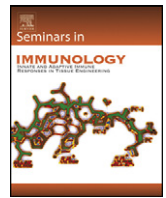




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Review

Autophagy as an emerging dimension to adaptive and innate immunity

Séamus Hussey^{a,b,c,d,1}, Leonardo H. Travassos^{c,1}, Nicola L. Jones^{a,b,d,*}^a Division of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, Canada^b Cell Biology Programme, Research Institute, Hospital for Sick Children, Toronto, Canada^c Department of Immunology, University of Toronto, Toronto, Canada^d Departments of Pediatrics and Physiology, University of Toronto, Toronto, Canada

ARTICLE INFO

Keywords:

Autophagy
 ATG16L1
 Innate immunity
 Antigen presentation
 Pattern recognition molecules

ABSTRACT

Autophagy is an evolutionary conserved cellular process during which cytoplasmic material is engulfed in double membrane vacuoles that then fuse with lysosomes, ultimately degrading their cargo. Emerging evidence, however, now suggests that autophagy can form part of our innate and adaptive immune defense programs. Recent studies have identified pattern recognition molecules as mediators of this process and shown that intracellular pathogens can interact with and even manipulate autophagy. Recent translational evidence has also implicated autophagy in the pathogenesis of several immune-mediated diseases, including Crohn disease. In this review, we present autophagy in the context of its role as an immune system component and effector and speculate on imminent and future research directions in this field.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Throughout evolution, facets of our cellular biology have been conserved and adapted. One process at the forefront of homeostasis and environmental interaction is autophagy. This complex process in eukaryotic cells involves the trafficking of cellular elements from the cytosol to the lysosome wherein they are degraded and processed. Ongoing developments in this field point to an inextricable link between autophagy and the innate and adaptive immune system. In this review, we summarize the pertinent research findings to date and suggest future research directions in this dynamic field, especially with respect to pattern recognition receptor interaction.

Three sub-types of autophagy have been described—chaperone-mediated autophagy, microautophagy and macroautophagy (hereafter called autophagy). The term ‘autophagy’ was first suggested by de Duve over 45 years ago [1]. Lamellar vesicles that encapsulated portions of the cytosol and organelle remnants had been described in early electron microscopy studies as vacuoles and lysosomes, and were speculated to arise from focal cytoplasmic degradation [2–4]. Such vesicles bore the hallmarks of what are now termed ‘autophagosomes’, the characteristic vacuoles synonymous with autophagy. Metabolic manipulation was shown to affect

autophagy induction, demonstrating that autophagy was a malleable rather than a static process. The catabolic hormone glucagon and deprivation of amino acids and nutrients were shown to induce autophagy while insulin and certain exogenous amino acids impaired autophagy and proteolysis, defining a role for autophagy in adaptation to cellular stresses [2,5–11]. The complexity of signaling molecules that influence autophagy is an ongoing focus of research and will be discussed later.

The stepwise process of autophagosome biogenesis is a cornerstone of autophagy. Over thirty governing autophagy genes (ATG) and their proteins (Atg) have been identified in elegant studies in yeast species [12–14]. While not all mammalian orthologs have been identified, some have numerous mammalian paralogs with striking similarities in structure and/or function to their yeast counterparts [15,16]. Ultra-structural studies of autophagosome membranes have shown that they harbor relatively few transmembrane proteins and are thinner than other cellular membranes e.g. the plasma membrane [17]. The earliest identifiable structure in the sequence of autophagosome formation is the disk-shaped, isolation membrane or phagophore (Fig. 1). Once formed, this membrane progressively elongates, encircling its cytosolic target, e.g. bacterium, within a portion of the cytosol, eventually sealing to complete the autophagosome. Speculation continues whether the foundation template for the isolation membrane originates from the endoplasmic reticulum, golgi, mitochondria, a pre-formed organelle membrane or even de novo [18–25]. The molecular mechanisms that lead to isolation membrane appearance continue to be elucidated in both yeast and mammalian cell systems.

* Corresponding author at: Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8. Tel.: +1 416 813 7734; fax: +1 416 813 6531.

E-mail address: nicola.jones@sickkids.ca (N.L. Jones).

¹ These authors contributed equally to this manuscript.

2. Machinery of autophagosome formation

Two ubiquitin-like conjugation systems are pivotal to autophagosome formation and completion. The first system modifies a core autophagy protein–microtubule-associated protein 1 light chain 3 (LC3). Multiple paralogs of Atg8 exist in mammals – LC3A, LC3B, GATE16, GABARAP – hereafter referred to collectively as LC3 [26,27]. LC3 has a diffuse cytosolic distribution. It is cleaved at its c-terminus by the cysteine protease Atg4 and in turn undergoes sequential ubiquitin-like modifications by the E1-like enzyme, Atg7, and the E2-like enzyme, Atg3, to form LC3-1. The c-terminal carboxyl group of LC3-1 is ultimately conjugated to the amine of phosphatidylethanolamine, forming LC3-II. This lipidation of LC3-1 to form LC3-II is notable in that LC3-II is exclusively found on autophagosome membranes. The conjugated yeast ortholog of LC3, Atg8, is known to have membrane tethering properties, which may explain one of its roles in autophagosome formation [28]. Atg4 also deconjugates LC3-II on the autophagosome membrane, releasing LC3, highlighting the plasticity of this process. The multifunctional protein p62 interacts with both ubiquitinated proteins and LC3, whereby it is incorporated into autophagosomes. p62 accumulates during autophagy inhibition and has been implicated in targeting proteins to autophagosomes, although it is not itself essential for autophagosome formation [29].

In the second conjugation system, the ubiquitin-like autophagy protein Atg12 is covalently conjugated to Atg5 via its c-terminal glycine, forming the dimeric Atg12–Atg5 complex, following ubiquitin-like reactions involving Atg7 and Atg10. The autophagy scaffold protein Atg16L1 is then conjugated to Atg5 via its N-terminus, forming the Atg12–Atg5–Atg16L1 complex. The Atg16L1 complex self-multimerizes, forming large 800 kDa complexes. These are found in the cytosol and on the evolving isolation membrane, and are likely necessary for the ultimate conjugation of LC3-I. Fujita et al. showed that the Atg16L1 complex behaves as an E3-like enzyme and targets LC3-I to its membrane site of lipid conjugation [30]. The Atg16L1 complexes dissociate from autophagosome membranes as they near completion.

Other essential groups of autophagy proteins participate in isolation membrane formation. The mammalian autophagy proteins ULK1 (Unc-51-like kinase), FIP200 (focal adhesion kinase family interacting protein) and Atg13 were recently identified in a complex which subsequently co-localized at the nascent isolation membrane on autophagy induction, similar to the Atg1–Atg13–Atg17 complex in yeast [31]. The c-terminus of ULK-1 binds to FIP200 and Atg13, and ULK-1 also interacts with LC3 [32]. Mammalian studies of the trans-membrane protein Atg9 have similarly underscored its essential role early in autophagosome formation. It associates with the trans-golgi network, late endosomes, LC3, the Rab-GTPase proteins (Rab7 and Rab9) and re-distributes following autophagy induction, localizing to the nascent autophagosome [33]. Yeast Atg9 was recently shown to self-multimerise via its c-terminus, facilitating its intra-cellular trafficking, independent of other autophagy proteins. This novel finding suggests a potential further role for such Atg9 complexes in contributing to early isolation membrane formation [25].

3. Controlling autophagy

The discovery of the target of rapamycin in yeast (TOR) and mammalian cells (mTOR) led to significant advances in understanding autophagy regulation, through the family of phosphatidylinositol kinase-related kinases [34–36]. These signaling networks are involved in broad cellular functions from metabolic responses to growth and proliferation.

The key serine/threonine kinase, Akt, links the mTOR and phosphatidylinositol-3 kinase (PI3K) pathways which are activated by a diverse array of stimuli, including cytokine receptors and toll-like receptors (TLR) [37]. Following receptor activation, class-I PI3Ks are recruited by receptor adaptor molecules to phosphorylate phosphatidylinositol-4,5-bisphosphate, which in turn phosphorylates and activates Akt [38,39]. The mTOR complexes, down-stream positive effectors of Akt, integrate multiple cellular signals, including those from growth factors, amino acids and intracellular ATP. mTOR activation increases cellular anabolic activity and protein translation [40–42]. Autophagy is under negative regulation by activated Akt and mTOR [43]. Recently, mTOR was shown to phosphorylate and therefore inhibit the ULK kinase-complex activity, disrupting autophagosome formation [44,45]. Rapamycin inhibition of mTOR and amino acid deprivation reversed these effects. mTOR may further affect autophagy through its control of autophagy gene transcription [40,46].

The class III PI3K enzyme, Vps34 (vacuolar protein sorting 34), solely phosphorylates phosphatidylinositol and is involved in regulating vesicular trafficking, nutrient sensing and autophagy [47,48]. The pharmacological agent 3-methyladenine (3-MA) inhibits its function *in vitro*. Together with Vps15 (another kinase), Beclin-1, UVRAG (ultraviolet radiation resistance associated gene) and Ambra-1, Vps34 forms a multiprotein complex that is necessary for early stages of autophagosome biogenesis and can up-regulate autophagy overall [49–51]. However, its seemingly paradoxical role in signal transduction to the mTOR complex following amino acid sensing suggests that its signaling function may depend on the nature of its interacting protein complexes [52,53].

Beclin-1, a tumor suppressor protein, is itself also involved in modulating autophagy through its interaction with Bcl-2, an anti-apoptotic protein that inhibits both autophagy and apoptosis. The Beclin-1/Bcl-2 interaction is an evolutionary conserved phenomenon, the balance of which determines either up- or down-regulation of autophagy. Silencing or over-expression of Bcl-2 was shown to enhance or suppress starvation-induced autophagy respectively [54]. These effects were specifically dependent on Beclin-1/Bcl-2 interaction, suggesting that nutrient sensing affects the equilibrium of the Beclin-1/Bcl-2 interaction. Bcl-2 dominant interactions with Beclin-1 likely disrupt Beclin-1/Vps34 complex formation, leading to autophagy suppression, although the mechanism has not been fully elucidated. Recently, the toll-like receptor (TLR) signaling molecules MyD88 and TRIF were shown to modulate the Beclin-1/Bcl-2 interaction, enhancing their interaction with Beclin-1 to induce autophagy [55].

A myriad of other signal transduction and effector molecules influence autophagy regulation, directly or indirectly. The Akt and JNK pathways have been shown to enhance or reduce expression of LC3 and Beclin-1 in response to tumor necrosis factor- α (TNF- α) and insulin-like growth factor-1 respectively [56]. The mammalian transcription factor, NF κ B, is a key regulator of gene expression, modulating physiological processes including inflammation, apoptosis and also autophagy. TNF α -induced NF κ B activation suppresses autophagy, while NF κ B suppression enhances starvation-induced autophagy [57,58]. NF κ B may signal through mTOR activation or by affecting enhanced Bcl-2 expression to modulate autophagy. Autophagy itself may in turn influence NF κ B activity since it is involved in degradation of I κ B kinase, the upstream activator of NF κ B, through association with the heat-shock protein, Hsp90 [59,60]. Reactive oxygen species (ROS) are highly reactive molecules generated from mitochondrial respiratory activity and the products of oxidase enzymes, including NADPH oxidase, and are capable of modulating autophagy [61,62]. Atg4 is redox-regulated via a conserved cysteine residue and, furthermore, starvation-induced autophagy depends on H₂O₂ signaling [62]. Starvation lead to local H₂O₂ formation, partly dependent on

class III PI3K activity, and anti-oxidant treatment in vitro attenuated autophagy induction. Recently, a transgenic mouse model harboring a mutant form of super-oxide dismutase, a key anti-oxidant enzyme, also showed increased autophagic activity due to ROS accumulation [63]. Evidence also suggests that autophagic (type II) cell death may stem from ROS accumulation, as seen following in vitro treatments with TNF α and LPS [57,64].

4. Pathogen recognition of autophagy

Microbial invasion of the cytosol presents a serious challenge to our innate defenses, including autophagy. While several agents succumb to autophagic destruction (xenophagy), others have evolved mechanisms of autophagy evasion and manipulation. Various Gram+ and Gram– bacteria, viruses and protozoa are known autophagy targets (Table 1). The mechanisms by which microbes are selectively sequestered in autophagosomes remain elusive and a combination of host and microbial factors are likely to be necessary. Microbial molecular motifs themselves may solicit autophagosome formation. Alternatively, the up-regulation of autophagy through activating multiple pattern recognition receptors could culminate in xenophagy or perhaps organelle or compartmental damage may lead to targeting by autophagic machinery. Microbial factors may be of equal importance for autophagy activation. For example, Group A streptococcus (GAS) is sequestered in autophagosomes following escape from its early endosomal compartment into the cytosol [65]. Lysosomal degradation of bacteria-containing autophagosomes ensues, effects not observed in autophagy deficient cells. Strains of GAS lacking the streptolysin O toxin remain within endosomes and avoid autophagic destruction indicating a role for streptolysin O in induction of autophagy.

The Gram– human diarrheal agent *Shigella flexneri* is a highly adapted pathogen harboring a type III secretion system (TTSS) for delivery of its effector proteins to host cells. In epithelial cells, wild-type (WT) strains secreting the effector IcsB are capable of evading entrapment in autophagosomes, in comparison to mutant strains lacking IcsB [66]. Interestingly, IcsB did not appear to confer autophagy protection in a subsequent study in murine marrow derived macrophages, suggesting a cell-type specific phenomenon

[67]. The intracellular bacterium *Burkholderia pseudomallei*, also avoids autophagic destruction in murine macrophages through secretion of its TTSS-delivered effector protein BopA, which shares some homology with IcsB [68].

Salmonella enterica serovar *Typhimurium* resides within salmonella-containing vacuoles following intracellular invasion. Salmonella employs its TTSS to disrupt these vacuoles, facilitating cytoplasmic entry. Autophagy promptly contributes to subsequent restriction of intracellular proliferation by targeting bacteria from damaged vacuoles—effects that were dependent on a functioning TTSS and reversed in autophagy deficient cells [69,70]. *Listeria monocytogenes*, a Gram+ bacillus, replicates within the host cytoplasm following phagosome escape, evading autophagic destruction [71,72]. The virulence factors listeriolysin O, ActA and phospholipase C were recently shown to be of importance in modulating *Listeria*-containing phagosomal compartments, blocking lysosomal degradation and facilitating replication and survival [73,74]. *Mycobacterium tuberculosis* has adapted to survive within host macrophages by interfering with and blocking phagosome fusion with lysosomes [75]. Autophagy up-regulation with rapamycin or IFN- γ overcame this evasion, and lead to phagosome degradation [76,77].

Secreted bacterial toxins are themselves capable of interacting with the autophagy pathway. The non-invasive pathogen, *Vibrio cholerae*, causes a potentially fatal secretory diarrhea. Its secreted exotoxin, VCC, induces vacuole formation consistent with autophagy induction [78]. Furthermore, cell viability was adversely affected following autophagy inhibition, suggesting that in this situation, autophagy may defend against cell toxicity. Our group has recently reported autophagy induction following infection with *Helicobacter pylori*, which was dependent on the vacuolating cytotxin, VacA. Autophagy limited the stability of intracellular VacA, again suggesting a cytoprotective function of autophagy in response to secreted toxins [79].

Viruses also interact with autophagy. The herpes virus HSV-1 evades autophagy in part through Beclin-1 inhibition by its neurovirulence protein ICP34.5 [80]. Poliovirus manipulates autophagic machinery following cellular infection, as evidenced by a marked reduction in viral release following pharmacological

Table 1
Microbial agents interacting with autophagy.

| Microbe | Host autophagy interaction | Biological factors and outcomes |
|-----------------------------------|----------------------------|---|
| Bacteria | | |
| <i>Streptococcus pyogenes</i> | Induction | Bacterial clearance |
| <i>Staphylococcus aureus</i> | Induction | Bacterial clearance |
| <i>Francisella tularensis</i> | Induction | Bacterial clearance |
| <i>Salmonella Typhimurium</i> | Induction | Bacterial clearance |
| <i>Rickettsia conorii</i> | Induction | Bacterial clearance |
| <i>Escherichia coli</i> | Induction | Bacterial clearance |
| <i>Mycobacterium tuberculosis</i> | Induction | IFN γ treatment enhances clearance |
| <i>Vibrio cholerae</i> (exotoxin) | Induction | Limits cytotoxicity, enhances survival |
| <i>Legionella pneumophila</i> | Manipulation | Autophagosome maturation delayed |
| <i>Brucella abortus</i> | Manipulation | Autophagy harnessed for replication |
| <i>Coxiella burnetii</i> | Manipulation | Autophagosome maturation delayed |
| <i>Listeria monocytogenes</i> | Evasion | Dependent on ActA, PLC |
| <i>Shigella flexneri</i> | Evasion | Bacterial escape, dependent on IcsB |
| <i>Burkholderia pseudomallei</i> | Evasion | Bacterial escape. Dependent on BopA |
| Viruses | | |
| Parvovirus B19 | Induction | Cell cycle arrest, virus sequestration |
| Herpes simplex virus | Evasion | Dependent on neurovirulence factor |
| Kaposi sarcoma-associated virus | Evasion | Viral Bcl-2 inhibits Beclin-1 |
| Rotavirus | Manipulation | Impaired autophagosome maturation |
| Human poliovirus | Manipulation | Autophagy harnessed for replication |
| Hepatitis C virus | Manipulation | Autophagosome maturation delayed |
| Coxsackievirus | Manipulation | Autophagy harnessed for replication |
| Protozoa | | |
| <i>Toxoplasma gondii</i> | Induction | Parasite elimination |

inhibition of autophagy and siRNA silencing of key autophagy proteins [81]. Rotavirus has been suggested to harness the autophagic apparatus to facilitate replication. Its enterotoxin, NSP4, was found to co-localize with LC3+ structures on immunofluorescence microscopy, and the study authors speculate that NSP4 may interfere with autophagosome–lysosome fusion, enabling viral recruitment of autophagosomes as replication niches [82]. The antiviral protein kinase, PKR, participates in viral induced autophagy, functioning upstream of Beclin-1. It is possible that other viruses which inhibit PKR function, including Influenza and Epstein–Barr virus, may in turn inhibit autophagy to enhance their own survival [83,84]. The possibility of a cell-type dependent autophagy response to viral infection was suggested by coronavirus studies, wherein mouse hepatitis virus replication was impaired in ATG5^{-/-} stem cells, but not in ATG5^{-/-} embryonic fibroblasts or marrow derived macrophages [85–87].

These diverse examples of autophagy–microbial interactions underpin the conserved primary innate role of autophagy as an anti-microbial, protective mechanism and how certain pathogenic organisms have evolved to recognize and commandeer this process for their own advantage.

5. Autophagy and innate immunity

The innate immune system is responsible for the early detection and destruction of pathogens. This first line of defense relies

mostly on a set of receptors called pattern recognition molecules (PRM) that sense molecular motifs that are common to a wide range of pathogens, triggering different signaling cascades that culminate with the elimination of pathogens and the initiation of an adaptive response [87,88]. The findings that autophagy can specifically target cytosolic pathogens immediately prompted the investigation of the role of PRM in the autophagic detection and elimination of intracellular microbes.

The TLRs are transmembrane proteins, mostly located at the cell surface, with a Toll-IL-1 receptor (TIR) domain facing the cytosol. This domain is able to recruit four different adaptor molecules: the myeloid differentiation primary response protein 88 (MyD88), the TIR domain-containing adaptor protein (TIRAP, also called MyD88 adaptor-like–MAL), the TIR domain-containing adaptor-inducing IFN- β -Trif, also called TIR-domain-containing adaptor molecule 1–TICAM-1) and the Trif-related adaptor molecule (TRAM or TICAM2) [88,89]. As we will see in this section, recent data suggest that induction of autophagy after TLR engagement requires the recruitment of specific adaptors (Fig. 2).

Eissa and colleagues provided the first evidence that TLRs are able to trigger an autophagic response by showing the formation of numerous autophagosomes in response to LPS stimulation in the murine macrophage RAW264.7 cell line [90]. Furthermore, silencing TLR4 using RNA interference resulted in significant reduction in autophagosomes. The TLR4-induced autophagic response was dependent on p38, RIP1 and Trif-, but not MyD88. As TLR4 can use

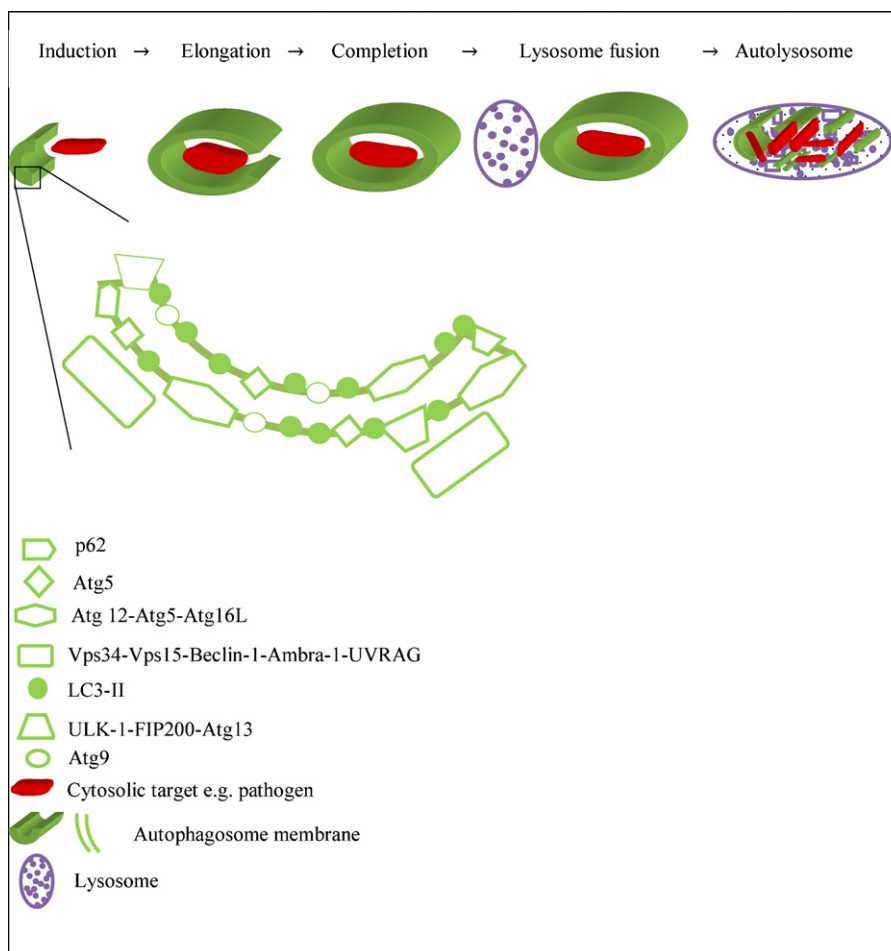


Fig. 1. Autophagosome biogenesis. The earliest identifiable structure in the initiation (nucleation) sequence of autophagosome formation is the crescent-shaped isolation membrane or phagophore. Key elements include Atg9, the ULK1–FIP200–Atg13 complex, LC3-II, the Atg12–Atg5–Atg16L complex. Once formed, this membrane progressively elongates (elongation), encircling its cytosolic target, e.g. bacterium, within a portion of the cytosol. The membrane tips fuse and eventually seal, forming the autophagosome (completion). The autophagosome may fuse with the endosomal compartment, forming an amphisome, prior to its ultimate maturation step, whereby its outer membrane fuses with the lysosome to form an autolysosome (also termed autophagolysosome). This facilitates degradation, processing and recycling of the contents of the autophagosome.

both MyD88 and Trif adapter molecules for downstream signaling, the authors proposed that by recruiting both signaling cascades, TLR4 could promote both a fast phagocytic response (through MyD88) and a slower autophagic response (via Trif).

Other TLR family members have also been implicated in the control of autophagy (Fig. 2). Deretic and colleagues have recently demonstrated that when RAW264.7 macrophages were stimulated with a panel of TLR ligands such as Pam₃CSK₄ (TLR2), flagellin (TLR5), CpG DNA (TLR9), poly (I:C) (TLR3) and ssRNA (TLR7), the latter three were able to up regulate autophagy [91]. In contrast to TLR3 (that recruits only Trif) and TLR4 (that recruits both MyD88 and Trif), TLR7 recruits only MyD88, suggesting that MyD88 may trigger autophagy after TLR7 activation. However, TLR9 activation by CpG DNA also activates MyD88 but did not induce autophagy. Therefore, a simple analysis of which downstream adaptor protein is recruited by TLRs does not fully explain the induction of autophagy by some pathogen associated molecular patterns (PAMPs) and not others. The mechanistic explanation is still elusive and seemingly conflicting evidence remains difficult to reconcile. For example, TLR7 recruitment of MyD88 also leads to the activation of NF κ B, which is thought to inhibit autophagy [57]. Trif-dependent signaling leads to the induction of type I interferon, which was previously shown not to affect autophagy [76,88]. Two recent studies have proposed a mechanism by which TLRs might regulate autophagy. Kehrl and Shi demonstrated that not only Trif, but also MyD88 targets Beclin1 and reduces its binding to Bcl-2, upon stimulation with an array of TLR ligands [54,55]. Alternatively, Wagner and colleagues observed that TLR activation leads to the activation of mTOR, which in turn interacts with the adaptor proteins MyD88 and interferon-regulatory factors (IRFs) 5 and 7, thus controlling the transcription of cytokines such as TNF- α , IL-10, IL12, type I interferons but, surprisingly, not IL-1 β [92]. These lines of evidence suggest a more elaborate TLR control of autophagy whereby TLR-adaptor molecules interact with proteins from the autophagic

pathway rather than by simply activating the classic hierarchical signaling cascades described heretofore.

In contrast with the general notion that TLR ligands up regulate autophagy, Green and colleagues suggested a model in which some TLRs, when engaged by their cognate ligands, usurp the autophagic pathway, recruiting LC3 to the phagosome membrane instead of forming classic autophagosomes [93]. However, as pointed out by the authors, it is not possible to exclude the possibility that the LC3 recruited to phagosomes has its origin in rapidly forming autophagosomes. If confirmed, these data would have a deep impact on the understanding on the role of autophagy in the enhancement of antigen presentation for example.

PAMP recognition as an autophagy trigger seems to be an evolutionary conserved feature. In *Drosophila*, peptidoglycan-recognition protein (PGRP) family members sense peptidoglycan (PG) from gram-negative bacteria [94]. One of the PGRP family members, PGRP-LE was recently implicated in PG sensing and induction of autophagy upon infection with *L. monocytogenes* thereby leading to clearance of bacteria [95]. Cytosolic PRMs have also been implicated in regulation of autophagy. Suzuki and colleagues demonstrated that Ipaf, a Nod-like protein previously shown to sense flagellin, down regulates autophagy during infection with the non-flagellated bacterium *S. flexneri* [67]. The down regulation of autophagy did not involve the ASC adaptor protein, normally required for the induction of IL-1 β after Ipaf activation. One can speculate that Nod proteins, which sense PG in mammalian cells, may play a similar role in the regulation of autophagy. Up-coming studies addressing this question are eagerly awaited.

In summary, the data above suggest a dynamic interaction between receptors from the innate immune system and regulation of autophagy. Additional studies with knockout mice are now needed in order to demonstrate a definitive role for TLR- or NLR in autophagy during infection with pathogens known to activate specific PRMs.

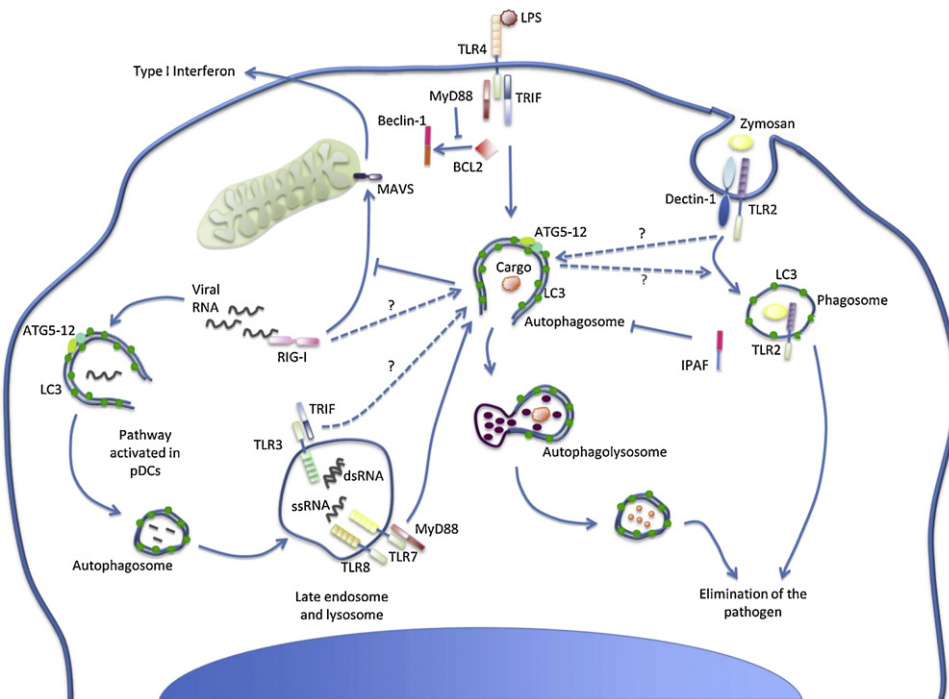


Fig. 2. TLR activation triggers autophagy. LPS triggers autophagy after recruitment of Trif (also RIP1 and p38, not shown) and MyD88. The latter seems to interact with Beclin-1, reducing its binding to the anti-autophagic molecule BCL-2. TLR2 engagement induces the incorporation of LC3 to phagosomes (unknown mechanism). Viruses are able to induce autophagy through TLR3, RIG-I (dsRNA) or TLR7/8-MyD88 (ssRNA). Conventional DCs sense viral ligands through the RIG-I/MAVS axis to secrete type I interferon, while basal autophagy. Plasmacytoid DCs deliver TLR7 ligands from the cytosol to the compartments containing TLR7 using IPAF. IPAF inhibits autophagy through an unclear mechanism.

6. Autophagy and cytokine responses

In the last few years, autophagy induction has been frequently reported as a consequence of innate immune system activation. However, there is compelling evidence that the relationship between autophagy and the immune system is reciprocal. Cytokines from the innate and adaptive systems regulate autophagy by different mechanisms. Two of the prototypical Th1 cytokines, IFN- γ and TNF- α , were shown to up-regulate autophagy. Gutierrez and colleagues first demonstrated that mouse macrophages harboring *Mycobacterium* within phagosomes were able to clear bacteria after stimulation with IFN- γ in an autophagy-dependent manner [76]. Follow up studies implicated GTPases in this process. The mouse genome contains 23 different immunity-related GTPases, most of which respond to IFN- γ stimulation and play a role in the defense against intracellular pathogens via a mechanism which is as yet unclear [96]. The studies from Deretic's group showed that both mouse immunity-related GTPase (*Irgm1*) and its human ortholog IRGM are the key molecules driving the induction of autophagy upon IFN- γ stimulation, leading to the clearance of *Mycobacterium* from infected macrophages [76,97].

The other Th1 cytokine shown to stimulate autophagy is TNF- α . Codogno and colleagues observed that cells stimulated with TNF- α are committed to die when NF κ B is blocked [57,59,60]. These findings are of great interest as the activation of autophagy may represent a way to overcome the resistance of cancer cells to anti-cancer drugs targeting NF κ B.

In contrast to the autophagy enhancing effect of some Th1 cytokines, Th2 cytokines such as IL-4 and IL-13, seem to counteract starvation and IFN- γ -induced autophagy by different pathways [97]. While IL-4 and IL-13 block starvation-induced autophagy by activating the Akt-mTOR axis, these cytokines inhibit IFN- γ -induced autophagy in an Akt-independent but STAT6-dependent manner.

The regulation of cytokine secretion by autophagy, has also been reported. Jounai and colleagues demonstrated that in response to infection with RNA viruses or immunostimulatory RNA, IFN- β levels were increased in ATG5 knockout embryonic fibroblasts [98]. The authors demonstrated that the Atg5-Atg12 conjugate negatively regulates the antiviral immune response by interacting with the RIG-I-like receptor (s protein retinoic acid-inducible gene I (RIG-I) and IFN- β promoter stimulator 1 (IPS-1) thus, implying autophagy contributes to viral replication. Iwasaki and colleagues showed that in autophagy-impaired cells the increased cytokine secretion in response to immune-stimulatory RNA is due to the accumulation of defective mitochondria and consequent IPS-1 and ROS accumulation, further strengthening the importance of autophagy in the maintenance of cellular homeostasis [99].

Autophagy has also been proposed to regulate cytokine secretion in Crohn disease. Crohn disease (CD) is a chronic inflammatory intestinal disease with a complex and multifactorial etiology. Several recent independent genome wide association studies have implicated a number of heretofore unappreciated biological pathways in CD pathogenesis, including autophagy [100–103]. Since the identification of a non-synonymous single nucleotide polymorphism in the ATG16L1 gene as a causal risk variant for CD, several groups have sought to elucidate its functional impact on development of CD. Akira and colleagues generated mice lacking the coiled-coil domain of ATG16L1 and observed aberrant IL-1 β secretion upon LPS stimulation of fetal derived liver macrophages. In contrast to previous studies, LPS did not induce autophagy in control macrophages indicating the enhanced IL-1 β was not due to disruption of LPS-mediated autophagy [104]. Chimeric mice with ATG16L1-deficient hematopoietic cells had an unremarkable baseline intestinal phenotype, but displayed increased susceptibility to

DSS-induced colitis compared with controls. Even though this study used mice expressing a truncated form of ATG16L1, rather than the ATG16L1 risk allele, the results point to the importance of functional autophagy machinery for normal intestinal function. Using an alternative mouse model hypomorphic for ATG16L1 protein expression, Cadwell and colleagues noted paneth cell-specific abnormalities including degenerating mitochondria, loss of lysozyme granule integrity and absence of apical microvilli [105]. Parallel findings were observed when intestinal ATG5 expression was suppressed. Transcriptional profiling analysis revealed that, among other differences, transcripts for the adipocytokines leptin and adiponectin were highly enriched. Similar increased expression profiles were observed previously in patients with CD [106,107]. The above findings, while not specific to ATG16L1 suppression, underscore the importance of autophagy pathway integrity to normal paneth cell function. Interestingly, ATG7 knockout of pancreatic islet cells resulted in abnormal cellular morphology on EM, including mitochondrial swelling, distension of the endoplasmic reticulum and a paucity of insulin granules when compared with controls [108]. It remains unclear why paneth cells, above others, are susceptible to autophagy interference and how autophagy is involved in maintaining integrity of its lysozyme exocytosis pathway. However, the interaction between autophagy and multivesicular body biogenesis may provide a potential explanation for abnormal granule formation and exocytosis. Once again, the paneth cell is placed at the convergence of several innate immune pathway aberrations and CD pathogenesis. Translational clinical data are keenly awaited.

7. Autophagy and antigen presentation

The products of the two main cellular degradation systems – the proteasome and the lysosome – are not merely unwanted material but are, instead, key molecules utilized to instruct the immune system. This instruction step is achieved by the presentation of these products to cells from both innate and adaptive immune systems. CD8⁺ T cells monitor mainly cytosolic and nuclear antigens degraded by the proteasome (a large cytosolic enzyme complex) and loaded into MHC class I. In contrast, CD4⁺ T cells respond to extracellular or membrane peptides generated by lysosomal degradation and presented in the context of MHC class II at the cell surface [109,110]. However, this paradigm has been challenged by the demonstration that dendritic cells (DCs) are also capable of presenting extracellular antigens on MHC class I, and not just on MHC class II as initially thought, through a mechanism called cross-presentation. Cross-presentation allows DCs to instruct also CD8⁺ T cells, generating a more efficient T-cell response [111].

Functional evidence for the presentation of endogenous antigens on MHC class II was first provided by Long and colleagues, who demonstrated that measles and influenza antigens could be presented in the context of MHC class II [112,113]. Indeed, the affinity purification of MHC class II from Epstein-Barr virus (EBV)-transformed B lymphoblastoid cells, murine B cell lymphoma and myeloid cells showed that more than 20% of natural MHC class II ligands had their origin in intracellular proteins [109]. Together these studies suggested that an alternative and unknown route could deliver antigens from the cytosolic compartment for presentation on MHC class II. Knecht and colleagues were the first to suggest a role for autophagy in this process by showing that glyceraldehyde-3-phosphate dehydrogenase, an important source of human MHC class II ligands, is degraded via chaperone-mediated autophagy [114]. Additionally, peptides from two Atg8 homologues, LC3 and GABARAP, have been isolated from human and mouse MHC class II molecules, respectively, providing further support to the notion of autophagy as an alternative route for delivery of cytosolic antigens for MHC class II.

More direct evidence came from studies using pharmacological inhibition of macroautophagy with PI3K inhibitors (such as 3-MA and wortmannin), which are thought to block the sequestration step of autophagy. Stockinger and colleagues demonstrated that over-expressed C5 was processed and loaded onto MHC class II in an autophagy-dependent manner, as the loading was decreased in the presence of 3-MA [114]. A similar approach was used to show that an endogenously expressed bacterial peptide, NeoR (neomycin-phosphotransferase II), was sequestered in autophagosomes and processed in endosomal/lysosomal compartments for loading onto MHC class II. Brossart and colleagues also used pharmacological inhibition to demonstrate that DCs electroporated with RNA coding for the tumor-associated antigen Muc-1 requires not only lysosomal antigen degradation and processing, but also autophagy in order to prime CD4⁺ T cells [115].

Further evidence that autophagy contributes to MHC class II presentation came from Munz and colleagues, in which they analyzed the endogenous MHC class II processing of the nuclear antigen 1 (EBNA 1) from EBV, the dominant EBV-latent antigen for CD4⁺ T cell. Inhibition of autophagy by Atg12siRNA in EBV-transformed B cells reduced recognition by EBNA1-specific CD4⁺ T cells [116]. In another study, the same group demonstrated that the fusion of influenza matrix protein 1 (MP1) with Atg8/LC3 drives this molecule to autophagosomes in different cell types and enhances recognition by antigen specific CD4⁺ T cells [117]. Knockdown of Atg12 confirmed that the localization of the fusion proteins with MHC class II molecules was dependent of autophagy. Importantly, these results represent great potential for vaccine design, since targeting antigens to autophagosomes induces a more robust T cell response. In support of this contention, Jagannath et al. demonstrated that induction of autophagy enhances BCG vaccine efficacy in a murine model [118].

In contrast to model or viral antigens, very little is known about bacterial antigens requiring autophagy for proper presentation on MHC class II. So far, only the 85B antigen from *M. tuberculosis* was shown to be presented more efficiently on MHC class II upon induction of autophagy [119]. Accordingly, Atg6 silencing dampened this process, while rapamycin treatment enhanced priming of 85B-specific CD4⁺ T cells, strongly suggesting a role for autophagy in MHC class II presentation of antigens of bacterial origin. Even though studies with other bacterial models are lacking, it is possible to speculate a role for autophagy in MHC class II presentation during infections with bacteria that escape from phagosomes, such as *L. monocytogenes*.

8. Conclusion

Autophagy is steadily emerging from its historic 'house-keeping' role as a new dimension in our host defense program. Taken together, the data highlighted above suggest that autophagy impacts on the development of both innate and adaptive immune responses to diverse pathogens and that, conversely, components of the immune system themselves also regulate autophagy. This biological process is now a major target for researchers who want to enhance understanding of and develop strategies to modulate immune responses in a variety of inflammatory and infectious conditions.

Acknowledgements

Funding sources: S.H. is supported by a research fellowship award from CIHR, Canadian Association of Gastroenterology and Crohn's and Colitis Foundation of Canada. L.H.T is supported by a research fellowship award from CIHR. N.L.J. is supported by operating grants from CIHR and CCFC.

References

- [1] Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 2007;8:931–7.
- [2] Ashford TP, Porter KR. Cytoplasmic components in hepatic cell lysosomes. *J Cell Biol* 1962;12:198–202.
- [3] Clark Jr SL. Cellular differentiation in the kidneys of newborn mice studied with the electron microscope. *J Biophys Biochem Cytol* 1957;3:349–62.
- [4] Hruban Z, Spargo B, Swift H, Wissler RW, Kleinfeld RG. Focal cytoplasmic degradation. *Am J Pathol* 1963;42:657–83.
- [5] Kovacs AL, Seglen PO. Inhibition of hepatocytic protein degradation by inducers of autophagosome accumulation. *Acta Biol Med Ger* 1982;41:125–30.
- [6] Mitchener JS, Shelburne JD, Bradford WD, Hawkins HK. Cellular autophagocytosis induced by deprivation of serum and amino acids in HeLa cells. *Am J Pathol* 1976;83:485–92.
- [7] Seglen PO, Gordon PB, Poli A. Amino acid inhibition of the autophagic/lysosomal pathway of protein degradation in isolated rat hepatocytes. *Biochim Biophys Acta* 1980;630:103–18.
- [8] Mortimore GE, Schworer CM. Induction of autophagy by amino-acid deprivation in perfused rat liver. *Nature* 1977;270:174–6.
- [9] Pfeifer U. Inhibition by insulin of the formation of autophagic vacuoles in rat liver. A morphometric approach to the kinetics of intracellular degradation by autophagy. *J Cell Biol* 1978;78:152–67.
- [10] Deter RL. Quantitative characterization of dense body, autophagic vacuole, and acid phosphatase-bearing particle populations during the early phases of glucagon-induced autophagy in rat liver. *J Cell Biol* 1971;48:473–89.
- [11] Seglen PO, Gordon PB. 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proc Natl Acad Sci USA* 1982;79:1889–92.
- [12] Klionsky DJ, Cregg JM, Dunn Jr WA, Emr SD, Sakai Y, Sandoval IV, et al. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 2003;5:539–45.
- [13] Kabeya Y, Kawamata T, Suzuki K, Ohsumi Y. Cis1/Atg31 is required for autophagosome formation in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 2007;356:405–10.
- [14] Suzuki K, Ohsumi Y. Molecular machinery of autophagosome formation in yeast, *Saccharomyces cerevisiae*. *FEBS Lett* 2007;581:2156–61.
- [15] Meijer WH, van der Klei IJ, Veenhuis M, Kiel JA. ATG genes involved in non-selective autophagy are conserved from yeast to man, but the selective Cvt and pexophagy pathways also require organism-specific genes. *Autophagy* 2007;3:106–16.
- [16] Yoshimori T, Noda T. Toward unraveling membrane biogenesis in mammalian autophagy. *Curr Opin Cell Biol* 2008;20:401–7.
- [17] Fengsrud M, Erichsen ES, Berg TO, Raiborg C, Seglen PO. Ultrastructural characterization of the delimiting membranes of isolated autophagosomes and amphisomes by freeze-fracture electron microscopy. *Eur J Cell Biol* 2000;79:871–82.
- [18] Dunn Jr WA. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. *J Cell Biol* 1990;110:1923–33.
- [19] Juhasz G, Neufeld TP. Autophagy: a forty-year search for a missing membrane source. *PLoS Biol* 2006;4:e36.
- [20] Ericsson JL. Studies on induced cellular autophagy. II. Characterization of the membranes bordering autophagosomes in parenchymal liver cells. *Exp Cell Res* 1969;56:393–405.
- [21] Novikoff AB, Shin WY. Endoplasmic reticulum and autophagy in rat hepatocytes. *Proc Natl Acad Sci USA* 1978;75:5039–42.
- [22] Locke M, Sykes AK. The role of the Golgi complex in the isolation and digestion of organelles. *Tissue Cell* 1975;7:143–58.
- [23] Yamamoto A, Masaki R, Tashiro Y. Characterization of the isolation membranes and the limiting membranes of autophagosomes in rat hepatocytes by lectin cytochemistry. *J Histochem Cytochem* 1990;38:573–80.
- [24] Glaumann H, Ericsson JL, Marzella L. Mechanisms of intralysosomal degradation with special reference to autophagocytosis and heterophagocytosis of cell organelles. *Int Rev Cytol* 1981;73:149–82.
- [25] He C, Baba M, Cao Y, Klionsky DJ. Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy. *Mol Biol Cell* 2008;19:5506–16.
- [26] Tanida I, Sou YS, Ezaki J, Minematsu-Ikeguchi N, Ueno T, Kominami E. HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and GABAA receptor-associated protein-phospholipid conjugates. *J Biol Chem* 2004;279:36268–76.
- [27] Tanida I, Tanida-Miyake E, Ueno T, Kominami E. The human homolog of *Saccharomyces cerevisiae* Apg7p is a protein-activating enzyme for multiple substrates including human Apg12p, GATE-16, GABARAP, and MAP-LC3. *J Biol Chem* 2001;276:1701–6.
- [28] Nakatogawa H, Ichimura Y, Ohsumi Y. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 2007;130:165–78.
- [29] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 2007;282:24131–45.
- [30] Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell* 2008;19:2092–100.

- [31] Ganley IG, Lam DH, Wang J, Ding X, Chen S, Jiang X. ULK1-ATG13-FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* 2009;284(18):12297–305.
- [32] Okazaki N, Yan J, Yuasa S, Ueno T, Kominami E, Masuho Y, et al. Interaction of the Unc-51-like kinase and microtubule-associated protein light chain 3 related proteins in the brain: possible role of vesicular transport in axonal elongation. *Brain Res Mol Brain Res* 2000;85:1–12.
- [33] Young AR, Chan EY, Hu XW, Kochl R, Crawshaw SG, High S, et al. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J Cell Sci* 2006;119:3888–900.
- [34] Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN. Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 1993;73:585–96.
- [35] Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD. Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* 1993;260:88–91.
- [36] Brunn GJ, Williams J, Sabers C, Wiederrecht G, Lawrence Jr JC, Abraham RT. Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J* 1996;15:5256–67.
- [37] Sekulic A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, et al. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res* 2000;60:3504–13.
- [38] Fruman DA. Towards an understanding of isoform specificity in phosphoinositide 3-kinase signalling in lymphocytes. *Biochem Soc Trans* 2004;32:155–9.
- [39] Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 1996;15:6541–51.
- [40] Diaz-Troya S, Perez-Perez ME, Florencio FJ, Crespo JL. The role of TOR in autophagy regulation from yeast to plants and mammals. *Autophagy* 2008;4:851–65.
- [41] Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;18:1926–45.
- [42] Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab* 2008;8:224–36.
- [43] Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, et al. Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. *Cancer Res* 2005;65:3336–46.
- [44] Chang YY, Juhasz G, Goraksha-Hicks P, Arsham AM, Mallin DR, Muller LK, et al. Nutrient-dependent regulation of autophagy through the target of rapamycin pathway. *Biochem Soc Trans* 2009;37:232–6.
- [45] Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 2009;20(7):1992–2003.
- [46] Rosenbluth JM, Mays DJ, Pino MF, Tang LJ, Pietenpol JA. A gene signature-based approach identifies mTOR as a regulator of p73. *Mol Cell Biol* 2008;28:5951–64.
- [47] Lindmo K, Stenmark H. Regulation of membrane traffic by phosphoinositide 3-kinases. *J Cell Sci* 2006;119:605–14.
- [48] Petiot A, Ogier-Denis E, Blommaert EF, Meijer AJ, Codogno P. Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J Biol Chem* 2000;275:992–8.
- [49] Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006;8:688–99.
- [50] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 1999;402:672–6.
- [51] Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* 2008;90:313–23.
- [52] Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, Gulati P, et al. Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. *Proc Natl Acad Sci USA* 2005;102:14238–43.
- [53] Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, Natt F, et al. Amino acids activate mTOR complex 1 via Ca²⁺/CaM signaling to hVps34. *Cell Metab* 2008;7:456–65.
- [54] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 2005;122:927–39.
- [55] Shi CS, Kehrl JH. MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J Biol Chem* 2008;283:3175–82.
- [56] Jia G, Cheng G, Gangahar DM, Agrawal DK. Insulin-like growth factor-1 and TNF- α regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. *Immunol Cell Biol* 2006;84:448–54.
- [57] Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Souquere S, et al. NF- κ B activation represses tumor necrosis factor- α -induced autophagy. *J Biol Chem* 2006;281:30373–82.
- [58] Fabre C, Carvalho G, Tasdemir E, Braun T, Ades L, Grosjean J, et al. NF- κ B inhibition sensitizes to starvation-induced cell death in high-risk myelodysplastic syndrome and acute myeloid leukemia. *Oncogene* 2007;26:4071–83.
- [59] Qing G, Yan P, Xiao G. Hsp90 inhibition results in autophagy-mediated proteasome-independent degradation of I κ B kinase (IKK). *Cell Res* 2006;16:895–901.
- [60] Qing G, Yan P, Qu Z, Liu H, Xiao G. Hsp90 regulates processing of NF- κ B p100 involving protection of NF- κ B-inducing kinase (NIK) from autophagy-mediated degradation. *Cell Res* 2007;17:520–30.
- [61] Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, et al. NF- κ B inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *EMBO J* 2003;22:3898–909.
- [62] Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* 2007;26:1749–60.
- [63] Dobrowolny G, Accello M, Rizzuto E, Beccafico S, Mammucari C, Boncompagni S, et al. Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. *Cell Metab* 2008;8:425–36.
- [64] Xu Y, Kim SO, Li Y, Han J. Autophagy contributes to caspase-independent macrophage cell death. *J Biol Chem* 2006;281:19179–87.
- [65] Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, et al. Autophagy defends cells against invading group A *Streptococcus*. *Science* 2004;306:1037–40.
- [66] Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. *Science* 2005;307:727–31.
- [67] Suzuki T, Franchi L, Toma C, Ashida H, Ogawa M, Yoshikawa Y, et al. Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in *Shigella*-infected macrophages. *PLoS Pathog* 2007;3:e111.
- [68] Cullinane M, Gong L, Li X, Lazar-Adler N, Tra T, Wolvetang E, et al. Stimulation of autophagy suppresses the intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines. *Autophagy* 2008;4:744–53.
- [69] Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH. Autophagy controls *Salmonella* infection in response to damage to the *Salmonella*-containing vacuole. *J Biol Chem* 2006;281:11374–83.
- [70] Birmingham CL, Brumell JH. Autophagy recognizes intracellular *Salmonella enterica* serovar *Typhimurium* in damaged vacuoles. *Autophagy* 2006;2:156–8.
- [71] Rich KA, Burkett C, Webster P. Cytoplasmic bacteria can be targets for autophagy. *Cell Microbiol* 2003;5:455–68.
- [72] Hamon M, Bierre H, Cossart P. *Listeria monocytogenes*: a multifaceted model. *Nat Rev Microbiol* 2006;4:423–34.
- [73] Birmingham CL, Canadien V, Gouin E, Troy EB, Yoshimori T, Cossart P, et al. *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. *Autophagy* 2007;3:442–51.
- [74] Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 2008;451:350–4.
- [75] Vergne I, Chua J, Deretic V. Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca²⁺/calmodulin-P13K hVps34 cascade. *J Exp Med* 2003;198:653–9.
- [76] 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;119:753–66.
- [77] Alonso S, Pethe K, Russell DG, Purdy GE. Lysosomal killing of *Mycobacterium* mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc Natl Acad Sci USA* 2007;104:6031–6.
- [78] Gutierrez MG, Saka HA, Chinen I, Zoppino FC, Yoshimori T, Bocco JL, et al. Protective role of autophagy against *Vibrio cholerae* cytotoxicity, a pore-forming toxin from *V. cholerae*. *Proc Natl Acad Sci USA* 2007;104:1829–34.
- [79] Terebiznik MR, Raju D, Vazquez CL, Torbrick K, Kulkarni R, Blanke SR, et al. Effect of *Helicobacter pylori*'s vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. *Autophagy* 2009;5:370–9.
- [80] Orvedahl A, Alexander D, Tallozy Z, Sun Q, Wei Y, Zhang W, et al. HSV-1 ICP34, 5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe* 2007;1:23–35.
- [81] Jackson WT, Giddings Jr TH, Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, et al. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol* 2005;3:e156.
- [82] Berkova Z, Crawford SE, Trugnan G, Yoshimori T, Morris AP, Estes MK. Rotavirus NSP4 induces a novel vesicular compartment regulated by calcium and associated with viroplasm. *J Virol* 2006;80:6061–71.
- [83] Poppers J, Mulvey M, Perez C, Khoo D, Mohr I. Identification of a lytic-cycle Epstein-Barr virus gene product that can regulate PKR activation. *J Virol* 2003;77:228–36.
- [84] Lu Y, Wambach M, Katze MG, Krug RM. Binding of the influenza virus NS1 protein to double-stranded RNA inhibits the activation of the protein kinase that phosphorylates the eIF-2 translation initiation factor. *Virology* 1995;214:222–8.
- [85] Prentice E, Jerome WG, Yoshimori T, Mizushima N, Denison MR. Coronavirus replication complex formation utilizes components of cellular autophagy. *J Biol Chem* 2004;279:10136–41.
- [86] Zhao Z, Thackray LB, Miller BC, Lynn TM, Becker MM, Ward E, et al. Coronavirus replication does not require the autophagy gene ATG5. *Autophagy* 2007;3:581–5.
- [87] Carneiro LA, Magalhaes JG, Tattoli I, Philpott DJ, Travassos LH. Nod-like proteins in inflammation and disease. *J Pathol* 2008;214:136–48.
- [88] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783–801.
- [89] Meylan E, Tschopp J, Karin M. Intracellular pattern recognition receptors in the host response. *Nature* 2006;442:39–44.

- [90] Xu Y, Liu XD, Gong X, Eissa NT. Signaling pathway of autophagy associated with innate immunity. *Autophagy* 2008;4:110–2.
- [91] Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *EMBO J* 2008;27:1110–21.
- [92] Schmitz F, Heit A, Dreher S, Eisenacher K, Mages J, Haas T, et al. Mammalian target of rapamycin (mTOR) orchestrates the defense program of innate immune cells. *Eur J Immunol* 2008;38:2981–92.
- [93] Sanjuan MA, Dillon CP, Tait SW, Moshiah S, Dorsey F, Connell S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 2007;450:1253–7.
- [94] Kaneko T, Yano T, Aggarwal K, Lim JH, Ueda K, Oshima Y, et al. PGRP-LC and PGRP-LE have essential yet distinct functions in the drosophila immune response to monomeric DAP-type peptidoglycan. *Nat Immunol* 2006;7:715–23.
- [95] Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, et al. Autophagic control of listeria through intracellular innate immune recognition in drosophila. *Nat Immunol* 2008;9:908–16.
- [96] Delgado M, Singh S, De Haro S, Master S, Ponpuak M, Dinkins C, et al. Autophagy and pattern recognition receptors in innate immunity. *Immunol Rev* 2009;227:189–202.
- [97] Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006;313:1438–41.
- [98] Jounai N, Takeshita F, Kobiyama K, Sawano A, Miyawaki A, Xin KQ, et al. The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc Natl Acad Sci USA* 2007;104:14050–5.
- [99] Tal MC, Sasai M, Lee HK, Yordy B, Shadel GS, Iwasaki A. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signalling. *Proc Natl Acad Sci USA* 2009;106:2770–5.
- [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] 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;39:207–11.
- [102] Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007;39:830–2.
- [103] Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;40:955–62.
- [104] Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 β production. *Nature* 2008;456:264–8.
- [105] 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;456:259–63.
- [106] Barbier M, Vidal H, Desreumaux P, Dubuquoy L, Bourreille A, Colombel JF, et al. Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases. *Gastroenterol Clin Biol* 2003;27:987–91.
- [107] Yamamoto K, Kiyohara T, Murayama Y, Kihara S, Okamoto Y, Funahashi T, et al. Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. *Gut* 2005;54:789–96.
- [108] Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, et al. Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. *Cell Metab* 2008;8:318–24.
- [109] Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S. SYF-PEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 1999;50:213–9.
- [110] Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 2000;404:770–4.
- [111] Lunemann JD, Munz C. Autophagy in CD4+ T-cell immunity and tolerance. *Cell Death Differ* 2009;16:79–86.
- [112] Jacobson S, Sekaly RP, Jacobson CL, McFarland HF, Long EO. HLA class II-restricted presentation of cytoplasmic measles virus antigens to cytotoxic T cells. *J Virol* 1989;63:1756–62.
- [113] Jaraquemada D, Marti M, Long EO. An endogenous processing pathway in vaccinia virus-infected cells for presentation of cytoplasmic antigens to class II-restricted T cells. *J Exp Med* 1990;172:947–54.
- [114] Brazil MI, Weiss S, Stockinger B. Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. *Eur J Immunol* 1997;27:1506–14.
- [115] Dorfel D, Appel S, Grunebach F, Weck MM, Muller MR, Heine A, et al. Processing and presentation of HLA class I and II epitopes by dendritic cells after transfection with in vitro-transcribed MUC1 RNA. *Blood* 2005;105:3199–205.
- [116] Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, et al. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 2005;307:593–6.
- [117] Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 2007;26:79–92.
- [118] Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter Jr RL, Eissa NT. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med* 2009;15:267–76.
- [119] Munz C. Enhancing immunity through autophagy. *Annu Rev Immunol* 2009;27:423–49.