Published in final edited form as:
Cell Microbiol. 2011 September ; 13(9): 1319–1327. doi:10.1111/j.1462-5822.2011.01632.x.

Eating the strangers within: host control of intracellular bacteria via xenophagy

Leigh A. Knodler and **Jean Celli**
Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, USA

Summary

Many bacterial pathogens rely on an intracellular cycle to ensure their proliferation within infected hosts, through their ability to avoid or circumvent host bactericidal pathways. Recent evidence supports an increasingly important role for the autophagy pathway in innate immune defenses against intracellular pathogens, as a mechanism of capture of either cytosol-adapted or vacuolar bacteria that redirect them to the lysosomal compartment for killing. Antibacterial autophagy, also referred to as xenophagy, involves selective recognition of intracellular bacteria and their targeting to the autophagic machinery for degradation. Here we review recent advances in our molecular understanding of these processes, and in how bacteria have adapted to avoid xenophagy or even take advantage of this innate immune process.

Bacterial pathogens with an intracellular lifestyle have evolved to not only escape from extracellular host immune effectors, but also circumvent intracellular bactericidal mechanisms that normally culminate in their delivery to the degradative lysosomal compartment. Typically, intracellular pathogens have been classified as vacuolar or cytosolic, depending on whether they dwell within a membrane-bound compartment or the cytosol following phagosomal escape. Vacuolar pathogens ensure their intracellular survival through remodelling of their initial phagosome into a pathogen-tailored vacuole, either by controlling endosomal maturation of their phagosome, as for *Mycobacterium tuberculosis* and *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) (Flannagan *et al*., 2009, Steele-Mortimer, 2008); through trafficking to, and conversion into, another intracellular compartment as exemplified by *Legionella pneumophila* and *Brucella* spp. (Roy *et al.*, 2006); through exclusion of host components and biogenesis of an idiosyncratic vacuole like for *Chlamydia* spp. (Cocchiaro *et al.*, 2009); or through resistance to the phagolysosomal environment like *Coxiella burnetii* (Flannagan *et al.*, 2009). Alternatively, cytosolic pathogens such as *Listeria monocytogenes*, *Shigella flexneri*, *Rickettsia* spp., *Mycobacterium marinum*, *Burkholderia* spp. and *Francisella tularensis* promote the disruption of their initial phagosome to gain access to the cytosol, where they proliferate. The arbitrary line between vacuolar and cytosolic bacterial lifestyles has been recently blurred by findings that vacuolar bacteria can become cytosolic (Beuzon *et al.*, 2000, Knodler *et al.*, 2010) and cytosolic bacteria can traffic through vacuolar compartments during their intracellular cycle (Checroun *et al.*, 2006). Regardless of classification, the numerous intracellular survival strategies developed by these bacteria highlight their efficient circumvention or avoidance of the degradative endosomal compartment. Nonetheless, a growing body of research has unraveled intracellular immune effector mechanisms related to mammalian macroautophagy

Corresponding author: Jean Celli, PhD, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, 903 South 4th Street, Hamilton, MT 59840, Phone: +1 406 375 9713, Fax: +1 406 375 9640, jcelli@niaid.nih.gov.

that counteract these survival strategies, delivering pathogens to a bactericidal lysosomal compartment.

Autophagy is a highly conserved eukaryotic cellular process that refers to the delivery of cytosolic material, such as soluble molecules, protein aggregates and organelles, to lysosomes for degradation. While this process includes chaperone-mediated autophagy, microautophagy and macroautophagy, we will only discuss selective processes of macroautophagy, hereafter referred to as autophagy. Autophagy was initially characterized in lower eukaryotes as a cytosolic bulk degradation pathway induced under nutrient starvation conditions. Yet it is also responsible for the disposal of damaged organelles and large protein aggregates, underlying its numerous roles in cellular biology processes such as energy homeostasis, protein recycling and organelle quality control (Levine *et al.*, 2008). Capture of cytosolic cargo into autophagosomes occurs via the formation of an isolation membrane called the phagophore, which is subsequently elongated and sealed into a double membrane autophagosome that undergoes maturation along the endocytic pathway and fuses with lysosomes (Figure 1). The molecular dissection of autophagy membrane dynamics has identified nucleation/initiation and elongation complexes that act sequentially to promote autophagosome formation. Upon induction of autophagy, the ULK1/2-ATG13-FIP200-ATG101 complex translocates from the cytosol to endoplasmic reticulum (ER) domains to recruit the class III phosphatidylinositol (PI)-3 kinase VPS34-Beclin1-VPS15-ATG14 complex that in turn generates phosphatidylinositol-3 phosphate (PI3P) on the outer leaflet of the ER (Itakura *et al.*, 2010). PI3P recruits the effector proteins DFCP1 and WIPI2, which generate specific ER-associated structures called omegasomes that act as cradle for phagophore formation (Axe *et al.*, 2008, Polson *et al.*, 2010). Elongation of the isolation membrane and autophagosomal closure involves the ATG7-ATG10 complex that conjugates ATG12-ATG5-ATG16L on autophagosomal membranes, and the ATG7-ATG3 complex that conjugates orthologues of the yeast Atg8 protein, such as LC3, whose active-membrane-bound form (LC3-II) is conjugated onto phosphatidyl-ethanolamine (PE) after cleavage by the ATG4 protease. While converging evidence indicates that autophagosomes originate from the ER, other compartments such as the Golgi apparatus, mitochondria and the plasma and nuclear membranes have been implicated in autophagosome formation (Levine *et al.*, 2011), leaving the question of their origin open.

In addition to its roles in cellular homeostasis and quality control, the autophagosomal degradation pathway has also been implicated in various aspects of innate and adaptive immunity (reviewed in (Virgin *et al.*, 2009, Deretic *et al.*, 2009, Levine *et al.*, 2011), among which the capture and degradation of intracellular viruses and bacteria has uncovered a specific antimicrobial role for autophagy (Deretic, 2005). While starvation-induced autophagy consists of the non-specific engulfment of cytosolic cargo, autophagosomal degradation of selective cargo such as organelles, protein aggregates and microorganisms requires their specific recognition by autophagic receptors for delivery into autophagosomes. As such, selective autophagosomal degradation of foreign microbes has been termed “xenophagy” (Levine, 2005). Here we will discuss recent developments in our understanding of xenophagic mechanisms acting against vacuolar and cytosolic bacteria, how they contribute to resistance to infections and how bacterial pathogens counteract and, in some cases, take advantage of xenophagy.

Xenophagic capture of intracellular bacteria

Given the cytosolic nature of the autophagic process, antibacterial autophagy should intuitively target cytosolic microorganisms. However, increasing evidence for autophagic capture of vacuolar bacteria has also emerged, which includes processes such as autophagy-mediated fusion of bacterial vacuoles with lysosomes and envelopment of damaged bacterial

vacuoles for delivery to lysosomes (Figure 2). Xenophagic control of intracellular bacteria was initially reported in the case of cytosolic bacteria. Rich *et al*. observed that cytosolic wild type and motility-deficient mutant (Δ*actA) L. monocytogenes* were localized within autophagic vacuoles in J774 murine macrophage-like cells when metabolically-arrested with chloramphenicol (Rich *et al.*, 2003). Antibacterial xenophagic capture was also demonstrated for cytoplasmic *Streptococcus pyogenes* in non-phagocytic cells (Nakagawa *et al.*, 2004). Upon streptolysin-mediated phagosomal escape, these bacteria are engulfed within large autophagosome-like vacuoles. This process requires ATG5 (Nakagawa *et al.*, 2004), providing the first molecular mechanistic evidence for antibacterial autophagy, and also depends upon the late endosomal small GTPase, Rab7 (Yamaguchi *et al.*, 2009). Additionally, the cytosol-adapted pathogen *F. tularensis* can also be sequestered into vacuoles with autophagic features following extensive replication in murine macrophages (Checroun *et al.*, 2006), indicating that xenophagic capture is not only restricted to cytosolic bacteria with limited intracellular survival capabilities, but can also target replication-competent cytosolic pathogens.

The first vacuolar bacterium found to be targeted by autophagy was *M. tuberculosis*. Upon IFNγ activation of RAW264.7 murine macrophage-like cells, the arrested mycobacterial phagosome resumes maturation and fuses with lysosomes through an autophagic process that depends upon the IFNγ-inducible p47-family GTPase, LRG47 (Gutierrez *et al.*, 2004). In U937 macrophage-like cells, the human orthologue to LRG47, IRGM, is required for autophagic control of intracellular mycobacteria (Singh *et al.*, 2006). These seminal findings demonstrated that autophagy can influence the intracellular fate of a vacuolar pathogen by overcoming its ability to control vacuole trafficking. Furthermore, autophagic bulk degradation of specific ribosomal and ubiquitinated cytosolic proteins (Ponpuak *et al.*, 2010) and subsequent proteolysis of ubiquitin itself (Alonso *et al.*, 2007) generates and delivers bactericidal peptides to *Mycobacterium*-containing autolysosomes, thereby contributing to bacterial killing via non-selective autophagy. Such mechanisms require the autophagic ubiquitin receptor p62 (also known as SQSTM1) (Ponpuak *et al.*, 2010), a protein involved in selective autophagy that binds ubiquitinated cargo via its UBA domain and ATG8/LC3 via its LIR domain (Randow, 2011).

Autophagy has also been assigned additional innate immune functions with the recognition of damaged pathogenic vacuoles (Figure 2). Birmingham *et al.* showed that in epithelial cells ~ 20% of *S.* Typhimurium becomes entrapped within multimembranous structures decorated with the autophagy marker LC3 in a ATG5-dependent manner (Birmingham *et al.*, 2006), in a process initiated on PI3P-enriched omegasomes that requires the GTPase Rab1 (Huang *et al.*, 2011). In support of this model, *S.* Typhimurium exhibits enhanced replication in ATG5-deficient mouse embryonic fibroblasts (MEFs) (Birmingham *et al.*, 2006). The type III secretion apparatus that some Gram-negative bacteria use to inject their proteins into host cells has been proposed to damage host cell membranes (Roy *et al.*, 2004). Since LC3 recruitment to the *Salmonella*-containing vacuole (SCV) was dependent upon the SPI-1 encoded Type III secretion system (T3SS1), it was proposed that the autophagic machinery recognizes the fraction of SCVs that are damaged by T3SS1 insertion, subsequently delivering these vacuoles to lysosomes (Birmingham *et al.*, 2006). This concept is challenged though by recent evidence that LC3 is recruited to intact SCVs independently of isolation membrane generation (Kageyama *et al.*, 2011).

The various examples of xenophagic capture of intracellular bacteria, whether cytosolic or vacuolar, involve the canonical ATG5- and LC3-dependent autophagic pathway. However, recent work on the intracellular fate of *M. marinum* suggests that unconventional or alternative autophagy processes (Nishida *et al.*, 2009) may also contribute to xenophagy. A fraction of cytosolic *M. marinum* that do not undergo actin-based motility are ubiquitinated

and captured into LC3-negative double membrane vacuoles in ATG5-deficient MEFs (Collins *et al.*, 2009). While the mechanisms underlying this process require further investigation, this suggests that xenophagy can proceed through mechanistically different capture processes.

Bacterial targeting to the autophagic pathway

With the increasing number of intracellular bacteria targeted by autophagy, an important question is how these organisms are recognized and directed to the autophagic pathway. The characterization of selective autophagy of cytosolic components such as mitochondria (Novak *et al.*, 2010), peroxisomes (Kim *et al.*, 2008) and protein aggregates has uncovered a role for molecular tagging via ubiquitination (Kim *et al.*, 2008, Kirkin *et al.*, 2009b) and various autophagy receptors. These include Nix, a mitochondrial protein that binds the Atg8 orthologs LC3 and GABARAP-L1 to promote mitophagy (Novak *et al.*, 2010) and p62/SQSTM1 (Pankiv *et al.*, 2007, Geisler *et al.*, 2010) and NBR1 (Kirkin *et al.*, 2009a), both of which bind ubiquitinated cargo and LC3-positive isolation membranes, thereby delivering cargo to forming autophagosomes for degradation. The demonstration of ubiquitination of *S.* Typhimurium (Perrin *et al.*, 2004), and their targeting to autophagy (Birmingham *et al.*, 2006) led to the identification of p62/SQSTM1 as an autophagic receptor of cytosolic bacteria in xenophagy (Zheng *et al.*, 2009), illustrating that degradation of both intracellular bacteria and misfolded proteins invokes a conserved pathway of selective autophagy. The ubiquitin-p62-LC3 pathway is also engaged by Δ*actA*, but not wild type, *L. monocytogenes* (Yoshikawa *et al.*, 2009, Perrin *et al.*, 2004), suggesting it is a general pathway of recognition of cytosol-reaching bacteria (Figure 2). Studies of antibacterial xenophagy have also uncovered a novel autophagy receptor, nuclear dot protein 52 kDa (NDP52), which senses and binds to ubiquitin-coated *S.* Typhimurium and *S. pyogenes* in epithelial cells and is required for their autophagic clearance (Thurston *et al.*, 2009). Interestingly, xenophagic control of bacterial replication involves the independent, yet cooperative, actions of p62 and NDP52 (Cemma *et al.*, 2011). These adaptors are recruited to different microdomains on the surface of cytosolic, ubiquitinated *S.* Typhimurium, which may allow for integration of additional signaling events that contribute to xenophagy, a hypothesis consistent with the role of NDP52 acting as a scaffold for the assembly of a Tank-binding protein (TBK1)-Sintbad-Nap1 signaling complex (Thurston *et al.*, 2009). TBK1 is required for maintaining the integrity of bacterial vacuolar membranes since there is increased release of *S.* Typhimurium and *S. pyogenes* into the host cell cytosol in TBK1 knockdown epithelial cells and TBK1-deficient MEFs (Radtke *et al.*, 2007, Thurston *et al.*, 2009). Recently, Dupont *et al*. expanded our knowledge of the crosstalk between autophagy and other host cellular pathways in response to cytosol-adapted pathogens (Dupont *et al.*, 2009). *S. flexneri* disrupts its phagosomal membrane shortly after entry into host cells and replicates extensively in the cytosol. These phagosomal membrane remnants are polyubiquitinated and targeted to the ubiquitin-p62-LC3 pathway for autophagic degradation in epithelial cells and macrophages (Figure 2). Surprisingly, p62 on phagosomal membrane remnants also colocalized with components of NF-κB signaling pathways such as TRAF6 and NOD1, thereby dampening the cellular inflammatory response (Dupont *et al.*, 2009). This established for the first time a direct link between autophagy and inflammation during infection.

In addition to the ubiquitin-p62-LC3 pathway, a newly described xenophagic pathway uses the lipid second messenger diacyglycerol (DAG) generated on damaged SCVs by phospholipase D and phosphatidic acid phosphatase to trigger antibacterial autophagy (Shahnazari *et al.*, 2010). Promotion of antibacterial autophagy by DAG requires activation of protein kinase Cδ (PKCδ) and its downstream targets JNK1 and NADPH oxidase, but is independent of ATG5. Therefore at least two independent mechanisms contribute to the recognition and xenophagic capture of cytosolic bacteria in mammalian cells. While it is

well-documented that recognition of cytosolic bacteria by the ubiquitin-p62-LC3 pathway is widespread, whether the DAG-dependent pathway is invoked by bacteria other than *S.* Typhimurium remains to be determined.

Mammalian recognition of bacteria and induction of autophagy

Given the prevalent role of xenophagy in cellular innate defenses, a key aspect of this process relies on sensing invading bacteria to trigger an autophagic response. Mammalian cells express a variety of either cell surface or cytosolic pattern recognition receptors (PRRs), such as Toll-like Receptors (TLRs) or cytosolic nucleotide-binding oligomerization domains (NOD)-like receptors (NLRs), which can recognize specific pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS) or peptidoglycans (PG) of Gram-negative bacteria (reviewed in (Franchi *et al.*, 2008)). Several studies have recently connected TLR and NLR sensing to autophagy induction, providing a missing link between pathogen recognition and initiation of autophagy. Initial evidence for bacterial sensing came from the discovery that TLR signaling induces recruitment of LC3 to forming phagosomes in a process that requires ATG5, ATG7 and the Beclin 1/PI3-kinase complex (Sanjuan *et al.*, 2007). The involvement of TLRs was confirmed by the findings that LPS can induce TLR4-mediated autophagy in phagocytic cells (Xu *et al.*, 2007) and single-stranded RNA induces antimycobacterial autophagy via TLR7 activation (Delgado *et al.*, 2008). Induction of autophagy at the site of microbial uptake is further supported by NADPH-oxidase dependent production of reactive oxygen species (ROS) upon TLR and Fcγ receptor-dependent phagocytosis recruiting the autophagic machinery to newly formed phagosomes (Huang *et al.*, 2009). The cell surface receptor SLAM functions as a microbial sensor that recruits the autophagy-associated PI3-kinase/Beclin 1 complex to forming phagosomes and regulate NADPH oxidase activity via PI3P generation (Berger *et al.*, 2010). Additionally, the cytosolic receptors NOD1 and NOD2, which detect bacterial peptidoglycan, recruit ATG16L1 to the site of *S. flexneri* entry (Travassos *et al.*, 2010). This finding is consistent with the role of the pattern recognition receptor PRGP-LE in driving autophagy-mediated control of *L. monocytogenes* intracellular growth in *Drosophila* (Yano *et al.*, 2008). Collectively these studies suggest that autophagic control of invading bacteria is determined at the onset of the bacterium-host cell interaction. However, subsequent TLR stimulation can also redirect preexisting pathogenic vacuoles to lysosomes via autophagy (Delgado *et al.*, 2008) and intracellular bacteria become targeted by xenophagy upon either vacuolar damage or entry into the cytosol (Birmingham *et al.*, 2006, Checroun *et al.*, 2006, Nakagawa *et al.*, 2004, Rich *et al.*, 2003, Yoshikawa *et al.*, 2009) indicating that not all antibacterial autophagic processes are determined at the time of entry. Future studies on the host cell mechanisms that sense intracellular microbes, for example, what proteins are required for the polyubiquitination of bacteria and/or pathogenic vacuoles, will certainly shed light on these processes.

Microbial interference with autophagy

Bacteria possess a remarkable capacity to adapt to their environment and have evolved strategies to not only circumvent host cell defenses, but also to take advantage of the underlying processes. Not surprisingly, given its potent role in controlling intracellular microbes, some bacteria have developed strategies to counteract autophagy. One strategy is a general, non-specific inhibition of autophagy, as exemplified by *F. tularensis* infection of human blood monocytes. *F. tularensis* infection leads to downregulation of the expression of several ATG genes (Butchar *et al.*, 2008), although whether this downregulation is sufficient to account for the lack of rapid autophagic recognition of cytosolic *F. tularensis* remains to be established. Kumar *et al*. recently reported that *M. tuberculosis* infection of human macrophage-like cells activates a network of regulatory pathways that converge in

negative regulation of autophagy (Kumar *et al.*, 2010). This non-specific inhibition of autophagy requires the mycobacterial protein Eis (Shin *et al.*, 2010) and illustrates *Mycobacterium*'s capacity to interfere with a pathway that promotes its intracellular killing (Gutierrez *et al.*, 2004). Interestingly, the ability of autophagy to control the intracellular replication of *S.* Typhimurium in epithelial cells appears to have a limited time frame since cytosolic bacteria are not decorated by LC3 after 1 h p.i. (Birmingham *et al.*, 2006) and hyper-replication of *S.* Typhimurium occurs in the cytosol at ≥ 8 h p.i. (Knodler *et al.*, 2010). Therefore *Salmonella* can block autophagic defenses at later stages of its infectious cycle, although this does not appear to be a generalized inhibition of autophagy (Birmingham *et al.*, 2006). More specific strategies of autophagy avoidance have been well characterized for the cytosolic pathogens *S. flexneri* and *L. monocytogenes*. *S. flexneri* secretes IcsB, a T3SS effector protein that competitively inhibits ATG5 binding to the actin-based motility surface protein VirG, thereby preventing induction of the autophagic cascade on the bacterial surface (Ogawa *et al.*, 2005). This molecular competition between a bacterial protein and the autophagic machinery suggested that ATG proteins may directly recognize bacterial surface proteins to initiate xenophagy. In a remarkable parallel, *L. monocytogenes* requires the actin-based motility surface protein ActA to avoid xenophagic killing (Birmingham *et al.*, 2007, Perrin *et al.*, 2004, Yoshikawa *et al.*, 2009). Yoshikawa *et al*. elegantly showed that actin-based motility *per se* was not responsible for autophagy avoidance, but instead that ActA-mediated recruitment of host cell cytoskeleton components accounted for the lack of autophagic recognition of cytosolic bacteria (Yoshikawa *et al.*, 2009). It was proposed that, by disguising itself as a host organelle through the initial steps of actin-based motility, *Listeria* prevents recognition of its surface PAMPs by the ubiquitin-p62-LC3 pathway.

While several intracellular pathogens inhibit xenophagic processes, others have evolved to take advantage of the autophagic pathway (Figure 2). For example, a bacterium living within a phagolysosomal environment would benefit from interacting with the autophagic bulk degradation pathway to scavenge cytosolic components for nutrient recycling. In support of this, *C. burnetii* resides within a highly fusogenic parasitophorous vacuole (PV) with phagolysosomal features and whose trafficking and intracellular growth is enhanced upon autophagy activation (Gutierrez *et al.*, 2005). The *Coxiella* PV is decorated with LC3 as early as 5 min p.i. and thereafter for many days, a process dictated by the bacteria (Romano *et al.*, 2007), but eventually loses it (Gutierrez *et al.*, 2005). Other intracellular bacteria modulate the trafficking of their vacuole to reside within compartments of autophagic origin, at the same time blocking their fusion with, and destruction by, degradative lysosomes. This is exemplified by *Porphyromonas gingivalis*, a bacterial periodontal pathogen which resides within arrested autophagosomes in endothelial cells (Dorn *et al.*, 2001) and *Yersinia pestis* (Pujol *et al.*, 2009) and *Yersinia pseudotuberculosis* (Moreau *et al.*, 2010), which reside within autophagosomes in macrophages. By preventing acidification of these vacuoles, bacteria avoid xenophagic killing. Additionally, a role for autophagy has been invoked in the intracellular trafficking of the ER-dwelling pathogens *Legionella pneumophila* and Brucella *abortus*. Early studies have linked trafficking of these bacteria to the autophagic pathway (Swanson *et al.*, 1995, Pizarro-Cerda *et al.*, 1998a, Pizarro-Cerda *et al.*, 1998b) and it was also recently proposed that autophagic events at the ER promote biogenesis of the ER-derived *Brucella* replicative vacuole (Qin *et al.*, 2008). Finally, the cytosol-adapted bacterium *L. monocytogenes* can also be contained within vacuoles called "spacious *Listeria*-containing phagosomes" (SLAPs) in macrophages (Birmingham *et al.*, 2008), the formation of which requires autophagy. While the pore-forming toxin Listeriolysin O (LLO) is required for SLAP formation, consistent with its ability to induce autophagy (Meyer-Morse *et al.*, 2010), these vacuoles seem to harbor bacteria that failed to lyse their phagosome due to low or inefficient LLO activity. SLAPs are evident in liver macrophages of immunodeficient SCID mice (Bhardwaj *et al.*, 1998) and thus posited to constitute a

survival niche during chronic infections (Birmingham *et al.*, 2008). With the exception of LLO, no specific bacterial factors have yet been identified that modulate vacuolar fusion with autophagosomes, but future studies will undoubtedly uncover their identity.

Concluding remarks

A number of recent studies have clearly established autophagy as a key pathway of intracellular innate immunity that can control the fate of many foreign microorganisms including bacteria but also viruses and protozoan parasites (Levine *et al.*, 2011). It is now becoming clear that autophagic processes and autophagy-associated proteins are integrated within pathogen innate sensing mechanisms to trigger selective autophagic recognition of invading microbes. While autophagy has long been seen as a cytosolic process and consequently uncovered as a defense response against cytosol-adapted pathogens, it also has the capacity to recognize and counteract the survival strategies of vacuolar microorganisms, a process often associated with vacuolar damage inflicted by bacterial activities. Whether they succumb to xenophagy or express mechanisms of avoidance, bacteria are useful cell biological tools that have allowed researchers to define many of the molecular mechanisms of selective autophagy. *In vivo* studies of the importance of xenophagy in infection have been limited and generally hampered by the lethality of most ATG gene knockout in mice (Levine *et al.*, 2008). Nonetheless, tissue-specific knockout of ATG5 confers an increased susceptibility to bacterial and protozoan pathogens (Zhao *et al.*, 2008, Polson *et al.*, 2010) and human population genetic studies have established a connection between autophagy and antimicrobial defenses (Deretic, 2010), clearly establishing a role for autophagy in controlling infections. Future studies will certainly expand our knowledge of the importance of this intracellular process in infectious diseases.

Acknowledgments

We are grateful to Anita Mora for graphics assistance. We apologize to those whose work we did not cite due to space limitations. This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases.

References

Alonso S, Pethe K, Russell DG, Purdy GE. Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:6031–6036. [PubMed: 17389386]

Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol. 2008; 182:685–701. [PubMed: 18725538]

Berger SB, Romero X, Ma C, Wang G, Faubion WA, Liao G, et al. SLAM is a microbial sensor that regulates bacterial phagosome functions in macrophages. Nat Immunol. 2010; 11:920–927. [PubMed: 20818396]

Beuzon CR, Meresse S, Unsworth KE, Ruiz-Albert J, Garvis S, Waterman SR, et al. Salmonella maintains the integrity of its intracellular vacuole through the action of SifA. Embo J. 2000; 19:3235–3249. [PubMed: 10880437]

Bhardwaj V, Kanagawa O, Swanson PE, Unanue ER. Chronic Listeria infection in SCID mice: requirements for the carrier state and the dual role of T cells in transferring protection or suppression. Journal of immunology. 1998; 160:376–384.

Birmingham CL, Canadien V, Gouin E, Troy EB, Yoshimori T, Cossart P, et al. Listeria monocytogenes evades killing by autophagy during colonization of host cells. Autophagy. 2007; 3:442–451. [PubMed: 17568179]

Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH. Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles. Nature. 2008; 451:350–354. [PubMed: 18202661]

Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH. Autophagy controls Salmonella infection in response to damage to the Salmonella-containing vacuole. J Biol Chem. 2006; 281:11374–11383. [PubMed: 16495224]

Butchar JP, Cremer TJ, Clay CD, Gavrilin MA, Wewers MD, Marsh CB, et al. Microarray analysis of human monocytes infected with Francisella tularensis identifies new targets of host response subversion. PLoS One. 2008; 3:e2924. [PubMed: 18698339]

Cemma M, Kim PK, Brumell JH. The ubiquitin-binding adaptor proteins p62/SQSTM1 and NDP52 are recruited independently to bacteria-associated microdomains to target Salmonella to the autophagy pathway. Autophagy. 2011; 7:22–26.

Checroun C, Wehrly TD, Fischer ER, Hayes SF, Celli J. Autophagy-mediated reentry of Francisella tularensis into the endocytic compartment after cytoplasmic replication. Proc Natl Acad Sci U S A. 2006; 103:14578–14583. [PubMed: 16983090]

Cocchiaro JL, Valdivia RH. New insights into Chlamydia intracellular survival mechanisms. Cellular microbiology. 2009; 11:1571–1578. [PubMed: 19673891]

Collins CA, De Maziere A, van Dijk S, Carlsson F, Klumperman J, Brown EJ. Atg5-independent sequestration of ubiquitinated mycobacteria. PLoS Pathog. 2009; 5:e1000430. [PubMed: 19436699]

Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. Embo J. 2008; 27:1110–1121. [PubMed: 18337753]

Deretic V. Autophagy in innate and adaptive immunity. Trends Immunol. 2005; 26:523–528. [PubMed: 16099218]

Deretic V. Autophagy in infection. Curr Opin Cell Biol. 2010; 22:252–262. [PubMed: 20116986]

Deretic V, Levine B. Autophagy, immunity, and microbial adaptations. Cell host & microbe. 2009; 5:527–549. [PubMed: 19527881]

Dorn BR, Dunn WA Jr, Progulske-Fox A. Porphyromonas gingivalis traffics to autophagosomes in human coronary artery endothelial cells. Infect Immun. 2001; 69:5698–5708. [PubMed: 11500446]

Dupont N, Lacas-Gervais S, Bertout J, Paz I, Freche B, Van Nhieu GT, et al. Shigella phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. Cell host & microbe. 2009; 6:137–149. [PubMed: 19683680]

Flannagan RS, Cosio G, Grinstein S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. Nat Rev Microbiol. 2009; 7:355–366. [PubMed: 19369951]

Franchi L, Park JH, Shaw MH, Marina-Garcia N, Chen G, Kim YG, Nunez G. Intracellular NOD-like receptors in innate immunity, infection and disease. Cellular microbiology. 2008; 10:1–8. [PubMed: 17944960]

Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nature cell biology. 2010; 12:119–131.

Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell. 2004; 119:753–766. [PubMed: 15607973]

Gutierrez MG, Vazquez CL, Munafo DB, Zoppino FC, Beron W, Rabinovitch M, Colombo MI. Autophagy induction favours the generation and maturation of the Coxiella-replicative vacuoles. Cell Microbiol. 2005; 7:981–993. [PubMed: 15953030]

Huang J, Birmingham CL, Shahnazari S, Shiu J, Zheng YT, Smith AC, et al. Antibacterial autophagy occurs at PI(3)P-enriched domains of the endoplasmic reticulum and requires Rab1 GTPase. Autophagy. 2011; 7:17–26. [PubMed: 20980813]

Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, Magalhaes MA, et al. Activation of antibacterial autophagy by NADPH oxidases. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:6226–6231. [PubMed: 19339495]

Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. Autophagy. 2010; 6:764–776. [PubMed: 20639694]

Kageyama S, Omori H, Saitoh T, Sone T, Guan JL, Akira S, et al. The LC3 recruitment mechanism is separate from Atg9L1-dependent membrane formation in the autophagic response against Salmonella. Mol Biol Cell. 2011

Kim PK, Hailey DW, Mullen RT, Lippincott-Schwartz J. Ubiquitin signals autophagic degradation of cytosolic proteins and peroxisomes. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:20567–20574. [PubMed: 19074260]

Kirkin V, Lamark T, Sou YS, Bjorkoy G, Nunn JL, Bruun JA, et al. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell. 2009a; 33:505–516. [PubMed: 19250911]

Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. Mol Cell. 2009b; 34:259–269. [PubMed: 19450525]

Knodler LA, Vallance BA, Celli J, Winfree S, Hansen B, Montero M, Steele-Mortimer O. Dissemination of invasive Salmonella via bacterial-induced extrusion of mucosal epithelia. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:17733–17738. [PubMed: 20876119]

Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, Rao KV. Genome-wide analysis of the host intracellular network that regulates survival of Mycobacterium tuberculosis. Cell. 2010; 140:731–743. [PubMed: 20211141]

Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell. 2005; 120:159–162. [PubMed: 15680321]

Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008; 132:27–42. [PubMed: 18191218]

Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature. 2011; 469:323–335. [PubMed: 21248839]

Meyer-Morse N, Robbins JR, Rae CS, Mochegova SN, Swanson MS, Zhao Z, et al. Listeriolysin O is necessary and sufficient to induce autophagy during Listeria monocytogenes infection. PLoS One. 2010; 5:e8610. [PubMed: 20062534]

Moreau K, Lacas-Gervais S, Fujita N, Sebbane F, Yoshimori T, Simonet M, Lafont F. Autophagosomes can support Yersinia pseudotuberculosis replication in macrophages. Cellular microbiology. 2010; 12:1108–1123. [PubMed: 20180800]

Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, et al. Autophagy defends cells against invading group A Streptococcus. Science. 2004; 306:1037–1040. [PubMed: 15528445]

Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, et al. Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature. 2009; 461:654–658. [PubMed: 19794493]

Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, et al. Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. 2010; 11:45–51. [PubMed: 20010802]

Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular Shigella from autophagy. Science. 2005; 307:727–731. [PubMed: 15576571]

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. The Journal of biological chemistry. 2007; 282:24131–24145. [PubMed: 17580304]

Perrin AJ, Jiang X, Birmingham CL, So NS, Brumell JH. Recognition of bacteria in the cytosol of Mammalian cells by the ubiquitin system. Curr Biol. 2004; 14:806–811. [PubMed: 15120074]

Pizarro-Cerda J, Meresse S, Parton RG, van der Goot G, Sola-Landa A, Lopez-Goni I, et al. Brucella abortus transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. Infect Immun. 1998a; 66:5711–5724. [PubMed: 9826346]

Pizarro-Cerda J, Moreno E, Sanguedolce V, Mege JL, Gorvel JP. Virulent Brucella abortus prevents lysosome fusion and is distributed within autophagosome-like compartments. Infect Immun. 1998b; 66:2387–2392. [PubMed: 9573138]

Polson HE, de Lartigue J, Rigden DJ, Reedijk M, Urbe S, Clague MJ, Tooze SA. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy. 2010; 6

Ponpuak M, Davis AS, Roberts EA, Delgado MA, Dinkins C, Zhao Z, et al. Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. Immunity. 2010; 32:329–341. [PubMed: 20206555]

Pujol C, Klein KA, Romanov GA, Palmer LE, Cirota C, Zhao Z, Bliska JB. Yersinia pestis can reside in autophagosomes and avoid xenophagy in murine macrophages by preventing vacuole acidification. Infection and immunity. 2009; 77:2251–2261. [PubMed: 19289509]

Qin QM, Pei J, Ancona V, Shaw BD, Ficht TA, de Figueiredo P. RNAi screen of endoplasmic reticulum-associated host factors reveals a role for IRE1alpha in supporting Brucella replication. PLoS Pathog. 2008; 4:e1000110. [PubMed: 18654626]

Radtke AL, Delbridge LM, Balachandran S, Barber GN, O'Riordan MX. TBK1 protects vacuolar integrity during intracellular bacterial infection. PLoS Pathog. 2007; 3:e29. [PubMed: 17335348]

Randow F. How cells deploy ubiquitin and autophagy to defend their cytosol from bacterial invasion. Autophagy. 2011; 7

Rich KA, Burkett C, Webster P. Cytoplasmic bacteria can be targets for autophagy. Cell Microbiol. 2003; 5:455–468. [PubMed: 12814436]

Romano PS, Gutierrez MG, Beron W, Rabinovitch M, Colombo MI. The autophagic pathway is actively modulated by phase II Coxiella burnetii to efficiently replicate in the host cell. Cellular microbiology. 2007; 9:891–909. [PubMed: 17087732]

Roy CR, Salcedo SP, Gorvel JP. Pathogen-endoplasmic-reticulum interactions: in through the out door. Nat Rev Immunol. 2006; 6:136–147. [PubMed: 16491138]

Roy D, Liston DR, Idone VJ, Di A, Nelson DJ, Pujol C, et al. A process for controlling intracellular bacterial infections induced by membrane injury. Science. 2004; 304:1515–1518. [PubMed: 15178804]

Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature. 2007; 450:1253–1257. [PubMed: 18097414]

Shahnazari S, Yen WL, Birmingham CL, Shiu J, Namolovan A, Zheng YT, et al. A diacylglycerol-dependent signaling pathway contributes to regulation of antibacterial autophagy. Cell host & microbe. 2010; 8:137–146. [PubMed: 20674539]

Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, Song CH, et al. Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS Pathog. 2010; 6:e1001230. [PubMed: 21187903]

Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science. 2006; 313:1438–1441. [PubMed: 16888103]

Steele-Mortimer O. The Salmonella-containing vacuole: moving with the times. Curr Opin Microbiol. 2008; 11:38–45. [PubMed: 18304858]

Swanson MS, Isberg RR. Association of Legionella pneumophila with the macrophage endoplasmic reticulum. Infection and immunity. 1995; 63:3609–3620. [PubMed: 7642298]

Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. Nat Immunol. 2009; 10:1215–1221. [PubMed: 19820708]

Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol. 2010; 11:55–62. [PubMed: 19898471]

Virgin HW, Levine B. Autophagy genes in immunity. Nat Immunol. 2009; 10:461–470. [PubMed: 19381141]

Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity. 2007; 27:135–144. [PubMed: 17658277]

Yamaguchi H, Nakagawa I, Yamamoto A, Amano A, Noda T, Yoshimori T. An initial step of GAS-containing autophagosome-like vacuoles formation requires Rab7. PLoS Pathog. 2009; 5:e1000670. [PubMed: 19956673]

Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, et al. Autophagic control of listeria through intracellular innate immune recognition in drosophila. Nat Immunol. 2008; 9:908–916. [PubMed: 18604211]

Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, et al. Listeria monocytogenes ActA-mediated escape from autophagic recognition. Nat Cell Biol. 2009; 11:1233–1240. [PubMed: 19749745]

Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, Miller BC, et al. Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. Cell Host Microbe. 2008; 4:458–469. [PubMed: 18996346]

Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH. The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. J Immunol. 2009; 183:5909–5916. [PubMed: 19812211]

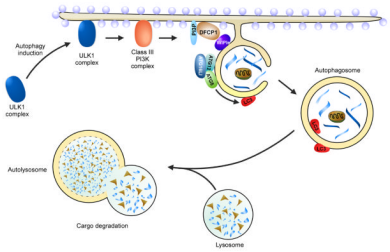

Figure 1.
Molecular cascade of autophagosome formation at the endoplasmic reticulum. Following autophagy induction, the ULK1 complex translocates to the ER and recruits the class III PI3-kinase complex generating the phosphoinositide, PI3P. PI3P recruits DFCP1 and WIPI proteins, contributing to the generation of an isolation membrane at specific sites called omegasomes. The isolation membrane is then elongated via conjugation of the ATG5-ATG12-ATG16L1 complex and LC3 to PE and closed into a double membrane autophagosome. The autophagosome then matures along the endocytic pathway and fuses with lysosomes, which degrades its intraluminal content for recycling.

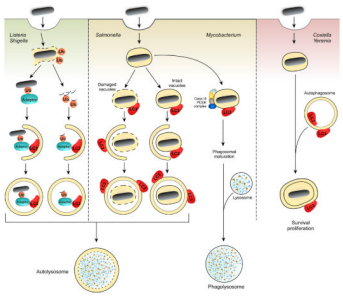

Figure 2.

The known xenophagic pathways of bacterial recognition and degradation. Cytosolic bacteria (*Listeria*, *Shigella*), phagosomal membrane remnants resulting from vacuolar disruption, and vacuolar bacteria (*Salmonella*) within intact or damaged phagosomes can be recognized and captured by the autophagy machinery via ubiquitin-dependent or -independent mechanisms. These are delivered to forming autophagosomes and trafficked to lysosomes for degradation. Vacuolar pathogens such as *Mycobacterium* can also be targeted for lysosomal degradation following autophagy-mediated resumption of their phagosome maturation upon immune activation. Alternatively, bacterial pathogens such as *Coxiella* and *Yersinia* can benefit from fusing with autophagosomes.