

# THE ROLE OF AUTOPHAGY IN IMMUNITY AND AUTOIMMUNE DISEASES

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## ULOGA AUTOFAGIJE U IMUNSKOM ODGOVORU I AUTOIMUNSKIM BOLESTIMA

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### ABSTRACT

*Autophagy is a catabolic mechanism in the cell that involves the degradation of unnecessary or dysfunctional cellular components by the lysosomal machinery. Recent studies have indicated that autophagy is a source of autoantigens, thus highlighting its potential role in the pathogenesis of autoimmunity. There are at least three different forms of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). The physiological role of autophagy is to maintain cellular homeostasis by removing long-lived, damaged proteins and dysfunctional organelles and by providing energy. Aberrant autophagy may contribute to chronic inflammatory diseases and autoimmune diseases.*

*An understanding of the complex relationships between autophagy and autophagy-related genes in each autoimmune disease creates the possibility of developing more specific and effective therapeutic strategies. Given the importance of autophagy in immune functions, this review article summarises current knowledge about the role of autophagy in the pathogenesis of autoimmune diseases.*

**Keywords:** Autophagy, Autoimmune diseases, mTOR, ATG16L1

### SAŽETAK

*Autofagija je lizosomalni katabolički proces razgradnje proteinskih agregata i oštećenih organela. Nedavne studije ukazuju na autofagiju kao izvor autoantigena i na taj način potvrđuju potencijalnu ulogu autofagije u patogenezi autoimunskih oboljenja. Postoje tri različite forme autofagije: makroautofagija, mikroautofagija i autofagija posredovana šaperonima. Fiziološka uloga autofagije se ogleda u održavanju ćelijske homeostaze, uklanjanjem dugoživećih oštećenih proteina, kao i disfunkcionalnih organela i održavanjem energije. Poremećaji procesa autofagije mogu da posreduju u hroničnim inflamatornim i autoimunskim bolestima.*

*Razumevanjem složenih odnosa između samog procesa autofagije i gena koji taj proces kodiraju u određenim autoimunskim bolestima otvaraju nove mogućnosti za razvoj specifičnijih i efektivnijih terapijskih agensa. U ovom revijskom radu smo sumirali već postojeće činjenice u vezi sa ulogom procesa autofagije u patogenezi autoimunskih bolesti.*

**Ključne reči:** Autofagija, autoimunske bolesti, mTOR, ATG16L1

### INTRODUCTION

Autophagy is a catabolic mechanism in the cell that involves the degradation of unnecessary or dysfunctional cellular components by the lysosomal machinery. The word "autophagy" is derived from the Greek words "auto," meaning "self," and "phagy," meaning "to eat." Initially, autophagy was recognised as a survival mechanism during starvation, but now, an ever-growing body of evidence shows that autophagy has a homeostatic function in many physiological processes, such as cell growth; cell development; and cell survival during hypoxia, oxidative stress

and DNA damage. Moreover, over the past few years, research in the field has demonstrated the involvement of autophagy in immunity and infection. This mechanism has important roles in the detection and clearance of pathogens, as well as in antigen presentation, in lymphocyte survival and homeostasis, and in the mediation of cytokine production (1-7). Recent studies indicated that autophagy is a source of autoantigens, thus highlighting its potential role in the pathogenesis of autoimmunity (8). Given the importance of autophagy in immune functions,



this review article summarises current knowledge about the role of autophagy in the pathogenesis of autoimmune diseases.

### *The autophagy process*

Autophagy is an evolutionarily conserved pathway in which long-lived, denatured proteins and damaged organelles are delivered to lysosomes for degradation. There are at least three different forms of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). CMA describes the chaperone-mediated, direct translocation of unfolded proteins across the lysosomal membrane, whereas during microautophagy, lysosomal uptake of cytosolic substrates is performed by invagination of the lysosomal membrane. In contrast, macroautophagy has several stages, starting with membrane isolation, or phagophore formation. The phagophore then expands across the cytosolic material and structures, resulting in a double-membrane cytosolic vesicle known as an autophagosome or autophagic vacuole. The completed autophagosome subsequently fuses with a lysosome to form a single-membrane autolysosome, allowing lysosomal hydrolases access to the inner autophagosomal membrane and its cargo, which is degraded and recycled. Among the three main types of autophagy, macroautophagy is the best-characterised process and is generally referred to as autophagy (9-15).

Autophagy is a tightly regulated process, and to date, at least 35 autophagy-related genes (*ATG*) involved in autophagosome formation have been defined (16,17). These genes are conserved from yeast to human. The main autophagy regulator is mammalian target of rapamycin (mTOR), which negatively regulates autophagy induction. Inhibiting mTOR kinase activity allows ULK1 activation, translocation of the mTOR substrate complex (ULK1/2, ATG13, FIP200 (also known as RB1CC1) and ATG101) and initiation of autophagy (18,19). This leads to recruitment of the class III phosphatidylinositol-3-OH kinase

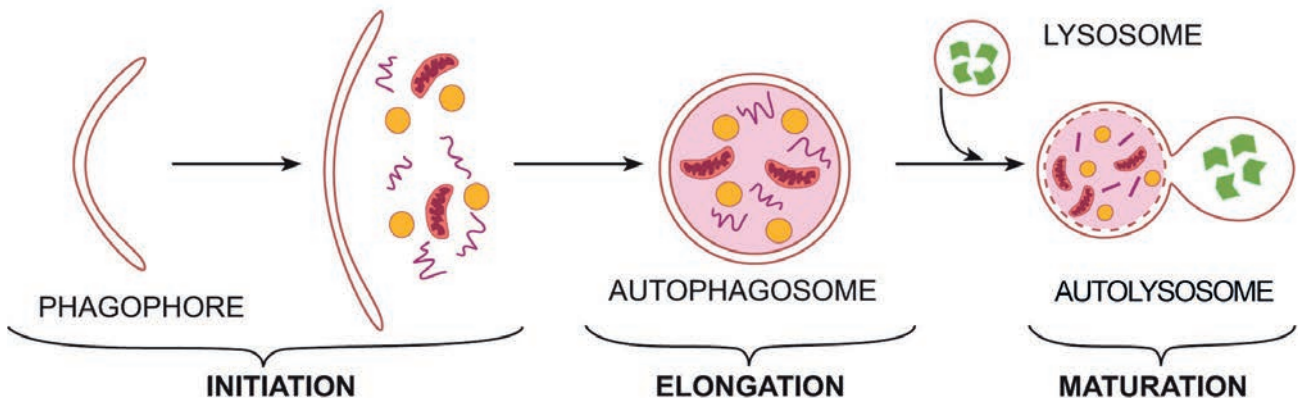
(PI(3)K) complex (PIK3C3/VPS34-BECN1) and *de novo* autophagosomal membrane formation (20). The source of membrane could be different organelles, including the ER, the Golgi complex, the mitochondria and the plasma membrane (21-23). The subsequent elongation and closure of autophagosomes involves the activation of two ubiquitin conjugation complexes, the ATG12 (ATG12-ATG5-ATG16L1) and LC3 (Atg 8) conjugation systems (24). The status of the autophagy repressor mTOR is controlled by two principal upstream regulatory pathways, or phosphatidylinositol 3-kinase (PI3K)-Akt and an AMP-activated protein kinase (AMPK)-dependent signalling network (25, 26).

Autophagy was originally regarded as a non-selective, bulk degradation process, but now, it has been demonstrated that autophagy degrades substrates in a selective manner and that proteins, such as sequestosome 1 (SQSTM1)/p62, NBR1, Nix and others, act as autophagic adaptors or cargo receptors. All autophagic adaptors contain an LC3-interacting domain through which they directly bind to the phagophore (27).

The physiological role of autophagy is to maintain cellular homeostasis by removing long-lived, damaged proteins and dysfunctional organelles and by providing energy. Under stress conditions, autophagy also represents a survival mechanism by providing energy and/or interfering with apoptosis. In contrast, during *Drosophila melanogaster* development or when there is excessive or abnormal cell proliferation in mammals, autophagy acts as a cell death pathway (programmed cell death type II) (28, 29). Recent data indicate that depending on the conditions, autophagy can either impair or promote cell survival (30, 31, 32).

### *Autophagy and innate immunity*

In addition to clearing endogenous substrates, autophagy plays an important role in the clearance of intracellular pathogens, and this form of selective autophagy is termed xenophagy. In xenophagy, pathogens are captured within autophagosomes and subsequently destroyed, which leads to the activation of pattern recognition receptors



**Figure 1.** The process of autophagy.



and enhanced MHC presentation of antigens (33-36). The precise membrane dynamics and specificity of xenophagy are not fully understood. Possible mechanisms of capturing autophagy-dependent viruses, bacteria and parasites involve wrapping of pathogen-containing phagosomes or endosomes with autophagosomal membranes, the fusion of pathogen-containing phagosomes or endosomes with autophagosomes, and the direct autophagic capture of a pathogen that has escaped into the cytoplasm (6). Different cellular adaptors are required for capturing intracellular bacteria and viruses than for endogenous substrates, such as the cellular factor nuclear dot protein 52 (NDP52), which plays a role in autophagosomal targeting of *Salmonella typhimurium* (37).

Signalling pathways that regulate the inflammatory response may also have a role in the regulation of autophagy, which may indicate the importance of autophagy in innate immunity. It has been demonstrated that autophagy is induced by different families of pathogen recognition receptors, including Toll-like receptors, Nod-like receptors, damage-associated molecular patterns (DAMPs), and pathogen receptors such as CD46; by interferon (IFN)- $\gamma$ ; and by downstream immunity-related guanosine triphosphatases (GTPases) (36, 38, 39). Additionally, autophagy-related proteins function in both stimulating and suppressing immune and inflammatory responses. Recent studies revealed a negative influence of autophagic proteins on inflammasome-associated proinflammatory cytokine maturation in macrophages. Induced transcription of pro-interleukin (IL)-1 $\beta$  and IL-18 is followed by inflammasome complex appearance and caspase 1 activation as a response to cellular infection. Inflammasomes are cytosolic complexes that contain NOD-like receptor proteins that serve as adaptors. It has been suggested that autophagy represents a feedback mechanism in macrophages to sustain the inflammatory process (40). For example, increased IL-1 $\beta$  and IL-18 secretion from Atg16L1- or Atg7-deficient mouse macrophages was observed after TLR4 stimulation. The same increased cytokine activation was also detected in LC3B- or beclin deficient macrophages (41). The relationship between autophagy and immunity/inflammation is complex and bidirectional.

### ***Autophagy and adaptive immunity***

Autophagy also participates in adaptive immunity, including the development/homeostasis of haematopoietic stem cells and lymphocytes and antigen presentation. Autophagy is involved in antigen processing for MHC class II presentation on professional antigen-presenting cells (APCs), or macrophages, B cells and dendritic cells. Dysfunction of Atg5 and Atg12, which are autophagy-related genes, during Epstein-Barr and herpes simplex 2 virus infections down-regulates antigen processing and MHC class II presentation on APCs (42-44). In contrast, inhibition of autophagy by pharmacological or gene manipulations does not affect the expression of MHC class

I molecules (45). Another crucial function of autophagy in adaptive immunity is the elimination of autoreactive T cells from the thymus. High levels of autophagy are present in thymic epithelial cells and participate in delivering endogenous proteins to MHC class II molecules. Transplantation of embryonic thymi from Atg5<sup>-/-</sup> mice into athymic nude mice was shown to induce systemic autoimmunity (46). It was also shown that autophagy specifically marks apoptotic cells for phagocytic clearance during embryonic development (47). Faulty removal of apoptotic cells in the embryonic lung and retina has been associated with an increased number of inflammatory cells, followed by endothelial damage, increased vascular permeability and oedema formation (48).

### ***Autophagy and cytokines***

Many cytokines are strong inducers of autophagy (49). A classic example of the involvement of cytokines in the regulation of autophagy is macrophages' response to *Mycobacterium tuberculosis*, demonstrating the stimulatory effect of tumour necrosis factor (TNF)- $\alpha$  and Th1 cytokines (IFN- $\gamma$  and IFN- $\alpha$ ) on autophagy in human and murine macrophages, human leukemic T lymphocytes, human vascular cells and rat epithelial cells (50, 51). In contrast, Th2 cytokines such as IL-4 and IL-13 antagonise autophagy through signal transducer and activator of transcription (STAT)-6 dependent stimulation of type I PI3-K, leading to the activation of mTOR, a serine/threonine protein kinase (50). Autophagy is regulated by cytokines, and this is not a unidirectional activity; the self-digestion process affects the transcription, selection and secretion of certain cytokines (51). Macrophages and dendritic cells with disrupted autophagy secrete IL-1 $\beta$  in response to TLR agonists (52). Rapamycin-induced autophagy inhibits release of IL-1 $\beta$  in murine dendritic cells activated by LPS, which establishes positive feedback for a self-sustaining inflammatory process.

### ***Autophagy and susceptibility to autoimmune diseases***

Aberrant autophagy may contribute to chronic inflammatory diseases and autoimmune diseases. There is a well-established connection between mutations in autophagy-related genes and Crohn's disease. Crohn's disease is a chronic inflammatory bowel disease associated with ulceration and neutrophil influx in the intestinal epithelia (53). Mutations in the autophagy-related genes autophagy-related 16-like 1 (ATG16L1) and immunity-related p47 GTPase family M (IRGM) are associated with an increased risk of Crohn's disease in humans (54-57). These two autophagy-related genes are believed to be important for both autophagy and antigen presentation (6). A single-nucleotide polymorphism (SNP) of ATG16L1 (threonine 300 is replaced with alanine, or T300A) has been associated with Crohn's disease, but the exact role of the human ATG16L1 protein and the consequences of this mutation have not been determined (58). One study suggest-



ed that dendritic cells expressing T300A from patients with Crohn's disease are defective in presenting bacterial antigen to CD4<sup>+</sup> T cells (49). Knocking out ATG16L1 in mice impairs autophagosome formation and enhances the generation of IL-1 $\beta$  by macrophages stimulated with endotoxin (59). ATG16L1-deficient mice die within the first day after delivery, but mice that express a low level of Atg16L1 exhibit Paneth cells with abnormal granule exocytosis and are more susceptible to experimentally induced colitis (60). Mice deficient in IRGM have a decreased capability to fight intracellular bacteria and therefore are more susceptible to infection (61). Two polymorphisms of IRGM have been correlated with Crohn's disease in humans (62). Another link between autophagy and pathogenesis in Crohn's disease has been demonstrated with the expression of NOD2 genetic variants (63). The induction of autophagy by muramyl dipeptide, a component of the bacterial peptidoglycan cell wall, requires NOD2-ATG16L1 interaction. NOD2 polymorphism associated with Crohn's disease results in impaired autophagy-dependent bacterial clearance and processing (64, 65).

Systemic lupus erythematosus (SLE) is a multifactorial disease with an unknown pathogenesis. The autophagy process has not yet been fully defined in SLE. Several SNPs in ATG5 (a key autophagy gene required for the formation of autophagosomes) seem to be predisposing factors for SLE. Loss of ATG5-dependent processes, including negative thymic selection, regulation of IFN and proinflammatory cytokine secretion, clearance of dying cells and antigen presentation, may contribute to autoimmunity and inflammation in SLE patients (66, 67). The impaired clearance of apoptotic cells may explain the accumulation of nuclear autoantigens in various tissues of SLE patients. It seems that dead cells are an abundant source of autoantigens in the setting of a clearance deficiency (68). Phagocytosis by macrophages (69) and phagocytosis by neutrophils are clearly depressed in SLE patients, and this deficient clearance of cellular debris may be a reason for the accumulation of apoptotic material in the tissues of certain SLE patients (68). Macroautophagy to remove cell debris may be insufficient. Autophagy was shown to be required for the activation of T cells and their survival after stimulation and differentiation (70, 71). A recent study clearly demonstrated that disrupted autophagy promoted the survival of autoreactive T cells in a lupus-prone mouse model and in lupus patients. One of the crucial roles of autophagy is in the regulation of peripheral T cell homeostasis in SLE patients (72). Other studies have suggested that an autophagy-independent role of ATG5 may contribute to the pathogenesis of SLE (73, 74).

Autophagy has a role in the pathophysiology of both type 1 and type 2 diabetes mellitus. It is assumed that the regulatory pathways of autophagy in insulin-generating  $\beta$ -cells in type 1 diabetes are different from those following the onset of insulin resistance in the tissues in type 2 diabetes. The role of autophagy in diabetes and metabolic disorders is critical because insulin and glucagon are well-known modulators of autophagy and are important to the function and survival of the pancreatic  $\beta$ -cells. Insulin and intracellular molecules

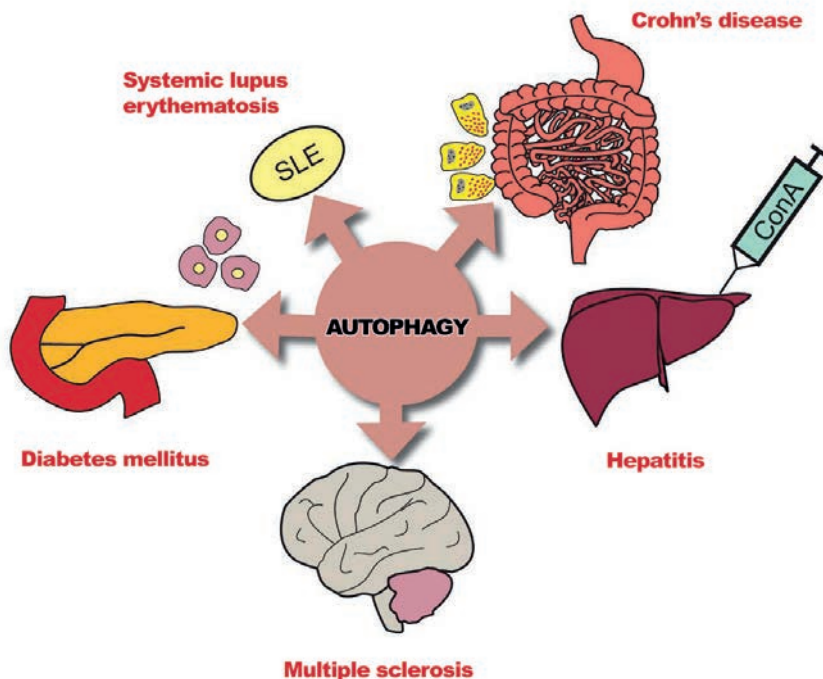
such as mTOR inhibit autophagy, whereas glucagon stimulates autophagy. Recent studies have strongly suggested that basal autophagy is important in the maintenance of  $\beta$ -cell volume and function. Deficiency of autophagy can lead to islet degeneration and reduced insulin secretion, which proves the crucial role of autophagy in islet function and survival (75). Atg7-deficient mice showed a reduction in  $\beta$ -cell mass and pancreatic insulin content as well as hypoinsulinaemia and hyperglycaemia (25). In addition, the role of an important autophagy-related protein, SQSTM1/p62, has been shown. SQSTM1/p62 knockout in mice causes metabolic disorders and insulin resistance, leading to type 2 diabetes (76). Suppression of autophagy can lead to the accumulation of reactive oxygen species in the mitochondria, and this may cause initiation of early diabetic nephropathy. Because CD4<sup>+</sup> and CD8<sup>+</sup> T cells are effector cells in the autoimmune destruction of  $\beta$ -cells (77), autophagy may initiate the pathogenesis of type 1 diabetes by affecting the function of T cells.

The association between autophagy and autoimmunity has also been detected in multiple sclerosis (MS). Increased ATG5 expression was detected in blood samples from mice with experimental autoimmune encephalomyelitis and in T cells from active relapsing-remitting MS patients (78).

Concanavalin A (Con A) can induce T cell-dependent and T cell-independent acute hepatitis in both immunocompetent and immunodeficient mice (79). Hepatocyte injury in NKT/CD4<sup>+</sup> T cell-mediated acute hepatitis is caused by apoptosis, whereas in Con A-induced T cell-independent acute hepatitis in SCID/NOD mice, hepatocyte death is mediated by autophagy (80). After binding to the mannose moiety of the cell membrane glycoprotein, Con A is internalised through endocytosis and accumulates preferentially on the mitochondria. Consequently, the mitochondrial membrane permeability is altered, and autophagy is activated, leading to lysosomal degradation of the affected mitochondria and caspase-independent cell death (80). Chang et al. reported that IFN- $\gamma$ , a potent Th1 cytokine, enhances autophagic flux and causes necrotic-type cell death in Con A-treated hepatocytes (81). The same group showed that the liver's blood vessels are the first target in both T cell-independent and T cell-dependent hepatitis. The infused Con A bonds to the hepatic vascular endothelial cells and causes damage to the liver's blood vessels before the induction of T cell-independent hepatitis via autophagy (82). Con A induces autophagy of endothelial cells, and haemorrhage is also enhanced by IFN- $\gamma$  (82). Necrostatin-1 (Nec-1), an inhibitor of programmed cell necrosis, inhibits Con A-induced acute liver injury and cell death. It seems that autophagy is one of the key mediators of this hepatoprotective effect. It was shown that autophagosome formation was significantly reduced following Nec-1 treatment, as was the expression of the autophagy-related proteins beclin-1 and LC3. The fact that Nec-1 treatment can reduce the level of autophagy in hepatocytes after Con A-induced injury provides new ideas and targets for the treatment of acute hepatitis (83). A recent study showed that metformin exacerbates Con A-induced hepatitis by promoting autophagy (84).



**Figure 2.** Pathological functions of autophagy.



## CONCLUSION

Over the past few years, there have been numerous reports on the functional correlation between autophagy and autoimmune diseases. This is not surprising, given the role of autophagy in T cell homeostasis and function. The potential implications of autophagy in autoimmune diseases could also explain the beneficial therapeutic effect of chloroquine, an autophagy inhibitor, in SLE and rheumatoid arthritis. There is a need for more studies in this field because autophagy has different roles in autoimmune diseases, depending on the pathophysiology. Understanding the complex relationships between autophagy and autophagy-related genes in each autoimmune disease creates the possibility of developing more specific and effective therapeutic strategies.

## CONFLICT OF INTEREST

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or a conflict of interest with respect to this manuscript.

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