MINIREVIEW

Autophagy as an innate defense against mycobacteria

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A contemporary, comprehensive and timely review of autophagy in mycobacterial infections with particular relevance to tuberculosis. A review of this proportion supported by excellent figures has long been needed.

Keywords
mycobacteria; autophagy; vitamin D; cytokines; autophagy receptors; innate immunity.

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Abstract

Over the past several years, much has been revealed about the roles of autophagy and the mechanisms by which the autophagic pathway activates the host innate effector response against Mycobacterium tuberculosis (Mtb) infection. In response to invading mycobacteria, the host innate immune system not only recognizes pathogen motifs through innate receptors, it also produces appropriate effector proteins, including cytokines. These innate signals activate or regulate autophagic pathways during infection. It is now clear that vitamin D and functional vitamin D receptor signaling are critical in the activation of autophagic defenses against Mtb in human cells. Immunity-related GTPase family M proteins, including the cationic antimicrobial protein cathelicidin and autophagic receptor p62, participate in autophagic pathways that enhance antimicrobial activity against mycobacteria. Moreover, reactive oxygen species mediate antibacterial autophagy and successful antimicrobial responses during antibiotic chemotherapy. Recent work has also shown that pathogenic Mtb can be targeted by selective autophagy through an ESX-1 type VII secretion system. Here, we review the triggers, host factors, and intracellular pathways that regulate host autophagy and its impact on antimicrobial host defenses during mycobacterial infection.

Introduction

Tuberculosis (TB) is one of the most devastating infectious diseases globally. It is estimated that around one-third of the population is latently infected. The WHO reported that there were an estimated 8–9 million TB cases in 2010. TB is the second leading cause of death caused by an infectious disease, with a death rate of 1.2–1.5 million worldwide (including deaths from TB among HIV-positive individuals). Serious concerns have been raised regarding an increase in the number of patients with MDR-TB (WHO, 2011). Autophagy is a ubiquitous intracellular process through which diverse cytoplasmic cargos are captured and destroyed to replenish amino acids and energy sources during metabolic stress. During the host response to intracellular parasites, autophagy plays two roles: effector and regulatory roles. During mycobacterial infection, autophagy is essential for mounting an effective host response (Gutierrez et al., 2004; Singh et al., 2006).

If the Mycobacterium tuberculosis (Mtb) phagosome is not appropriately acidified through the selective exclusion of late endosomal Rab7 (Via et al., 1997), it persists in immature phagosomes within host macrophages. Autophagy acts as an immune effector, resulting in phagosomal maturation that mediates mycobacteria clearance (Gutierrez et al., 2004). Interferon (IFN)-inducible effector proteins, including immunity-related GTPase family M (Irgm) proteins, play a host protective function against mycobacterial infection through IFN-γ-induced autophagy (Singh et al., 2006). Additionally, human genetic approaches have identified Irgm gene variants that protect against Mtb infection (Intemann et al., 2009).

Recently, vitamin D receptor (VDR), a nuclear receptor that mediates various biological functions of 1,25(OH)2D3 (1,25D3), has been found to play an essential role in antimycobacterial responses through the activation of autophagy (Liu & Modlin, 2008; Fabri & Modlin, 2009; Jo, 2010). Cargo receptors, including p62/SQSTM1, have been featured in selective autophagy through the connection of ubiquitinated intracytoplasmic cargos into autophagic machinery (Kirkin et al., 2009). Moreover, the roles of reactive oxygen species (ROS) in antibacterial autophagy
have been emphasized (Huang et al., 2009; Shin et al., 2010a). Antibiotic-mediated triggering of bacterial and cellular ROS generation is essential for controlling the activation of autophagy, which is critical for a successful antimicrobial response to mycobacterial infection (Kim et al., 2012a). Thus, autophagy and its interactions with antimicrobial pathways can restrict mycobacterial pathogens in a coordinated and cooperative manner.

Although numerous previous studies showed that exogenous induction of autophagy can target mycobacteria, recent work has also revealed that Mtb phagosomal permeabilization through ESX-1 secretion system induces the ubiquitin-mediated autophagy pathway during natural infection of Mtb through the selective autophagic receptors p62 and NDP52 (Watson et al., 2012). In this review, we focus on autophagy as a host defensive strategy against mycobacterial infection and provide a recent update on the pathways and mechanisms by which autophagy connects with innate immunity to enhance antimicrobial responses against Mtb. We also emphasize the regulatory roles of autophagy in the prevention of inflammation during mycobacterial infection.

Overview of autophagy and innate immune receptors in mycobacterial infection

Autophagy is a process for maintaining intracellular quality control in the face of various stressors that mainly play a housekeeping role. So far, three autophagic pathways have been identified: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (referred to as autophagy throughout this review) initiates the formation of a new vesicle, the phagophore, which is enlarged, elongated, and generated into a double-membraned organelle, the autophagosome. The autophagosome fuses with lysosomes, maturing into an autolysosome. Here, cytoplasmic cargos are degraded. This mechanism is recognized as an essential host defense mechanism able to eliminate bacteria in macrophages and nonphagocytic cells infected with numerous bacteria, including Streptococcus, Shigella, Legionella, and Salmonella typhimurium (Nakagawa et al., 2004; Amer et al., 2005; Ogawa et al., 2005; Birmingham et al., 2006). Various bacterial pathogens have evolved strategies to escape from or subvert host autophagy to persist in or invade host cells.

Autophagy can also function as an innate effector and regulate innate immunity to various viruses and bacteria, including Mtb (Levine & Deretic, 2007; Schmid & Müñz, 2007; Yuk et al., 2012). Mtb is a successful intracellular pathogen that has evolved a successful strategy for evading host defenses by arresting phagosome maturation in infected host cells (Fratti et al., 2001; Vergne et al., 2004). The activation of autophagy induces co-localization of the mycobacterial phagosome with LC3 autophagosomes and delivers antimicrobial proteins to autolysosomal compartments, achieving enhanced bactericidal activity in phagocytes (Gutierrez et al., 2004; Singh et al., 2006). Understanding autophagy’s role in regulating innate immunity and vice versa will help elucidate the molecular basis of host defenses against mycobacteria (Fig. 1).

During mycobacterial infection, toll-like receptor (TLR) signaling plays an important role in the recognition of pathogens and elicits an inflammatory response, including the production of cytokines and antimicrobial effectors (Kleinnijenhuis et al., 2011; Yuk & Jo, 2011). Among theTLRs, TLR2, TLR9, and, possibly, TLR4 recognize Mtb and are involved in activation of the innate immune response (Kleinnijenhuis et al., 2011). Although there are controversial reports regarding the susceptibility of TLR2-deficient mice, in vivo studies using gene knockout mice of myeloid differentiation factor 88 (MyD88) and mice lacking both TLR2 and TLR9 revealed that these components are involved in host defensive pathways during mycobacterial infection (reviewed in Kleinnijenhuis et al., 2011; Saiga et al., 2011; Yuk & Jo, 2011). The protective role of a non-TLR, Nod-like receptor (NOD)2, was demonstrated in NOD2-deficient mice, which showed poor innate and adaptive immune responses and increased bacterial loads (Divangahi et al., 2008). Moreover, NOD2 participates in nonredundant mechanisms and promotes the production of cytokines leading to TLR2 activation (Ferwerda et al., 2005). Additionally, NOD2 plays a crucial role in the production of the inflammatory cytokines TNF-α and IL-1β in response to Mtb and Mycobacterium bovis BCG in human macrophages (Brooks et al., 2011).

Several atypical mycobacteria and their unknown components induce Dectin-1 signaling (Yadav & Shorey, 2006; Shin et al., 2008; Lee et al., 2009). Further, trehalose-6,6′-dimycolate (TDM, also called cord factor), a mycobacterial cell wall glycolipid, induces macrophage activation and the induction of Th17 immune responses through C-type lectin Mincle receptors (Ishikawa et al., 2009; Schoenen et al., 2010). TDM-induced inflammatory signaling is mediated through a class A scavenger receptor called macrophage receptor with collagenous structure (MARCO) and TLR2/CD14 (Bowdish et al., 2009). These and future studies of the nature and shaping of innate immune responses through the recognition of various innate receptors by mycobacteria and their components are important for understanding the role of autophagy and the molecular mechanisms by which autophagy combats the pathogenic strategies of intracellular pathogens.

Live Mtb infection and the Mtb protein ESAT-6 were shown to induce IL-1β and NLRP3 transcription and to activate the NLRP3/ASC inflammasome in human macrophages (Mishra et al., 2010). This was not observed in murine macrophages (Master et al., 2008). NLRP3 inflammasome activation may have suppressive roles in the promotion of autophagy and vice versa (Jo et al., 2012). It is beyond the scope of this review to describe the relationship between autophagy and the inflammasome. It is noteworthy, however, that specific mycobacterial components can induce inflammasome activation, thus accelerating the escape from autophagic pathways.

Crosstalk between autophagy and innate immunity in the fight against mycobacteria

TLR signaling and autophagy are linked: TLR signaling recognizes pathogen-associated molecular patterns through
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Pattern-recognition receptors (PRRs), and autophagic pathways can eliminate harmful pathogens. As many Mtb molecules act as ligands for TLRs and non-TLRs (reviewed in Jo et al., 2007; Kleinnijenhuis et al., 2011; Saiga et al., 2011), the recognition of mycobacterial molecules may initiate or regulate autophagy. Various TLR stimuli, in addition to Nod-like receptor-induced signals or CD40, can activate autophagic responses to induce host innate immunity and overcome a mycobacterial phagosome arrest (Sanjuan et al., 2007, 2009; Xu et al., 2007; Delgado et al., 2008; Orvedahl & Levine, 2009). It is of interest that, regardless of the TLR ligand stimulus, host autophagy activation can lead to the clearance of mycobacteria-containing phagosomes through the recruitment of autophagosomes and induction of phagosomal maturation (Xu et al., 2007; Delgado et al., 2008). Further, several crucial components of TLR signaling such as MyD88, TIR-domain-containing adapter-inducing interferon-β (TRIF), and mitogen-activated protein kinase (MAPK) are involved in the regulation of autophagy activation (Xu et al., 2007; Delgado et al., 2008; Shi & Kehrl, 2008).

Indeed, several mycobacterial components per se can activate or modulate host autophagy (Fig. 1). The well-known mycobacterial TLR2 ligand, 19-kDa lipoprotein, robustly activates autophagic responses in monocytes/macrophages. This only occurs with sufficient amounts of active vitamin D metabolites or with Cyp27b1 gene activation, followed by functional vitamin D signaling (Shin et al., 2010b). In contrast, Mtb eis downregulates autophagy, which is mediated by cellular- and NADPH oxidase-derived ROS generation (Shin et al., 2010a). Recent studies have revealed that Mtb eis is an efficient N(ε)-acetyltransferase that acetylates Lys55 of dual-specificity protein phosphatase 16 (DUSP16)/MAPK phosphatase-7 (MKP-7). ESAT-6 protein and Zmp1 may modulate autophagy through positive and negative regulation of the NLRP3 inflammasome, respectively. LpqH/19-kDa lipoprotein of mycobacteria promotes antibacterial autophagy through functional VDR signaling activation.

Fig. 1 Crosstalk between autophagy and innate immune signaling in mycobacterial infection. Mycobacteria trigger innate immune signaling through pathways involving diverse innate receptors, including TLRs (TLR2, TLR4, and TLR9) and non-TLRs (Dectin-1, Mincle, and NOD2). Several mycobacterial ligands activate innate immune pathways, thus affecting antibacterial autophagic responses in Mtb-infected macrophages. Mtb eis inhibits autophagy through the modulation of ROS and acetylation of dual-specificity protein phosphatase 16 (DUSP16)/MAPK phosphatase-7 (MKP-7). ESAT-6 protein and Zmp1 may modulate autophagy through positive and negative regulation of the NLRP3 inflammasome, respectively. LpqH/19-kDa lipoprotein of mycobacteria promotes antibacterial autophagy through functional VDR signaling activation.
tionally, mutant mice harboring variants of NOD2 (3020insC) showed elevated NF-κB activation in response to MDP and upregulated IL-1β secretion (Maeda et al., 2005). Moreover, mutant NOD2 (Cohn’s disease-associated NOD2 frame-shift mutation) failed to recruit ATG16L1 to the plasma membrane and induce autophagy, suggesting that NOD2 and autophagic pathways are functionally linked to increase bacterial killing and to maintain host immune homeostasis.

The mycobacterial NOD2 ligand MDP, which is N-glycolylated by N-acetyl muramic acid hydroxylase, shows more potent NOD2-stimulating immune activation in mouse models (Coulombe et al., 2008). Thus, mycobacterial N-glycolylated MDP may induce autophagy to a greater extent than N-acetylated MDP, the most common form in bacteria. It remains unclear whether mycobacterial signaling induced by the C-type lectin receptor Dectin-1 or Mincle, and by scavenger receptors, including MARCO, is involved in the activation or regulation of the autophagic pathway during infection. An understanding of the receptor-specific regulation and induction of autophagy by individual mycobacterial ligands or antigens may facilitate the development of effective therapeutic and vaccine strategies against human TB.

**Autophagy and cytokines in mycobacterial infection**

Expression of the Th1 cytokine IFN-γ, mainly by CD4+ T cells, is central to host defenses against TB (Harding & Boom, 2010). TNF-α plays a role in promoting host protective immunity during TB, as shown in cases of TB in patients treated with anti-TNF-α agents in a range of inflammatory/autoimmune diseases, including rheumatoid arthritis (Harris & Keane, 2010). Recent studies have provided insight into the roles of these protective cytokines in the activation of autophagy, thus promoting antimycobacterial immune defenses. IFN-γ enhances the autophagic control of Mtb, whereas the Th2 cytokines IL-4 and IL-13 inhibit those effects (Harris et al., 2007). IFN-γ and its effector mechanisms are mediated through multiple pathways: the induction of antimicrobial effectors, including inducible nitric oxide synthase 2, which may produce nitric oxide; the IFN-inducible GTPase family LRG47 (also known as Irgm) in mice (Macmicking et al., 2003); and upregulated expression of MHC class II molecules, which play a central role in antigen processing and presentation (Harding & Boom, 2010). Autophagy activation promotes antigen processing and presentation by MHC class II molecules and results in the bridging of innate and adaptive immune responses. In this aspect, IFN-γ may facilitate host protective immune responses through the connection of autophagy activation and innate immune activation for controlling Mtb infection. Mycobacteria also inhibit the Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway (Hussain et al., 1999), and STAT1 is required for protective immunity against mycobacteria (Sugawara et al., 2004; Vairo et al., 2011). It is unknown whether JAK-STAT signaling also promotes host autophagy activation and antimicrobial clearance.

Proinflammatory TNF-α plays an important role in the activation of phagosome maturation because TNF-α neutralization suppresses IFN-γ-induced phagosome maturation in primary human peripheral blood monocyte-derived macrophages (Harris et al., 2008). These results indicate that TNF-α may synergize IFN-γ-induced antimicrobial responses and autophagic responses against intracellular mycobacteria (Harris, 2011). Many other cytokines, including IL-2, CCL2, IL-6, and TGF-β, play key roles in positively regulating autophagy in various cell types (e.g. IL-2 in CD4+ T cells, CCL2 and IL-6 in CD11b+ peripheral blood mononuclear cells, and TGF-β in hepatocarcinoma cell lines) (reviewed in Harris, 2011). A variety of innate cytokines, including CCL2, IL-6, and TNF-α, are increased during the initiation of mycobacterial infection. These cytokines are involved in the development of granuloma, pathophysiology, and local immunity during TB (Cooper et al., 2011). Thus, it is important to determine the roles of each and/or combined cytokines in the control of TB.

Mtbc-induced IL-12p40 and IL-23p19 expression is negatively regulated in human macrophages by the mammalian targets of rapamycin (mTOR)/70-kDa ribosomal S6 kinase 1 (S6K1) pathway (Yang et al., 2006). These data suggest that IL-12/IL-23 is likely induced during autophagic pathway activation in mycobacterial infection. Additionally, IL-23 production by Mtbc in dendritic cells is mediated by the combined sensing of NOD2 and TLR2, whereas IL-12p70 production requires IFN-γ or the TLR7/8 ligand R848 (Gerosa et al., 2008). All of these signals play a crucial role in the activation of autophagy, suggesting a mechanistic link between protective cytokines and the induction of autophagy.

Autophagy negatively regulates the activation of transcription, processing, and secretion of several proinflammatory cytokines, including IL-1α, IL-1β, and IL-18 (Saitoh et al., 2008; Crişan et al., 2011; Harris et al., 2011; Nakahira et al., 2011; Zhou et al., 2011). It is beyond the scope of this review to comprehensively discuss the role of autophagy in the inhibition of inflammasome activation. However, the autophagic pathway negatively controls IL-1β production by at least two mechanisms (i.e. by targeting pro-IL-1β for degradation in lysosomal compartments and by inhibiting NLRP3 inflammasome activation) (Harris et al., 2011; Jo et al., 2012). Conversely, it was also reported that autophagy may play an important role in the induction of TNF-α transcription and secretion (Crişan et al., 2011). Another recent paper showed an appreciable contribution of autophagy to the synthesis and secretion of the proinflammatory cytokine IL-1β (Dupont et al., 2011). Multiple cytokines and mediators participate in the regulation of autophagic pathways to promote or inhibit host defenses (Fig. 2). The crosstalk between autophagic pathways and various activating/inhibiting cytokine stimuli may be influenced by the microenvironment, which affects the behavior of immune cells, or by functional properties of the innate receptors or signaling molecules that contribute to cytokine generation and the autophagic process.
Vitamin D and autophagy during mycobacterial infection

Global vitamin D insufficiency and proper VDR signaling are important in a variety of human diseases, including TB (Bouillon et al., 2008; Hewison, 2011a, 2012a). Recent studies have emphasized a role for the coordinated function of VDR signaling in the regulation of innate and adaptive immunity (Baek et al., 2010; Hewison, 2011a, b, 2012b). Through the autocrine or paracrine synthesis of 1,25D3 from the precursor 25-hydroxyvitamin D(3), vitamin D plays an important role in antibacterial activities through the induction of antibacterial proteins in various cell types, including phagocytes (Adams et al., 2007; Liu & Modlin, 2008; Jo, 2010). Several signals, including TLR2/1 stimulation, activate innate immune cells to express the enzyme 25-hydroxyvitamin D-1α-hydroxylase (Cyp27b1), which is critical for the intracrine conversion of 25-hydroxyvitamin D to active 1,25D3 (Liu et al., 2006; Krutzik et al., 2008; Liu & Modlin, 2008; Jo, 2010; Fabri et al., 2011a, b).

Vitamin D-induced human antimicrobial activity against Mtb is mediated through the cationic antibacterial protein cathelicidin LL-37 (Liu et al., 2006, 2007; Yuk et al., 2009; Shin et al., 2010b). Cathelicidins and defensins are part of innate immune antibiotics found in many species. These antimicrobial peptides function not only in the innate host defense against pathogens, especially in the airway, but also in the regulation of a variety of physiological functions, including immune activation, wound healing, angiogenesis, and migration (Zanetti, 2004; Bowdish et al., 2006; Teclé et al., 2010; Shin & Jo, 2011). Additionally, human cathelicidin LL-37/hCAP-18 mediates the induction of vitamin D-dependent autophagy and TLR-mediated vitamin D-dependent autophagy through the transcriptional regulation of the essential autophagy-related genes Beclin-1 and Atg5 and is recruited into autophagosomes after 1,25D3 or TLR2/1 stimulation (Yuk et al., 2009; Shin et al., 2010b). Further, the Th1 cytokine IFN-γ, but not the Th2 cytokine IL-4, upregulates cathelicidin and β-defensin 2 in human monocyte-derived macrophages cultured in media containing 25-D3 (Fabri et al., 2011a, b). In the same study, it was demonstrated that IFN-γ-induced autophagy and phagosome–lysosome fusion require vitamin D sufficiency and the activation of functional VDR signaling (Fabri et al., 2011a, b). These data suggest that vitamin D-dependent autophagy activation and antimicrobial responses are important for innate (TLR) and acquired (IFN-γ-dependent) mechanisms (Fig. 3), although these two immune arms use distinct receptors and signaling pathways (i.e. MyD88 and STAT1, respectively) (Yuk et al., 2009; Shin et al., 2010b; Fabri et al., 2011a, b).

Co-infection with Mtb and HIV potentiates the deterioration of immune functions and leads to premature death (Pawlowski et al., 2012). Additionally, both infections are highly associated with vitamin D insufficiency (Campbell & Spector, 2011; Luong & Nguyen, 2011; Khoo et al., 2012). In human macrophages, vitamin D induces the activation of autophagy, which is dependent on pathways involving phosphatidylinositol 3-kinase, Atg5, and Beclin-1. Autophagic pathways are required for the suppression of HIV-1 replication in vitro (Campbell & Spector, 2011). Black Africans in Cape Town are highly susceptible to active TB.
in the presence and absence of HIV infection, and they have a reciprocal relationship between seasonal variations in serum 25D3 status and the incidence of TB (Martineau et al., 2011). A meta-analysis demonstrated that certain VDR gene polymorphisms are significantly linked with the risk of TB (Gao et al., 2010). Moreover, multiple genes, including IFNG, NRAMP1, and NOS2A, have been suggested as candidate genes in association studies (Möller & Hoal, 2010). Depending on the vitamin D concentration, it would be interesting to examine whether certain VDR polymorphisms and the activation of autophagy are strongly associated with TB incidence and/or susceptibility. A combination of genetic and physiological knowledge is essential to understand the key factors driving pathogenesis and protective immunity against TB. This knowledge will improve the therapeutic development of customized patient profiles.

### Immunity-related GTPases and autophagy in mycobacterial infection

Immunity-related GTPases (IRG proteins) are an important innate resistance system against a variety of intracellular pathogens, including Mtb and Toxoplasma gondii (Mackmicking et al., 2003; Bekpen et al., 2010; Hunn et al., 2011). The IRG protein family consists of IFN-inducible GTPases belonging to two subfamilies (i.e. GMS and GKS) (Boehm et al., 1998; Bekpen et al., 2010). Among them, the GMS subfamily (Irgm1, Irgm2, and Irgm3) has a methionine (M) instead of a lysine (K) in the G1 motif, and it plays an essential function in the regulation of the GTPase cycle of GKS proteins and their normal accumulation at the parasitophorous vacuolar membrane after intracellular parasitic infection (Hunn et al., 2008; Bekpen et al., 2010). The best characterized IRG protein, Irgm1, plays an essential role in IFN-γ-dependent intracellular bacterial elimination through the activation of autophagy (Gutierrez et al., 2004). Additionally, it has various roles in immune regulation such as controlling macrophage motility by positioning of the GKS IRG protein (Irgb6) to the advancing lamellipodia (Henry et al., 2010) and in the protection of mature effector CD4+ T lymphocytes from IFN-γ-induced autophagic cell death (Feng et al., 2008). The GKS protein Irga6 also plays a role in the IFN-γ-mediated fusion of intracellular pathogens with autophagosomes and bacterial elimination (Al-Zeer et al., 2009).

Human Irgm, the sole human IRG, plays a key function in the induction of autophagy (Fig. 3). Several pathways involving rapamycin (mTOR inhibition) and IFN-γ lead to the activation of Irgm-mediated autophagy (Singh et al., 2006, 2010). Irgm contributes to the activation of autophagy by translocating to mitochondria, and it is required for mitochondrial fission, which is crucial for the autophagic control of intracelluarmycobacteria (Singh et al., 2010). Irgm also affects the localization of a second family of IFN-induced GTP-binding proteins, guanylate-binding proteins (GBPps). This leads to the accumulation of Gbp2 in LC3/p62-expressing intracellular compartments (Traver et al., 2011). Several GBP families, including Gbp1, Gbp2, and Gbp7, also play a key role in antimicrobial immunity against mycobacterial infection within phagocytes and in mice through coordination with multiple defense systems, including phagoctye oxidase, antimicrobial peptides, and autophagy effector activation (Kim et al., 2011). GBPps promote autophagy via an interaction with specific autophagy proteins; Gbp1 interacts with the SLR p62, while Gbp7 interacts with Atg4 (Kim et al., 2011). IRG proteins may contribute to host antibacterial defenses via the enforcement of an autophagy activation flux, the terminal stage of autophagic degradation, through mediating vesicular trafficking, fusion between organelles, and lysosomal function to clear intracellular cargos (Howard, 2008).

Human genetic studies have indicated the potential role of Irgm in the pathogenesis of TB and Crohn’s disease.
Genetic analysis has shown that an Irgm1 allele with two single-nucleotide polymorphisms (rs13361189 T/C and rs10065172 C/T) and a 20-kb deletion upstream of the coding region are associated with Crohn’s disease (Parkes et al., 2007). The allele with the 20-kb deletion is also associated with decreased Irgm levels (Mccarroll et al., 2008). These data suggest that defective Irgm expression affects the autophagic process against intracellular bacteria, resulting in the pathogenesis of Crohn’s disease (Mccarroll et al., 2008). Moreover, the Crohn’s disease-related T allele of rs10065172 is associated with TB susceptibility among African-Americans (King et al., 2011). The Irgm variant -261TT genotype is also significantly related to protection against human pulmonary TB caused by Mtb, but not M. africanum strains (Intemann et al., 2009). This polymorphism Irgm -261TT is associated with enhanced expression of mature Irgm, suggesting that increased Irgm promotes the autophagic degradation of intracellular bacteria (Intemann et al., 2009).

Although these studies suggest the pivotal roles of IRG and GBP proteins in host innate immunity, the detailed mechanisms by which IRG proteins influence immune resistance against infectious and inflammatory diseases are unknown. In addition, it is interesting how the same protein is involved in host susceptibility to both TB and Crohn’s disease. A better understanding of the roles of Irgm will be gained by comprehensive clinical genetic studies.

**Autophagy receptors during mycobacterial infection**

Recent studies have suggested the roles of autophagy receptors, including p62, NBR1 and NDP52, in selective antibacterial autophagy (von Muhlinen et al., 2010; Mostowy et al., 2011; Randow, 2011; Deretic, 2012; Kuballa et al., 2012). Ubiquitin-decorated bacteria are recognized by the autophagic adapters p62 and NBR1, which act as cytosolic bridges for pathogens/proteins to autolysosomes (Johansen & Lamark, 2011; Randow, 2011). p62 and NBR1 have domains that recognize cargo and participate in inflammatory responses. These proteins can also be selectively degraded by autophagy [reviewed in (Deretic, 2012)]. During mycobacterial infection, the autophagy receptor p62 eliminates Mtb through processing and the generation of antimicrobial peptides from cytosolic components (Ponpuak et al., 2010). In addition, p62 participates in the activation of the M. abscessus-induced NLRP3 inflammasome in human macrophages (Lee et al., 2011), suggesting a role for p62 in the induction of inflammatory processes. Although numerous studies have elucidated the roles of optineurin, NDP52, or NBR1 in bacterial targeting to autophagosomes (reviewed in Kuballa et al., 2012), much less is known about the roles of these autophagic adaptors during mycobacterial infection. Recent report has revealed that selective autophagy pathway targets phagosomal Mtb via recognition of Mtb DNA, which is translocated into cytosol through Mtb ESX-1 secretion system (Watson et al., 2012). Interestingly, cytosolic access of Mtb DNA leads to ubiquitination of bacteria that can be targeted by autophagy system via autophagic receptors p62 and NDP51, as well as TBK1 (Watson et al., 2012). Additional studies are needed to reveal the exact functions and mechanisms of several autophagic receptors and the crosstalk between innate immune signaling during mycobacterial infection.

**Role of ROS in the activation of host autophagy by antibiotic chemotherapy against mycobacteria**

An essential role for host autophagy activation has been reported in successful antimicrobial responses during antibiotic chemotherapy (Kim et al., 2012a). Isoniazid or pyrazinamide treatment significantly induces the autophagic flux in macrophages infected with Mtb. Antibiotics initiate the fusion of mycobacterial phagosomes with autophagosomes (Kim et al., 2012a), suggesting that antibiotic treatment leads to phagosomal maturation in Mtb-infected cells. As antimicrobial killing effects have been observed at later times (e.g. 3 days after), the activation of autophagy by antibiotic treatment plays an important role in intracellular killing in murine macrophages in vitro and in M. marinum-infected flies in vivo (Kim et al., 2012a). Previous studies have shown that bactericidal drug action is potentiated via hydroxyl radicals and oxidative damage of bacterial death pathways, regardless of the drug targets (Kohanski et al., 2007, 2008). Importantly, ROS derived from antibiotic-killed bacteria triggered the release of cellular ROS originating from NOX2 and mitochondria, which activated host cell autophagy (Kim et al., 2012a).

These findings provide new insight into the initial mechanism of pyrazinamide inside phagocytes and in vivo. Pyrazinamide does not have sterilizing activity against Mtb, unless it is activated in an acidic environment (pH 5.0–5.5) in vitro (Mcdermott & Tompsett, 1954). However, it is a paradox how pyrazinamide can be activated inside cells and in vivo. Pyrazinamide action may occur as drug-triggered hydroxyl radicals and host autophagy lead to the targeting of bacteria to more acidic compartments, which constitute a more appropriate environment for pyrazinamide action as well as autolysosomal degradation (Fig. 4). These data indicate that antituberculosis drugs promote host autophagy through ROS generation in infected cells and that the activation of autophagy is required for efficient bacterial clearance during chemotherapy against mycobacterial infection.

**Concluding remarks**

Studies of antimycobacterial autophagy have made considerable progress. Studies of innate immunity during mycobacterial infection have unveiled the crosstalk between PRR signaling and autophagic pathways. Essential roles for immune regulators such as cytokines in the modulation of autophagy and a new role for antimicrobial proteins in controlling autophagic pathways are gaining support. The activation of functional VDR signaling in macrophages represent a mechanism for inducing autophagy and antimicrobial responses against mycobacterial infection. Addition-
ally, IRG proteins play a crucial role in the activation of autophagy, and the actions of these proteins have clear clinical relevance to humans. Newly described cargo receptors such as p62 are being recognized to mediate xenophagy against mycobacteria through the selective clearance of ubiquitinated target bacteria or proteins. Progress in understanding the roles of ROS and antibacterial autophagy may help answer key questions such as how to develop new therapeutics targeting host autophagy against mycobacterial infection. Antibacterial autophagy either leads to the resolution of intracellular bacterial invasion or regulates excessive host inflammatory responses during mycobacterial infection. Future efforts will be directed not only toward further elucidation of the basic mechanisms of the autophagic pathways in innate immunity to mycobacteria, but also toward achieving protective and therapeutic benefits in human TB.

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Fig. 4 Mechanisms of antibiotic-induced autophagy in mycobacteria-infected cells. During chemotherapy, antibiotic-mediated autophagy activation is required to mount a successful antimicrobial response against mycobacteria. Antimycobacterial antibiotics, including isoniazid (INH) and pyrazinamide (PZA), can activate host autophagy to induce phagosomal maturation in Mtb-infected host cells. The treatment of Mtb-infected macrophages with antibiotics triggers bacterial hydroxyl radical generation, which activates ROS production, mainly through NADPH oxidase 2 and mitochondria. ROS-triggered signaling and intracellular increases in Ca$^{2+}$ and AMPK pathways are involved in the activation of antibiotic-mediated autophagy in Mtb-infected cells.

Fig. 4

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