal muscle,[94] in atherosclerotic vascular smooth cells[59] and in trophoblastic cells.[95] The effect of TNFα on mitophagy, a specific type of autophagy that involves the degradation of mitochondrial proteins and the mitochondrial organelle, has also been demonstrated in mouse macrophages.[96] This study found that macrophages activated by TNFα have increased mitophagy, resulting in increased mitochondrial protein degradation and presentation to T cells via MHC I on the cell surface of the macrophages. As macrophages play a crucial role in innate immunity and inflammation within the gastrointestinal tract, further investigation of the effects of TNFα on autophagy in this cell type will be particularly relevant to IBD.

Taken together, these studies suggest that anti-TNF agents would inhibit autophagy [Figure 3]. Although there are no studies that have directly confirmed this, there is support for this hypothesis; anti-TNF agents can induce reactivation of *Mycobacterium tuberculosis*, at least partially due to decreased autophagy.[97] This effect is likely due to the protective antibacterial and anti-inflammatory roles of autophagy in epithelial cells infected with this non-motile bacillus.[98] It is worth noting however, that TNFα can also have inhibitory effects on autophagy in some contexts. A study investigating the effects of elevated TNFα on congestive heart failure in H9C2 rat cardiomyoblasts found that, although TNFα induces autophagy, autophagic protein degradation is disrupted, as evidenced by accumulation of p62 and increased ubiquitin-proteasome pathway activity.[99] Additionally, *Andrographis paniculata* plant extract [HMPL-400], which is currently being studied in IBD trials for reduction of TNFα, IL-1β, IFN-γ and IL-22 expression, has been shown to inhibit autophagy in cancer.[100] This may be due to the reduction of cytokines or another mechanism affected by HMPL-400.

4. Conclusions

The modulation of autophagy represents an exciting therapeutic option for the treatment of IBD, and evidence is already emerging that drugs currently used for the treatment of IBD can affect the autophagy pathway. The cross-talk between autophagy and ER stress offers new options for how IBD could be targeted, and combination treatments aimed at modulating both the UPR and autophagy warrant further investigation. However, to date there is little evidence that modulation of autophagy can be directly linked to amelioration of disease, with only one published case study of the mTORC1 inhibitor sirolimus [rapamycin] improving symptoms and healing in a patient with severe refractory CD.[101] A major caveat is that autophagy is cell type specific, which makes it difficult to mechanistically link drug-induced autophagy to modulation of disease. Irrespective of this there is a pressing need to determine how these drugs modulate the autophagy pathway, specifically in patients with known mutations in the genes regulating the autophagy apparatus, and this must begin with consolidating studies in an *in vitro* setting in cell types directly relevant to IBD. A more comprehensive understanding of their mechanisms of action will undoubtedly allow for better-informed decisions regarding suitability of drug treatment for IBD on a patient-to-patient basis.

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Conflict of Interest

None.

Author Contributions

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References

1. NHS England. *NHS Standard Contract For Colorectal: Complex Inflammatory Bowel Disease [Adult]*. London: NHS England, 2013/14.
2. Bassi A, Dodd S, Williamson P, Bodger K. Cost of illness of inflammatory bowel disease in the UK: A single centre retrospective study. *Gut* 2004;**53**:1471–8.
3. Diefenbach KA, Breuer CK. Pediatric inflammatory bowel disease. *World J Gastroenterol* 2006;**12**:3204–12.
4. Bernstein CN, Loftus EV Jr, Ng SC, Lakatos PL, Moum B. Hospitalisations and surgery in Crohn's disease. *Gut* 2012;**61**:622–9.
5. Denson LA, Long MD, McGovern DP, *et al*. Challenges in IBD research: Update on progress and prioritization of the CCFA's research agenda. *Inflamm Bowel Dis* 2013;**19**:677–82.
6. Fiocchi C. Tailoring treatment to the individual patient will inflammatory bowel disease medicine be personalized? *Dig Dis* 2015;**33**[Suppl 1]82–9.
7. Boyapati R, Satsangi J, Ho GT. Pathogenesis of Crohn's disease. *F1000Prime Rep* 2015;**7**:44.
8. Jostins L, Ripke S, Weersma RK, *et al*. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;**491**:119–24.
9. Kaser A, Lee AH, Franke A, *et al*. Xbp1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 2008;**134**:743–56.
10. Yang Z, Klionsky DJ. Eaten alive: A history of macroautophagy. *Nat Cell Biol* 2010;**12**:814–22.
11. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;**132**:27–42.
12. Lamb CA, Yoshimori T, Tooze SA. The autophagosome: Origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 2013;**14**:759–74.

13. Fujita N, Itoh T, Omori H, *et al*. The atg16l complex specifies the site of lc3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell* 2008;**19**:2092–100.
14. Kroemer G, Marino G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010;**40**:280–93.
15. Kim YC, Guan KL. Mtor: A pharmacologic target for autophagy regulation. *J Clin Invest* 2015;**125**:25–32.
16. Kim J, Kundu M, Viollet B, Guan KL. Ampk and mtor regulate autophagy through direct phosphorylation of ulk1. *Nat Cell Biol* 2011;**13**:132–41.
17. Russell RC, Tian Y, Yuan H, *et al*. Ulk1 induces autophagy by phosphorylating beclin-1 and activating vps34 lipid kinase. *Nat Cell Biol* 2013;**15**:741–50.
18. Levine B, Sinha S, Kroemer G. Bcl-2 family members: Dual regulators of apoptosis and autophagy. *Autophagy* 2008;**4**:600–6.
19. Liang XH, Kleeman LK, Jiang HH, *et al*. Protection against fatal sindbis virus encephalitis by beclin, a novel bcl-2-interacting protein. *J Virol* 1998;**72**:8586–96.
20. Pattingre S, Tassa A, Qu X, *et al*. Bcl-2 antiapoptotic proteins inhibit beclin 1-dependent autophagy. *Cell* 2005;**122**:927–39.
21. Maiuri MC, Le Toumelin G, Criollo A, *et al*. Functional and physical interaction between bcl-x[l] and a bh3-like domain in beclin-1. *Embo J* 2007;**26**:2527–39.
22. Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. Jnk1-mediated phosphorylation of bcl-2 regulates starvation-induced autophagy. *Mol Cell* 2008;**30**:678–88.
23. Bassik MC, Scorrano L, Oakes SA, Pozzan T, Korsmeyer SJ. Phosphorylation of bcl-2 regulates er ca[2+] homeostasis and apoptosis. *EMBO J* 2004;**23**:1207–16.
24. Wei Y, Sinha S, Levine B. Dual role of jnk1-mediated phosphorylation of bcl-2 in autophagy and apoptosis regulation. *Autophagy* 2008;**4**:949–51.
25. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: Crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007;**8**:741–52.
26. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013;**13**:722–37.
27. Delgado M, Singh S, De Haro S, *et al*. Autophagy and pattern recognition receptors in innate immunity. *Immunol Rev* 2009;**227**:189–202.
28. Hugot JP, Chamaillard M, Zouali H, *et al*. Association of nod2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;**411**:599–603.
29. Hampe J, Cuthbert A, Croucher PJ, *et al*. Association between insertion mutation in nod2 gene and Crohn's disease in German and British populations. *Lancet* 2001;**357**:1925–8.
30. Ogura Y, Bonen DK, Inohara N, *et al*. A frameshift mutation in nod2 associated with susceptibility to Crohn's disease. *Nature* 2001;**411**:603–6.
31. Niess JH, Klaus J, Stephani J, *et al*. Nod2 polymorphism predicts response to treatment in Crohn's disease first steps to a personalized therapy. *Dig Dis Sci* 2012;**57**:879–86.
32. Lesage S, Zouali H, Cézard J-P, *et al*. Card15/nod2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002;**70**:845–57.
33. Cooney R, Baker J, Brain O, *et al*. Nod2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010;**16**:90–7.
34. Hampe J, Franke A, Rosenstiel P, *et al*. A genome-wide association scan of nonsynonymous snps identifies a susceptibility variant for Crohn disease in atg16l1. *Nat Genet* 2007;**39**:207–11.
35. Cadwell K, Patel KK, Maloney NS, *et al*. Virus-plus-susceptibility gene interaction determines Crohn's disease gene atg16l1 phenotypes in intestine. *Cell* 2010;**141**:1135–45.
36. Cadwell K, Liu JY, Brown SL, *et al*. A key role for autophagy and the autophagy gene atg16l1 in mouse and human intestinal paneth cells. *Nature* 2008;**456**:259–63.
37. Murthy A, Li Y, Peng I, *et al*. A Crohn's disease variant in atg16l1 enhances its degradation by caspase 3. *Nature* 2014;**506**:456–62.
38. Homer CR, Richmond AL, Rebert NA, Achkar JP, McDonald C. Atg16l1 and nod2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. *Gastroenterology* 2010;**139**:1630–41.
39. Saitoh T, Fujita N, Jang MH, *et al*. Loss of the autophagy protein atg16l1 enhances endotoxin-induced il-1beta production. *Nature* 2008;**456**:264–8.
40. Plantinga TS, Crisan TO, Oosting M, *et al*. Crohn's disease-associated atg16l1 polymorphism modulates pro-inflammatory cytokine responses selectively upon activation of nod2. *Gut* 2011;**60**:1229–35.
41. Strisciuglio C, Miele E, Wildenberg ME, *et al*. T300a variant of autophagy atg16l1 gene is associated with decreased antigen sampling and processing by dendritic cells in pediatric Crohn's disease. *Inflamm Bowel Dis* 2013;**19**:2339–48.
42. Travassos LH, Carneiro LA, Ramjeet M, *et al*. Nod1 and nod2 direct autophagy by recruiting atg16l1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* 2010;**11**:55–62.
43. McCarroll SA, Huett A, Kuballa P, *et al*. Deletion polymorphism upstream of irgm associated with altered irgm expression and Crohn's disease. *Nat Genet* 2008;**40**:1107–12.
44. Brest P, Lapaquette P, Souidi M, *et al*. A synonymous variant in irgm alters a binding site for mir-196 and causes deregulation of irgm-dependent xenophagy in Crohn's disease. *Nat Genet* 2011;**43**:242–5.
45. Gardet A, Benita Y, Li C, *et al*. Lrrk2 is involved in the ifn-gamma response and host response to pathogens. *J Immunol* 2010;**185**:5577–85.
46. Liu B, Gulati AS, Cantillana V, *et al*. Irgm1-deficient mice exhibit paneth cell abnormalities and increased susceptibility to acute intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2013;**305**:G573–84.
47. Liu Z, Lee J, Krummey S, *et al*. The kinase lrrk2 is a regulator of the transcription factor nfat that modulates the severity of inflammatory bowel disease. *Nat Immunol* 2011;**12**:1063–70.
48. Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 2008;**8**:663–74.
49. McGuckin MA, Eri RD, Das I, Lourie R, Florin TH. ER stress and the unfolded protein response in intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2010;**298**:G820–32.
50. Adolph TE, Tomczak MF, Niederreiter L, *et al*. Paneth cells as a site of origin for intestinal inflammation. *Nature* 2013;**503**:272–6.
51. Ogata M, Hino S, Saito A, *et al*. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 2006;**26**:9220–31.
52. Deuring JJ, Fuhler GM, Konstantinov SR, *et al*. Genomic atg16l1 risk allele-restricted paneth cell ER stress in quiescent Crohn's disease. *Gut* 2014;**63**:1081–91.
53. Keestra-Gounder AM, Byndloss MX, Seyffert N, *et al*. Nod1 and nod2 signalling links ER stress with inflammation. *Nature* 2016;**532**:394–7.
54. Kuenzig ME, Rezaie A, Seow CH, *et al*. Budesonide for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2014;**8**:CD002913.
55. Naves JE, Manye J, Loren V, *et al*. P042 response to corticosteroids in ulcerative colitis may be related to modulation of mtor signaling pathway genes by micrornas. *J Crohns Colitis* 2015;**9**[Suppl 1]:S98.
56. Polman JA, Hunter RG, Speksnijder N, *et al*. Glucocorticoids modulate the mtor pathway in the hippocampus: Differential effects depending on stress history. *Endocrinology* 2012;**153**:4317–27.
57. Wang H, Kubica N, Ellisen LW, Jefferson LS, Kimball SR. Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of redd1. *J Biol Chem* 2006;**281**:39128–34.
58. Fatkhullina AR, Abramov SN, Skibo Iu V, Abramova ZI. [Dexamethasone affect on the expression of bcl-2 and mtor genes in t-lymphocytes from healthy donors]. *Tsitologiia* 2014;**56**:459–61.
59. Jia G, Cheng G, Gangahar DM, Agrawal DK. Insulin-like growth factor-1 and tnf-alpha regulate autophagy through c-jun n-terminal kinase and akt pathways in human atherosclerotic vascular smooth cells. *Immunol Cell Biol* 2006;**84**:448–54.
60. Xia X, Kar R, Gluhak-Heinrich J, *et al*. Glucocorticoid-induced autophagy in osteocytes. *J Bone Miner Res* 2010;**25**:2479–88.
61. Chen HC, Fong TH, Lee AW, Chiu WT. Autophagy is activated in injured neurons and inhibited by methylprednisolone after experimental spinal cord injury. *Spine* 2012;**37**:470–5.
62. Harr MW, McColl KS, Zhong F, Molitoris JK, Distelhorst CW. Glucocorticoids downregulate fyn and inhibit ip[3]-mediated calcium signaling to promote autophagy in t lymphocytes. *Autophagy* 2010;**6**:912–21.

63. Swerdlow S, McColl K, Rong Y, *et al*. Apoptosis inhibition by bcl-2 gives way to autophagy in glucocorticoid-treated lymphocytes. *Autophagy* 2008;**4**:612–20.
64. Laane E, Tamm KP, Buentke E, *et al*. Cell death induced by dexamethasone in lymphoid leukemia is mediated through initiation of autophagy. *Cell Death Differ* 2009;**16**:1018–29.
65. Kyrmizi I, Gresnigt MS, Akoumianaki T, *et al*. Corticosteroids block autophagy protein recruitment in aspergillus fumigatus phagosomes via targeting dectin-1/syk kinase signaling. *J Immunol* 2013;**191**:1287–99.
66. Turner D, Levine A, Escher JC, *et al*. Management of pediatric ulcerative colitis: Joint ECCO and ESPGHAN evidence-based consensus guidelines. *J Pediatr Gastroenterol Nutr* 2012;**55**:340–61.
67. Schoepfer AM, Bortolotti M, Pittet V, *et al*. The gap between scientific evidence and clinical practice: 5-aminosalicylates are frequently used for the treatment of Crohn's disease. *Aliment Pharmacol Ther* 2014;**40**:930–7.
68. Crohn's and Colitis Foundation of America. *Aminosalicylates*. 2013. www.ccfa.org/assets/pdfs/aminosalicylates.pdf
69. Campregher C, Gasche C. Aminosalicylates. *Best Pract Res Clin Gastroenterol* 2011;**25**:535–46.
70. Chacon-Cabrera A, Fermoselle C, Urtreger AJ, *et al*. Pharmacological strategies in lung cancer-induced cachexia: Effects on muscle proteolysis, autophagy, structure, and weakness. *J Cell Physiol* 2014;**229**:1660–72.
71. Salminen A, Hyttinen JMT, Kauppinen A, Kaarniranta K. Context-dependent regulation of autophagy by ikk-nf-κb signaling: Impact on the aging process. *Int J Cell Biol* 2012;**2012**:849541.
72. Han HY, Kim H, Jeong SH, Lim DS, Ryu MH. Sulfasalazine induces autophagic cell death in oral cancer cells via akt and erk pathways. *Asian Pac J Cancer Prev* 2014;**15**:6939–44.
73. Guijarro LG, Roman ID, Fernandez-Moreno MD, Gisbert JP, Hernandez-Breijo B. Is the autophagy induced by thiopurines beneficial or deleterious? *Curr Drug Metab* 2012;**13**:1267–76.
74. Gisbert JP, Chaparro M, Gomollon F. Common misconceptions about 5-aminosalicylates and thiopurines in inflammatory bowel disease. *World J Gastroenterol* 2011;**17**:3467–78.
75. Stocco G, Cuzzoni E, De Iudicibus S, *et al*. Thiopurine metabolites variations during co-treatment with aminosalicylates for inflammatory bowel disease: Effect of n-acetyl transferase polymorphisms. *World J Gastroenterol* 2015;**21**:3571–8.
76. Tiede I, Fritz G, Strand S, *et al*. Cd28-dependent rac1 activation is the molecular target of azathioprine in primary human cd4+ t lymphocytes. *J Clin Invest* 2003;**111**:1133–45.
77. Gardiner SJ, Gearry RB, Burt MJ, Ding SL, Barclay ML. Severe hepatotoxicity with high 6-methylmercaptopurine nucleotide concentrations after thiopurine dose escalation due to low 6-thioguanine nucleotides. *Eur J Gastroenterol Hepatol* 2008;**20**:1238–42.
78. Petit E, Langouet S, Akhdar H, *et al*. Differential toxic effects of azathioprine, 6-mercaptopurine and 6-thioguanine on human hepatocytes. *Toxicol In Vitro* 2008;**22**:632–42.
79. Wildenberg M, Koelink P, Diederen K, *et al*. Autophagy regulates dendritic cell migration through rac1: Implications for thiopurine therapy. *J Crohns Colitis* 2016;**10**[Suppl 1]:S3–S4.
80. Oancea I, Das I, Aguirre de Carcer D, *et al*. Bacterial activation of thioguanine results in lymphocyte independent improvement in murine colitis. *J Crohns Colitis* 2016;**10**[Supp 1] S117.
81. Markowitz J, Grancher K, Kohn N, Daum F. Immunomodulatory therapy for pediatric inflammatory bowel disease: Changing patterns of use, 1990–2000. *Am J Gastroenterol* 2002;**97**:928–32.
82. Nuki Y, Esaki M, Asano K, *et al*. Comparison of the therapeutic efficacy and safety between tacrolimus and infliximab for moderate-to-severe ulcerative colitis: A single center experience. *Scand J Gastroenterol* 2016;**51**:700–5.
83. Ciechomska IA, Gabrusiewicz K, Szczepankiewicz AA, Kaminska B. Endoplasmic reticulum stress triggers autophagy in malignant glioma cells undergoing cyclosporine a-induced cell death. *Oncogene* 2013;**32**:1518–29.
84. Pallet N, Bouvier N, Legendre C, *et al*. Autophagy protects renal tubular cells against cyclosporine toxicity. *Autophagy* 2008;**4**:783–91.
85. Kimura T, Takahashi A, Takabatake Y, *et al*. Autophagy protects kidney proximal tubule epithelial cells from mitochondrial metabolic stress. *Autophagy* 2013;**9**:1876–86.
86. Kim HS, Choi S-I, Jeung E-B, Yoo Y-M. Cyclosporine a induces apoptotic and autophagic cell death in rat pituitary gh3 cells. *PLoS One* 2014;**9**:e108981.
87. Liu J, Albers MW, Wandless TJ, *et al*. Inhibition of t cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. *Biochemistry* 1992;**31**:3896–901.
88. Ge D, Han L, Huang S, *et al*. Identification of a novel mtor activator and discovery of a competing endogenous rna regulating autophagy in vascular endothelial cells. *Autophagy* 2014;**10**:957–71.
89. Amiot A, Peyrin-Biroulet L. Current, new and future biological agents on the horizon for the treatment of inflammatory bowel diseases. *Ther Adv Gastroenterol* 2015;**8**:66–82.
90. Nakagaki T, Satoh K, Ishibashi D, *et al*. Fk506 reduces abnormal prion protein through the activation of autolysosomal degradation and prolongs survival in prion-infected mice. *Autophagy* 2013;**9**:1386–94.
91. Nys K, Agostinis P, Vermeire S. Autophagy: A new target or an old strategy for the treatment of Crohn's disease? *Nat Rev Gastroenterol Hepatol* 2013;**10**:395–401.
92. de Ridder L, Waterman M, Turner D, *et al*. Use of biosimilars in paediatric inflammatory bowel disease: A position statement of the ESPGHAN Paediatric IBD Porto group. *J Pediatr Gastroenterol Nutr* 2015;**61**:5038.
93. Connor AM, Mahomed N, Gandhi R, Keystone EC, Berger SA. Tnfalpha modulates protein degradation pathways in rheumatoid arthritis synovial fibroblasts. *Arthritis Res Ther* 2012;**14**:R62.
94. Keller CW, Fokken C, Turville SG, *et al*. Tnf-α induces macroautophagy and regulates mhc class ii expression in human skeletal muscle cells. *J Biol Chem* 2011;**286**:3970–80.
95. Cha HH, Hwang JR, Kim HY, *et al*. Autophagy induced by tumor necrosis factor alpha mediates intrinsic apoptosis in trophoblastic cells. *Reprod Sci* 2014;**21**:612–22.
96. Bell C, English L, Boulais J, *et al*. Quantitative proteomics reveals the induction of mitophagy in tumor necrosis factor-α-activated [tnfα] macrophages. *Mol Cell Proteomics* 2013;**12**:2394–407.
97. Harris J, Keane J. How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clin Exp Immunol* 2010;**161**:1–9.
98. Castillo EF, Dekonenko A, Arko-Mensah J, *et al*. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc Natl Acad Sci U S A* 2012;**109**:E3168–76.
99. Opperman CM, Sishi BJ. Tumor necrosis factor alpha stimulates p62 accumulation and enhances proteasome activity independently of ros. *Cell Biol Toxicol* 2015;**31**:83–94.
100. Zhou J, Hu SE, Tan SH, *et al*. Andrographolide sensitizes cisplatin-induced apoptosis via suppression of autophagosome-lysosome fusion in human cancer cells. *Autophagy* 2012;**8**:338–49.
101. Massey DC, Bredin F, Parkes M. Use of sirolimus [rapamycin] to treat refractory Crohn's disease. *Gut* 2008;**57**:1294–6.