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Authors

KimmeY, Jacqueline M
Stallings, Christina L

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Bacterial Pathogens versus Autophagy: Implications for Therapeutic Interventions

Jacqueline M. Kimmey and Christina L. Stallings*

Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, USA

Abstract

Research in recent years has focused significantly on the role of selective macroautophagy in targeting intracellular pathogens for lysosomal degradation, a process termed xenophagy. In this review we evaluate the proposed roles for xenophagy in controlling bacterial infection, highlighting the concept that successful pathogens have evolved ways to subvert or exploit this defense, minimizing the actual effectiveness of xenophagy in innate immunity. Instead, studies in animal models have revealed that autophagy-associated proteins often function outside of xenophagy to influence bacterial pathogenesis. In light of current efforts to manipulate autophagy and the development of host-directed therapies to fight bacterial infections, we also discuss the implications stemming from the complicated relationship that exists between autophagy and bacterial pathogens.

Keywords

Autophagy; Xenophagy; bacterial pathogens; *Mycobacterium tuberculosis*; host-directed therapies

Xenophagy as an Immune Defense against Intracellular Bacteria

The rise in the prevalence of antibiotic-resistant infections and the dearth of new antibiotics in the pipeline has raised significant interest in developing host-directed therapies that harness the immune response to control bacterial infection as an alternative therapeutic strategy[1]. One attractive cellular target for immunotherapy is the stimulation of **macroautophagy** (herein referred to as autophagy, see Glossary), and more specifically **xenophagy**. Autophagy is a dynamic process that targets cellular cytoplasmic contents for lysosomal degradation and serves myriad roles in eukaryotic cells. Xenophagy is a type of selective macroautophagy that specifically targets intracellular pathogens to lysosomes, restricting their replication and survival.

Xenophagy involves the same steps as canonical autophagy, which are often delineated as initiation, elongation, substrate targeting, and maturation/lysosomal fusion (resulting in degradation of cargo)(Fig. I, Box 1)[2]. In the case of bacterial infection, substrate targeting involves ubiquitination of bacteria or colocalization of ubiquitin to bacteria, and the

*Correspondence: stallings@wustl.edu (C.L. Stallings).

recognition of the ubiquitin by autophagy receptors that interact with LC3 (microtubule-associated protein 1 light chain 3) to recruit the bacteria to autophagosomes, which then fuse to lysosomes to degrade the cargo [2] (Box 2). The first report indicating that bacteria could be eliminated by xenophagy was published in 1984 when Rikihisa observed autophagosome formation in neutrophils infected by *Rickettsia conorii*[3]. Since then, the role of xenophagy in cell culture infection experiments has been investigated for all widely studied intracellular bacterial pathogens. Using mammalian cell culture approaches (Box 3), researchers have gained tremendous insight into key players and mechanisms of xenophagy. However, no single experimental approach captures the complete dynamics of xenophagy and, despite the immense focus in this area, these studies often raise uncertainty with regard to the role of xenophagy in controlling intracellular bacterial infections. Therefore, a more critical and comprehensive evaluation of the role of xenophagy and autophagy-related proteins during bacterial infection is necessary to understand the range of effects that might occur while intervening with **autophagic flux**. In this review we evaluate the data available regarding the interplay between xenophagy and different intracellular bacterial pathogens, highlighting *Mycobacterium tuberculosis*, which has historically been used as a model of xenophagic control of bacterial replication used as a model of xenophagic control of bacterial replication (for different intracellular bacterial pathogens, see Table S1 in the supplemental information online). Detailed analysis of the data reveals that successful pathogens have evolved ways to subvert or exploit xenophagy, but autophagy-associated proteins can function outside of xenophagy to influence bacterial pathogenesis. Finally, we discuss the implications of the complicated relationship between autophagy and bacterial pathogens which may impact the development of host-directed therapies to treat these infections.

Xenophagy and *Mycobacterium tuberculosis* Pathogenesis

The role of xenophagy in controlling *M. tuberculosis* replication in immune cells has been extensively studied in cell culture by multiple groups. As a result, *M. tuberculosis* is often cited as an example of a bacterial pathogen controlled by xenophagy. The survival of *M. tuberculosis* in phagocytes is dependent on its ability to inhibit phagolysosome fusion. Interferon- γ (IFN- γ) activation of cultured macrophages results in increased trafficking of *M. tuberculosis* to the lysosome and subsequent killing[4], and xenophagy is often implicated in this process. Many studies have demonstrated that autophagy factors including LC3 are targeted to a subset of intracellular *M. tuberculosis* bacteria [4–8]. There are also reports that knockdown (KD) of Unc 51-like kinase 1 *Ulk1*[9], *Beclin1*[10], *Atg5*[10], *Atg7*[11], or *p62*[6] autophagy genes can result in improved *M. tuberculosis* survival in cell cultures. It is notable that at most, 30% of *M. tuberculosis* bacteria appear to be targeted by LC3 at any given timepoint, and only twofold to threefold changes in bacterial survival are observed following modulation of autophagy [4–8].

In 2012, two groups reported that mice lacking *Atg5* in myeloid derived cells (*Atg5^{fl/fl}-LysM-Cre*) succumb to *M. tuberculosis* infection within the first 40 days of infection[6,12], a phenotype that is similar to mice completely lacking IFN- γ signaling[13] and among the most severe phenotypes described for a knockout (KO) mouse infected with *M. tuberculosis*. Control of *M. tuberculosis* is extremely impaired in *Atg5^{fl/fl}-LysM-Cre* mice, resulting in 1–

2 logs higher bacterial burden and more severe inflammation in lungs than control mice. Given the focus of previous cell culture studies, a prevailing theory was that this effect was due to loss of xenophagy-dependent control of bacterial replication. However, a recent study found that while ATG5 is required in **LysM⁺ cells** for mice to survive acute *M. tuberculosis* infection, other essential autophagy factors are not. In fact, mice lacking ATG3, ATG7, ATG12, ATG14L, or ATG16L1 in LysM⁺ cells or lacking ULK1, ULK2, p62, or ATG4B in all cells effectively control *M. tuberculosis* burden and survive for more than 80 days post-infection with no overt signs of morbidity[14]. This demonstrates that neither xenophagy nor other autophagy-mediated processes are required in myeloid cells to control *M. tuberculosis* infection in mice. Instead, ATG5 plays a specific role in limiting neutrophil-mediated inflammation through a mechanism that is independent of all pathways involving autophagy or LC3-lipidation[14]. These data demonstrate a lack of correlation between *in vitro* and *in vivo* findings of the roles of autophagy genes in controlling *M. tuberculosis* replication and highlight the importance of studying the roles for xenophagy *in vivo*. These *in vivo* genetic analyses also argue for a shift in focus onto macroautophagy-independent roles of ATG5 in controlling resistance to *Mtb* infection *in vivo*.

A separate study investigated the role of PARKIN, a ubiquitin ligase important for mitophagy (autophagic targeting of mitochondria), in controlling *M. tuberculosis* infection[15]. Similar to other cell culture experiments involving autophagy-associated proteins, the PARKIN protein colocalized to approximately 12% of *M. tuberculosis* and loss of the gene encoding PARKIN, *Park2*, resulted in a 2 fold increase in *M. tuberculosis* survival in murine macrophages. Moreover, *Park2*^{-/-} mice infected with *M. tuberculosis* had a 10 fold increase in bacterial titers by 21 days post-infection and succumbed to infection by 85 days post-infection. Given that mice deficient in autophagy survived past this time point[14], PARKIN likely plays an autophagy-independent role in controlling *M. tuberculosis* infection. Furthermore, as the susceptibility of *Park2*^{-/-} mice was not as severe as that of *Atg5*^{fl/fl}-*LysM-Cre* mice, PARKIN is not required for the autophagy-independent role of ATG5, highlighting that these factors have diverse functions beyond canonical autophagy. Notably, genetic polymorphisms in the *Park2* regulatory region are associated with increased susceptibility to *Mycobacterium leprae* and *Salmonella enterica* serovar Typhi in humans[16,17] and *Park2*^{-/-} mice are more susceptible to *Listeria monocytogenes* infection[15], indicating that PARKIN may function in general antibacterial responses.

Recent mouse studies challenging the paradigm that xenophagy is important in controlling *M. tuberculosis* infection, along with the modest effects of xenophagy in cell cultures, suggest that *M. tuberculosis* has evolved ways to evade host defense. In fact, a study characterizing autophagic flux over a time course of *M. tuberculosis* infection in cell cultures found that although *M. tuberculosis* was targeted by xenophagy, virulent strains of *M. tuberculosis* inhibited autophagosome maturation, thereby avoiding fusion with the lysosome and subsequent degradation[18] (Fig. 1). Several *M. tuberculosis* virulence factors that alter host signaling and vesicle trafficking also decrease autophagy, and this may explain the mechanism by which *M. tuberculosis* manipulates autophagic flux. Specifically, *M. tuberculosis* prevents phagolysosome fusion in cultured murine macrophages by interfering with fusion in cultured murine macrophages by interfering with phosphatidylinositol 3-

phosphate (PI3P) levels PIP₃ levels through the activity of **mannose-capped lipoaribomannan** (ManLAM)[19]. ManLAM also inhibits of Ca²⁺ influx, which can alter **mammalian target of rapamycin (mTOR)** signaling and ULK1-activation[19]. Consistent with this, ManLAM-coated beads have been shown to decrease LC3-targeting in cultured murine macrophages[20], but the role this plays during *M. tuberculosis* infection remains to be elucidated. *M. tuberculosis* also recruits the host factor Coronin1a to phagosomes within cultured murine macrophages, which blocks autophagosome formation through an unknown mechanism[21]. *M. tuberculosis* expresses Eis, an N-acetyltransferase that inactivates JUN N-terminal kinase (JNK), leading to a decrease in reactive oxygen species (ROS) that normally activates autophagy in cultured murine macrophages[22,23]. However, the *eis* mutant does not present a survival defect in cell cultures or mice, suggesting that this is not a critical mechanism of xenophagy evasion, and that *M. tuberculosis* likely employs multiple factors to inhibit xenophagy.

Xenophagy beyond *M. tuberculosis* as Revealed by Cell Culture

Approaches

M. tuberculosis is not an exception in its evolution of ways to evade xenophagy. Although many cell culture studies have suggested roles for xenophagy in controlling replication of certain pathogenic bacteria, for all intracellular bacterial pathogens that have been investigated, there is evidence of strategies that allow them to subvert or exploit (Figures 1 and 2) autophagy to promote pathogenesis. In fact, one may conclude that in order to become a successful intracellular pathogen, a bacterium must evolve mechanisms to avoid this ancient innate immune defense.

Shielding to Prevent Detection

One strategy used by multiple pathogens to avoid xenophagy is to shield themselves from being recognized by autophagy factors. *Francisella tularensis* invades macrophages and, after a transient interaction with the endocytic pathway, the bacteria escape into the cytosol, proliferate, and avoid being targeted by LC3 by at least two mechanisms. First, *F. tularensis* surface polysaccharidic O-antigen acts as a shield against ubiquitinylation and subsequent xenophagic recognition during infection of cultured murine macrophages[24]. Second, antioxidant superoxide dismutase enzymes of *F. tularensis* inhibit ROS-mediated induction of xenophagy and **LC3-associated phagocytosis** (LAP) during infection of cultured murine macrophages[25]. In addition, *F. tularensis* exploits an ATG5-independent autophagy pathway in fibroblasts and macrophages to obtain nutrients released from degraded cellular constituents to promote its replication[26]. Following replication within the cytosol of macrophages, *F. tularensis* re-enters the endocytic pathway and resides in *Francisella*-containing vacuoles (FCV) that colocalize with LC3[27]. FCV formation is critical for *F. tularensis* to complete its replication cycle and **3-methyladenine (3-MA)** treatment, which among other effects inhibits autophagy (Box 3), blocks FCV formation and *F. tularensis* replication. *Listeria monocytogenes* replicates rapidly in the cytosol of macrophages and epithelial cells and avoids being targeted by xenophagy by decorating its surface with host proteins through the activity of ActA and InlK[28,29]. *L. monocytogenes* also expresses phospholipases that prevent the formation of PI3P, thus stalling autophagosome formation in

macrophages and epithelial cells[30,31]. *Shigella flexneri* avoids recognition by autophagy factors while it replicates in the cytosol of epithelial cells and fibroblasts by expressing the effector IcsB, which masks a region of the *S. flexneri* surface protein IcsA that is recognized by ATG5. The effector VirA, a GTPase-activating protein (GAP) for Rab1, also prevents LC3 recruitment to *S. flexneri* [32–35]. These shielding strategies allow these bacteria to replicate undetected by the autophagy machinery, thus subverting this type of immune defense.

Blocking Induction of Autophagy, Autophagosome Formation, and LC3Targeting

Other bacteria block autophagosome formation or targeting by autophagy factors without known mechanisms of shielding the pathogen. After entry into epithelial, endothelial cells, macrophages, and **dendritic cells (DC)**, *Orientia tsutsugamushi* escapes from the endosomal pathway and replicates in the cytosol of eukaryotic host cells. *O. tsutsugamushi* infection induces autophagy, however, most of the bacteria do not colocalize with autophagosomes, even when further stimulated with **rapamycin**. Therefore, *O. tsutsugamushi* must block autophagic recognition[36,37]. Another pathogen, *Burkholderia pseudomallei* promotes its replication in the cytosol of murine macrophages by evading LC3 targeting through an undefined process involving the type III secretion system ATPase BpscN[38]. In addition, infection of macrophages and epithelial cells with *B. pseudomallei* or *Burkholderia cenocepacia* leads to downregulation of certain autophagy genes, resulting in decreased levels of autophagy[39,40]. Group B streptococcus (GBS) induces autophagy during infection of EC, but still resides primarily in single membrane-bound compartments, suggesting that GBS evades targeting to autophagosomes[41]. *Streptococcus suis* blocks xenophagy during infection of macrophages by secreting superoxide dismutase to scavenge cellular ROS[42]. *Salmonella enterica* serovar Typhimurium uses a type III secretion system (T3SS-1) to invade epithelial cells and form the *Salmonella*-containing vacuole (SCV). However, the T3SS-1 also damages the SCV, and this induces xenophagy and recruits the autophagic machinery to the SCV. *S. Typhimurium* exploits the autophagic machinery to repair damage to SCV membranes caused by T3SS-1, thereby allowing compartment maturation and subsequent expression of a second type III secretion system, T3SS-2, which together promote intracellular survival[43]. *S. Typhimurium* then downregulates LC3 colocalization in epithelial cells, fibroblasts, and macrophages by restoring mTOR activation, which in turn inhibits autophagy[44–47]. Clinically important *Salmonella*, including Typhi and Typhimurium, also express SpvB from the virulence plasmid pRST98, which can suppress autophagosome formation in macrophages by depolymerizing actin and enhancing bacterial survival[48]. The diverse mechanisms employed by these bacteria to avoid clearance by xenophagy facilitate their ability to replicate in host cells.

Blocking Autophagosome Maturation

Another strategy commonly used by bacteria to evade xenophagy is to block autophagosome maturation and fusion with lysosomes, thus allowing for continued bacterial replication and survival. The obligate intracellular pathogen *Chlamydia trachomatis* induces autophagy during infection, but then blocks the fusion of its vacuole to the lysosome in human epithelial cells, murine fibroblasts, and murine macrophages[49]. *C. trachomatis* also co-opts LC3 for an autophagy-independent function that promotes bacterial infection in human

epithelial cells[50]. Adherent and invasive *Escherichia coli* (AIEC) infection in enterocytes leads to up-regulation of microRNAs that reduce the levels of ATG5 and ATG16L1, and inhibit autophagy[51]. AIEC also blocks autophagy at the autolysosomal step during infection of neutrophil-like PLB-985 cells, allowing for intracellular survival of these bacteria[52]. *Legionella pneumophila* survives within a vacuole that is targeted by LC3 during infection in macrophages, but blocks acidification by cleaving membrane-conjugated LC3 via the effector protein RavZ[53]. In addition, *L. pneumophila* blocks autophagy during infection of macrophages by translocating the effector protein sphingosine-1 phosphate lyase (LpSpl), and this triggers a reduction of several host sphingolipids critical for autophagy[54]. *Serratia marcescens* replicates and persists inside large membrane-bound compartments in epithelial cells that colocalize with LC3 but are non-acidic, indicating that the bacteria prevent fusion of the vesicles with lysosomes to generate a suitable niche for proliferation inside the host cell[55]. Similarly, *Yersinia pestis* and *Yersinia pseudotuberculosis* replicate in LC3-positive vacuoles within macrophages, but prevent vacuole acidification to allow sustained bacterial replication[56,57]. Therefore, although xenophagy is triggered in infected host cells and bacteria are targeted by autophagy proteins, the ability of these pathogens to block autophagosome maturation allows them to survive and replicate within the cell.

Group A Streptococcus (GAS)- Employing Cell Type-Specific Evasion Mechanisms

Following internalization, GAS escapes into the cytosol, which results in stimulation of autophagy. This leads to co-localization of LC3 to a subset of intracellular bacteria, but the degree of targeting depends on the cell type infected. In HeLa cells, 80% of GAS were shown to colocalize with LC3, correlating with a fivefold decrease in bacterial titers, suggesting that xenophagy might contribute to the control of bacterial killing within epithelial cells. However, by 24 h post-infection, ~50% of the infected cells underwent **apoptosis**, suggesting that xenophagic killing of GAS is not sufficient to protect host cells[58]. During infection of macrophages, LC3 is only targeted to approximately 26% of GAS; however, proficient replication of GAS continues despite the recruitment of autophagy receptors[59]. In addition, because not all cytosolic bacteria are targeted by LC3, there is likely a subpopulation of GAS within macrophages that evades recognition by autophagy factors[59]. In keratinocytes, 80–90% of GAS was found to localize in LC3 vesicles, but translocation of *Streptococcus pyogenes* NAD⁺ glycohydrolase (SPN) into the cytosol prevented maturation of the GAS-containing autophagosome-like vacuoles[60]. Similarly, in endothelial cells, the low pH is not maintained in GAS-containing autophagosomes, and the bacteria replicate efficiently[61]. In addition, the globally disseminated GAS serotype M1T1 secretes SpeB, a protease that degrades ubiquitin adaptors within the host cell cytosol to avoid recognition by LC3 in epithelial cells[62]. Thus, GAS employs multiple mechanisms to evade xenophagy and promote its replication in diverse types of host cells.

Exploiting Autophagy Proteins to Promote Replication

Other bacterial pathogens exploit autophagy or autophagy-associated proteins for intracellular replication. During infection of human promyelocytic leukemia cells, *Anaplasma phagocytophilum* replicates in membrane-bound compartments devoid of lysosomal markers, indicating that the pathogen inhibits fusion to lysosomes[63]. A.

phagocytophilum secretes an effector, Ats-1, to promote autophagosome nucleation and fusion to its vesicle, stimulating its own growth by using the nutrients contained in the autophagosomes[64]. The obligate intracellular bacterium *Coxiella burnetii* survives and replicates within large, acidified, vacuoles that colocalize with LC3 during infection of epithelial cells and macrophages[65–67]. *C. burnetii* exploits these vacuoles as a favorable replication niche while also recruiting autophagosomes to deliver nutrients and provide a membrane for the expanding vacuole[68]. *C. burnetii* also secretes CvpB, which manipulates PI3P metabolism, promoting replication[69]. *Porphyromonas gingivalis* avoids targeting to lysosomes in human DCs and instead traffics to vesicles resembling autophagosomes where it replicates. Indeed, selective engagement of DC-SIGN by *P. gingivalis* during uptake promotes evasion of lysosome fusion[70]. Induction of autophagy promotes the replication of *A. phagocytophilum*, *C. burnetii*, and *P. gingivalis*, while inhibition of autophagy blocks the replication of these pathogens in cell culture systems[63,67,71–73].

Staphylococcus aureus exploits autophagy factors to form a niche for replication that resembles an autophagosome but is blocked for maturation and acidification[74,75]. *S. aureus* depends on this vacuole for replication and, as such, is unable to survive and replicate in *Atg5*^{-/-} mouse embryonic fibroblasts (MEFs)[74]. Expression of IsaB and agr by *S. aureus* is important for exploiting the autophagic machinery through undefined mechanisms[74,75]. The intracellular bacterium *Brucella abortus* also co-opts autophagy factors to support its replication, but is unique in that this process does not involve the LC3-conjugation machinery. After initial entry into fibroblasts or macrophages, *B. abortus* forms a *Brucella*-containing vacuole (BCV) that traffics from the endocytic compartment to the endoplasmic reticulum (ER), where the bacterium proliferates[76]. Following *B. abortus* replication, the BCV is converted into a compartment with autophagic features in a process that is dependent upon the autophagy-initiation proteins, but independent of autophagy-elongation or LC3-conjugation machinery. These autophagosome-like vesicles are required to complete the *B. abortus* lifecycle[77]. *Brucella melitensis* also requires autophagic flux for replication in macrophages[78]. Autophagy has also been proposed to be beneficial for the survival of uropathogenic *E. coli* (UPEC) in macrophages, based on the observation that ATG16L1-deficient macrophages display increased uptake and enhanced killing of UPEC[79].

A handful of studies have also explored the roles of xenophagy in controlling bacterial pathogens that are primarily extracellular but may have intracellular stages during infection, including *Helicobacter pylori*[80–82], *Streptococcus pneumoniae*[83], *Pseudomonas aeruginosa*[84], and *Citrobacter rodentium*[85]. Even for these pathogens that may be less adapted to an intracellular environment, there is evidence for xenophagy evasion strategies[80–85]. However, more studies will be necessary to understand the relevance of xenophagy during infection of these mostly extracellular pathogens.

The reoccurring theme is that xenophagy is insufficient to fully control bacterial replication of successful intracellular bacterial pathogens and most, if not all, intracellular bacterial pathogens employ multiple strategies to avoid clearance by xenophagy (Figures 1 and 2). These studies also highlight that colocalization of autophagy factors does not equate to the role of xenophagy in controlling bacterial replication, and may actually represent evasion

strategies instead. Thus, detailed mechanistic studies will be necessary to understand the relationship between autophagy-associated proteins and a particular bacterial pathogen. In addition, roles for autophagy proteins can vary based on cell type and species (i.e., human versus mouse), and it is important to consider these caveats when interpreting the significance of findings.

Autophagy Associated Proteins during Bacterial Infection in Animal Models

Although xenophagy has been proven to be limited in its impact on bacterial pathogenesis, autophagy-associated proteins can still influence bacterial infections in other autophagy-dependent and -independent ways[86–88] (Box 4). Due to the diverse roles for autophagy-associated proteins, detailed *in vivo* studies are necessary to fully understand how autophagy-related genes and proteins impact on bacterial infection. Work with *M. tuberculosis* highlights the importance of *in vivo* studies to delineate how processes observed in cell culture models relate to mechanisms of pathogen control *in vivo*. Unfortunately, *in vivo* approaches may be limited by the animal models available for specific pathogens as well as by the degree to which a particular animal model represents human infection. Nonetheless, the generation of genetically engineered mice with mutations in the autophagy pathway has facilitated the discovery and study of myriad roles for autophagy proteins during bacterial pathogenesis. Mice with germline deletions in redundant autophagy factors have been generated, but these are limited by the ability of other factors to compensate for the absence of the targeted gene. Germline deletions of essential autophagy factors are embryonically or perinatally lethal and are often studied using conditional deletion Cre/lox recombination systems. As with all methods for studying autophagy, the use of conditional KOs comes with its own complications. For instance, autophagy-associated proteins also play roles in cell differentiation, proliferation, and survival[89], which may complicate analyses. To reconcile some of these issues and discern the function of specific pathways, different complementary mouse strains and approaches should be used in combination.

In addition to *M. tuberculosis*, mouse studies with other pathogens have revealed roles for autophagy-associated proteins in controlling bacterial infections by showing that loss of autophagy-associated proteins is detrimental to the host. One study reported that mice lacking ATG5 in myeloid cells presented a modest increase in susceptibility to *L. monocytogenes*, as measured by bacterial levels in spleen and liver 3 days after infection[90]. Mice in which *Atg5* or *Atg161l* were conditionally deleted from the intestinal epithelium, presented abnormalities in granule secretion from Paneth cells and were more susceptible to *Salmonella enterica* serovar Typhimurium infection[91–93]. Mice with conditionally-deleted *Atg7* in the intestinal epithelium also exhibited increased susceptibility to *C. rodentium* infection[94], possibly due to a similar mechanism as in *Salmonella* infection. Another study showed that mice, globally hypomorphic for ATG16L1 expression (ATG16L1^{HM}), displayed increased lethality during methicillin-resistant *Staphylococcus aureus* (MRSA) infection. However, this effect did not seem to be due to the role of ATG16L1 in reducing bacterial burden, but rather to protection of host cells from α -toxin-induced damage[95].

Consistent with these results, induction of autophagy has been shown to be beneficial to the host during some bacterial infections. For instance, deletion of RNF5, which decreases the stability of ATG4B to limit LC3 processing, enhances autophagy and improves survival of mice during GAS infection[96]. Induction of autophagy in myeloid cells during *S. Typhimurium* infection also appears to be beneficial to the host. **Focal adhesion kinase (FAK)** is normally recruited to the surface of the *Salmonella*-containing vacuole (SCV) where it amplifies signaling through the Akt-mTOR axis to inhibit autophagy; when mice do not express FAK in myeloid cells, autophagy is enhanced and *S. Typhimurium* is cleared faster from infected tissues [45].

In other cases, mouse studies have revealed roles for autophagy-associated proteins in promoting bacterial infections. ATG16L1^{HM} mice infected with UPEC showed more rapid bacterial clearance and epithelial recovery[97], indicating that ATG16L1 usually promotes UPEC infection. Loss of ATG16L1 leads to higher levels of IL-1 β production from macrophages in response to UPEC, and this could contribute to the protection of ATG16L1^{HM} mice[79]. These same ATG16L1^{HM} mice are also resistant to intestinal disease induced by *C. rodentium*, at least in part as a result of enhanced immune responses[98]. *Beclin1* heterozygous-deficient mice have been reported to be resistant to *A. phagocytophilum* infection [64], supporting the requirement for autophagy in *A. phagocytophilum* replication, as shown in cell cultures.

These animal studies have revealed the impact of autophagy associated proteins beyond xenophagy and highlight the complicated roles of autophagy proteins in regulating bacterial pathogenesis. The animal data further demonstrate how a single autophagy protein can have different effects on bacterial infection depending on the pathogen and the cell in which it functions. For many cases, whether the susceptibility of these mice is due to loss of autophagy, loss of an autophagy-independent role for the particular ATG protein, or effects on a combination of these processes remains unknown since conclusions about the role of autophagy in the immune response to a pathogen cannot be made based on data from a single autophagy protein.

Targeting Autophagy as a Host-Directed Therapy to Treat Bacterial Infections

There has been growing interest in modulating autophagy as a therapeutic intervention for a number of ailments, including bacterial infections (Box 5)[99]. Given the ability of intracellular bacterial pathogens to evade and exploit xenophagy and the impact of autophagy-associated proteins on diverse immune responses, pursuing autophagy as a therapeutic target requires the consideration of a number of factors. For example, some infections will be exacerbated by induction of autophagy (including those discussed in this review (Figure 2)), as well as by co-infections with some viruses or parasites[100,101]. In addition, manipulating the activity of autophagy proteins may affect both autophagy-dependent and -independent processes that have myriad effects on disease outcomes. Furthermore, any attempts to stimulate xenophagy may be futile if the pathogen efficiently blocks or avoids a downstream step in the pathway (Figures 1 and 2). Importantly, if a

pathogen blocks a downstream step of autophagy— such as autophagolysosome formation— then induction of autophagy may not be effective and could result in a buildup of intermediates (undegraded autophagosomes) that may be detrimental to the cell. Therefore, a mechanistic understanding of such evasion mechanisms is critical to the design of successful therapeutic strategies.

The first strategy being pursued to target autophagy in the treatment of bacterial infections is the repurposing of pharmacologic agents that are approved by the FDA and present pleiotropic effects, including the induction of autophagy[102]. Notably, it is unknown whether any of these agents exert their clinical benefits through autophagy. The most widely studied example is rapamycin, an agent used to prevent transplant rejection. The use of rapamycin in cell cultures has been shown to decrease the survival of various intracellular bacterial pathogens including GBS[41], *P. aeruginosa*[103], *Salmonella*[44], *B. pseudomallei*[104], and *M. tuberculosis*[105]. However, the differences in bacterial burden tend to be less than 2.5 fold[41,44,103,104]. In addition, a recent study found that in macrophages coinfecting with HIV and *M. tuberculosis*, stimulation of autophagy with rapamycin actually led to increased *M. tuberculosis* survival[106]. Thus, it will be critical to determine the impact of comorbidities such as HIV when perusing options for clinical intervention. Rapamycin has been tested in mouse models of intranasal *P. aeruginosa* infection, resulting in a twofold reduction in bacterial burdens in the lungs compared to control mice, but there were no differences in survival between rapamycin and control-treated mice[107]. Therefore, it is unclear if this would lead to better outcomes following infection. In addition, rapamycin diminished the bactericidal activity of neutrophils and increased the bacterial burden in *P. aeruginosa*-infected corneas in a mouse model of *P. aeruginosa* keratitis[108]. This may be related to the immunosuppressive effects of rapamycin, which could also cause reactivation and progression of some infections, outweighing any benefit of activating autophagy.

AR-12, identified as a suppressor of tumor cell viability, is another agent that modulates several pathways within mammalian cells including autophagy, and has been tested for effects on bacterial infections in mouse models. Administration of AR-12 to mice during *S. Typhimurium* infection resulted in lower bacterial burdens in the liver and spleen in some, but not all, mice, and a significant but incremental improvement in survival[109]. More impressive results were found when AR-12 was combined with aminoglycosides, and AR-12 improved the efficacy of this class of antibiotics in terms of prolonging survival of *S. Typhimurium*-infected mice[110]. Other FDA approved pharmacologic agents that induce autophagy, in addition to their other effects, have been shown to improve control of *M. tuberculosis* in mouse models, and include Gefitinib[111], an epidermal growth factor receptor (EGFR) inhibitor used to treat cancer, and statins[112]. However, the effects of these treatments on bacterial burden have been very modest in cultured macrophages and in mice, and it is unclear whether the effects on bacterial burden are due to autophagy. Furthermore, because *M. tuberculosis* blocks Rab7-mediated fusion with lysosomes[18], a process that is shared between autophagy, phagocytosis, and LAP, it is unknown whether stimulating upstream steps of these pathways (i.e., inducing autophagosome formation) will

be able to increase killing of *M. tuberculosis* to a level that is clinically significant (without including a direct bacterial inhibitor).

The likely complications resulting from the pleiotropic effects of these pharmacologic agents have raised interest in more specific ways to modulate autophagy with limited side effects. One such approach is lentivirus- or adenovirus-based gene therapy for tissue-specific expression of autophagy genes. Positive results for gene therapy targeting autophagy have been obtained in animal models for a number of non-communicable diseases[99], but it is unknown whether these approaches will prove to be effective for the treatment of infectious diseases. Another approach being pursued is the use of cell-penetrating autophagy-inducing peptides. Tat-beclin 1 peptide is the best-developed agent in this class, and is believed to stimulate autophagy by interfering with a negative regulator of autophagy (GAPR-1)[113]. Tat-beclin 1 peptide treatment reduces the replication of an *L. monocytogenes actA* strain during infection of macrophages, although the *actA* mutant is unable to shield itself from autophagic recognition[113]. Development of therapeutics that specifically interfere with the molecules used by bacteria to restrict autophagy may be an effective approach, and could avoid problems associated with targeting autophagy at a systemic level. Furthermore, this approach may be effective in combination with autophagy-inducing therapeutics to provide a more potent, shorter course of treatment [102].

Concluding Remarks

The rise in incidence of antibiotic-resistant bacterial infections and the dearth of antibiotics in the pipeline has raised interest in applying host-directed therapies to the treatment of bacterial infections. In particular, there are efforts currently underway to develop modulators of autophagy as a therapeutic strategy. Much of the focus in these areas constitutes identifying agents that induce xenophagy to target intracellular bacterial pathogens for degradation. Although there has recently been an upsurge in these investigations, many open questions remain that will need to be addressed to be successful in this approach (See Outstanding Questions). A major obstacle is that most intracellular bacterial pathogens have evolved to avoid or exploit targeting by xenophagy. Thus far, xenophagy has not proved effective in controlling bacterial pathogens, at least in cell culture experiments and inbred mouse models. It remains to be seen whether stimulation of xenophagy will overcome these defenses to a clinically relevant degree, or whether simultaneously targeting bacterial defense mechanisms will be necessary. It is also necessary to establish whether cell culture experiments represent an accurate model for testing the efficacy of targeting autophagy, particularly since phenotypes observed in cell culture have thus far been modest and do not always correlate well with results from *in vivo* studies. Alternative therapeutic strategies could focus on roles for autophagy proteins outside of xenophagy that may have a significant impact on the outcomes of infection and investigating the synergy between autophagy-modulators and antibiotics may be fruitful. These areas, similar to targeting xenophagy, still require a significant amount of research to fully understand their clinical potential, but may hold promise for future therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

3-Methyladenine(3-MA)

prevents autophagosome formation by interfering with class III phosphatidylinositol3-kinases (PI3Ks)

Apoptosis

a highly regulated and controlled process of programmed cell death. Generally results in very little inflammation

AR-12

an antitumor celecoxib derivative that is used in clinical trials as an anticancer agent. Has many effects, including stimulation of autophagy

Autophagic cell death

a form of cell death dependent on autophagy genes, and is associated with morphological features of increased autophagic vacuoles. Distinct from autosis and apoptosis

Autophagic flux

a measure of autophagic degradation activity, that is, the rate of substrate sequestration and degradation by autophagy. Distinct from the number of autophagosomes in the cell, which can be caused by increased autophagosome formation or decreased autophagosome degradation

Autosis

a mode of cell death dependent on autophagy genes. Mediated by the Na⁺, K⁺-ATPase pump and is distinct from autophagic cell death, apoptosis, and necrosis

B cell

adaptive immune cells which respond to infection in an antigen-specific manner, and are important in the production of antibodies

Bafilomycin A1

prevents maturation of autophagic vacuoles by inhibiting vacuolar H⁺ ATPase (V-ATPase), and decreases fusion between autophagosomes and lysosomes

Chloroquine

prevents endosomal acidification and increases lysosomal pH, leading to inhibition of both fusion of autophagosome with lysosome and lysosomal protein degradation. Used to inhibit autophagosomal degradation

Damage-associated molecular patterns (DAMPs)

host biomolecules that are released from cells upon damage that can initiate and perpetuate a noninfectious inflammatory response

Dendritic cell (DC)

innate immune cells which are important for antigen presentation

Efferocytosis

the process by which apoptotic cells are removed by phagocytosis (most often by macrophages)

Entosis

a process by which a living cell is internalized by another living cell, often observed in epithelial cells after detachment from the extracellular matrix

Focal adhesion kinase (FAK)

a non-receptor tyrosine kinase that is recruited to the *Salmonella*-containing vacuole and promotes intracellular survival in macrophages. Leads to inhibition of autophagy through effects on signaling through the Akt–mTOR axis

LC3-associated phagocytosis (LAP)

a type of phagocytosis which induces components of the autophagy machinery (including LC3) to associate with the phagosome, promoting its fusion to lysosomes

LysM+ cells

cells which express lysozyme M, that is, myeloid-derived cells such as macrophages, monocytes, neutrophils, and myeloid-derived dendritic cells. Typically referenced in the context of LysM–Cre, which results in conditional deletion in LysM+ cells

Macroautophagy

a process in which cellular contents are sequestered by double-membraned structures called autophagosomes and degraded by lysosomes. Specific subtypes of macroautophagy are named based on the substrate targeted for degradation such as ribophagy (ribosomes), mitophagy (mitochondria), and pathogen (xenophagy)

Macrophage

innate immune cells are important for phagocytosis of pathogens and cell debris

Mammalian target of rapamycin (mTOR)

a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy, transcription

Mannose-capped lipoaribomannan (ManLAM)

a glycolipid primarily produced by pathogenic mycobacterial species, and represents an important immunomodulatory virulence factor for *Mycobacterium tuberculosis*

Mitophagy

a type of selective macroautophagy that specifically targets mitochondria to lysosomes

Necrosis

a form of cell injury which results in the premature death of cells by autolysis. Causes inflammation and is typically detrimental to the host

Pathogen-associated molecular patterns (PAMPs)

molecules associated with groups of pathogens that are recognized by the innate immune system and can modulate inflammation

Phosphatidylinositol3-kinases (PI3Ks)

a family of enzymes involved in initiation of autophagy, as well as diverse cellular functions including cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking

Rapamycin

inhibitor of the Ser/Thr protein kinase mTOR, which regulates cell growth and metabolism in response to environmental cues. Often used to induce autophagy, as inhibition of mTOR mimics cellular starvation by blocking signals required for cell growth and proliferation

Retinoic acid-inducible gene 1 (RIG-I)

a pattern recognition receptor that recognizes double-stranded RNA

Stimulator of interferon genes (STING)

functions to increase Type I interferon production through its action as a direct cytosolic DNA sensor and an adaptor protein in Type I interferon

T cells

adaptive immune cells which respond to infection in an antigen-specific manner. Can be cytotoxic (cause lysis of target cells) or support immune functions through the production of cytokines

Toll-like receptor (TLR)

a class of proteins that play a key role in the innate immune system by recognizing structurally conserved molecules derived from microbes

Wortmannin

an inhibitor of autophagy that functions through inhibition of phosphatidylinositol3-kinase

Xenophagy

a type of selective macroautophagy that specifically targets intracellular pathogens to lysosomes

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Box 1**Steps of Canonical Macroautophagy**

Initiation of autophagy involves the sequestration of a small portion of the cytoplasm in a membranous sac referred to as a phagophore (Figure I) [2,114]. Phagophore formation is mediated by translocation of the ULK1 complex (ULK1/ULK2, ATG13, FIP200, ATG101) from the cytoplasm to the endoplasmic reticulum (ER) and, as such, the ER is thought to be the source of the autophagosome membrane. The ULK1 complex functions in the recruitment of the autophagosome specific [phosphatidylinositol3-kinase(PI3K)] complex consisting of ATG14L, BECLIN1, VPS15, and VPS34[2,114]. The PI3K complex produces phosphatidylinositol 3-phosphate (PI3P), which is not normally found in the ER and is essential for canonical autophagy[2,87,115].

Elongation of the autophagosomal double membrane depends on two ubiquitin-like conjugation systems. In the first system, ATG12 (ubiquitin-like protein) is synthesized with an exposed C-terminal glycine, activated by ATG7 (E1-like)[2,87,115], and transferred to ATG10 (E2-like), which facilitates its final ligation to ATG5. ATG5 non-covalently interacts with ATG16L1, resulting in an ATG5-ATG12—ATG16L1 complex that is present on the phagophore and elongating membrane, but dissociates upon completion of the autophagosome[2,116,117]. The second ubiquitin like component is LC3 (microtubule-associated protein 1 light chain 3)[2,87,115]. Upon synthesis, LC3 (pro-LC3) is rapidly processed by the cysteine-protease ATG4 to generate a cytoplasmic form called LC3-I. LC3-I is conjugated to phosphatidylethanolamine, generating the membrane bound form called LC3-II through the actions of ATG7 (E1-like) and ATG3 (E2-like). ATG5-ATG12 facilitates LC3 lipidation through its interactions with ATG3, while ATG16L1 specifies the localization of LC3 conjugation to the autophagosome membrane.

Upon completion of the autophagosome membrane, removal of external LC3 by ATG4 facilitates autophagosome maturation as well as recycling of LC3, while LC3 on the interior of the autophagosome is inaccessible to ATG4 and is degraded. Autophagosome maturation occurs through SNARE-mediated fusion of autophagosomes with lysosomes, degrading the cargo[2].

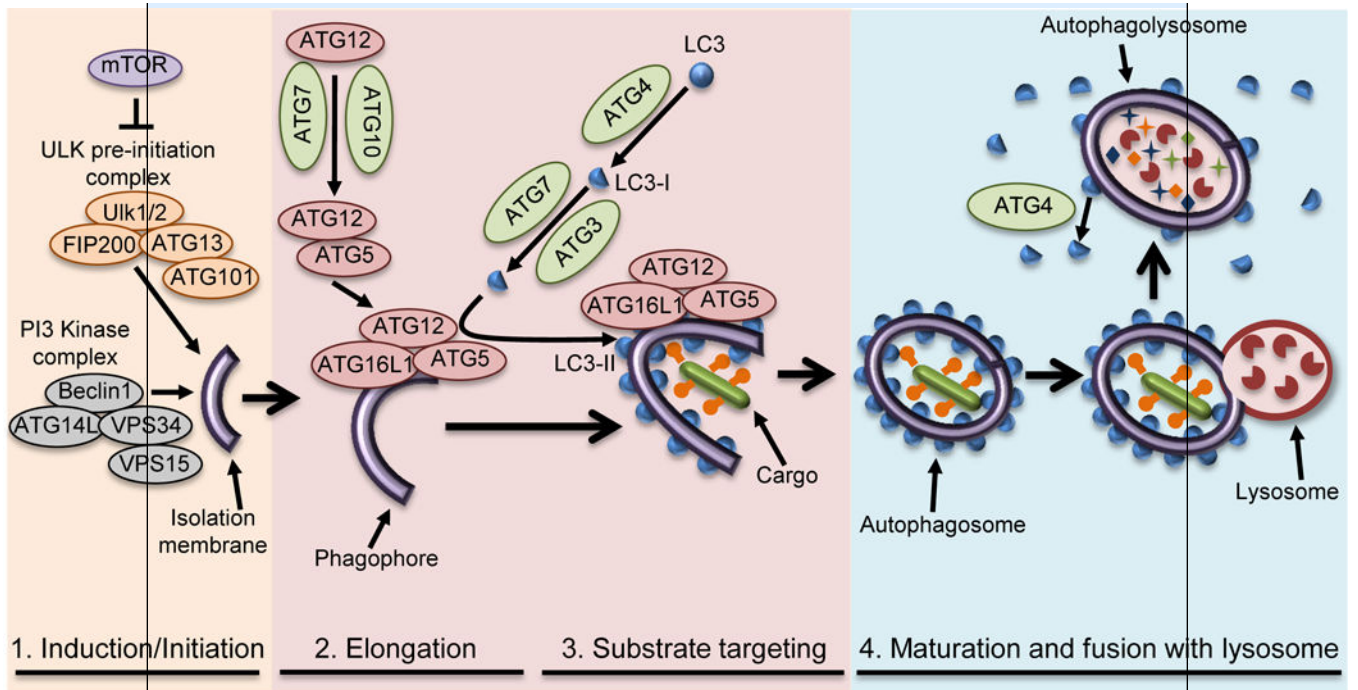


Figure I. Schematic of the Stages of Canonical Macroautophagy

(1) Induction/initiation, (2) elongation, (3) substrate targeting, and (4) maturation/lysosomal fusion and targeting of bacterial pathogens (substrate targeting) as detailed in Box 2.

Box 2**Targeting Bacteria by Xenophagy**

In general, once a bacterial pathogen either escapes into the cytosol or is exposed to the cytosol through damage to the vesicle in which it resides, xenophagy is induced by cytoplasmic recognition of **pathogen-associated molecular patterns (PAMPs)** or **damage-associated molecular patterns (DAMPs)** [6,15,118–120]. Xenophagy occurs through the same basic steps as canonical macroautophagy (Box 1, Figure I).

Once phagophore formation is induced, bacteria are targeted by the autophagosome via ubiquitination of the bacteria or colocalization of ubiquitin to the bacteria via ubiquitin ligases such as PARKIN[15]. Ubiquitin is then recognized and bound by autophagy adaptors such as p62 (also called Sequestosome-1, SQSTM1), neighbor of BRCA1 gene 1 (NBR1), nuclear dot protein 52 kDa (NDP52) and optineurin (OPTN). These same adaptors interact with LC3 to recruit the bacteria to autophagosomes[87].

Autophagy adaptors are regulated by a variety of mechanisms including expression, spatial organization, cellular localization, and post-translational modifications[121]. In particular, regulation of autophagy adaptors by phosphorylation is emerging as an important theme for control of selective autophagy. For example, p62 can be phosphorylated at S403 by casein kinase 2, and this leads to increased affinity of p62 for polyubiquitin chains and plays a role in regulation of mitophagy[122].

TANK-binding kinase 1 (TBK1) can also phosphorylate p62 on S403, and has been shown to play a role in control of *Mycobacterium bovis* BCG infection[123]. During *S. Typhimurium* infection, NDP52 recruits TBK1, which constitutively associates with a second autophagy adaptor OPTN. TBK1-mediated phosphorylation of OPTN on S177 regulates its LC3-binding affinity and subsequent targeting of *Salmonella* by xenophagy[124]. Following bacterial targeting to the autophagosome, the autophagosome matures and fuses with lysosomes, killing invading bacteria.

Box 3**Approaches for Studying Xenophagy in Mammalian Cell Cultures:
Necessary Considerations**

Most studies into the role of xenophagy in controlling bacterial infections have used cell culture models of infection. Broadly, there are two approaches used to investigate whether bacterial pathogens are targeted by xenophagy[125,126]: 1) Observation of hallmarks of autophagy including the colocalization of autophagy factors to the bacteria and 2) Experimental manipulation of autophagy and analysis of the impact on bacterial replication.

To investigate the presence of hallmarks of autophagy, electron microscopy can be used to visualize double membrane autophagosomes around targeted bacteria. Alternatively, fluorescent microscopy can be used to visualize colocalization of autophagy-associated proteins with the bacterium. LC3 colocalization is the most common assay performed because autophagosomes will remain LC3⁺ until they are degraded in the lysosome. However, a common misconception is that increased numbers of LC3-associated autophagosomes represent an increase in autophagy[126]. Autophagy is a dynamic process and increases in LC3 puncta can be caused by either increased LC3 lipidation (autophagosome biogenesis) or decreased LC3 turnover (autophagosome degradation). Furthermore, because many LC3 molecules are degraded upon lysosomal fusion, extended stimulation of autophagy will result in a decrease in cellular LC3, which could be misinterpreted as decreased autophagy. LC3 is also central to an ever-growing list of processes that are distinct from canonical autophagy, but still result in vacuole-associated LC3-II. Therefore, measurement of LC3 puncta alone is no longer sufficient to demonstrate a role for autophagy[126].

For the second approach, autophagic flux can be manipulated by targeting autophagosome formation or degradation. Chemically, autophagy is most often induced by inhibiting mTOR through nutrient starvation or rapamycin treatment. mTOR is the main negative regulator of autophagy but also regulates other processes including cell growth, proliferation, motility, survival, protein synthesis, and transcription. Autophagosome formation can be inhibited by PI3K inhibitors such as **wortmannin** or **3-methyladenine (3-MA)**, which also inhibit kinases involved in diverse cell signaling and membrane-trafficking processes. Drugs such as **bafilomycin A1** or **chloroquine** block autophagosome degradation by inhibiting lysosomal acidification, but also affect processes such as endocytosis and mitosis. Because of such diverse effects, these drugs should be used as complementary approaches to identify if an observed effect is actually the result of interference with autophagy[126]. Autophagy can also be manipulated through genetic approaches by knockdown, knockout, or ectopic expression of autophagy genes. While targeting individual autophagy proteins may have fewer pleiotropic effects than the chemical approaches listed, it is still important to consider the effects of each gene in autophagy-independent pathways as well as dominant negative effects or off-target effects, all of which have been documented[126].

Importantly, when used individually, none of these methods are able to capture the complete dynamics of xenophagy. Therefore, it is critical to employ multiple approaches when measuring autophagic flux and to consider the effects of cell type, culture conditions, and timing of analysis, which can all impact the results.

Box 4**Additional Roles of Autophagy-Associated Proteins in Host Immunity to Bacteria**

In addition to xenophagy, autophagy-associated proteins can influence bacterial infections in many other autophagy-dependent and -independent ways[86–88]. First, autophagic flux cross-regulates phagocytosis. One mechanism for this occurs when autophagy is downregulated: the adaptor protein p62 accumulates, resulting in increased Nrf2-mediated expression of scavenger receptors and increased phagocytosis of certain bacteria[127]. Many autophagy proteins also participate in LC3-associated phagocytosis (LAP), which also traffics pathogens to the lysosome for degradation. In contrast to canonical macroautophagy, LAP uses RUBICON and UV irradiation resistance-associated gene A (UVRAG) instead of ATG14L in the PI3K complex and does not depend on ULK1[88,128]. During LAP, LC3 is recruited to the phagosome, resulting in LC3⁺ single-membrane vesicles instead of the double membrane characteristic of autophagosomes. Because LAP was discovered relatively recently, previous studies describing pathogen-associated LC3⁺ vesicles now require additional investigation to determine if these are generated through xenophagy or LAP.

Once the pathogen invades the cell, autophagy can increase **Toll-like receptor (TLR)** signaling by delivering nucleic acids to TLRs, activating the production of type I IFN[129]. By contrast, ATG5-ATG12 can negatively regulate **RIG-I**, and ATG9A can negatively regulate DNA receptor **stimulator of interferon genes (STING)**, leading to a decrease in type I IFN responses[87]. Type I IFN signaling can be detrimental or protective to the host, depending on the bacterial pathogen[130]. Autophagy also affects the production and secretion of IL-1 β , a potent pro-inflammatory molecule, typically important for control of bacterial infections, but can also lead to significant immunopathology, and ultimately be detrimental to the host[131–135].

Autophagy can also directly increase host cell survival by acting as a starvation response, as well as decrease cell survival through **autophagic cell death** or **autosis**[136]. The mechanism by which an infected cell dies can have a major impact on immune responses since DAMPs released from dying cells will trigger inflammation[137]. Notably, ATG5 also impacts on cell death in neutrophils independently of autophagy when it is cleaved by calpain and translocates to the mitochondria where it exerts pro-apoptotic effects[138]. Autophagy is also important for the removal of apoptotic cells by phagocytic cells (**efferocytosis**) or endothelial cells (**entosis**)[139]. During *M. tuberculosis* infection, efferocytosis facilitates antigen presentation by DCs, and macrophages are better able to kill *M. tuberculosis* when it is acquired through efferocytosis as opposed to phagocytosis of free bacteria[140,141]. Autophagy-related genes are also associated with the maintenance and function of numerous immune cell populations including DCs, **B cells**, **T cells**, and fetal hematopoietic stem cells[87,89,115]. Thus, autophagy-associated proteins have diverse effects on inflammation and immunity that, depending on the pathogen and timing of the response, can exert both positive and negative effects for the host.

Box 5**Clinician's Corner**

- Autophagy is an intracellular process that plays diverse roles in the host, including affecting cell adaptation, starvation, as well as eliminating intracellular organisms (a process called xenophagy).
- There is great interest in targeting autophagy for the treatment of non-communicable diseases as well as infectious diseases.
- Several currently FDA-approved agents induce autophagy and may increase xenophagic targeting of bacteria. However, these drugs also impact other pathways in the cell, leading to broad-spectrum effects that may negate the benefits. Furthermore, it is currently unknown whether the clinical benefits of any of these are due to autophagy or alternative effects of these drugs.
- Ongoing efforts focus on developing tissue-specific modulators of autophagy, including gene therapy strategies.
- Most, if not all, intracellular bacterial pathogens encode specific inhibitors of autophagy and, therefore a better understanding of bacterial evasion mechanisms is warranted for the development of potential specific therapeutic approaches targeting evasion.
- Autophagy has been shown to play a role in various common bacterial infection comorbidities including HIV-1 infection, cancer, and diabetes. It will be critical to determine the impact of manipulating autophagy on these comorbidities when perusing options for clinical intervention.

Trends

Xenophagy is a type of selective macroautophagy that targets intracellular pathogens for lysosomal degradation.

Intracellular bacterial pathogens have evolved mechanisms of evading xenophagy, minimizing its effectiveness as an innate immune response to these infections.

Mycobacterium tuberculosis is an example of a pathogen that is not effectively targeted by xenophagy in myeloid cells. In fact, no autophagy-dependent pathway is required in myeloid cells to control *M. tuberculosis* infection in mouse models. However, ATG5 functions independently of autophagy in myeloid cells to limit neutrophil-mediated inflammation, suggesting that autophagy-related proteins can function in multiple ways to control infection.

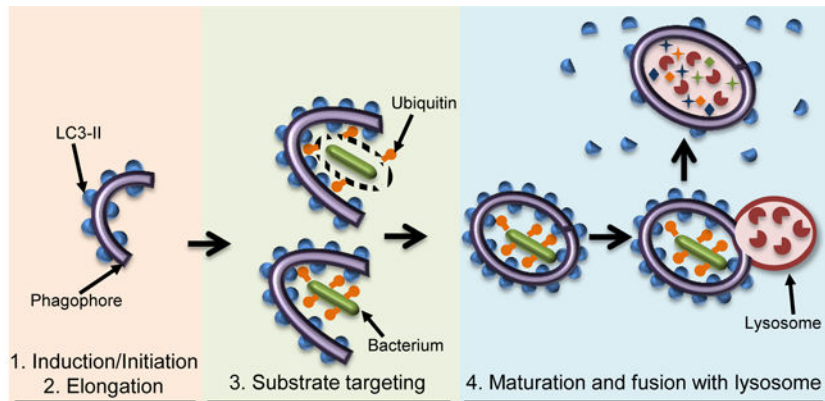
Some intracellular bacteria exploit autophagy to promote pathogenesis, requiring autophagic flux for survival and replication.

Autophagy-associated proteins play diverse roles in inflammation and immunity and can exert both positive and negative effects for the host. Many of these effects result from autophagy-independent functions and can vary depending on the cell type, host species, and interactions with particular pathogens.

There is interest in developing novel therapeutic strategies aimed at targeting autophagy to treat bacterial infection; however, this will require a better understanding of the roles of autophagy-associated proteins during infection and how bacteria evade these defenses.

Outstanding Questions

- Can we overcome bacterial defense mechanisms by activating autophagy using host-directed therapies?
- Are there other pathways involving autophagy-associated proteins that would be more effective targets in host-directed therapies than xenophagy?
- What is the mechanistic basis for the autophagy-independent role of ATG5 in controlling *Mycobacterium tuberculosis* infection, and can this be exploited in therapeutic interventions?
- Can we engineer specific modulators of pathways involving autophagy-associated proteins to avoid off-target effects during system-wide autophagy manipulation?
- Can differences in pharmacologically-induced autophagy observed in cell cultures correlate with *in vivo* efficacy?



	Blocking initiation and autophagosome formation	Shielding that interferes with recognition	Block LC3-targeting or recognition of bacteria	Blocking maturation or fusion with lysosome
Adherent and Invasive <i>E. coli</i>	Blocks autophagy gene expression			unknown mechanism
<i>B. cenocepacia</i>	Blocks autophagy gene expression			
<i>B. pseudomallei</i>	Blocks autophagy gene expression		BpscN-unknown mechanism	
<i>C. trachomatis</i>				Unknown mechanism
<i>F. tularensis</i>	inhibits ROS-mediated induction	O-antigen shields recognition		
Group A <i>Streptococcus</i>			SpeB degrades ubiquitin adaptors	SPN prevents maturation of vesicles
Group B <i>Streptococcus</i>			unknown mechanism	
<i>L. pneumophila</i>	LpSpl reduces host sphingolipids			RavZ cleaves LC3 to prevent maturation
<i>L. monocytogenes</i>	phospholipases prevent PI ₃ P formation	acquires host proteins to shield		
<i>M. tuberculosis</i>	Inhibits ROS-induced induction, may alter mTOR activation, ManLAM inhibits PI ₃ P formation, Coronin1a-unknown mechanism			blocks Rab7-mediated fusion
<i>O. tsutsugamushi</i>			unknown mechanism	
<i>S. Typhi</i>	depolymerization of actin			
<i>S. Typhimurium</i>	restores mTOR function, depolymerization of actin			
<i>S. marcescens</i>				unknown mechanism
<i>S. flexneri</i>		IcsB shields recognition	VirA interferes with Rab1	
<i>S. suis</i>	inhibits ROS-mediated induction			
<i>Y. pestis</i> and <i>Y. pseudotuberculosis</i>				unknown mechanism

Figure 1. Intracellular Bacterial Pathogens Avoid or Block Xenophagy-Mediated Clearance
 The schematic images above the table depict the relevant stages of xenophagy: 1. Initiation of xenophagy via phagophore formation. 2. Elongation of the autophagosomal double membrane. LC3-II (blue half-circles) is formed and localizes to the inner and outer autophagosomal membranes. 3. Bacteria (green rods) within a damaged vesicle (top) or cytosolic bacteria (bottom) colocalize with ubiquitin (orange shapes), which is then recognized by autophagy receptors. The latter also interact with LC3-II, thus targeting bacteria to autophagosomes. 4. Autophagosomes mature and fuse with lysosomes (pink

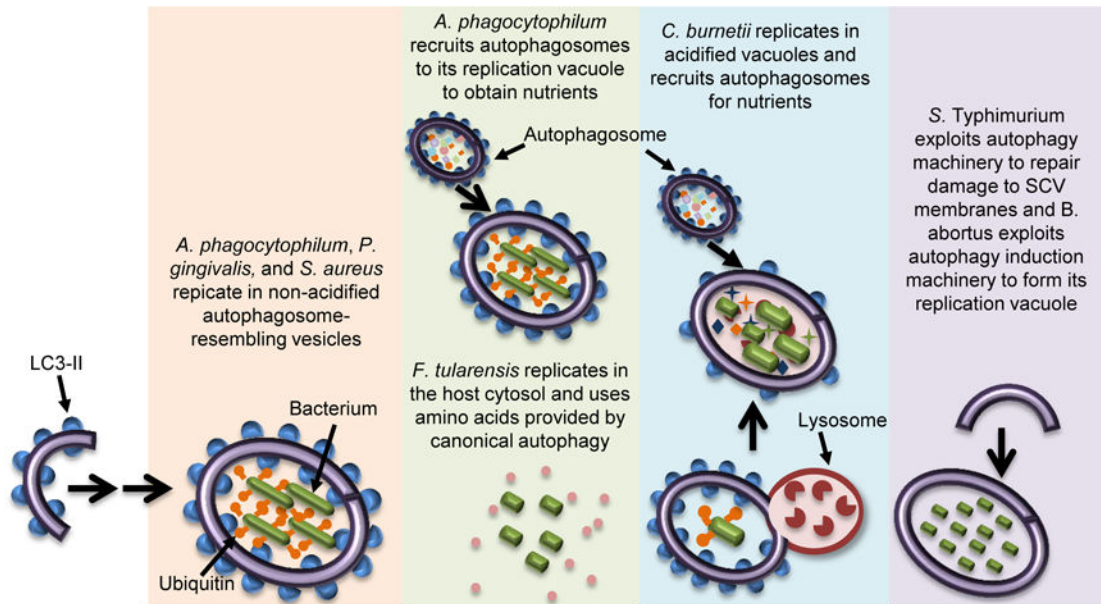
circle with red partial circle shapes inside). The cargo, including the bacteria, is then degraded. The ways in which bacteria interfere with each step of xenophagy are listed in the first column for each step. Many bacterial species use multiple strategies to interfere with autophagic flux at multiple steps. Some of these mechanisms of evasion may be cell type or host species specific. If a cell in the table is blank, there is no current evidence that the pathogen interferes with that stage of xenophagy. References are within the main text.

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	Replicates in a non-acidified autophagosome-resembling vesicle	Obtaining nutrients	Replicates in acidified vacuole	Other
<i>A. phagocytophilum</i>	✓	✓, Ats-1 promotes autophagosome nucleation		
<i>B. abortus</i>				Utilizes induction machinery (LC3-independent)
<i>C. trachomatis</i>				utilizes LC3 to promote infection, unknown mechanism
<i>C. burnetii</i>		✓	✓	
<i>F. tularensis</i>		✓, amino acids from the host cytosol		
<i>P. gingivalis</i>	✓			
<i>S. aureus</i>	✓, IsaB and agr exploit autophagy, unknown mechanism			
Uropathogenic <i>E. coli</i>				unknown mechanism
<i>S. Typhimurium</i>				exploits the autophagy machinery to repair damage to vacuole

Figure 2. Intracellular Bacterial Pathogens Exploit Autophagy-Associated Proteins for Replication and/or Survival

For the pathogens listed in this table, inhibiting autophagy would block infection and inducing autophagy would promote the infection. A check mark signifies that a given pathogen uses the mechanism denoted at the top of that column. If a cell in the table is blank, there is no current evidence that the pathogen exploits that mechanism. The schematic image above depicts each stage exploited by various bacteria and describes how the bacterium uses autophagy, with the shapes designating autophagy factors and bacteria as

green rods. Note that *A. phagocytophilum* both replicates in a non-acidified vesicle and uses nutrients derived from autophagic flux. References are within the main text.

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