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Autophagy and checkpoints for intracellular pathogen defense

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Abstract

Purpose of review—Autophagy plays a crucial role in intracellular defense against various pathogens. Xenophagy is a form of selective autophagy that targets intracellular pathogens for degradation. In addition, several related yet distinct intracellular defense responses depend on autophagy-related (ATG) genes. This review gives an overview of these processes, pathogen strategies to subvert them, and their crosstalk with various cell death programs.

Recent findings—The recruitment of ATG proteins plays a key role in multiple intracellular defense programs, specifically xenophagy, LC3-associated phagocytosis (LAP), and the IFNγ-mediated elimination of pathogens such as *Toxoplasma gondii* and murine norovirus. Recent progress has revealed methods employed by pathogens to resist these intracellular defense mechanisms and/or persist in spite of them. The intracellular pathogen load can tip the balance between cell survival and cell death. Further, it was recently observed that LAP is indispensable for the efficient clearance of dying cells.

Summary—Autophagy-dependent and ATG gene-dependent pathways are essential in intracellular defense against a broad range of pathogens.

Keywords

autophagy; xenophagy; LC3-associated phagocytosis; IFNγ-mediated pathogen elimination; pathogen survival strategies

Introduction

Classical autophagy refers to an evolutionarily conserved catabolic process in eukaryotic cells in which cytoplasmic components are engulfed by a double-membraned autophagosome and degraded after fusion with a lysosome [1, 2]. The canonical autophagy machinery consists of more than 30 autophagy-related (ATG) genes, originally identified in yeast [2]. An important marker of autophagy is the recruitment of microtubule-associated

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protein light-chain 3 (LC3), the mammalian orthologue of yeast Atg8, to the autophagosome [2].

Selective autophagy of pathogens, also referred to as xenophagy, is a key defense mechanism against a broad range of infections [3]. Additionally, genome-wide association studies have revealed associations between inflammatory bowel diseases (IBD) and single-nucleotide polymorphisms (SNPs) in genes involved in autophagy. Most notably, SNPs in *ATG16L1* and *IRGM* specifically increase the risk for Crohn's disease and genetic variants in *ATG4* and *FNBP1L* are associated with the presence of granulomas in Crohn's disease patients, a marker of a more aggressive disease course [1, 4, 5]. Other genes, like *SMURF1*, are found in risk loci for ulcerative colitis [5].

In addition, noncanonical forms of autophagy, which rely on select components of the autophagy machinery, are implicated in intracellular pathogen defense. For instance, in a process termed LC3-associated phagocytosis (LAP), a subset of ATG proteins assists in the digestion of phagocytosed pathogens. Central to this process is the conjugation of LC3 to the single-membraned phagosomes [6, 7]. Further, certain ATG proteins take part in the interferon gamma (IFNγ)-mediated defense against pathogens such as *Mycobacterium tuberculosis*, *Toxoplasma gondii*, and murine norovirus, whose human equivalent is the most common cause of viral gastroenteritis [8,9,10].

Autophagy in pathogen defense

The canonical macroautophagy pathway, hereafter referred to as autophagy, is the most studied and well-understood form of autophagy.

Different ATG protein conjugation complexes act at the different stages of the autophagy pathway [2,11]. First, an isolation membrane (phagophore) is generated *de novo* from the endoplasmic reticulum, Golgi apparatus, mitochondria, or other plasma membrane-derived endocytic organelles [11]. The initiation of the phagophore is mediated by the ULK1/FIP200/ATG101/ATG13 protein kinase complex and Vps34/Beclin-1/Vps15/ATG14 lipid kinase complex. These complexes induce the formation of the ATG5/ATG12/ATG16L1 complex (formed with the help of ATG7 and ATG10), which promotes the elongation of the phagophore that eventually closes around cytoplasmic cargo, forming a double-membraned vacuole called the autophagosome. Aided by ATG3 and ATG7, these complexes facilitate the addition of a phosphatidylethanolamine group to the mammalian LC3 homologues (LC3A, LC3B, LC3C, GABARAP, GABARAP-L1, GABARAP-L2) [12]), which are then recruited to the autophagosome. This conversion from the cytosolic to the lipid-bound form of LC3 is commonly referred to as LC3-I to LC3-II conversion. Finally, the autophagosome matures and fuses with the lysosome, forming a single-membraned vesicle termed the autolysosome, wherein the contents are degraded. This step is effectuated by several lysosomal membrane proteins, including the GTPase Rab7 and LAMP2, as well as by SNARE proteins such as syntaxin 17 and SNAP29. Another Beclin1/VPS34 complex assists in this final step, only here instead of ATG14 the UV radiation resistance-associated gene (UVRAG) is required.

Apart from their role in autophagosome formation, the LC3/GABARAP proteins provide docking sites for receptors that deliver cargo to phagophores during *selective* autophagy. To date, eight mammalian cargo receptors have been identified: p62, its paralogue NBR1, NDP52, its paralogue T6BP, optineurin [13, 14], Nix [15], NCOA4 [16, 17] and recently TRIM5α [18]. They all share an LC3-interacting region (LIR), typically a WxxL sequence N-terminally preceded by negatively charged residues. Variations in their domain structure allow them to interact with specific members of the LC3/GABARAP family and specific pathogen-associated substrates [14, 19, 20]. Recent studies in yeast identified a LIR-independent interaction between ATG19 (a relative of mammalian p62 and NBR1) and ATG8 and ATG11 [21, 22]. ATG19 contains multiple binding sites for ATG8 and its interaction with ATG11 is achieved through kinase Hrr25 phosphorylation [22].

Xenophagy is a specific form of selective autophagy where the cargo captured by the autophagosome is an intracellular pathogen [23-26] (Fig. 1a). Xenophagy is an important immune response, through the direct and selective elimination of intracellular pathogens. Studies involving *M. tuberculosis* and *Salmonella* Typhimurium infection indicate that approximately 30% of intracellular bacteria are associated with autophagy markers [27, 28].

When bacteria enter the cell, they are engulfed by the host plasma membrane. The resulting single-membrane pathogen-containing vacuole can mature and fuse with the lysosome, forming a phagolysosome in a process termed phagocytosis. In order to evade this process and facilitate their survival and replication, most bacteria (e.g., *Coxiella, Chlamydia, Salmonella, Legionella, Mycobacterium*) adapt to or modulate the conditions of the phagosome, thereby establishing a vacuolar replication niche, and often concurrently block the fusion with the lysosome [29]. A percentage of these bacteria eventually become cytosolic because of failure to maintain vacuolar integrity, to obtain additional nutrients, or to spread to adjacent cells [27]. Other bacteria, including *Shigella, Listeria,* and *Francisella* quickly escape the vacuole and attempt to resist cytosolic host immune defense strategies.

When intracellular pathogens become cytosolic, their vacuole membrane ruptures, thereby exposing the previously inward-facing glycans to the host cytosol. The exposed glycans are damage-associated molecular patterns (DAMPs) that can be recognized by cargo receptors [3, 30]. For example, upon infection of epithelial cells with *S.* Typhimurium, galectin-8 interacts with exposed glycans on the ruptured membranes, accumulates, and recruits the cargo receptor NDP52 [31]. Additionally, xenophagy typically involves the poly-ubiquitination of either exposed phagosomeglycans [32] or of cytosolic bacteria themselves [33, 34]. This process is governed by E3 ligases such as LRSAM1 [35, 36] and PARKIN [35]. Similarly, viruses are commonly targeted for ubiquitin-mediated xenophagy. The transmembrane protein STING (stimulator of interferon genes) can detect double-stranded DNA viruses such as herpes simplex virus 1 (HSV1) and human cytomegalovirus and target them for autophagy [37]. The E3 ligase SMURF1 causes the clearance of Sindbis virus and HSV1 [38].

Pathogen secretion products can also induce xenophagy. For instance, *M. tuberculosis* can permeabilize its vacuole and release extracellular DNA (eDNA) to assist bacterial adhesion to epithelial cells. STING can detect this eDNA and cause ubiquitination of the *M.*

tuberculosis-containing vacuole [9, 37, 39]. The resulting ubiquitin coat is subsequently recognized by cargo receptors NDP52, p62, NBR1, and optineurin [20].

In comparison to the insight in the recognition of pathogens as autophagic cargo, much less is understood about the signals inducing phagophore formation upon infection. One study reported that bacteria with access to the cytosol activate xenophagy by nutrient competition with the host [40]. Specifically, amino acid starvation in the host cell inhibits the mammalian target of rapamycin complex 1 (mTORC1), which negatively regulates the ULK1 complex. Additionally, nutrient starvation of the host cell can induce nuclear translocation of the transcription factor EB (TFEB), a master regulator of lysosome biogenesis, driving mTORC1-independent autophagy [41, 42].

Non-canonical autophagy programs in pathogen defense

Several intracellular defense mechanisms have been identified that depend on a subset of ATG genes.

LC3-associated phagocytosis

As discussed above, many microorganisms inside the phagosome have developed mechanisms to disrupt vesicular trafficking and avoid phagosome-lysosome fusion [29]. A complementary pathogen defense mechanism to xenophagy, coined LAP, involves the recruitment of LC3 to the still-intact phagosome (Fig. 1b) [6, 7, 43]. This process is independent of the ULK1 initiation complex, consistent with the absence of phagophore formation in LAP. LC3 is mobilized by TLRs or Fcγ receptors (for bacterial infections) [44] or CLEC7A (for fungal infections) [45]. The main function of LAP appears to be promoting rapid fusion of phagosomes with lysosomes, thereby accelerating conventional phagocytosis [46].

The discovery of LAP illustrates the importance of not solely relying on LC3 as a marker for autophagy. The mere presence of LC3 on pathogen-containing vacuoles cannot distinguish between canonical xenophagy and LAP. At present, the two mechanisms can be discerned using electron microscopy, which allows verification of whether LC3 is associated with a single or double membrane. Further, since LAP targets pathogens already contained in a phagosome, it does not depend on selective cargo receptors and is ULK1-independent. Moreover, in xenophagy the beclin-1 binding protein rubicon suppresses autophagosome maturation, whereas in LAP it positively regulates the generation of an oxidative burst, a key antimicrobial component of phagocytosis [47]. Regardless of the present discrimination methods, it would be beneficial to identify LAP-specific genetic markers.

IFNγ-mediated pathogen elimination

A related intracellular innate immune response involves the IFNγ-mediated control of pathogens, for instance the parasite *T. gondii* in macrophages [8]. In the acute stage of the infection, tachyzoites (fast-replicating forms of the parasite) actively penetrate the host cell at a moving junction and create a non-fusogenic vacuole (Fig. 1c). Inside this membrane-bound parasitophorous vacuole, the parasites replicate by endodyogeny. Meanwhile, extracellular profilin, a *T. gondii* protein crucial for its motility and host cell invasion is

recognized by TLR11 and TLR12 in murine dendritic cells. This causes the dendritic cells to secrete IL-12 in a MyD88-dependent manner, which promotes IFNγ production from natural killer cells. Once the latter is detected by the infected cell, it can halt *T.gondii* replication through multiple mechanisms. The induction of indoleamine 2,3 dioxygenase (IDO) results in reduced tryptophan levels, an essential amino acid without which *T.gondii* replication is decelerated. Second, IFNγ can produce inducible nitric oxide synthase (iNOS), resulting in decreased arginine levels and consequently increased nitric oxide (NO) levels. The former once more induces starvation of the parasite, whereas NO has direct microbicidal effects. Most importantly, IFNγ upregulates p47 GTPases, also known as IRGs, the murine equivalent of human IRGM. During the infection, the IRGs target the parasitophorous vacuole, leading to its disruption as well as the destruction of the parasite's plasma membrane. Subsequently, the stripped parasites are eliminated via lysosome-mediated degradation. Understanding the mechanism for the human immune response to *T. gondii* requires more work. First, humans lack functional TLR11. Supportive roles for TLR7 and TLR9 have been identified in mice, which could play a larger role in humans. Additionally, the human immune response can be mediated more strongly by neutrophils, not dendritic cells. Finally, human IRGM is not regulated by IFNγ, indicating a potentially larger role for NO/IDO-mediated pathogen destruction.

It was recently shown that the canonical autophagy initiation genes ATG14, ULK1, and ULK2 are not required for the IFNγ-mediated immune response to *T. gondii* [25]. However, it does require the downstream ATG5/ATG12/ATG16L1 complex and the presence of ATG3 and ATG7, responsible for LC3-I to LC3-II conversion. No autophagosomes are formed; however, LC3 associates directly with the parasitophorous vacuole membrane. This colocalization is required for the recruitment of the IFNγ-effectors to the parasitophorous vacuole during *T.gondii* infection, suggesting that the destruction of *T.gondii* requires same machinery as the aforementioned LAP. In fact, IFNγ-mediated LAP is observed as an alternative defense mechanism against *M. tuberculosis* [9].

The ATG5/ATG12/ATG16L1 complex also assists the IFNγ-mediated control of murine norovirus by inhibiting the formation of its viral replication complex. In contrast, LC3 is dispensable for this activity [10].

Non-canonical autophagosome formation

Certain studies report the manifestation of autophagosomes resulting in xenophagy in the absence of key ATG genes involved in phagophore formation. Notably, α-hemolysin is a toxin secreted by *Staphylococcus aureus* that is reported to induce a beclin 1-independent autophagy response [48]. Similarly, it was deduced that infection with the foot-and-mouth disease virus can activate autophagy in the absence of VPS34 [49].

The bigger picture: interaction of autophagy and ATG genes with other branches of intracellular immunity

There is accumulating evidence for complex cell type-specific interplay between ATG proteins and other cellular immune responses, yet mechanistic insight is often incomplete

[23, 50]. Pattern recognition receptors (PRRs) such as NOD-like receptors (NLRs), Toll-like receptors (TLRs), and RIG-I-like receptors (RLRs) are central players of innate immunity, capable of activating phagocytosis and various inflammatory processes, as well as chemokines and antimicrobial molecules. ATG16L1 is recruited by NOD1 and NOD2 to the bacterial entry site at the plasma membrane in epithelial cells, and silencing of NOD2 in monocyte-derived dendritic cells decreases LC3-II levels after treatment with the NOD2 ligand muramyldipeptide [51]. Recent data show that infected bone marrow-derived macrophages lacking caspase-1 show increased autophagy, attributed to caspase-1's ability to cleave the TLR adaptor TRIF [52]. The study of *M. tuberculosis*-infected macrophages reveals how xenophagy can intricately modulate inflammatory responses and inflammasome signaling. The infection induces interleukin-1β (IL-1β), IFNγ, and tumor necrosis factor (TNF) levels, all T helper 1 (T_H1) cell-derived cytokines, which evoke xenophagy. On the other hand, T_H2 cytokines such as IL-4 and IL-13 have the opposite effect and inhibit starvation- and IFNγ-induced autophagosome formation [3, 53]. A recent study demonstrates the activation of the DNA damage-regulated autophagy modulator DRAM1 by the TLR/MYD88/NF-κB signaling cascade, which subsequently activates autophagy [54]. Viri activate RLRs, which negatively regulate xenophagy, likely to prevent excessive IFN levels and control inflammation [55].

Autophagy assists in the secretion of antimicrobial peptides and proteins (AMPs) and intracellular viral restriction factors [23, 50]. For instance, an intestinal epithelial-specific deletion of vitamin D3 receptor (VDR), an IBD risk gene that can activate the AMP LL-37, induces a significant reduction of both ATG16L1 and the antimicrobial enzyme lysozyme as well as an accumulation of p62 *in vivo* [56]. Moreover, recent data showed that the viral restriction factor TRIM5α doubles as a viral autophagy cargo receptor [18]. Additionally, it was recently observed that TFEB induces the expression of multiple antimicrobial and autophagy proteins in *Caenorhabditis elegans* and activates several proinflammatory cytokines and chemokines in mice after infection with *S. aureus* [57].

Finally, xenophagy assists the adaptive immune response via the fusion of autophagosomes with antigen-loading compartments for major histocompatibility complex class II (MHC-II) molecules. Similarly, a study in macrophages and dendritic cells showed LC3-positive phagosomes enable prolonged antigen presentation on MHC-II molecules [58]. Here, the delay of the fusion of the LC3-decorated phagosome with the lysosome allows the mounting of an adaptive immune response against the sequestered pathogen. Lastly, deletions of key ATG proteins are associated with the reduced development, activity, and survival of lymphocytes [28, 59, 60].

Mouse models provide an opportunity to study the cell type-specific effects of xenophagy, either in isolation from one another (e.g., using a conditional knockout mouse) or in connection with one another (e.g., using a conventional knockout or knock-in mouse). For instance, consider the coding SNP Thr300Ala (T300A) in ATG16L1, which is associated with increased risk for Crohn's disease. Consistent with the human phenotype, Paneth cells of T300A knock-in mice show abnormal lysozyme distribution, accompanied by reduced secretion of their antimicrobial compounds. Additionally, the knock-in mice showed altered goblet cell morphology. These phenotypes contribute to reduced pathogen clearance.

Establishing a vicious cycle, nearby dendritic cells and macrophages display more paracrine signaling of pro-inflammatory molecules [28, 61, 62]. This is a plausible mechanism for the observed chronic infection and altered microbial ecology in patients with Crohn's disease.

Pathogen resistance and persistence to LC3-associated intracellular immunity

Disrupting a crucial step in the host defense pathway is a common pathogen strategy to resist autophagy programs [23, 29, 63-65]. For example, cytosolic *Shigella* produces IcsB that binds to ATG5, thereby averting phagophore elongation [23], and the viral phosphoprotein P of parainfluenza virus type 3 (HPIV3) binds to SNAP29, preventing the SNARE complex from ensuring autophagosome-lysosome fusion [64].

Alternatively, certain pathogens use key elements of xenophagy or LAP to their advantage. *Coxiella burnetti* for instance does not merely resist the lysosomal degradative environment, but exploits the acidic pH for its own metabolic activation [66]. In influenza A, a LIR motif in a viral protein can redirect LC3 to the plasma membrane, where the budding of stable viruses is then promoted [67]. Similarly, vesicular stomatitis virus customizes UVRAG to hijack and redirect virus-bearing endosomes to specific cellular locations where it can deliver its genetic material [68, 69]. Hepatitis C virus engages autophagy to degrade mitochondria (mitophagy). It is hypothesized that the resulting increase in cellular ATP levels causes the observed reduced HCV-induced apoptosis [70].

Some pathogens exploit other intracellular defense mechanisms to reduce xenophagy. Specifically, adherent Invasive *Escherichia coli* (AIEC), associated with Crohn's disease, use the infection-induced NF-κB response to upregulate microRNAs in the host cell. This reduces ATG5 and ATG16L1 levels, leading to an increased bacterial load and inflammation level [71].

A persistent infection is characterized by a subpopulation of intracellular pathogens hiding from the immune system by slowing or halting their replication altogether, thus assuming a dormant or latent state. For instance, *Listeria monocytogenes* depend on a pore-forming toxin, listeriolysin O (LLO), to escape the phagosome and replicate in the cytosol. However, a fraction of the bacteria that infects murine macrophages is characterized by reduced LLO activity, trapping them inside the phagosome. The lower LLO levels are nevertheless sufficient to block acidification of the phagosome and its fusion with a lysosome, thereby establishing the formation of a spacious *Listeria*-containing phagosome (SLAP) [43]. Inside, the bacteria reduce their replication rate, presumably due to a lack of nutrients. SLAPs have also been observed in a mouse model of *L. monocytogenes* persistent infection.

Similarly, mice infected with *S.* Typhimurium displayed a significant subpopulation of non-replicating bacteria in several organs, even 24 hours after infection [72]. Since most antibiotics target the enzymes of metabolically active and dividing bacteria, persisters can survive antibiotic treatment [73]. Indeed, after 5 days of antibiotic treatment that started 24 hours after inoculation, a small subset of non-replicating persisters was retrieved from the mesenteric lymph nodes, a known site of persistence [72].

Studies in *E. coli* have attributed the ability to develop persistence to the toxin gene of the toxin/antitoxins (TA) operons such as hipA/B, RelE/B, MazF/E, YafQ/DinJ, vapB/C, phd/doc, MqsR/A, HigB/A, and CcdA/B [73, 74]. Either stochastically or due to an external circumstance, such as the presence of antibiotics, oxidative stress, or starvation, the bacterial stress alarmone ppGpp accumulates and mediates the degradation of the operon's antitoxin compound, thereby creating persistence. Analysis of the *Salmonella* genome revealed 14 TA operons, the majority of which contributed to the persister formation [72], and proteomic profiling of *M. tuberculosis*, also capable of establishing latent infection, has revealed multiple nutrient starvation responsive TA systems [75]. Since culturing under nutrient starvation can mediate antitoxin degradation, it is plausible that the nutrient-poor conditions inside bacteria-containing vacuoles induce persister formation. Furthermore, in light of this discussion, it would be interesting to verify whether LLO is part of a TA operon (or comparable system) in the *Listeria* genome.

It should be noted that analogous phenomena have been observed as well for fungi, parasites, and viruses [76]. After the trigger for persistence is removed (e.g., aggressive immune response or antibiotic treatment), the persisters start replicating again and form the basis of a new population, thereby strongly contributing to the occurrence of chronic and recurrent infections [73].

A life-or-death situation on the cellular level

It is insightful to understand how the level of cellular stress mediates the LC3-mediated immune response (Fig. 2). Below, only concepts observed directly in the context of intracellular defense or potentially applicable thereto are described.

Initially, xenophagy and its variants perform a cytoprotective function; however, prolonged and irreversible stress will lead to cell death [77-80]. There is complex crosstalk between autophagy systems and cell death programs. For example, in the absence of cellular stress, beclin-1 is bound to anti-apoptotic proteins of the Bcl-2 family and both autophagy and apoptosis are inactive. At low to moderate stress levels, activated autophagy genes typically present a barrier for programmed cell death (apoptosis). For instance, high-mobility group box 1 (HMGB1), a DAMP, disrupts the beclin-1/Bcl-2 complex. While this does not alter the anti-apoptotic potential of Bcl-2, the now free beclin-1 both induces autophagy and inhibits apoptosis [79, 81, 82]. Past a critical stress level, complex regulatory mechanisms induce programmed cell death, where the cell breaks into multiple apoptotic bodies. Once this happens, autophagy is inactivated in part due to the caspase- or calpain-mediated cleavage of essential autophagy proteins such as ATG3 and beclin-1 [79]. Further, truncated ATG proteins can exhibit pro-apoptotic properties. For instance, antimicrobial peptides can cause an influx of calcium, which allows calpain-mediated cleavage of ATG5, actively contributing to apoptosis [83]. Meanwhile, studies have shown the potential of key autophagy proteins involved in autophagosome formation induce apoptosis via caspase activation [79].

Autophagosome accumulation can result in programmed cell necrosis, characterized by a loss of cell membrane integrity and release of cellular contents in the cytoplasm [79].

Finally, recent work showed that autophagy triggers, such as starvation and autophagy-inducing peptides, can induce a form of cell death with distinct morphological features, including the increased presence of autophagosome/autolysomes. This autophagic cell death is termed “autosis” and is regulated by the sodium-potassium pump Na^+/K^+-ATPase [80].

LAP has been observed to efficiently clear apoptotic corpses and necrotic cells [10, 84-86]. Here, LAP is triggered by the engagement of TIM4, a receptor for phosphatidylserine, displayed by dying cells. LAP-defective macrophages produce more pro- and fewer anti-inflammatory cytokines [86], which could contribute to disease.

Conclusion

Multiple ATG gene-dependent intracellular defense mechanisms have been identified, although to date, studies have largely focused on xenophagy, the autophagic clearance of pathogens. Additionally, a clearer picture of the complex interplay between cytoprotective autophagy-dependent systems and cell death cassettes is emerging.

Pathogens continually evolve to develop strategies to resist and persist ATG-dependent destruction. This fact, combined with their roles in numerous human infectious diseases and gastrointestinal disorders, underscores the importance of mechanistic studies of the pathways and regulatory networks involved in both the immune response and pathogen's survival strategies. The recent successful feasibility studies of conducting genome-wide CRISPR-Cas9 knockout screens in human cells pave the way for their application in this area [87, 88]. Relevant mouse models can help piece together a holistic picture of the consequences of impaired immunity *in vivo*.

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Key points

- Autophagy and ATG gene-dependent processes such as IFNγ-induced pathogen elimination and LC3-associated phagocytosis (LAP) are vital host immune responses against a broad range of intracellular pathogens.
- Pathogens can resist these processes by a variety of mechanisms, including disrupting key steps in the degradation pathway, adapting to the associated degradative conditions, and exploiting host immune responses to promote their replication and/or survival.
- A persistent infection is characterized by a subpopulation of intracellular pathogens slowing or halting their replication inside a vacuole, thereby evading both immune responses and antibiotic drug action.
- Autophagy and various cell death programs show mutual regulation; in addition, LAP is essential for the efficient clearance of apoptotic and necrotic cells.

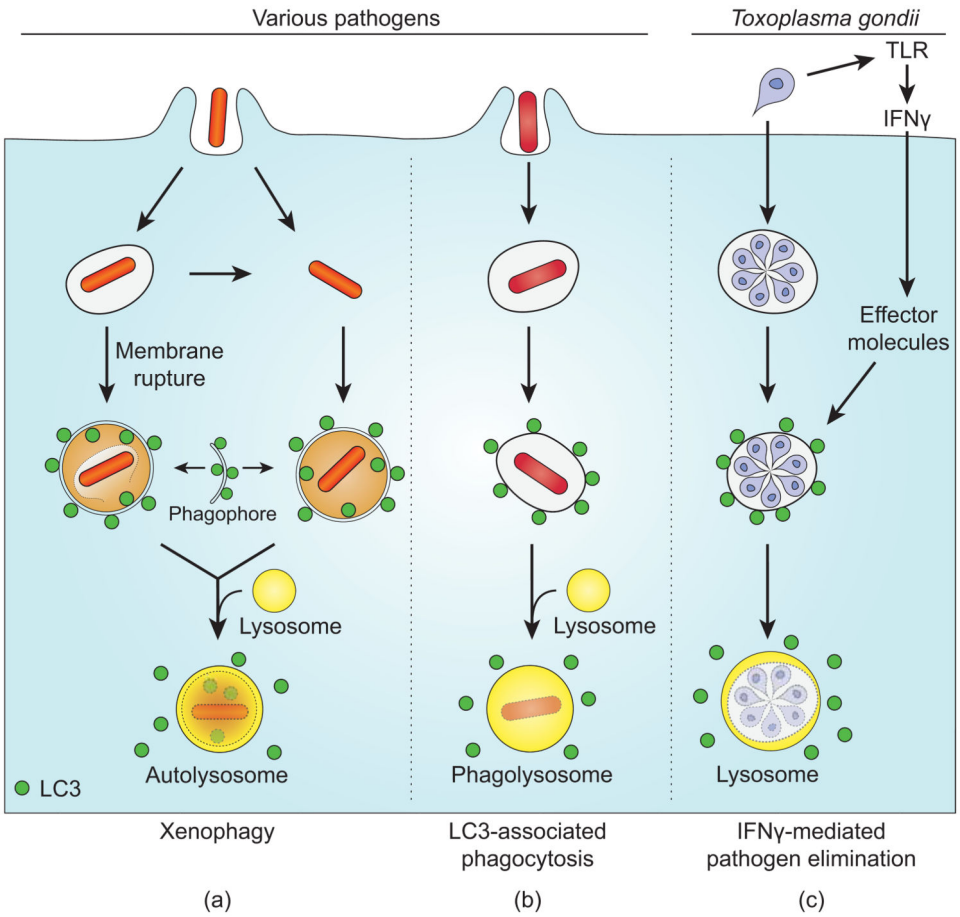

Figure 1. Pathways for LC3-associated pathogen defense

(a) Xenophagy, (b) LC3-associated phagocytosis, (c) IFNγ-induced pathogen degradation illustrated for *T. gondii* infection.

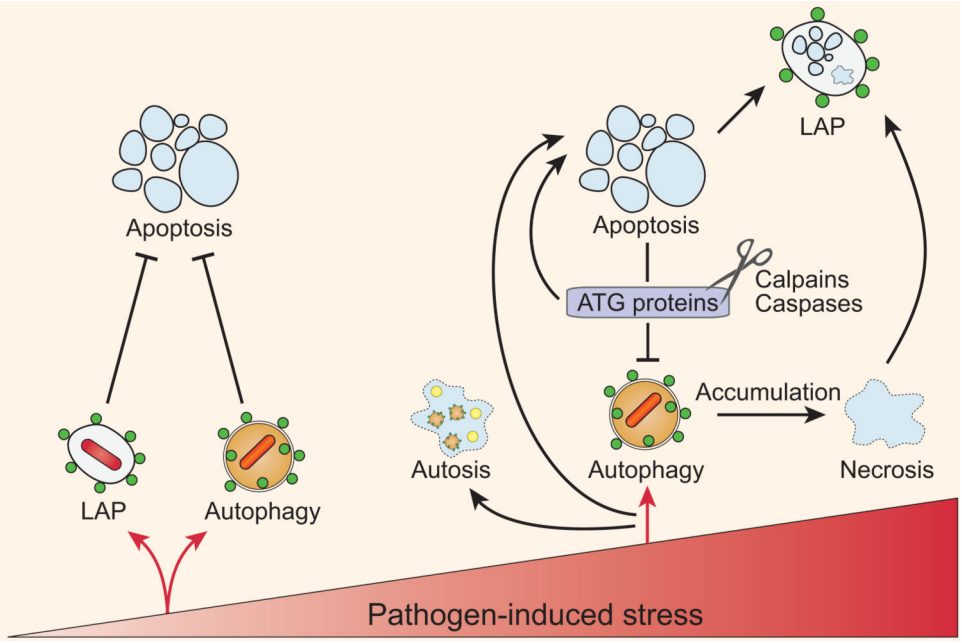

Figure 2. Complex stress level-dependent interplay between xenophagy, LAP, apoptosis, autosis, and necrosis

At low stress levels, autophagy proteins such as beclin-1 inhibit apoptosis. At higher stress intensity, apoptosis negatively regulates autophagy by cleaving ATG proteins. The truncated ATG proteins either are non-functional or obtain pro-apoptotic properties. Genes involved in autophagosome formation can induce apoptosis by activating caspases. Autophagy triggers can activate a morphologically distinct form of cell death, termed autosis. Finally, an accumulation of autophagosomes can induce necrosis. LC3-associated phagocytosis (LAP) efficiently removes apoptotic and necrotic cells.