Autophagy and inflammatory diseases

Sarah A Jones[1,2], Kingston HG Mills[1,2] and James Harris[2]

Autophagy is a cellular mechanism for the sequestration and degradation of intracellular pathogens and compromised organelles, particularly damaged mitochondria. Autophagy also clears other cellular components, such as inflammasomes and cytokines, thus providing an important means of regulating inflammation. Defects in autophagy have been found by genetic association studies to confer susceptibility to several autoimmune and inflammatory disorders, particularly inflammatory bowel disease. Thus, the manipulation of autophagy in disease situations is of growing interest for therapeutic targeting; however, the involvement of autophagy in cellular homoeostasis, in normal immune function and in inflammation is manifold. An appreciation of the intricacies of the contributions of this process to inflammation, and how these are altered by various immune and environmental stimuli, is essential for the understanding and interpretation of studies of inflammation and the design of therapeutics exploiting the manipulation of autophagy. This review focuses on the known roles of autophagy in the induction and maintenance of inflammation and on its role in the aetiology and regulation of inflammatory and autoimmune disorders.

Many damaged cellular constituents are cleared through the process of macroautophagy, in which a nascent double-membraned autophagosome forms around protein aggregates and organelles in the first step of a process that ultimately results in lysosomal degradation and recycling of components for use by the cell. In a quiescent cell, macroautophagy occurs at a basal level to remove defective organelles, such as dysfunctional mitochondria and peroxisomes, as well as misfolded proteins in response to endoplasmic reticulum (ER) stress (reviewed elsewhere[1]). Thus, autophagy is required for normal cell functioning and survival. Macroautophagy (hereafter referred to as autophagy) is characterised by the formation of an isolation membrane, or phagophore, which elongates around its target and fuses with itself to form a double-membraned autophagosome. This can then fuse with lysosomes to form an autolysosome, leading to the degradation of its luminal contents. This process is controlled by the products of numerous autophagy-specific genes (Atg) and by the mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that regulates cell growth, proliferation, motility and survival, gene transcription and protein synthesis. Inhibition of mTOR is essential for autophagy to initiate, and allows the translocation of a complex containing Atg1/unc-51-like kinase (ULK)1/2, Atg13, FIP200 and Atg101 from the cytosol to the ER, a process dependent on the interaction between ULK1 and AMP-activated protein kinase (AMPK).[2,3] This leads to the recruitment of the type III phosphatidylinositol-3-kinase, VPS34, in a complex with other proteins, including beclin 1, to the developing autophagosome.

Generation of phosphatidylinositol-3-phosphate by this complex is crucial for the recruitment of proteins required for initiation of autophagosome formation[4,5] (Figure 1). Inhibitors of phosphatidylinositol-3-kinase, including 3-methyladenine (3-MA), are commonly used to inhibit autophagy in *in vitro* studies, although such studies must be interpreted with caution due to other effects of the compounds used (Box 1).

Autophagy regulates energy and nutrient homoeostasis and has an essential role in tissue development.[6] Autophagic activity is amplified in times of deprivation of oxygen, growth factors or nutrients, and this is essential for cell survival.[7–9] Increased autophagy in hypoxic or starved cells facilitates a shift from aerobic respiration to glycolysis and provides a means by which cellular components can be hydrolysed to provide fuel for metabolism. This glycolytic shift also occurs in proliferating myeloid cells and lymphocytes and increased levels of autophagy are characteristic of activated immune cells.[10] In addition, autophagy is an important clean-up mechanism following the respiratory burst in leucocytes, clearing reactive oxygen species and mitochondrial debris and protecting against damage and death.[11–13] Although a moderate level of autophagy is required to maintain a healthy cytosolic environment, excessive autophagy can lead to autophagic cell death.[14]

Autophagy also shapes immune responses by directly participating in immune cell function. For example, autophagy-degraded cellular components can be loaded onto MHC (major histocompatibility complex) class I and II molecules for presentation to T cells.[15–17]
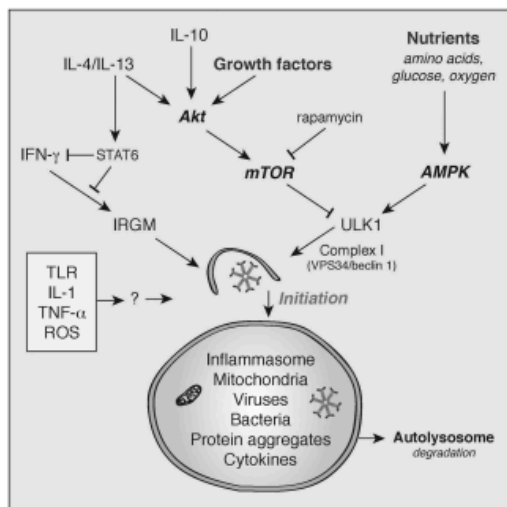
Figure 1 Pathways involved in autophagy regulation. Autophagy is regulated by numerous stimuli, including nutrient starvation, growth factors, cytokines, reactive oxygen species (ROS), pharmacological inhibitors and danger/pathogen-associated molecular patterns (DAMPs and PAMPs). Autophagosome formation is largely controlled by mTOR; inhibition of mTOR leads to the interaction between the serine/threonine protein kinase ULK1 and AMPK, which, in turn, recruits the type III PI3 kinase VPS34, in complex with other proteins, including beclin 1, to the developing autophagosome. Nutrient deprivation activates AMPK and may also inhibit mTOR activation, leading to autophagosome formation. Activation of the AKT pathway by growth factors and cytokines, including IL-4, IL-13 and IL-10, leads to activation of mTOR and inhibition of autophagosome formation. Other cytokines induce autophagy. IFN-γ promotes autophagosome formation through an IRGM (Irgm1 in mice)-dependent mechanism. This pathway is not well understood but does involve mitochondrial fission. This process is inhibited by IL-4 and IL-13 through a STAT6 (signal transducer and activator of transcription factor 6)-dependent mechanism. TNF-α, IL-1, ROS and engagement of Toll-like receptors (TLR) also induce autophagy, although these pathways are not well characterised. Autophagosomes can sequester and deliver cytosolic constituents to lysosomes for degradation and recycling. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

Intracellular pathogens can be killed by autophagy, including *Mycobacterium tuberculosis*, *Candida albicans*, adherent-invasive *Escherichia coli* and group A Streptococcus.[18–21] Similarly, autophagy is involved in host-protective immune responses against infection with viruses, such as Sindbis virus,[22] Epstein Barr virus[23] and vesicular stomatitis virus.[24] The rate of autophagy can be modulated in lymphocytes by antigen receptor stimulation and in macrophages following activation of Toll-like receptors (TLRs) and pattern-recognition receptors with pathogen- and danger-associated molecular patterns.[25,26–28] Furthermore, T helper type 1 (Th1) and pro-inflammatory cytokines, including interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-23, induce autophagy,[29–32] while Th2 and regulatory cytokines, including IL-4, IL-13 and IL-10, are inhibitory.[33–36] Importantly, autophagy is now recognised to be a major mechanism for regulating the secretion of cytokines and chemokines, particularly in macrophages, facilitating macrophage-mediated control of cell recruitment and orchestration of immune responses.[37–40] The influence of the metabolic state of

Box 1 An important cautionary point in the interpretation of studies using the autophagy inhibitor, 3-methyladenine (3-MA).

3-MA inhibits the class III PI3K VPS34 and thus blocks the early stages of autophagosome biogenesis. However, 3-MA also inhibits the class I phosphatidylinositol-3-kinase (PI3K), which disrupts the AKT pathway and can affect cell viability. Importantly, 3-MA can have different temporal patterns of inhibition; its effect on VPS34 is relatively short-lived, whereas its effect on the class I PI3K is more long-term, potentially resulting in an increase in autophagy over longer time periods.[155] Wortmannin, which inhibits both PI3Ks on a more equal basis, can be used in place of 3-MA in such studies. In addition, effects on the class I PI3K and the AKT pathway can have autophagy-independent effects on cytokine secretion. In our own studies, we have found that 3-MA can inhibit lipopolysaccharide (LPS)-induced tumour necrosis factor-α, interleukin (IL)-12p40 and IL-6 secretion by murine macrophages, but these effects are not seen in cells transfected with siRNA against autophagy genes.[32,64] A recent study has also suggested that, in the murine RAW264.7 macrophage cell line, 3-MA enhances IL-1β transcription and secretion in an autophagy-independent manner.[156] In this study, 3-MA, used at sub-optimal dose (1 mM), induced autophagy and increased pro-inflammatory responses through the inhibition of AKT and glycogen synthase kinase 3β. However, given that RAW264.7 cells do not express ASC (apoptotic speck protein containing a caspase recruitment domain),[157] this may represent a different mechanism of caspase-1-independent IL-1β processing, unrelated to that seen in previous studies using 3-MA, wortmannin and genetic deletion of autophagy genes.[32,64–67] Moreover, in murine bone marrow-derived dendritic cells, LPS-induced IL-1β secretion is increased by treatment with 3-MA, Ly294002 and wortmannin but not by class I PI3Kα/δ inhibitors or an AKT inhibitor.[32,64] These studies clearly emphasise the importance of using different, corroborative methods and diverse cell systems for monitoring the regulation of autophagy.

immune cells on inflammatory responses is an area of growing interest, and the roles of autophagy in this process are of considerable potential importance.

STARVATION-INDUCED AUTOPHAGY AND INFLUENCES ON INFLAMMATION

In conditions of low cellular energy and essential amino-acid deprivation, the induction of autophagy is driven by AMPK, which is antagonised by mTOR when nutrients are sufficient.[41–43] Activation of AMPK occurs through an increase in the ratio of AMP to ATP, indicative of a state of oxygen deprivation, as well as phosphorylation by CaMKKβ when cytosolic Ca^{2+} accumulates, which occurs during amino-acid deprivation.[42,44] AMPK activation stimulates pathways that correct imbalances in glucose and lipid concentrations and return energy levels to normal. When energy levels are low, AMPK halts cell growth and migration and supports cell survival by driving autophagic degradation of damaged mitochondria.[45] Small molecule activators of AMPK can induce autophagic clearance of β-amyloid plaques in models of Alzheimer's disease,[46] and there is emerging evidence that AMPK is a key modulator of immune responses; it can reduce the severity of inflammation and tissue damage in colitis[47,48] and experimental autoimmune encephalomyelitis[49] and airway inflammation in asthma.[50] In addition, AMPK can drive the induction of regulatory T cells,[51] the differentiation and inhibitory activity of myeloid-derived suppressor cells[52] and, in macrophages, AMPK activation is a critical point at which anti-inflammatory signals converge to elicit suppressive responses. For example, IL-10 and transforming growth factor β activate AMPK in macrophages and inhibition of AMPK in macrophages results in excessive production of IL-6, TNF-α and cyclooxygenase-2 in response to lipopolysaccharide

(LPS).[53] Conversely, AMPK suppresses LPS-induced IL-6 and TNF-α and inhibits the respiratory burst in neutrophils.[11,54] Thus, activation of AMPK is predominantly anti-inflammatory and autophagy may represent one mechanism through which AMPK exerts these effects.

AUTOPHAGY REGULATES CYTOKINE SECRETION

As well as regulating responses to pathogens within cells, autophagy can influence immune responses in microenvironments through its role as a regulator of cytokine secretion, particularly within antigen-presenting cells. In particular, autophagy can modulate the secretion of members of the IL-1 cytokine family, IL-23 and, as a consequence, IL-17.

Autophagy and IL-1 family cytokines

The IL-1 cytokine family, including IL-1α, IL-1β, IL-18, IL-33, IL-36, IL-37 and IL-38, orchestrate a wide range of immune and physiological effects. In particular, IL-1α and IL-1β, which signal through the IL-1 type I receptor (IL-1R1), are pro-inflammatory, acting partly through the induction of cyclooxygenase-2, type 2 phospholipase A and inducible nitric oxide synthase.[55] IL-1α and IL-1β also recruit myeloid cells, including neutrophils, to sites of inflammation.[56] Like IL-1α and IL-1β, IL-18 promotes inflammation, stimulating IFN-γ production by natural killer cells and Th1 cells and IL-17 production by γδ T cells.[57] IL-1β and IL-18 are produced as inactive pro-forms that are cleaved by caspase-1 to form the mature, bioactive cytokines. Caspase-1 is itself activated by an inflammasome, a large multimeric structure that includes an intracellular sensor, such as the NOD-like receptor (NLR) NLRP3 or the DNA sensor, absent in melanoma 2 (AIM2).[58] Recently, findings have suggested that IL-1β can drive the secretion of both IL-1α and IL-23,[59,60] further highlighting the importance of this cytokine in regulating inflammatory responses.

The activity of IL-1α and IL-1β is regulated by a naturally occurring IL-1 receptor antagonist IL-1Ra and by the decoy receptor IL-1RII,[61] whereas IL-18 is regulated by IL-18-binding protein.[62,63] It has also been demonstrated that autophagy can regulate IL-1β, IL-1α and IL-18 at the levels of transcription, processing and secretion. This occurs through at least two distinct mechanisms (Figure 2). Firstly, autophagy suppresses TLR-induced secretion of IL-1β, IL-1α and IL-18 in macrophages and dendritic cells (DC).[64–67] Production of biologically active IL-1β typically requires two signals. The initial signal is provided by pathogen-associated molecular patterns, such as LPS, or danger-associated molecular patterns, such as HMGB1, and results in transcription of pro-IL-1. This is followed by activation of inflammasome assembly by a second stimulus, such as reactive oxygen species, mitochondrial DNA, ATP, particulates (for example, silica, alum), protein aggregates and lysosomal rupture. Autophagy suppresses inflammasome assembly by degrading numerous endogenous stimuli, including mitochondrial DNA and reactive oxygen species,[65,67] that would otherwise induce inflammasome activation and processing of pro-IL-1β into the mature cytokine. Thus, inhibition of autophagy under these conditions leads to an increase in inflammasome activation and subsequent processing of IL-1β and IL-18.

The second mechanism by which autophagy negatively influences IL-1 and IL-18 secretion is more direct; autophagosomes can sequester and degrade inflammasome components and pro-IL-1β.[64,68] In mouse DC, induction of autophagy can prevent IL-1β secretion in response to LPS with alum or ATP, while in LPS-stimulated mouse macrophages, in the absence of an inflammasome-inducing signal, autophagosomes sequester and degrade pro-IL-1β.[64] More recently, Shi et al.[68] have demonstrated that activation of the NLRP3 and AIM2 inflammasomes induces autophagy in human macrophages. In
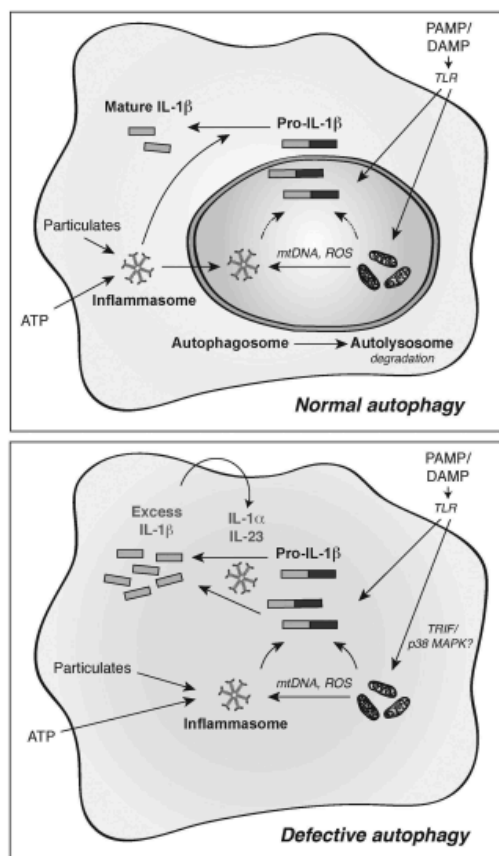

Figure 2 Regulation of IL-1β secretion by autophagy. Secretion of IL-1β by macrophages and dendritic cells requires the production of an inactive precursor (pro-IL-1β) and the assembly and activation of an inflammasome, which, in turn, activates caspase-1 to process pro-IL-1β into the mature, active cytokine. Stimulation of TLRs by endogenous danger signals (DAMPs) and pathogen-associated molecules (PAMPs) stimulates the production of pro-ILβ but also induce mitochondrial dysfunction or instability, leading to the release of reactive oxygen species (ROS) and mitochondrial DNA (mtDNA). Inflammasome assembly is activated by numerous stimuli, including ATP and particulates, such as silica and uric acid acrystals. In normal cells, autophagosomes can sequester and degrade pro-IL-1β, damaged mitochondria and inflammasome components, thus limiting IL-1β secretion. However, in cells defective in autophagy, all of these stimuli can remain uncontrolled, leading to excessive IL-1β secretion. This, in turn, may stimulate the autocrine secretion of other pro-inflammatory cytokines, particularly IL-1α and IL-23. MAPK, mitogen-activated protein kinase; TRIF, TIR-domain-containing adapter-inducing interferon-β. A full colour version of this figure is available at the *Immunology and Cell Biology* journal online.

addition, inflammasome components have been observed to co-localise with autophagosome components,[68] indicating that, similar to pro-IL-1β, inflammasomes are degraded within autophagosomes. These data suggest that autophagy is induced by inflammatory stimuli and acts a self-regulatory mechanism for the control of inflammatory

cytokine secretion, thereby downregulating potentially deleterious inflammatory responses.

Autophagic regulation of the IL-23–IL-17 pathway

Through regulating IL-1β secretion, autophagy also moderates the production of another inflammatory cytokine, IL-23.[32,60] IL-23 is an IL-12 family cytokine that, synergistically with IL-1α, IL-1β or IL-18, induces the differentiation and expansion of Th17 cells from naive CD4 T cells, as well as the secretion of IL-17 by γδ T cells and other innate lymphoid cells.[57,69] Both IL-23 and IL-17 are closely linked with a number of autoimmune diseases, including psoriasis and multiple sclerosis, as well as asthma and ankylosing spondylitis (reviewed elsewhere[70]). In both mouse and human macrophages and DC, inhibition of autophagy allows excessive IL-23 secretion, whereas induction of autophagy has the opposite effect.[32] IL-1β can drive IL-23 secretion,[60] and this appears to be the mechanism through which autophagy exerts its effects on IL-23. Supporting this, IL-23 production in autophagy-impaired human macrophages is dependent on NF-κB signalling and is inhibited by IL-1-neutralising antibodies. As IL-1, IL-18 and IL-23 have a major role in promoting IL-17 production by T cells,[71] regulation of these cytokines by autophagy can affect IL-17 secretion. Indeed, supernatants from mouse DC primed with LPS and cultured in the presence of the autophagy inhibitor 3-MA contained high levels of IL-1β and IL-23 and potently induced IL-17, IL-22 and IFN-γ secretion by γδ T cells *in vitro*.[32] This may also operate *in vivo*, as mice lacking the autophagy protein Atg5 in myeloid cells secrete higher levels of IL-1α, IL-12p70, CXCL1 (C-X-C motif chemokine ligand 1) and IL-17 in response to infection with *M. tuberculosis*.[72] These data indicate that autophagy in innate immune cells has the potential to influence T-cell polarisation, suggesting an important role in the control of both inflammation and innate regulation of adaptive immune responses.

AUTOPHAGY IN INFLAMMATORY DISEASES

The regulation of IL-1β and IL-23 secretion by autophagy may be of critical importance in the prevention of the autoimmune diseases in which hyperactivation of this pathway is a major driver of pathology. Polymorphisms in the IL-23R locus confer susceptibility to inflammatory bowel disease (IBD), including Crohn's disease (CD)[73] and ulcerative colitis,[74] psoriasis,[75] rheumatoid arthritis[76] and ankylosing spondylitis.[77,78] In addition, systemic lupus erythematosus (SLE) patients produce excess IL-17 and IL-23 that may exacerbate their disease.[79,80] In agreement with this, SLE-prone MRL[lpr/lpr] mice that lack the IL-23R or are treated with neutralising anti-IL-23 have significantly reduced clinical symptoms of disease.[81] Rapamycin or other mTOR-inhibiting drugs, which enhance autophagy, have successfully treated severe refractory CD,[82] lessened tissue damage in rheumatoid arthritis (RA) patients,[83–85] reduced disease activity in refractory SLE patients[86] and attenuated intestinal inflammation in a mouse model of colitis.[87] It should be noted that these drugs exert other, autophagy-independent immunosuppressive effects, particularly the inhibition of T-cell proliferation, and thus the induction of autophagy may not be their only mode of action. Further studies using specific inhibitors of autophagy are therefore warranted based on successes reported using these autophagy-inhibiting, but also broad-acting, drugs.

As well as its wider role in limiting the release of pro-inflammatory cytokines that drive disease progression in a general manner, autophagy has been implicated in more cell type-specific dysfunctions in particular autoimmune and inflammatory diseases. For example, in cystic fibrosis (CF), overexpression of beclin 1, required for autophagosome formation, rescues defective autophagy in airway epithelia and restores normal trafficking of CF transmembrane conductance regulator (CFTRF508del) to the cell surface. This prevents the CF phenotype in mouse models and in human CF biopsies.[88] Also, autophagy removes β-amyloid plaques in mouse models of Alzheimer's disease[46] and inhibits NLRP3 activity in response to cholesterol crystals,[89] which otherwise drives atherosclerosis.[90] Defective autophagy has also been implicated in cardiac disease; ineffective autophagy results in inadequate sequestration and degradation of mitochondrial DNA that accumulates, activates TLR9 and thus triggers heart inflammation.[91]

Crohn's Disease

It is well established that compromised autophagy is linked with CD, a chronic inflammatory condition that is a common form of IBD. Polymorphisms in the genes encoding the autophagy-related proteins Atg2a, Atg4a, Atg4d, death-associated protein, immunity-related GTPase family M protein (IRGM) and ULK-1 have been associated with susceptibility to CD.[92,93–95] The mouse ortholog of IRGM, Irgm1 (formerly LRG47), is an IFN-γ-inducible GTPase involved in immune responses to *M. tuberculosis*[96] and has been shown to induce autophagy in macrophages in response to IFN-γ.[29,97] Human IRGM, although not IFN-γ-inducible, also regulates autophagy in response to IFN-γ.[97,98] In addition to these identified loci, genome-wide association studies of CD patients have identified a strong susceptibility locus, the T300A polymorphism in the Atg16L1 gene, which produces a hypomorphic allele that severely impairs autophagic activity.[99,100] Macrophages from mice with the Atg16L1 T300A mutation display uncontrolled production of IL-1β and are more susceptible to dextran sodium sulphate-induced colitis.[66] As well as impaired macrophage functions, patients with this mutation and mice engineered to bear the same allele have striking defects in autophagy in Paneth cells of the intestinal epithelium. Paneth cells are located in the crypts of Lieberkühn in the small intestine and are specialized to produce lysozyme and antimicrobial peptides. Besides the accumulation of ER and mitochondria that would be expected in Paneth cells bearing the autophagy-compromising T300A mutation, these cells fail to secrete lysozyme and thus lysozyme is absent in the ileal mucous layer of these patients, defects that are also seen when autophagy is impaired by deletion of Atg5 in mice.[101] Thus, a deficiency in lysozyme-mediated control of intestinal microbiota may contribute to the development of pathology in patients bearing variants of Atg16L1, although this has not been clearly delineated.

Indeed, the effective limitation of symbionts and pathogens in the gut environment is critical for the prevention of IBD and particularly CD,[102] which is illustrated by the finding that CD patients have abnormal gut microbiotic profiles.[101,103] Altered autophagic activity downstream of microbial sensors in the gut appears to be a key reason for the failure of IBD patients to control intestinal microbiota and prevent gut pathology. In particular, defects in autophagy-related genes permit the establishment of adherent-invasive *E. coli*, common in lesions in the intestinal epithelia of patients with CD.[104] In addition, genetic linkage data have firmly established polymorphisms in the bacterial sensor and inducer of autophagy, NOD2, in susceptibility to CD.[103,105,106] Several NOD2 variants have been identified, including a frameshift mutation, that confer susceptibility to CD.[103,105,106] Following bacterial infection of host cells, NOD2 recruits Atg16L1 to the cell membrane, initiating autophagosome induction and bacterial clearance, a process that is impaired when NOD2 contains a CD-associated mutation.[107] Similarly, the NOD2 ligand muramyl dipeptide induces autophagy and killing of

pathogenic Salmonella, both dependent on functional NOD2 and ATG16L1.[108]

Although autophagic degradation of invasive bacteria is crucial for controlling bacterial infection, autophagy appears to have additional anti-inflammatory effects in the gut microenvironment. Autophagy stimulated in response to NOD2 activation also controls IL-1β and IL-6 release, and peripheral blood mononuclear cells from CD patients bearing the Atg16L1 susceptibility allele secrete more of these pro-inflammatory cytokines.[109,110] As well as controlling NOD2-dependent inflammatory cytokine release, autophagy also modulates intestinal inflammation by promoting non-inflammatory DC–T-cell interactions. In the intestine, DC sample antigens by extending protrusions through the epithelial cell layer, a process that itself depends on autophagy.[111] These antigens are then presented on MHC class II complexes and if they are derived from commensal bacteria, elicit non-activating, self-recognition T-cell responses. NOD2-stimulated autophagy in DC results in tolerogenic presentation of commensal bacterial components on MHC class II complexes.[112] Inhibiting autophagy prevents sampling and results in enhanced HLA-DR and CD86 expression and downregulation of IL-10 production by DC. These changes produce pro-inflammatory DCs that stimulate T-cell proliferation.[111] When T cells and DC interact, an immunological synapse is formed and must be stably maintained to result in T-cell activation.[113] In a recent study, T-cell–DC interactions result in autophagosome formation in DC, which was orientated towards the synapse and destabilised the synapse. When autophagy was blocked, the immunological synapse persisted and resulted in excess activation of T cells and induction of a Th17 phenotype.[114] DC from CD patients bearing the Atg16L1 T300A mutation had similarly persistent synapses.[114]

Thus, there are multiple points at which autophagy can influence immune responses that lead to the development and pathologies of CD and IBD. Autophagy can regulate the microbial profile in the gut and limit invasion of pathogenic bacteria. In addition, autophagy appears to promote tolerance to commensal bacteria by influencing the outcome of T-cell interactions with antigen-presenting cells and by directing the cytokine profile in the gut environment away from an excessive pro-inflammatory response. These findings may, in part, explain the initial results demonstrating that mTOR-inhibiting drugs are protective against intestinal inflammation, both in mouse models[87] and human patients.[82]

SLE

SLE is an autoantibody-mediated autoimmune disease that can affect multiple organs and tissues, including the skin, joints, kidneys and brain. As the pathological mechanisms driving the initiation and progression of SLE are diverse and complex, the points at which autophagy influences these mechanisms are poorly defined. Nonetheless, genetic association studies have established autophagy as an important process in SLE, as several mutations have been identified in autophagy-related genes that confer susceptibility to this disease, including IRGM.[115,116] IRGM is required for the autophagic destruction of mycobacteria,[97] and a Taiwanese study suggests that tuberculosis and SLE development are correlated.[117] It has been suggested that this may not only be due to perturbations in the IL-23/IL-17 axis in patients,[118] but could also be due to direct defects in autophagy pathways. In addition to IRGM, the locus containing Atg5 and the PRDM1 genes is a susceptibility locus for SLE.[119,120] The PRDM1 gene encodes the plasma cell differentiation factor Blimp-1 and although variations in this locus may affect plasma cell differentiation and behaviour to influence SLE development, a more specific genetic association study has clarified a protective role for Atg5 against SLE and also confirmed the autophagy gene Atg7 as a SLE susceptibility locus.[115]

SLE is driven by the formation of immune complexes of autoantibodies bound to autoantigens; many of these autoantigens are thought to be exposed to B cells when apoptotic debris fails to be cleared effectively, a process that requires autophagy in macrophages.[121] Immune complexes accumulate in tissues, such as the fine capillaries of the glomerulus, where they precipitate complement deposition and damaging inflammatory responses. In addition, immune complexes stimulate TLR7 and TLR9 on B cells and DC and, particularly in immature plasmacytoid DC, this stimulates the production of IFN-α which, in turn, activates and induces maturation of the B cells and other cells that participate in the disease process.[122]

Autophagy is also particularly important in T-cell development, function and homoeostasis, and defects in autophagy genes may alter the activity of T cells in the context of SLE. In particular, deficiencies in the autophagy pathway cause defects in ER and leave T cells more prone to cell death.[123,124] Interestingly, naive CD4 T cells from patients with SLE have lower constitutive levels of autophagy than those from healthy donors, and these cells are also resistant to induction of autophagy by serum starvation.[125] The functional relevance of these findings is not clear, although this resistence to autophagy may increase the susceptibility of lymphocytes to apoptosis, which could contribute to the accumulation of apoptotic debris that provides a source of autoantigens and drives autoimmune pathology.[125,126] Similarly, if autophagy is impaired in macrophages or DCs, this could affect the regulation of pro-inflammatory cytokine secretion and further promote pathology.

As well as its putative protective effects, autophagy may have roles in facilitating the initiation of SLE by stimulating processes that promote the activation of self-reactive B cells to produce autoantibodies. For example, autophagy is required for human neutrophil extracellular DNA trap (NET) release in response to PMA stimulation[127] and in gout.[128] NET release allows the exposure of multiple typical B-cell nuclear autoantigens containing TLR ligands and may exacerbate disease by precipitating complement deposition and tissue damage.[129] NETs activate plasmacytoid DC production of IFN-α[130] and, in the presence of type I IFN, autoantibodies further stimulate NET release, potentially driving ongoing disease. As well as promoting NET formation and thus autoantigen display, autophagy may aid in the activation of auto-reactive B cells once they encounter antigen. B-cell receptor (BCR) stimulation by cognate antigen triggers autophagosome formation and antigen processing, which promotes B-cell acquisition of T-cell help.[131] In autoreactive B cells, DNA-containing autoantigens stimulate BCR internalisation and recruitment of TLR9-containing endosomes to autophagosomes, a process that results in the B-cell hyper-responsiveness that is characteristic of autoimmune B cells.[132] As well as enabling the induction of autoantibody production, autophagy may promote cytokine release in response to immune complexes. TLR7 ligation induces autophagy,[28] which is required for IFN-α production by plasmacytoid DC in response to an ssRNA virus.[24] However, the direct relevance of autophagy in modulating IFN-α secretion by plasmacytoid DC and other cells has not been assessed in the context of autoimmunity.

Although the roles of autophagy in SLE disease processes are still unclear, it appears that autophagy has an overall protective effect in the disease. Therapeutic interventions that stimulate autophagy, particularly mTOR inhibitors, are of growing interest for the treatment of SLE and appear to be well tolerated by patients. In

(NZB/NZW)$_{F1}$ lupus-prone mice, rapamycin prevented development of nephritis, inhibiting lymphoproliferation and MCP-1 expression in kidneys,[133] reducing autoantibody production and enhancing survival.[134] Moreover, rapamycin treatment of older (NZB/NZW)$_{F1}$ female mice with established nephritis improved survival; splenomegaly was reduced and anti-nuclear antibodies were diminished, while renal function was significantly preserved compared with control mice.[135] Low-dose rapamycin prevented deterioration of renal function in immunoglobulin A nephropathy patients[136] and is currently being tested in a phase II trial in SLE (NCT00779194). Thus, autophagy represents a significant therapeutic target for the treatment of SLE, but further studies on the precise mechanisms involved are essential to maximise the potential of such treatments.

Arthritis

Autophagy appears to promote the survival of cells that actively drive RA, whereas in osteoarthritic (OA) joints, the pro-survival effects of autophagy can prevent the death of cells that maintain joint structure. In RA, joint destruction is mediated primarily by TNF-α, which stimulates synovial fibroblast production of the growth factors, chemokines, proteinases and adhesion molecules that are characteristic of the arthritic joint environment. Autophagy in these fibroblasts is enhanced to counter acute ER stress and maintain cell survival.[137] As well as stimulating fibroblasts to produce effector molecules, TNF-α potently activates murine osteoclasts to resorb the bone matrix,[138] and autophagy is a critical point at which osteoclast activity and bone degradation are regulated. TNF-α stimulates autophagy in osteoclasts, promoting their differentiation, and inhibition of autophagy in TNF-α transgenic mice reduced osteoclast differentiation and joint damage.[139] In addition to TNF-α, other factors that promote arthritic progression include MCP-1, IL-1β and IL-8, all of which induce MCP-1-induced protein, MCPIP. MCPIP contributes to the pathology in RA by promoting angiogenesis[140] and osteoclastogenesis,[141] and these may act via induction of autophagy. Conversely, another study found that the mTOR inhibitor everolimus, which induces autophagy, inhibited osteoclast differentiation and activity and induced osteoclast apoptosis. However, treatment of RA patients with everolimus resulted in only a transient and modest improvement in clinical signs of disease.[142] The data so far would suggest a negative role for autophagy in RA, although this is largely based on studies that focused specifically on osteoclasts. The role, if any, of autophagy in immune cells in the rheumatic joint has yet to be elucidated.

In contrast to RA, autophagy appears to be protective against joint destruction in OA,[143] and rapamycin reduces disease severity.[144] Autophagic activity is increased in cartilage and in cartilage-producing chondrocytes, and inhibiting autophagy results in similar gene expression changes to those seen in OA joints.[145] Moreover, induction of autophagy with rapamycin cleared reactive oxygen species and prevented IL-1β-dependent transcriptional changes that drive OA.[145] Thus, the role of autophagy in arthritis may be very much disease- and context-specific and requires further study to elucidate the mechanisms at play.

Autophagic regulation of autoantigen presentation

In autoantibody-mediated autoimmune diseases such as SLE and RA, autophagy may facilitate antigen presentation and thus enable the switching and maturation of B cells to plasma cells that secrete pathogenic, T-cell-dependent antibody isotypes. Interestingly, blocking autophagy may specifically prevent the presentation of modified peptides that are common autoantigens. Anti-self antibodies against citrullinated self-antigens are markers of autoantibody-mediated diseases, particularly RA.[146] Citrullination occurs in inflamed tissues[147] and in antigen-presenting cells, where it has recently been found to occur in autophagosomes. In addition, peptidylarginine deiminase, which deiminates arginine to form citrulline, is found in autophagosomes in B cells, macrophages and DC.[148] DC and macrophages can present citrullinated peptides without extra stimuli, whereas B cells must receive BCR stimulation to present citrullinated peptides. The autophagy inhibitor 3-MA blocked the presentation of citrullinated, but not unmodified, peptides.[148] Thus, excessive autophagy may potentiate autoantigen exposure and thus autoimmune disease initiation. However, in a more controlled environment, these effects may be balanced by other autophagy-dependent effects, such as cell survival, increased clearance of apoptotic bodies and regulation of pro-inflammatory cytokine secretion.

Sepsis

Considering the systemic inflammation and cell death that characterise sepsis, it is perhaps not surprising that autophagy markers increase in septic tissues and their expression is correlated with cell survival, both in animal models and in humans.[149–152] Inhibition of autophagy in septic mice boosts inflammatory cytokine levels and increases mortality, probably due to the failure to clear damaged or dysfunctional mitochondria, which activate the NLRP3 inflammasome.[65] Similarly, in mice in which Atg7 is specifically deleted in the intestinal epithelium, LPS induces high levels of IL-1β mRNA,[153] while LC3B$^{-/-}$ mice produce more IL-1β and IL-18 in response to LPS- or caecal ligation and puncture-induced sepsis.[65] Conversely, induction of autophagy with rapamycin inhibits the release of IL-1β and IL-23 into the serum of mice injected intraperitoneally with LPS[32,64] and protects mice against Staphylococcus enterotoxin-induced septic shock[154] and against cardiac dysfunction following caecal ligation and puncture.[152] Thus, sepsis may represent a condition where the control of pro-inflammatory cytokine secretion by autophagy has a clear protective role to play.

CONCLUSIONS

It is evident that autophagy has diverse functions and may contribute to altered cell behaviour in disease situations in a variety of ways. A thorough understanding of the effects of altering autophagic activity is therefore necessary for the design of therapeutics that aim to target this process to improve disease outcomes in patients. Altering autophagy systemically will affect all autophagy-dependent events and thus determining the balance of these effects will be important in assessing whether therapeutic intervention of autophagy will produce an overall positive, or negative, outcome for patients. Evidence from animal models and early clinical trials suggest that the generalised induction of autophagy may be beneficial in the treatment of CD, some cases of SLE and in OA. The effects of modulating autophagy have not yet been adequately tested for potential therapeutic in other diseases, although considering the conceivable protective role of autophagy in situations of dysregulated inflammation, specifically enhancing autophagy may be predicted to be an effective means of targeting many inflammatory and autoimmune diseases.

CONFLICT OF INTEREST

1 Ding WX, Yin XM . recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome. *Autophagy* 2008; **4**: 141–150.

2 Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell biol* 2010; **22**: 132–139.

3 Lee JW, Park S, Takahashi Y, Wang HG. The association of AMPK with ULK1 regulates autophagy. *PloS one* 2010; **5**: e15394.

4 Filimonenko M, Isakson P, Finley KD, Anderson M, Jeong H, Melia TJ *et al.* The selective macroautophagic degradation of aggregated proteins requires the PI3P-binding protein Alfy. *Mol Cell* 2010; **38**: 265–279.

5 Simonsen A, Birkeland HC, Gillooly DJ, Mizushima N, Kuma A, Yoshimori T *et al.* Alfy, a novel FYVE-domain-containing protein associated with protein granules and autophagic membranes. *J Cell Sci* 2004; **117**: 4239–4251.

6 Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. *Nat Cell Biol* 2010; **12**: 814–822.

7 Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T *et al.* Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* 2005; **120**: 237–248.

8 Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S *et al.* Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 2006; **26**: 9220–9231.

9 Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. *J Biol Chem* 2006; **281**: 30299–30304.

10 Newell MK, Harper ME, Fortner K, Desbarats J, Russo A, Huber SA. Does the oxidative/glycolytic ratio determine proliferation or death in immune recognition? *Ann New York Acad Sci* 1999; **887**: 77–82.

11 Alba G, El Bekay R, Alvarez-Maqueda M, Chacon P, Vega A, Monteseirin J *et al.* Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils. *FEBS lett* 2004; **573**: 219–225.

12 Pua HH, Guo J, Komatsu M, He YW. Autophagy is essential for mitochondrial clearance in mature T lymphocytes. *J Immunol* 2009; **182**: 4046–4055.

13 Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J Exp Med* 2007; **204**: 25–31.

14 Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev. Mol Cell Biol* 2008; **9**: 1004–1010.

15 Munz C. Antigen processing via autophagy—not only for MHC class II presentation anymore? *Curr Opin Immunol* 2010; **22**: 89–93.

16 English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D *et al.* Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat Immunol* 2009; **10**: 480–487.

17 Crotzer VL, Blum JS. Autophagy and its role in MHC-mediated antigen presentation. *J Immunol* 2009; **182**: 3335–3341.

18 Harris J, Hope JC, Lavelle EC. Autophagy and the immune response to TB. *Transbound Emerg Dis* 2009; **56**: 248–254.

19 Nicola AM, Albuquerque P, Martinez LR, Dal-Rosso RA, Saylor C, De Jesus M *et al.* Macrophage autophagy in immunity to *Cryptococcus neoformans* and Candida albicans. *Infect Immun* 2012; **80**: 3065–3076.

20 Lapaquette P, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 2010; **12**: 99–113.

21 Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T *et al.* Autophagy defends cells against invading group A Streptococcus. *Science* 2004; **306**: 1037–1040.

22 Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G *et al.* Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J Virol* 1998; **72**: 8586–8596.

23 Severa M, Giacomini E, Gafa V, Anastasiadou E, Rizzo F, Corazzari M *et al.* EBV stimulates TLR- and autophagy-dependent pathways and impairs maturation in plasmacytoid dendritic cells: Implications for viral immune escape. *Eur J Immunol* 2013; **43**: 147–158.

24 Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 2007; **315**: 1398–1401.

25 Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 2007; **27**: 135–144.

26 Tang D, Kang R, Cheh CW, Livesey KM, Liang X, Schapiro NE *et al.* HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* 2010; **29**: 5299–5310.

27 Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P *et al.* Endogenous HMGB1 regulates autophagy. *J Cell Biol* 2010; **190**: 881–892.

28 Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *EMBO J* 2008; **27**: 1110–1121.

29 Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 2004; **119**: 753–766.

30 Harris J, Keane J. How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clin Exp Immunol* 2010; **161**: 1–9.

31 Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal* 2010; **3**: ra42.

32 Peral de Castro C, Jones SA, Ni Cheallaigh C, Hearnden CA, Williams L, Winter J *et al.* Autophagy Regulates IL-23 Secretion and Innate T Cell Responses through Effects on IL-1 Secretion. *J Immunol* 2012; **189**: 4144–4153.

33 Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M *et al.* T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis. *Immunity* 2007; **27**: 505–517.

34 Ni Cheallaigh C, Keane J, Lavelle EC, Hope JC, Harris J. Autophagy in the immune response to tuberculosis: clinical perspectives. *Clin Exp Immunol* 2011; **164**: 291–300.

35 Park HJ, Lee SJ, Kim SH, Han J, Bae J, Kim SJ *et al.* IL-10 inhibits the starvation induced autophagy in macrophages via class I phosphatidylinositol 3-kinase (PI3K) pathway. *Mol Immunol* 2011; **48**: 720–727.

36 Van Grol J, Subauste C, Andrade RM, Fujinaga K, Nelson J, Subauste CS. HIV-1 inhibits autophagy in bystander macrophage/monocytic cells through Src-Akt and STAT3. *PloS one* 2010; **5**: e11733.

37 Harris J. Autophagy and cytokines. *Cytokine* 2011; **56**: 140–144.

38 Narita M, Young AR, Arakawa S, Samarajiwa SA, Nakashima T, Yoshida S *et al.* Spatial coupling of mTOR and autophagy augments secretory phenotypes. *Science* 2011; **332**: 966–970.

39 Li YY, Ishihara S, Aziz MM, Oka A, Kusunoki R, Tada Y *et al.* Autophagy is required for toll-like receptor-mediated interleukin-8 production in intestinal epithelial cells. *Int J Mol Med* 2011; **27**: 337–344.

40 Hoermannsperger G, Clavel T, Hoffmann M, Reiff C, Kelly D, Loh G *et al.* Post-translational inhibition of IP-10 secretion in IEC by probiotic bacteria: impact on chronic inflammation. *PloS one* 2009; **4**: e4365.

41 Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011; **13**: 132–141.

42 Ghislat G, Patron M, Rizzuto R, Knecht E. Withdrawal of essential amino acids increases autophagy by a pathway involving Ca2+/calmodulin-dependent kinase kinase-β (CaMKK-β). *J Biol Chem* 2012; **287**: 38625–38636.

43 Shang L, Chen S, Du F, Li S, Zhao L, Wang X. Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc Natl Acad Sci USA* 2011; **108**: 4788–4793.

44 Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T *et al.* Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. *Mol Cell* 2007; **25**: 193–205.

45 Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W *et al.* Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 2011; **331**: 456–461.

46 Vingtdeux V, Chandakkar P, Zhao H, d'Abramo C, Davies P, Marambaud P. Novel synthetic small-molecule activators of AMPK as enhancers of autophagy and amyloid-β peptide degradation. *FASEB J* 2011; **25**: 219–231.

47 Bai A, Yong M, Ma AG, Ma Y, Weiss CR, Guan Q *et al.* Novel anti-inflammatory action of 5-aminoimidazole-4-carboxamide ribonucleoside with protective effect in dextran sulfate sodium-induced acute and chronic colitis. *J Pharm Exp Ther* 2010; **333**: 717–725.

48 Bai A, Ma AG, Yong M, Weiss CR, Ma Y, Guan Q *et al.* AMPK agonist downregulates innate and adaptive immune responses in TNBS-induced murine acute and relapsing colitis. *Biochem Pharmacol* 2010; **80**: 1708–1717.

49 Nath N, Khan M, Rattan R, Mangalam A, Makkar RS, de Meester C *et al.* Loss of AMPK exacerbates experimental autoimmune encephalomyelitis disease severity. *Biochem Biophys Res Commun* 2009; **386**: 16–20.

50 Park CS, Bang BR, Kwon HS, Moon KA, Kim TB, Lee KY *et al.* Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. *Biochem Pharmacol* 2012; **84**: 1660–1670.

51 Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF *et al.* Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* 2011; **186**: 3299–3303.

52 Hammami I, Chen J, Murschel F, Bronte V, De Crescenzo G, Jolicoeur M. Immunosuppressive activity enhances central carbon metabolism and bioenergetics in myeloid-derived suppressor cells *in vitro* models. *BMC Cell Biol* 2012; **13**: 18.

53 Sag D, Carling D, Stout RD, Suttles J. Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J Immunol* 2008; **181**: 8633–8641.

54 Zhao X, Zmijewski JW, Lorne E, Liu G, Park YJ, Tsuruta Y *et al.* Activation of AMPK attenuates neutrophil proinflammatory activity and decreases the severity of acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**: L497–L504.

55 Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002; **20**(5 Suppl 27): S1–13.

56 Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E *et al.* IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. *J Immunol* 2011; **187**: 4835–4843.

57 Lalor SJ, Dungan LS, Sutton CE, Basdeo SA, Fletcher JM, Mills KH. Caspase-1-processed cytokines IL-1β and IL-18 promote IL-17 production by γδ and CD4 T cells that mediate autoimmunity. *J Immunol* 2011; **186**: 5738–5748.

58 Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* 2011; **29**: 707–735.

59 Fettelschoss A, Kistowska M, LeibundGut-Landmann S, Beer HD, Johansen P, Senti G et al. Inflammasome activation and IL-1β target IL-1α for secretion as opposed to surface expression. *Proc Natl Acad Sci USA* 2011; **108**: 18055–18060.

60 Harris KM, Fasano A, Mann DL. Cutting edge: IL-1 controls the IL-23 response induced by gliadin, the etiologic agent in celiac disease. *J Immunol* 2008; **181**: 4457–4460.

61 Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 1993; **261**: 472–475.

62 Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc Natl Acad Sci USA* 2000; **97**: 1190–1195.

63 Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* 1999; **10**: 127–136.

64 Harris J, Hartman M, Roche C, Zeng SG, O'Shea A, Sharp FA et al. Autophagy controls IL-1β secretion by targeting pro-IL-1β for degradation. *J Biol Chem* 2011; **286**: 9587–9597.

65 Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol* 2011; **12**: 222–230.

66 Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. *Nature* 2008; **456**: 264–268.

67 Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 2011; **469**: 221–225.

68 Shi CS, Shenderov K, Huang NN, Kabat J, Abu-Asab M, Fitzgerald KA et al. Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat Immunol* 2012; **13**: 255–263.

69 Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from γδ T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009; **31**: 331–341.

70 Jones SA, Sutton CE, Cua D, Mills KH. Therapeutic potential of targeting IL-17. *Nat Immunol* 2012; **13**: 1022–1025.

71 Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003; **421**: 744–748.

72 Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, Jiang S et al. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc Natl Acad Sci USA* 2012; **109**: E3168–E3176.

73 Amre DK, Mack D, Israel D, Morgan K, Lambrette P, Law L et al. Association between genetic variants in the IL-23R gene and early-onset Crohn's disease: results from a case-control and family-based study among Canadian children. *Am J Gastroenterol* 2008; **103**: 615–620.

74 Hayatbakhsh MM, Zahedi MJ, Shafiepour M, Nikpoor AR, Mohammadi M. IL-23 Receptor Gene rs7517847 and rs1004819 SNPs in Ulcerative Colitis. *Iran J Immunol* 2012; **9**: 128–135.

75 Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; **80**: 273–290.

76 Song GG, Bae SC, Choi SJ, Ji JD, Lee YH. Associations between interleukin-23 receptor polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. *Mol Biol Rep* 2012; **39**: 10655–10663.

77 Lee YH, Choi SJ, Ji JD, Song GG. Associations between interleukin-23 R polymorphisms and ankylosing spondylitis susceptibility: a meta-analysis. *Inflamm Res* 2012; **61**: 143–149.

78 Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J et al. IL-23 induces spondyloarthropathy by acting on ROR-gammat+ CD3+CD4-CD8- entheseal resident T cells. *Nat Med* 2012; **18**: 1069–1076.

79 Huang X, Hua J, Shen N, Chen S. Dysregulated expression of interleukin-23 and interleukin-12 subunits in systemic lupus erythematosus patients. *Mod Rheumatol* 2007; **17**: 220–223.

80 Wong CK, Lit LC, Tam LS, Li EK, Wong PT, Lam CW. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity. *Clin Immunol* 2008; **127**: 385–393.

81 Kyttaris VC, Zhang Z, Kuchroo VK, Oukka M, Tsokos GC. Cutting edge: IL-23 receptor deficiency prevents the development of lupus nephritis in C57BL/6-lpr/lpr mice. *J Immunol* 2010; **184**: 4605–4609.

82 Massey DC, Bredin F, Parkes M. Use of sirolimus (rapamycin) to treat refractory Crohn's disease. *Gut* 2008; **57**: 1294–1296.

83 Laragione T, Gulko PS. mTOR regulates the invasive properties of synovial fibroblasts in rheumatoid arthritis. *Mol Med* 2010; **16**: 352–358.

84 Cejka D, Hayer S, Niederreiter B, Sieghart W, Fuereder T, Zwerina J et al. Mammalian target of rapamycin signaling is crucial for joint destruction in experimental arthritis and is activated in osteoclasts from patients with rheumatoid arthritis. *Arthritis Rheum* 2010; **62**: 2294–2302.

85 Kneissel M, Luong-Nguyen NH, Baptist M, Cortesi R, Zumstein-Mecker S, Kossida S et al. Everolimus suppresses cancellous bone loss, bone resorption, and cathepsin K expression by osteoclasts. *Bone* 2004; **35**: 1144–1156.

86 Fernandez D, Perl A. mTOR signaling: a central pathway to pathogenesis in systemic lupus erythematosus? *Discov Med* 2010; **9**: 173–178.

87 Bhonde MR, Gupte RD, Dadarkar SD, Jadhav MG, Tannu AA, Bhatt P et al. A novel mTOR inhibitor is efficacious in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G1237–G1245.

88 Luciani A, Villella VR, Esposito S, Brunetti-Pierri N, Medina D, Settembre C et al. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nat Cell Biol* 2010; **12**: 863–875.

89 Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S et al. Autophagy links inflammasomes to atherosclerotic progression. *Cell Metab* 2012; **15**: 534–544.

90 Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010; **464**: 1357–1361.

91 Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T et al. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* 2012; **485**: 251–255.

92 Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; **43**: 246–252.

93 Brinar M, Vermeire S, Cleynen I, Lemmens B, Sagaert X, Henckaerts L et al. Genetic variants in autophagy-related genes and granuloma formation in a cohort of surgically treated Crohn's disease patients. *J Crohns Colitis* 2012; **6**: 43–50.

94 Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, Robson S et al. Genome-wide association study of CNVs in 16 000 cases of eight common diseases and 3,000 shared controls. *Nature* 2010; **464**: 713–720.

95 Henckaerts L, Cleynen I, Brinar M, John JM, Van Steen K, Rutgeerts P et al. Genetic variation in the autophagy gene ULK1 and risk of Crohn's disease. *Inflamm Bowel Dis* 2011; **17**: 1392–1397.

96 MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science* 2003; **302**: 654–659.

97 Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006; **313**: 1438–1441.

98 Singh SB, Ornatowski W, Vergne I, Naylor J, Delgado M, Roberts E et al. Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 2010; **12**: 1154–1165.

99 Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207–211.

100 Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596–604.

101 Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; **456**: 259–263.

102 Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119–124.

103 Nell S, Suerbaum S, Josenhans C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nat Rev Microbiol* 2010; **8**: 564–577.

104 Lapaquette P, Bringer MA, Darfeuille-Michaud A. Defects in autophagy favour adherent-invasive Escherichia coli persistence within macrophages leading to increased pro-inflammatory response. *Cell Microbiol* 2012; **14**: 791–807.

105 Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599–603.

106 Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S et al. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001; **357**: 1925–1928.

107 Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG 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.

108 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**: 1630–1641.

109 Ferwerda G, Kramer M, de Jong D, Piccini A, Joosten LA, Devesaginer I et al. Engagement of NOD2 has a dual effect on proIL-1β mRNA transcription and secretion of bioactive IL-1β. *Eur J Immunol* 2008; **38**: 184–191.

110 Plantinga TS, Crisan TO, Oosting M, van de Veerdonk FL, de Jong DJ, Philpott DJ et al. Crohn's disease-associated ATG16L1 polymorphism modulates pro-inflammatory cytokine responses selectively upon activation of NOD2. *Gut* 2011; **60**: 1229–1235.

111 Strisciuglio C, Duijvestein M, Verhaar AP, Vos AC, den Brink GR, Hommes DW et al. Impaired autophagy leads to abnormal dendritic cell-epithelial cell interactions. *J Crohns Colitis* 2012; **S1873-9946**: 00371–00376.

112 Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010; **16**: 90–97.

113 Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM et al. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999; **285**: 221–227.

114 Wildenberg ME, Vos AC, Wolfkamp SC, Duijvestein M, Verhaar AP, Te Velde AA et al. Autophagy attenuates the adaptive immune response by destabilizing the immunologic synapse. *Gastroenterology* 2012; **142**: 1493–1503.

115 Zhou XJ, Lu XL, Lv JC, Yang HZ, Qin LX, Zhao MH et al. Genetic association of PRDM1-ATG5 intergenic region and autophagy with systemic lupus erythematosus in a Chinese population. Ann Rheum Dis 2011; 70: 1330–1337.

116 Ramos PS, Criswell LA, Moser KL, Comeau ME, Williams AH, Pajewski NM et al. A comprehensive analysis of shared loci between systemic lupus erythematosus (SLE) and sixteen autoimmune diseases reveals limited genetic overlap. PLoS Genet 2011; 142: 1493–1503.

117 Lin YC, Liang SJ, Liu YH, Hsu WH, Shih CM, Sung FC et al. Tuberculosis as a risk factor for systemic lupus erythematosus: results of a nationwide study in Taiwan. Rheumatol Int 2012; 32: 1669–1673.

118 Park SJ, Kim JH, Ha TS, Shin JI. The role of interleukin-23/interleukin-17 axis in the development of systemic lupus erythematosus among patients with tuberculosis: Comment on: Lin YC, Liang SJ, Liu YH et al.: Tuberculosis as a risk factor for systemic lupus erythematosus: results of a nationwide study in Taiwan. Rheumatol Int 2013; 33: 271–272.

119 Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, Hu Z et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet 2009; 41: 1234–1237.

120 Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet 2008; 40: 204–210.

121 Zang L, Xu Q, Ye Y, Li X, Liu Y, Tashiro S et al. Autophagy enhanced phagocytosis of apoptotic cells by oridonin-treated human histocytic lymphoma U937 cells. Arch Biochem Biophys 2012; 518: 31–41.

122 Ronnblom L, Alm GV, Eloranta ML. The type I interferon system in the development of lupus. Semin Immunol 2011; 23: 113–121.

123 Jia W, Pua HH, Li QJ, He YW. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. J Immunol 2011; 186: 1564–1574.

124 Jia W, He YW. Temporal regulation of intracellular organelle homeostasis in T lymphocytes by autophagy. Immunity 2011; 186: 5313–5322.

125 Alessandri C, Barbati C, Vacirca D, Piscopo P, Confaloni A, Sanchez M et al. T lymphocytes from patients with systemic lupus erythematosus are resistant to induction of autophagy. FASEB J 2012; 26: 4722–4732.

126 Gaipl US, Kuhn A, Sheriff A, Munoz LE, Franz S, Voll RE et al. Clearance of apoptotic cells in human SLE. Curr Dir Autoimmun 2006; 9: 173–187.

127 Remijsen Q, Vanden Berghe T, Wirawan E, Asselbergh B, Parthoens E, De Rycke R et al. Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. Cell Res 2011; 21: 290–304.

128 Mitroulis I, Kambas K, Chrysanthopoulou A, Skendros P, Apostolidou E, Kourtzelis I et al. Neutrophil extracellular trap formation is associated with IL-1β and autophagy-related signaling in gout. PLoS one 2011; 6: e29318.

129 Leffler J, Martin M, Gullstrand B, Tyden H, Lood C, Truedsson L et al. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. J Immunol 2012; 188: 3522–3531.

130 Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci. Transl Med 2011; 3: 73ra20.

131 Watanabe K, Ichinose S, Hayashizaki K, Tsubata T. Induction of autophagy by B cell antigen receptor stimulation and its inhibition by costimulation. Biochem Biophys Res Commun 2008; 374: 274–281.

132 Chaturvedi A, Dorward D, Pierce SK. The B cell receptor governs the subcellular location of Toll-like receptor 9 leading to hyperresponses to DNA-containing antigens. Immunity 2008; 28: 799–809.

133 Lui SL, Yung S, Tsang R, Zhang F, Chan KW, Tam S et al. Rapamycin prevents the development of nephritis in lupus-prone NZB/W F1 mice. Lupus 2008; 17: 305–313.

134 Ramos-Barron A, Pinera-Haces C, Gomez-Alamillo C, Santiuste-Torcida I, Ruiz JC, Buelta-Carrillo L et al. Prevention of murine lupus disease in (NZBxNZW)F1 mice by sirolimus treatment. Lupus 2007; 16: 775–781.

135 Lui SL, Tsang R, Chan KW, Zhang F, Tam S, Yung S et al. Rapamycin attenuates the severity of established nephritis in lupus-prone NZB/W F1 mice. Nephrol Dial Transplant 2008; 23: 2768–2776.

136 Cruzado JM, Poveda R, Ibernon M, Diaz M, Fulladosa X, Carrera M et al. Low-dose sirolimus combined with angiotensin-converting enzyme inhibitor and statin stabilizes renal function and reduces glomerular proliferation in poor prognosis IgA nephropathy. Nephrol Dial Transplant 2011; 26: 3596–3602.

137 Connor AM, Mahomed N, Gandhi R, Keystone EC, Berger SA. TNFα modulates protein degradation pathways in rheumatoid arthritis synovial fibroblasts. Arthritis Res Ther 2012; 14: R62.

138 Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000; 191: 275–286.

139 Lin NY, Beyer C, Giessl A, Kireva T, Scholtysek C, Uderhardt S et al. Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. Ann Rheum Dis (e-pub ahead of print 12 September 2012; doi:10.1136/annrheumdis-2012-201671).

140 Roy A, Kolattukudy PE. Monocyte chemotactic protein-induced protein (MCPIP) promotes inflammatory angiogenesis via sequential induction of oxidative stress, endoplasmic reticulum stress and autophagy. Cell Signal 2012; 24: 2123–2131.

141 Wang K, Niu J, Kim H, Kolattukudy PE. Osteoclast precursor differentiation by MCPIP via oxidative stress, endoplasmic reticulum stress, and autophagy. J Mol Biol 2011; 3: 360–368.

142 Bruyn GA, Tate G, Caeiro F, Maldonado-Cocco J, Westhovens R, Tannenbaum H et al. Everolimus in patients with rheumatoid arthritis receiving concomitant methotrexate: a 3-month, double-blind, randomised, placebo-controlled, parallel-group, proof-of-concept study. Ann Rheum Dis 2008; 67: 1090–1095.

143 Carames B, Taniguchi N, Otsuki S, Blanco FJ, Lotz M. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. Arthritis Rheum 2010; 62: 791–801.

144 Carames B, Hasegawa A, Taniguchi N, Miyaki S, Blanco FJ, Lotz M. Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. Ann Rheum Dis 2012; 71: 575–581.

145 Sasaki H, Takayama K, Matsushita T, Ishida K, Kubo S, Matsumoto T et al. Autophagy modulates osteoarthritis-related gene expression in human chondrocytes. Arthritis Rheum 2012; 64: 1920–1928.

146 Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000; 43: 155–163.

147 Makrygiannakis D, af Klint E, Lundberg IE, Lofberg R, Ulfgren AK, Klareskog L et al. Citrullination is an inflammation-dependent process. Ann Rheum Dis 2006; 65: 1219–1222.

148 Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. J Exp Med 2011; 208: 2625–2632.

149 Watanabe E, Muenzer JT, Hawkins WG, Davis CG, Dixon DJ, McDunn JE et al. Sepsis induces extensive autophagic vacuolization in hepatocytes: a clinical and laboratory-based study. Lab Invest 2009; 89: 549–561.

150 Lo S, Yuan SS, Hsu C, Cheng YJ, Chang YF, Hsueh HW et al. Lc3 transgene improves Survival and Attenuates Lung Injury Through Increasing Autophagosomal Clearance in Septic Mice. Ann Surg 2013; 257: 352–363.

151 Carchman EH, Rao J, Loughran PA, Rosengart MR, Zuckerbraun BS. Heme oxygenase-1-mediated autophagy protects against hepatocyte cell death and hepatic injury from infection/sepsis in mice. Hepatology 2011; 53: 2053–2062.

152 Hsieh CH, Pai PY, Hsueh HW, Yuan SS, Hsieh YC. Complete induction of autophagy is essential for cardioprotection in sepsis. Ann Surg 2011; 253: 1190–1200.

153 Fujishima Y, Nishiumi S, Masuda A, Inoue J, Nguyen NM, Irino Y et al. Autophagy in the intestinal epithelium reduces endotoxin-induced inflammatory responses by inhibiting NF-kappaB activation. Arch Biochem Biophys 2011; 506: 223–235.

154 Krakauer T, Buckley M, Issaq HJ, Fox SD. Rapamycin protects mice from staphylococcal enterotoxin B-induced toxic shock and blocks cytokine release in vitro and in vivo. Antimicrob Agents Chemother 2010; 54: 1125–1131.

155 Wu YT, Tan HL, Shui G, Bauvy C, Huang Q, Wenk MR et al. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. J Biol Chem 2010; 285: 10850–10861.

156 Lin YC, Kuo HC, Wang JS, Lin WW. Regulation of inflammatory response by 3-methyladenine involves the coordinative actions on akt and glycogen synthase kinase 3β rather than autophagy. J Immunol 2012; 189: 4154–4164.

157 Pelegrin P, Barroso-Gutierrez C, Surprenant A. P2 × 7 receptor differentially couples to distinct release pathways for IL-1β in mouse macrophage. J Immunol 2008; 180: 7147–7157.