Chapter 10
Autophagy: A Potential Antibacterial Therapeutic Target

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Abstract Bacterial infections caused by pathogenic bacteria, like tuberculosis by *Mycobacterium tuberculosis*, listeriosis by *Listeria monocytogenes*, and gastroenteritis by *Salmonella typhimurium*, are on the rise. With the increase in pathogen resistance to antibiotics, novel approaches are required for therapeutic interventions to treat bacterial infections. Autophagy is an essential host defense mechanism against infections and, in recent times, has shown promising potential as a therapeutic target in this regard. This article reviews the role of autophagy during infection with pathogenic bacteria and recent studies which highlight the importance of autophagy as a prospective therapeutic target.

Keywords Autophagy · Bacterial infections · Therapeutic target

10.1 Introduction

Autophagy is an evolutionarily conserved cellular defense mechanism which involves the cloistering of cargo molecules (viz., damaged cellular organelles, protein aggregates, or pathogens) in a double-membrane vacuole, known as an autophagosome, which are ultimately degraded by lysosomal hydrolases. Autophagy can be triggered by a variety of factors, such as damaged cellular organelles, withdrawal of growth factors, amino acid deprivation, oxidative stress, hypoxia, endoplasmic reticulum stress, low cellular energy levels, and infection (Lin and Baehrecke 2015). The autophagy of cellular organelles and protein aggregates is an essential part of the maintenance of cellular homeostasis, whereas that of pathogens acts as a defense mechanism against infections (termed xenophagy) (Samson 1981).

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Autophagy can be classified into different types. In selective autophagy, molecules called autophagy adaptors or cargo receptors recognize and bind to a specific target cargo molecule and subsequently lead to its degradation by autophagy. Nonselective autophagy involves the indiscriminate entrapment of cargo into developing autophagosomes (Moy and Cherry 2013). Autophagy adaptors like optineurin (OPTN), sequestosome 1 (SQSTM1), neighbor of BRCA1 gene 1 (NBR1), nuclear dot protein 52 (NDP52), Toll-interacting protein (Tollip), TAX1-binding protein 1 (TAX1BP1), and nuclear receptor subfamily 1, group D, member 1 (NR1D1) have been identified, and most of them have been shown to be involved in xenophagy (Bjørkøy et al. 2005; Kirkin et al. 2009; Thurston et al. 2009; Zheng et al. 2009; Dupont et al. 2009; Ogawa et al. 2011; Osawa et al. 2011; Newman et al. 2012; Khweek et al. 2013; Lu et al. 2014; Chandra et al. 2015). Autophagy can also be further divided into three categories: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy is often referred to as conventional autophagy, wherein cytoplasmic cargo is cloistered into autophagosomes, which is followed by fusion of lysosomes with autophagosomes to form autolysosomes and subsequent degradation of cargo by lysosomal hydrolases (Fig. 10.1). Microautophagy involves the uptake of cytosomal components directly

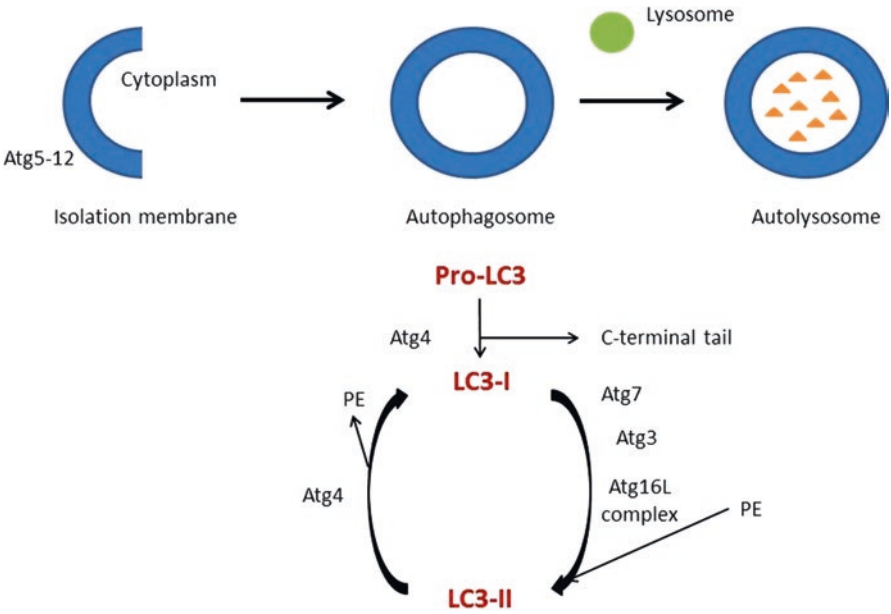

Fig. 10.1 Stages in autophagy. The process of autophagosomal membrane formation starts when the autophagy-related genes (Atg) Atg5 and Atg12 conjugate to form an isolation membrane. The membrane then envelops cargo molecules and closes to form an autophagosome. Lysosomes fuse with autophagosomes to form autolysosomes, wherein the autophagic cargo is degraded via lysosomal hydrolases. In the LC3 pathway, Atg4 cleaves the C-terminal tail of sequence of the pro-LC3 molecule to generate LC3-I (cytosolic). Atg7 activates LC3-I and is conjugated to Atg3. This Atg3-LC3-I conjugate binds to the Atg16L complex, and LC3-I binds to phosphatidylethanolamine (PE), thereby generating LC3-II (membrane-bound). The PE of LC3-II is subsequently cleaved by Atg4 to produce LC3-I. (Modified from Noda et al. 2009)

by the lysosomes by invagination, without forming intermediate autophagosomal structures. Chaperone-mediated autophagy is mediated by chaperone proteins recognized by the lysosomal membrane receptor lysosome-associated membrane protein 2A, which form a complex with cargo and are translocated across the lysosomal membrane (Glick et al. 2010).

At the molecular level, autophagy is mediated by autophagy-related genes (Atg), which are present both in yeast and mammals (Mizushima et al. 2011). The induction of autophagy is responsible for the activation of the Unc-51-like autophagy activating kinase 1 (ULK1), which, in turn, activates Beclin-1/Atg6 (Russell et al. 2013). The class III phosphatidylinositol 3-phosphate kinase Vps34 phosphorylates phosphatidylinositol to produce phosphatidylinositol 3-phosphate (PtdIns(3)P) which provides a docking site for WD-repeat protein which then interacts with phosphoinositides (WIPI) protein family (Proikas-Cezanne et al. 2015). Atg12 binds to Atg5 and then to Atg16L1 to form a complex which binds and activates Atg3 (Hanada et al. 2007). Atg3 attaches Atg8 (microtubule-associated protein 1 light chain 3 [LC3]), which is first processed by Atg4, to phosphatidylethanolamine (PE) on the surface of autophagosomes leading to the closure of autophagosomes (Fujita et al. 2008; Kirisako et al. 2000). The fusion of lysosomes with closed autophagosomes results in cargo degradation.

Autophagy is both pro- and antibacterial during infections. This review discusses the role of autophagy during bacterial infections and also if autophagy can act as a target for therapeutic interventions during bacterial infections.

10.2 Role of Autophagy in Bacterial Pathogenesis

Several pathogenic bacteria are known to induce autophagy during infection, and some have also devised various strategies to evade autophagic recognition (Table 10.1). The following sections discuss these aspects in greater detail.

Table 10.1 Bacteria and bacterial factors that are involved in the induction or evasion of autophagy

Bacterium	Bacterial factor(s)	Autophagy induction	Autophagy evasion
Listeria monocytogenes	LLO, ActA, InlK	Yes	Yes
Salmonella typhimurium	SipB, TTSS, SseL	Yes	Yes
Mycobacterium tuberculosis	EspB, EIS	Yes	Yes
Shigella flexneri	IcsB, IcsA	Yes	Yes
Legionella pneumophila	RavZ, LpSpl	Yes	Yes
Streptococcus pyogenes	SLO, NADase, SpeB	Yes	Yes
Streptococcus pneumoniae	PLY	Yes	n.d.
Pseudomonas aeruginosa	Phycocyanin, TpIE	Yes	n.d.
Francisella tularensis	dipA, O-antigen	Yes	Yes

10.2.1 Mechanisms of Autophagy Induction by Bacteria During Infection

10.2.1.1 Virulence Factors of Bacteria

Bacterial virulence factors play an essential role in mediating the recognition of pathogenic bacteria by the host autophagy machinery. The type III secretion system (TTSS) of *Salmonella typhimurium* ruptures the cytosolic compartments in which intracellular *S. typhimurium* are contained (termed as *Salmonella*-containing vacuoles [SCV]), and the entrapped bacteria are ubiquitinated and subsequently targeted by autophagy (Birmingham et al. 2006). The key virulence factor of the Gram-positive bacterium *Listeria monocytogenes* reported to be involved in autophagy induction is the pore-forming toxin listeriolysin O (LLO) (Py et al. 2007). Amino acid starvation can also be triggered by LLO-dependent phagosomal lysis during *L. monocytogenes* infection, which can result in induction of autophagy (Tattoli et al. 2013). LC3-associated phagocytosis (LAP) is also induced by LLO and facilitates the formation of spacious *Listeria*-containing phagosomes (SLAPs: LC3-positive *L. monocytogenes*-containing phagosomes). These LC3-positive single-membrane compartments allow listerial survival and their slow replication (Lam et al. 2013; Birmingham et al. 2008).

10.2.1.2 Regulation of Host Autophagy Signaling

Autophagy can also be induced when bacteria regulate host signaling pathways during infection. Autophagy is activated when amino acid starvation is triggered by the infection of epithelial cells with *S. typhimurium* (Tattoli et al. 2012). It is already established that macrophage scavenger protein apoptosis inhibitor of macrophages (AIM) enhances the mycobactericidal activity of macrophages by increasing the levels of processed LC3 form and Beclin 1 (Sanjurjo et al. 2013). It has also been reported that during infection of macrophages with *Mycobacterium tuberculosis*, the cytosolic DNA sensor cyclic GMP-AMP synthase triggers STING/TBK1/IRF3-dependent interferon production (Watson et al. 2015). Autophagy is regulated by eukaryotic microRNAs including miR-155 in macrophages. Thus, during infection with *M. tuberculosis*, miR-155 enhances bacterial elimination and, via binding to the Ras homologue enriched in brain (Rheb), a negative regulator of autophagy, accelerates autophagy (Wang et al. 2013). It is well established that infection with *L. monocytogenes* induces autophagy in host cells (Rich et al. 2003). Toll-like receptor 2 (TLR2) and Nod-like receptors 1 and 2, acting via the downstream extracellular signal-regulated kinases, have been shown to play a crucial role for autophagy in *Listeria*-infected cells (Anand et al. 2011). The role of histone deacetylase 6

(HDAC6) during *L. monocytogenes* infection has been studied recently, and HDAC6 has been reported to control innate immune and autophagy responses to TLR-mediated signaling during infection with *L. monocytogenes* (Morenzo-Gonzalo et al. 2017). Additionally, Gluschko et al. (2018) have very recently reported that the in vivo infection of macrophages by *L. monocytogenes* leads to their interaction with the β-2 integrin macrophage-1 antigen (Mac-1), which activates Nox2 and induces the production of reactive oxidation species that subsequently leads to the recruitment of LC3 to *L. monocytogenes*-containing phagosomes.

10.2.1.3 Recruitment of Autophagy Receptors

Numerous studies have reported on the recruitment of autophagy receptors to intracellular bacteria in order to mediate their recognition by the host autophagy machinery. Intracellular *L. monocytogenes* is ubiquitinated and detected by the autophagy receptors SQSTM1 and NDP52 (Yoshikawa et al. 2009; Mostowy et al. 2011). In response to *M. tuberculosis* infection, SQSTM1 is phosphorylated by TBK1 which also coordinates the assembly and function of the autophagic machinery. The transmembrane protein STING recognizes *M. tuberculosis* extracellular DNA which is ubiquitinated, and the autophagy receptors SQSTM1 and NDP52 are recruited to it (Watson et al. 2012). The autophagy receptors SQSTM1 and NDP52 have been shown to be recruited to intracellular *S. typhimurium* independently of each other and with similar kinetics (Zheng et al. 2009; Thurston et al. 2009). The depletion of either of the receptors hampers autophagy. It has also been reported that SQSTM1 and NDP52 have convergent roles in mediating antibacterial autophagy (Cemma et al. 2011). Moreover, NDP52 has been reported to target bacteria to autophagosomes and thereby promote the maturation of *Salmonella*-containing autophagosomes by binding to LC3A, LC3B, GABARAPL2, and myosin VI (Verlhac et al. 2015). Thurston et al. (2012) have reported that galectin-8 (a danger receptor) recruits NDP52 to damaged SCVs and restricts the growth of *S. typhimurium* by autophagy. We have recently reported the involvement of another autophagy receptor, OPTN, in the growth inhibition of *L. monocytogenes* and that OPTN phosphorylation by TBK1 enhances the growth restriction of intracellular *L. monocytogenes* in an LLO-dependent manner (Puri et al. 2017). Moreover, OPTN and TAX1BP1 restrict the growth of *S. typhimurium* (Wild et al. 2011; Tumbarello et al. 2015). It has also been shown that the expression of the autophagy receptor NR1D1 increases the number of acidic vacuoles and the levels of processed LC3 and also modulates lysosome biogenesis during *M. tuberculosis* infection (Chandra et al. 2015).

10.2.2 Mechanisms of Autophagy Evasion by Bacteria During Infection

Several infection-causing bacteria have also devised strategies to evade autophagy during infection. *S. typhimurium* produces the virulence protein SseL which deubiquitinates *S. typhimurium*-induced aggregates which accumulate at SCV (Thomas et al. 2012). Another mechanism is the suppression of the overall autophagy by acting on the Akt-mTOR signaling pathway (Owen et al. 2014). *L. monocytogenes* expresses two phospholipases C, PlcA and PlcB, which allow escape from autophagosomes (Birmingham et al. 2007; Py et al. 2007). Additionally, it produces the surface-located protein actin assembly-inducing protein (ActA) which binds to host cell actin machinery. This, on the one hand, allows bacterial intracellular movement and, on the other hand, disguises the pathogen as a host cell organelle and thereby allows autophagosomal evasion (Yoshikawa et al. 2009). In the absence of ActA, *L. monocytogenes* harbors another protein, internalin K (InlK), which camouflages the pathogen from autophagic recognition as it interacts with the major vault protein (MVP) (Dortet et al. 2011). An interesting study by Mitchell et al. (2018) has reported that upon *L. monocytogenes* infection, noncanonical autophagy is activated, whereas growth-restricting xenophagy is inhibited in a FIP200- and TBK1-dependent manner. *M. tuberculosis* is capable of evading autophagy by various mechanisms including the expression of the early secretory antigenic target 6 (ESAT-6) system 1 (ESX-1) secretion-associated protein B (EspB) of *M. tuberculosis* which suppresses LC3B expression and autophagosome formation (Huang and Bao 2016). *M. tuberculosis* blocks also phagosomal maturation (via IL-27 induction) and can promote the intracellular growth of *M. tuberculosis* by the inhibition of IFN-γ- and starvation-induced autophagy (Sharma et al. 2014). *M. tuberculosis* growth is facilitated by the inhibition of autophagy by the overexpression of miR-30A (Chen et al. 2015). Another mechanism includes the enhanced intracellular survival (EIS) gene-dependent upregulation of IL-10 which acts, via acetylation of histone H3, on mTOR pathway and thereby suppresses autophagy (Duan et al. 2016). The phospholipase A2-dependent phagosome escape by some strains of *M. tuberculosis* is crucial because of their reduced capacity to tolerate phagosomal stresses, and it serves as a "virulence-rescue" mechanism which favors suppression of autophagy in macrophages (Jamwal et al. 2016).

10.2.3 Role of Autophagy in Crohn's Disease

Genome-wide association studies have implicated autophagy as an essential part in the pathogenesis of Crohn's disease (Hampe et al. 2007; Barrett et al. 2008). In particular, Rioux et al. 2007 have reported that the autophagy gene ATG16L1 is expressed in intestinal epithelial cells and its knockdown revokes the autophagy of *S. typhimurium*. Moreover, mice deficient in Nod2 have decreased expression of

α-defensins associated with Paneth cells and a severe defect in handling orally administered *L. monocytogenes* (Kobayashi et al. 2005). However, Atg16l1 hypomorphic mice are not deficient in handling *L. monocytogenes* despite differences in Paneth cell granule structure and composition (Cadwell et al. 2008). ATG16L1 T300A variant-transfected epithelial cells show impaired capture of internalized *Salmonella* within autophagosomes (Kuballa et al. 2008).

10.3 Autophagy as a Potential Therapeutic Target

With a plethora of studies on bacterial infections and autophagy, the current research should focus on the potential of autophagy as a therapeutic target for bacterial infections. A promising strategy in this direction could be to target bacterial factors that antagonize the functions of autophagy or autophagy factors. The inhibition of bacterial virulence factors which enable intracellular bacteria to escape autophagic recognition – such as ActA of *L. monocytogenes* or EspB of *M. tuberculosis* – could possibly enhance the xenophagic degradation of these bacteria and thereby provide an adjuvant therapy against bacterial infection. This approach may prove to be more specific and effective in the treatment of infections as it avoids the potential drawbacks associated with the manipulation of autophagy itself. Another strategy that can be employed to control bacterial infections is to exploit the autophagy receptor-bacteria interaction. Several autophagy receptors are known to bind to ubiquitinated bacteria and deliver them to autophagosomes like SQSTM1, NDP52, NBR1, optineurin, and TAX1BP1. Therefore, approaches that increase the interaction of autophagy receptors and bacteria, and also which augment certain modifications of autophagy receptors, like the phosphorylation of OPTN by TBK1 increases its LC3-binding affinity, may prove to be effective in enhancing the autophagic degradation of intracellular pathogens. Deciphering the molecular mechanisms of how autophagy receptors function could provide new avenues for the development of compounds that selectively enhance microbial autophagy. However, the downside of this strategy is that most autophagy receptors also mediate the selective autophagy of damaged cellular organelles and have other autophagy-independent functions; therefore, such manipulating these receptors may have undesired repercussions for the host.

Another plausible approach to target autophagy for antibacterial therapy involves the identification of novel autophagy-inducing compounds. Toward this end, chemical compounds can be screened on the basis of measurements of autophagosomal fluorescence (green fluorescent protein-LC3-positive puncta) by live-cell imaging methods, and the total LC3 levels can be determined by FACS analysis. Proteomic mapping methods like spatially restricted enzymatic tagging in living cells can be employed for the identification of autophagy-specific regulatory steps (Rhee et al. 2013). A caveat for compounds which modulate autophagy is that they usually induce other effects which may be unrelated to autophagy, thereby making it

difficult to determine the contribution of the autophagy to their therapeutic effects. It is known that some autophagy-inducing agents fail to induce their beneficial effects in host organisms lacking autophagy genes (Levine et al. 2015). The upregulation of autophagy has been shown to have promising effects in preclinical models of diseases, viz., trifluoperazine in *Salmonella* infection and statins in *M. tuberculosis* infection (Conway et al. 2013; Parihar et al. 2014). It is unknown whether the therapeutic effects shown by the clinically recommended concentrations of these agents correspond to considerable increase in autophagy induction.

Several compounds have been shown to modulate the autophagy of pathogenic bacteria. The treatment with isoniazid has been shown to activate autophagy and decrease the pro-inflammatory responses induced by *M. tuberculosis* in macrophages (Kim et al. 2012). The intracellular growth of *M. tuberculosis* has also been shown to be inhibited by autophagy induction upon treatment with the antiprotozoan drug nitazoxanide and its active metabolite tizoxanide (Lam et al. 2012). Lieberman and Higgins (2009) have shown that a small molecule called pimozide, which promotes autophagy (Zhang et al. 2007) and is used as an antipsychotic drug, inhibits *L. monocytogenes* infection. They have also reported that the antipsychotic drug thioridazine, also known to induce autophagy (Chen et al. 2015), inhibits vacuolar escape and the intracellular growth of *L. monocytogenes* in murine macrophages (Lieberman and Higgins 2010). It is, therefore, imperative to further examine the connection between antipsychotic drugs and their antibacterial and pro-autophagy effects. Simvastatin, a drug known to modulate cholesterol turnover and to enhance autophagy, also prevents the phagosomal escape of *L. monocytogenes* and thereby decreases infection in mice (Parihar et al. 2013).

10.4 Conclusions

Autophagy is an integral part of the host defense mechanism against infections. With current antibiotics being prone to drug resistance, alternate strategies should be adopted for the treatment of bacterial infections. Targeting autophagy as an additional novel therapeutic target apart from conventional antibacterial therapy has a promising potential, and that should be the focus of upcoming research in the field of pathogenic bacterial infections.

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References

Anand PK, Tait SW, Lamkanfi M, Amer AO, Nunez G, Pagès G, Pouysségur J et al (2011) TLR2 and RIP2 pathways mediate autophagy of *Listeria monocytogenes* via extracellular signal-regulated kinase (ERK) activation. J Biol Chem 286:42981–42991

Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD et al (2008) Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40:955–962

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

Birmingham CL, Canadien V, Gouin E, Troy EB, Yoshimori T, Cossart P, Higgins DE, Brumell JH (2007) *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. Autophagy 3:442–451

Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH (2008) Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. Nature 451:350–354

Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T (2005) p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171:603–614

Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK et al (2008) A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 456:259–263

Cemma M, Kim PK, Brumell JH (2011) The ubiquitin-binding adaptor proteins p62/SQSTM1 and NDP52 are recruited independently to bacteria-associated microdomains to target *Salmonella* to the autophagy pathway. Autophagy 7:341–345

Chandra V, Bhagyaraj E, Nanduri R, Ahuja N, Gupta P (2015) NR1D1 ameliorates *Mycobacterium tuberculosis* clearance through regulation of autophagy. Autophagy 11:1987–1997

Chen Z, Wang T, Liu Z, Zhang G, Wang J, Feng S et al (2015) Inhibition of autophagy by miR-30A induced by *Mycobacteria tuberculosis* as a possible mechanism of immune escape in human macrophages. Jpn J Infect Dis 68:420–424

Conway KL, Kuballa P, Songh JH, Patel KK, Castoreno AB, Yilmaz OH et al (2013) Atg16l1 is required for autophagy in intestinal epithelial cells and protection of mice from *Salmonella* infection. Gastroenterology 145:1347–1357

Dortet L, Mostowy S, Samba-Louaka A, Gouin E, Nahori MA, Wiemer EA, Dussurget O, Cossart P (2011) Recruitment of the major vault protein by InlK: a *Listeria monocytogenes* strategy to avoid autophagy. PLoS Pathog 7:e1002168

Duan L, Yi M, Chen J, Li S, Chen W (2016) *Mycobacterium tuberculosis* EIS gene inhibits macrophage autophagy through up-regulation of IL-10 by increasing the acetylation of histone H3. Biochem Biophys Res Commun 473:1229–1234

Dupont N, Lacas-Gervais S, Bertout J, Paz I, Freche B, Van Nhieu GT, van der Goot FG, Sansonetti PJ, Lafont F (2009) *Shigella* phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. Cell Host Microbe 6:137–149

Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T (2008) The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol Biol Cell 19:2092–2100

Glick D, Barth S, Macleod KF (2010) Autophagy: cellular and molecular mechanisms. J Pathol 221:3–12

Gluschko A, Herb M, Wiegmann K, Krut O, Neiss WF, Utermohlen O et al (2018) The β2 integrin Mac-1 induces protective LC3-associated phagocytosis of *Listeria monocytogenes*. Cell Host Microbe 23:324–337

Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K et al (2007) A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 39:207–211

Hanada T, Noda NN, Satomi Y et al (2007) The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. J Biol Chem 282:37298–37302

Huang D, Bao L (2016) *Mycobacterium tuberculosis* EspB protein suppresses interferon-γ-induced autophagy in murine macrophages. J Microbiol Immunol Infect 49:859–865

Jamwal SV, Mehrotra P, Singh A, Siddiqui Z, Basu A, Raoy KV (2016) Mycobacterial escape from macrophage phagosomes to the cytoplasm represents an alternate adaptation mechanism. Sci Rep 6:23089. https://doi.org/10.1038/srep23089

Khweek AA, Caution K, Akhter A, Abdulrahman BA, Tazi M, Hassan H, Majumdar N et al (2013) A bacterial protein promotes the recognition of the *Legionella pneumophila* vacuole by autophagy. Eur J Immunol 43:1333–1344

Kim JJ, Lee HM, Shin DM, Kim W, Yuk JM, Jin HS et al (2012) Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug action. Cell Host Microbe 11:457–468

Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, Ohsumi M, Takao T, Noda T, Ohsumi Y (2000) The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. J Cell Biol 151:263–276

Kirkin V, Lamark T, Sou YS, Bjørkøy G, Nunn JL, Bruun JA, Shvets E et al (2009) A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell 33:505–516

Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G et al (2005) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307:731–734

Kuballa P, Huett A, Rioux JD, Daly MJ, Xavier RJ (2008) Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. PLoS One 3(10):e3391. https://doi.org/10.1371/journal.pone.0003391 Epub 2008 Oct 13

Lam KK, Zheng X, Forestieri R, Balgi AD, Nodwell M, Vollett S et al (2012) Nitazoxanide stimulates autophagy and inhibits mTORC1 signaling and intracellular proliferation of *Mycobacterium tuberculosis*. PLoS Pathog 8:e1002691. https://doi.org/10.1371/journal.ppat.1002691

Lam GY, Cemma M, Muise AM, Higgins DE, Brumell JH (2013) Host and bacterial factors that regulate LC3 recruitment to *Listeria monocytogenes* during the early stages of macrophage infection. Autophagy 9:985–995

Levine B, Packer M, Codogno P (2015) Development of autophagy inducers in clinical medicine. J Clin Invest 125:14–24

Lieberman LA, Higgins DE (2009) A small-molecule screen identifies the antipsychotic drug pimozide as an inhibitor of *Listeria monocytogenes* infection. Antimicrob Agents Chemother 53:756–764

Lieberman LA, Higgins DE (2010) Inhibition of *Listeria monocytogenes* infection by neurological drugs. Int J Antimicrob Agents 35:292–296

Lin L, Baehrecke EH (2015) Autophagy, cell death, and cancer. Mol Cell Oncol 2:e985913. https://doi.org/10.4161/23723556.2014.985913

Lu K, Psakhye I, Jentsch S (2014) A new class of ubiquitin-Atg8 receptors involved in selective autophagy and polyQ protein clearance. Autophagy 10:2381–2382

Mitchell G, Cheng MI, Chen C, Nguyen BN, Whiteley AT, Kianian S, Cox JS et al (2018) *Listeria monocytogenes* triggers noncanonical autophagy upon phagocytosis, but avoids subsequent growth-restricting xenophagy. Proc Natl Acad Sci USA 115:E210–E217

Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107–132

Morenzo-Gonzalo O, Ramirez-Huresca M, Blas-Rus N, Cibrian D, Saiz ML, Jorge I, Camafeita E et al (2017) HDAC6 controls innate immune and autophagy responses to TLR-mediated signalling by the intracellular bacteria *Listeria monocytogenes*. PLoS Pathog 13:e1006799. https://doi.org/10.1371/journal.ppat.1006799 eCollection 2017 Dec

Mostowy S, Sancho-Shimizu V, Hamon MA, Simeone R, Brosch R, Johansen T, Cossart P (2011) p62 and NDP52 proteins target intracytosolic *Shigella* and *Listeria* to different autophagy pathways. J Biol Chem 286:26987–26995

Moy RH, Cherry S (2013) Antimicrobial autophagy: a conserved innate immune response in *Drosophila*. J Innate Immun 5:444–455

Newman AC, Scholefield CL, Kemp AJ, Newman M, McIver EG, Kamal A, Wilkinson S (2012) TBK1 kinase addiction in lung cancer cells is mediated via autophagy of Tax1bp1/Ndp52 and non-canonical NF-κB signaling. PLoS One 7:e50672

Noda T, Fujita N, Yoshimori T (2009) The late stages of autophagy: how does the end begin? Cell Death Differ 16:984–990

Ogawa M, Yoshikawa Y, Mimuro H, Hain T, Chakraborty T, Sasakawa C (2011) Autophagy targeting of *Listeria monocytogenes* and the bacterial countermeasure. Autophagy 7:310–314

Osawa T, Mizuno Y, Fujita Y, Takatama M, Nakazato Y, Okamoto K (2011) Optineurin in neurodegenerative diseases. Neuropathol Off J Japan Soc Neuropathol 31:569–574

Owen KA, Meyer CB, Bouton AH, Casanova JE (2014) Activation of focal adhesion kinase by *Salmonella* suppresses autophagy via an Akt/mTOR signaling pathway and promotes bacterial survival in macrophages. PLoS Pathog 10:e1004159. https://doi.org/10.1371/journal.ppat.1004159 eCollection 2014

Parihar SP, Guler R, Lang DM, Harukazu S, David Marais A, Brombacher F, Ratner AJ (2013) Simvastatin enhances protection against Listeria monocytogenes infection in mice by counteracting listeria-induced Phagosomal escape. PLoS One 8(9):e75490

Parihar SP, Guler R, Khutlang R, Lang DM, Hurdayal R, Mhlanga MM et al (2014) Statin therapy reduces the *Mycobacterium tuberculosis* burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. J Infect Dis 209:754–763

Proikas-Cezanne T, Takacs Z, Dönnes P, Kohlbacher O (2015) WIPI proteins: essential PtdIns3P effectors at the nascent autophagosome. J Cell Sci 128:207–217

Puri M, La Pietra L, Mraheil MA, Lucas R, Chakraborty T and Pillich H. 2017. Listeriolysin O regulates the expression of optineurin, an autophagy adaptor that inhibits the growth of *Listeria monocytogenes*. Toxins (Basel) 9:273. pii: E273. https://doi.org/10.3390/toxins9090273

Py BF, Lipinski MM, Yuan J (2007) Autophagy limits *Listeria monocytogenes* intracellular growth in the early phase of primary infection. Autophagy 3:117–125

Rhee HW, Zou P, Udeshi ND, Martell JD, Mootha VK, Carr SA et al (2013) Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. Science 339:1328–1331

Rich KA, Burkett C, Webster P (2003) Cytoplasmic bacteria can be targets for autophagy. Cell Microbiol 5:455–468

Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A et al (2007) Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39:596–604

Russell RC, Tian Y, Yuan H et al (2013) ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 15:741–750

Samson E (1981) Xenophagy. Br Dent J 150:136

Sanjurjo L, Amézaga N, Vilaplana C, Cáceres N, Marzo E, Valeri M et al (2013) The scavenger protein apoptosis inhibitor of macrophages (AIM) potentiates the antimicrobial response against *Mycobacterium tuberculosis* by enhancing autophagy. PLoS One 8:e79670. https://doi.org/10.1371/journal.pone.0079670 eCollection 2013

Sharma G, Dutta RK, Khan MA, Ishaq M, Sharma K, Malhotra H (2014) IL-27 inhibits IFN-γ induced autophagy by concomitant induction of JAK/PI3 K/Akt/mTOR cascade and up-regulation of Mcl-1 in *Mycobacterium tuberculosis* H37Rv infected macrophages. Int J Biochem Cell Biol 55:335–347. https://doi.org/10.1016/j.biocel.2014.08.022 Epub 2014 Sep 4

Tattoli I, Sorbara MT, Vuckovic D, Ling A, Soares F, Carneiro LA, Yang C, Emili A, Philpott DJ, Girardin SE (2012) Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program. Cell Host Microbe 11:563–575

Tattoli I, Sorbara MT, Yang C, Tooze SA, Philpott DJ, Girardin SE (2013) *Listeria* phospholipases subvert host autophagic defenses by stalling pre-autophagosomal structures. EMBO J 32:3066–3078

Thomas M, Mesquita FS, Holden DW (2012) The DUB-ious lack of ALIS in *Salmonella* infection: a *Salmonella* deubiquitinase regulates the autophagy of protein aggregates. Autophagy 8:1824–1826

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

Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482:414–418

Tumbarello DA, Manna PT, Allen M, Bycroft M, Arden SD, Kendrick-Jones J, Buss F (2015) The autophagy receptor TAX1BP1 and the molecular motor myosin VI are required for clearance of *Salmonella typhimurium* by autophagy. PLoS Pathog 11:e1005174. https://doi.org/10.1371/journal.ppat.1005174 eCollection 2015

Verlhac P, Viret C, Faure M (2015) Dual function of CALCOCO2/NDP52 during xenophagy. Autophagy 11:965–966. https://doi.org/10.1080/15548627.2015.1046672

Wang J, Yang K, Zhou L, Minhaowu WY, Zhu M et al (2013) MicroRNA-155 promotes autophagy to eliminate intracellular mycobacteria by targeting Rheb. PLoS Pathog 9:e1003697. https://doi.org/10.1371/journal.ppat.1003697 Epub 2013 Oct 10

Watson RO, Manzanillo PS, Cox JS (2012) Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 150:803–815. https://doi.org/10.1016/j.cell.2012.06.040

Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J (2015) The cytosolic sensor cGAS detects *Mycobacterium tuberculosis* DNA to induce type I interferons and activate autophagy. Cell Host Microbe 17:811–819. https://doi.org/10.1016/j.chom.2015.05.004 Epub 2015 Jun 2

Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B et al (2011) Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth. Science 333:228–233

Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, Mimuro H et al (2009) *Listeria monocytogenes* ActA-mediated escape from autophagic recognition. Nat Cell Biol 11:1233–1240

Zhang L, Yu J, Pan H, Hu P, Hao Y, Cai W, Zhu H, Yu AD, Xie X, Ma D, Yuan J (2007) Small molecule regulators of autophagy identified by an image-based high-throughput screen. Proc Natl Acad Sci USA 104:19023–19028

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