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AUTOPHAGY AND IMMUNITY

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1.1 INTRODUCTION

Autophagy is a highly controlled process in which cellular components are self-degraded and subsequently recycled. This pathway in part plays a "house cleaning" role in the cell, directing numerous cargoes to the lysosome (or the vacuole in yeast and plants) for degradation. Depending on the specific conditions, the cargoes include random portions of cytoplasm, protein aggregates, and damaged or superfluous organelles such as mitochondria and peroxisomes. Dysfunction of autophagy is linked with many pathologies, including cancer, diabetes, myopathies, heart, liver and lung diseases, and certain types of neurodegenerative disease (Castets et al., 2013; Gonzalez et al., 2011; Klionsky and Codogno, 2013; Murrow and Debnath, 2013; Rubinsztein et al., 2012; Yang and Klionsky, 2010).

Emerging studies have revealed that autophagy plays important roles in immunity. In 2004, independent studies demonstrated for the first time that invading pathogens can be cargoes for autophagy (Gutierrez et al., 2004; Nakagawa et al., 2004). Today it is well accepted that autophagy can directly eliminate intracellular pathogens, including bacteria, fungal parasites, and viruses. Autophagy can also activate innate immune signaling cascades such as Toll-like receptor (TLR) signaling to attack invading pathogens (Lee et al., 2007; Xu et al., 2007). However, microbes constantly undergo strong selective pressure to develop strategies to block host defense mechanisms. Indeed, studies indicate that some adaptations that confer pathogenicity involve microbial inactivation or subversion of

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autophagy through distinct mechanisms (Deretic and Levine, 2009; Kuballa et al., 2012; Levine et al., 2011; Yuk et al., 2012; Zhou and Zhang, 2012).

Autophagy's role in immunity is not limited to controlling infection by direct elimination of pathogens. For example, autophagy facilitates MHC (major histocompatibility complex) antigen presentation, indicating that autophagy is involved in adaptive as well as innate immunity (English et al., 2009; Paludan et al., 2005). Moreover, defects in autophagy are associated with autoimmune diseases such as Crohn disease (Levine et al., 2011; Schroder and Tschopp, 2010; Shi et al., 2012). Thus, autophagy is an integral part of our response to infection and plays a key role in immunity. A comprehensive understanding of autophagy as it pertains to microbial infection and the molecular mechanisms that underlie the interplay between autophagy and immune signaling pathways may enable us to unravel the pathogenesis of many infectious and immune diseases, and develop more effective therapeutic strategies for their treatment.

1.2 AUTOPHAGY

1.2.1 Types of autophagy

There are three main types of autophagy: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Figure 1.1). CMA is a process where a cytosolic chaperone protein, HSPA8/HSC70, specifically recognizes its cargo proteins through a KFERQ-like

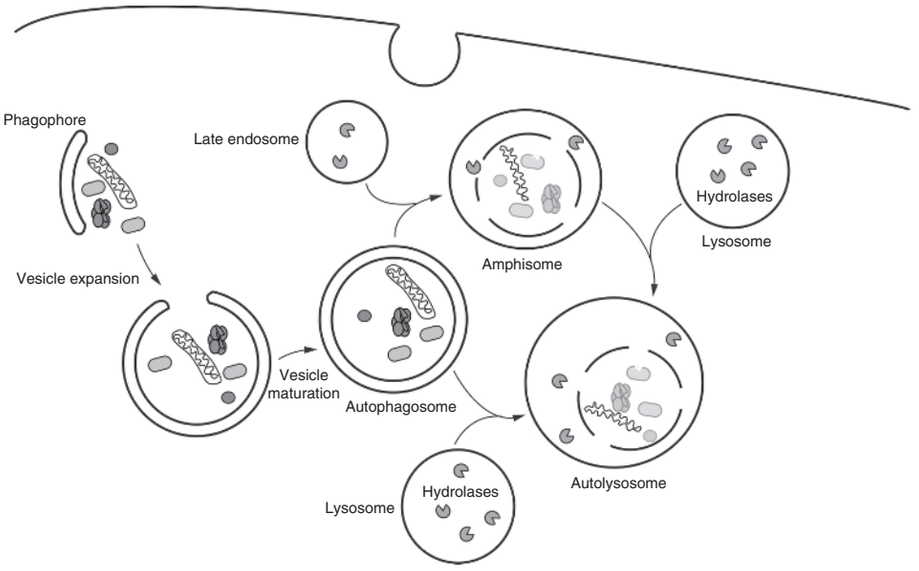

Figure 1.1. Schematic model of mammalian autophagy. Cargoes including cytosolic proteins, protein aggregates, and damaged organelles are sequestered by a phagophore, which will expand and mature to form a complete autophagosome. The outer membrane of the autophagosome fuses with either a late endosome (forming an amphisome, which then fuses with a lysosome) or lysosome, forming an autolysosome. Finally, the cargoes together with the inner membrane are degraded and the breakdown products are released back into the cytosol for reuse.

motif and facilitates their translocation directly across the lysosomal membrane for degradation (Dice, 2007; Kaushik and Cuervo, 2012). Microautophagy involves the uptake of portions of cytoplasm by the direct invagination or protrusion of the lysosomal or vacuolar membrane (Mijaljica et al., 2011). The third process, macroautophagy, hereafter referred to as autophagy, is the best characterized and will be the focus of this chapter.

1.2.2 Morphology

The morphological hallmark of autophagy involves the *de novo* formation of a double-membrane organelle named the autophagosome; however, this structure is essentially an end product of the sequestration process and as such is not really the primary functional unit of autophagy. Rather, the precursor to the autophagosome, the phagophore, is the dynamic membrane structure that is responsible for sequestering the cargos such as damaged organelles and invading pathogens (Figure 1.1). The phagophore expands with the addition of membrane, the sources of which are suggested to include almost every intracellular organelle. Upon completion, the phagophore seals and becomes a completed autophagosome. The autophagosome may fuse directly with a lysosome or, first, with a late endosome to form an intermediate amphisome. The subsequent fusion of the outer membrane of the autophagosome or the amphisome limiting membrane with a lysosome generates an autolysosome and exposes the cargoes to the degradative lysosomal enzymes. The degradation products, especially amino acids, are subsequently released back into the cytosol and are used in generating energy or as substrates for biosynthetic pathways.

1.2.3 Molecular machinery

Even though autophagosomes have been observed by electron microscopy as early as the 1950s, the molecular mechanisms of autophagy have been poorly studied until the past two decades (Stromhaug and Klionsky, 2001). The molecular machinery was first identified through studies in budding yeast, *Saccharomyces cerevisiae*, and to date more than 30 autophagy-related (*ATG*) genes have been identified as being involved in this process (Harding et al., 1995; Klionsky et al., 2003; Thumm et al., 1994; Tsukada and Ohsumi, 1993). Subsequent work with mammalian cells has revealed homologs of the core autophagy machinery (Xie and Klionsky, 2007), supporting the notion that autophagy is evolutionarily conserved. At the same time, there are also increasing numbers of ATG proteins being identified in mammals and other model systems such as *Caenorhabditis elegans* that lack yeast homologs, suggesting an increased complexity and diversity of function in higher eukaryotes (Klionsky and Codogno, 2013). For ease of discussion, the protein machinery of autophagy is subdivided into four major complexes in the following sections, and we focus on the mammalian autophagy machinery.

ULK1/ULK2 complex Autophagy occurs at a basal level in cells under normal conditions. Upon stress or other stimuli, autophagy can be induced, and defects in regulation that prevent proper induction can lead to aberrant cell physiology; however, too much autophagy activity can also be detrimental to the cell. Thus, the level of autophagy must be tightly controlled. Accordingly, there are various factors that regulate autophagy induction, and studies have shown that the ULK1/ULK2 (unc-51 like autophagy activating kinase 1/2) complex functions in part in an early stage of autophagy regulation.

ULK1 and ULK2 are kinases and the other components of the complex include ATG13, RB1CC1/FIP200 (RB1-inducible coiled-coil 1), and ATG101. ATG13 directly interacts

with ULK1/ULK2 and RB1CC1 regardless of the nutrient availability (Hosokawa et al., 2009; Jung et al., 2009); however, the phosphorylation status of these proteins changes under different conditions. In nutrient-rich conditions, a key upstream negative regulator of autophagy, the mechanistic target of rapamycin complex 1 (MTORC1) interacts with the complex and phosphorylates ULK1/ULK2 and ATG13, inhibiting ULK1/ULK2 kinase activity. Upon starvation, MTORC1 is released from the complex. ULK1/ULK2 and ATG13 are then partially dephosphorylated, leading to activation of ULK1/ULK2 kinase activity, which in turn leads to phosphorylation of ATG13 (presumably on distinct sites from those used by MTORC1) and RB1CC1 to induce autophagy (Chan, 2009; Hara et al., 2008; Hosokawa et al., 2009). AMPK (AMP-activated protein kinase) also binds ULK1/ULK2 and positively regulates autophagy through phosphorylation upon glucose starvation; as expected, AMPK and MTORC1 phosphorylate ULK1 at different sites (Kim et al., 2011; Zhao and Klionsky, 2011).

Class III phosphatidylinositol 3-kinase complexes The class III phosphatidylinositol 3-kinase (PtdIns3K) is generally thought to act downstream of the ULK1/ULK2 complex, mediating formation of phosphatidylinositol-3-phosphate (PtdIns3P) on the phagophore membrane, an event essential for autophagy. PtdIns3P serves to recruit downstream factors such as WIPI1 (WD repeat domain, phosphoinositide interacting 1) and WIPI2, which are involved in the trafficking of ATG9 and promote autophagosome maturation (Polson et al., 2010). In mammals, there are multiple class III PtdIns3K complexes with the core components being PIK3C3/VPS34 (phosphatidylinositol 3-kinase, catalytic subunit type 3), BECN1/Beclin 1 (beclin 1, autophagy related), and PIK3R4/VPS15/p150 (phosphoinositide-3-kinase, regulatory subunit 4). BECN1 can interact with several proteins, including AMBRA1 (autophagy/beclin-1 regulator 1), ATG14/ATG14L/Barkor, UVRAG (UV radiation resistance associated), KIAA0226/Rubicon and BCL2 (B-cell CLL/lymphoma 2) to form distinct complexes (Furuya et al., 2005; Itakura et al., 2008; Matsunaga et al., 2009; Petiot, 2000). BECN1 was first identified as a BCL2 binding protein. The interaction between BECN1 and BCL2 inhibits the binding of the former with PIK3C3, thus inhibiting autophagy. The ATG14–BECN1–PIK3C3–PIK3R4–AMBRA1 complex is specific for autophagy; ATG14 may direct this complex to the phagophore to promote autophagosome biogenesis (Itakura et al., 2008; Matsunaga et al., 2009), whereas the SH3GLB1 (SH3-domain GRB2-like endophilin B1)–UVRAG–BECN1–PIK3C3–PIK3R4 complex functions at a later step to promote autophagosome maturation (Itakura et al., 2008). In contrast, the KIAA0226–UVRAG–BECN1–PIK3C3–PIK3R4 complex localizes to late endosomes and negatively regulates autophagosome maturation (Matsunaga et al., 2009).

ATG9 trafficking system The Atg9 trafficking system is best characterized in yeast, although even in that model organism there are many questions that remain to be answered. The current model is that the transmembrane protein Atg9 cycles between the phagophore assembly site (PAS) and peripheral (i.e., non-PAS) sites, and that this process is needed for the proper delivery of membrane from various donor organelles to the expanding phagophore (Noda et al., 2000; Reggiori et al., 2005). Atg23 and Atg27 interact with Atg9 and facilitate its anterograde traffic from the peripheral sites to the PAS, whereas Atg2–Atg18 and the Atg1–Atg13 complex (yeast homolog of the ULK1/ULK2 complex) are required for its retrograde transport from the PAS back to the peripheral sites (Guan et al., 2001; Reggiori et al., 2004; Wang et al., 2001; Yen et al., 2007).

In mammals, ATG9 localizes to the *trans*-Golgi network and endosomes in nutrient-rich conditions. A pool of ATG9 translocates to MAP1LC3 (microtubule-associated protein 1 light chain 3)/LC3-positive compartments upon starvation. This translocation is dependent on ULK1 and PIK3C3 kinase activity (Young et al., 2006). The dynamic movement between ATG9 and the phagophore membrane during autophagy suggests a conserved role for ATG9 in membrane movement during phagophore expansion. Similar to yeast, ATG9 retrieval from the phagophore membrane is dependent on WIPI2, a homolog of yeast Atg18, but movement in this direction is ULK1 kinase independent (Orsi et al., 2012).

Ubiquitin-like conjugation systems There are two ubiquitin-like (Ubl) conjugation systems, which involve the Ubl proteins ATG12 and LC3. These systems are quite well-studied, playing important roles in phagophore expansion and maturation (Ichimura et al., 2000; Mizushima et al., 1998, 2001). ATG12 is conjugated with ATG5 in a manner that is similar to canonical ubiquitination (Mizushima et al., 1998). The E1-like enzyme ATG7 activates ATG12 via a thioester bond (Tanida et al., 2001). ATG12 is then transferred to an E2-like enzyme, ATG10, before it is finally conjugated to an internal lysine of ATG5. ATG5 then noncovalently binds ATG16L1 (autophagy related 16-like 1 (*S. cerevisiae*)), which subsequently dimerizes. During autophagy, ATG5 directs the ATG12—ATG5–ATG16L1 complex to the phagophore (Mizushima, 2003).

The different isoforms of LC3 (and the related GABARAP (GABA(A) receptor-associated protein) subfamily proteins) are conjugated to the lipid phosphatidylethanolamine (PE), and this modification is required for association with the phagophore membrane (Kabeya et al., 2004; Tanida et al., 2003). Initially, the cysteine protease ATG4B removes the C-terminal amino acids of pro-LC3 to reveal a glycine residue, generating a cytosolic form named LC3-I. LC3-I is then sequentially activated by ATG7 and conjugated via the E2-like enzyme ATG3, resulting in the membrane-associated form, LC3-II (Tanida et al., 2001, 2002). The PE group can ultimately be cleaved by ATG4B in a deconjugation step, which is important for maintaining the proper level of autophagy activity (Tanida et al., 2006).

1.2.4 Physiological roles

Autophagy has many physiological roles. First, autophagy is a protective mechanism against cellular stress (Kuma et al., 2004; Yang and Klionsky, 2010). For example, autophagy's role in supplying essential building blocks or metabolic substrates such as amino acids under conditions of nutrient deprivation is critical for maintaining cell viability under adverse conditions; autophagic degradation and recycling enable cells to maintain the synthesis of essential proteins and to generate ATP.

Recent studies indicate that autophagy is also indispensible during development. One example of such a role is seen after oocyte fertilization in *C. elegans*, where autophagy is involved in the elimination of maternal mitochondria (Al Rawi et al., 2011; Sato and Sato, 2011); however, this does not appear to be the case in mammals (Luo et al., 2013). In addition, during embryonic development, clearance of apoptotic cells is achieved through autophagy (Qu et al., 2007). Autophagy is also implicated in life span extension; induction of autophagy increases longevity in several model organisms (Rubinsztein et al., 2011) and its role in clearing aggregate-prone proteins and damaged mitochondria might be relevant to its antiaging effects.

As autophagy acts to eliminate many harmful components in a cell, malfunction of autophagy has also been suggested to correlate with or be the cause of a variety of diseases, such as cancer, neurodegeneration, cardiovascular myopathies, and lysosomal storage disorders (Klionsky and Codogno, 2013). For example, the selective degradation of damaged mitochondria is suggested to underlie the tumor suppressive effects of autophagy, possibly through reducing oxidative stress and preventing DNA damage (Narendra et al., 2008). Several lines of evidence suggest that the role of autophagy in clearing toxic aggregate-prone proteins is critical to prevent certain types of neurodegeneration, including those associated with Huntington, Alzheimer, and Parkinson diseases (Bjørkøy et al., 2005; Ravikumar et al., 2002).

1.3 AUTOPHAGY AND IMMUNITY

1.3.1 Xenophagy: autophagic clearance of intracellular microorganisms

For decades, scientists have explored how our body fights against invading pathogens. Even though an understanding of our immune systems has steadily increased, a major problem, how a cell breaks down an intracellular pathogen without harming itself, has been overlooked or at least unanswered. Only recently have researchers realized that autophagy plays a vital role in this process. This specific type of autophagy is termed "xenophagy."

Autophagic degradation of bacteria and parasites Several independent studies have revealed that xenophagy acts to eliminate many different bacteria and other microbes (Levine et al., 2011; Yuk et al., 2012). A good example of parasite clearance is seen with *Toxoplasma gondii* (Andrade et al., 2006). This parasite is able to survive within macrophages by residing in parasitophorous vacuoles that are modified to avoid fusion with lysosomes. However, stimulation of *T. gondii*-infected macrophages with CD40 (CD40 molecule, TNF receptor superfamily member 5), a member of the TNF (tumor necrosis factor) receptor superfamily, causes colocalization of parasitophorous vacuoles and LC3. Conversely, treatment of infected cells with the autophagy inhibitor 3-methyladenine (3-MA) or knockdown of BECN1 blocks the fusion of parasitophorous vacuoles with lysosomal compartments (Andrade et al., 2006). Thus, these results suggest that phagophores capture parasites that are residing within these vacuoles and direct them to the lysosome for degradation.

As for bacterial clearance, evidence indicates that autophagosomes can sequester both bacteria that reside within membranous compartments and those present free within the cytosol, through mechanisms that are overlapping, but distinct (Figure 1.2) (Levine and Deretic, 2007). The clearance of *Mycobacterium tuberculosis* is a good example of engulfment of bacteria residing within phagosomes (Gutierrez et al., 2004). After entering the cell through endocytosis, *M. tuberculosis* can actively survive in a host cell and evade the host defense by inhibiting phagosomal maturation. However, if autophagy is induced by either nitrogen starvation or rapamycin treatment, the inhibition of phagosomal maturation by *M. tuberculosis* is suppressed and intracellular bacterial survival is significantly decreased. Also, a substantial colocalization of *M. tuberculosis*-containing phagosomes with autophagosomes is observed upon autophagy induction, supporting the idea that phagophores capture bacteria residing within phagosomes and target them to lysosomal compartments for degradation (Gutierrez et al., 2004).

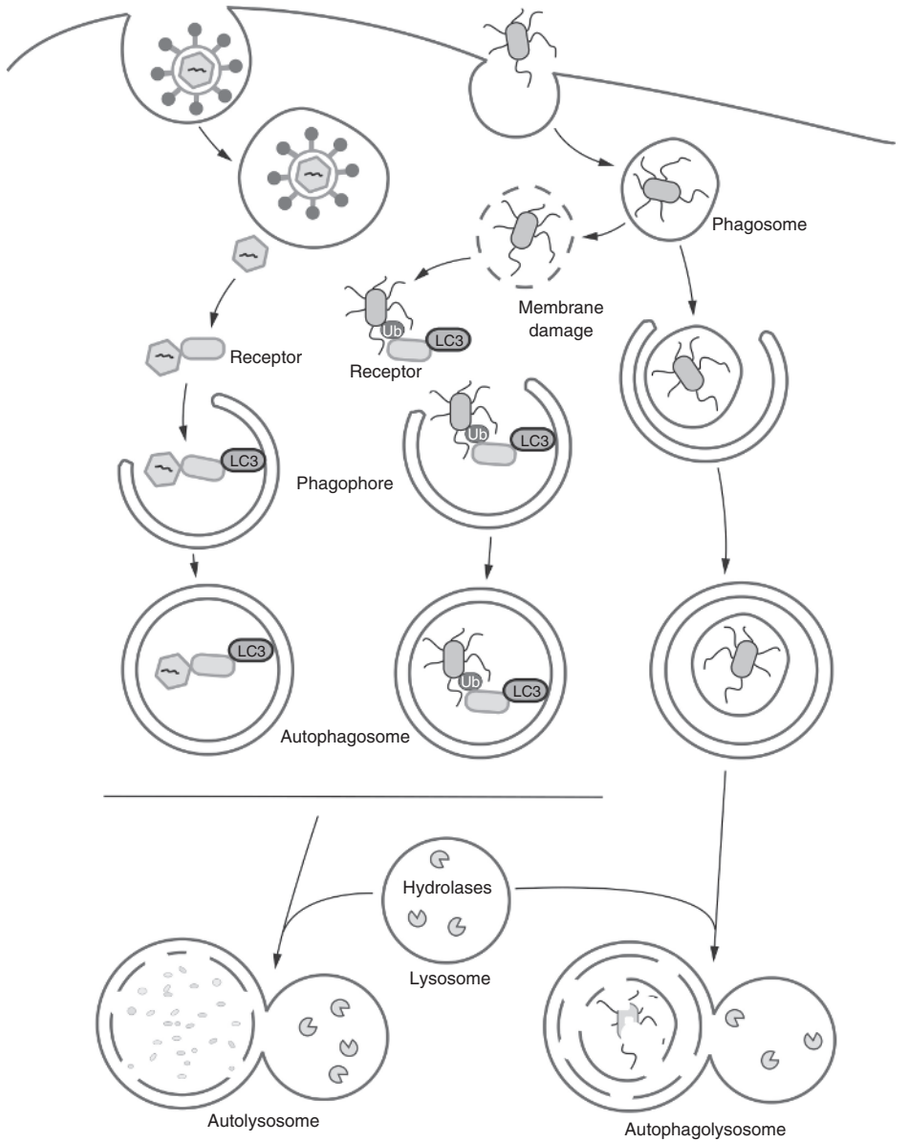

Figure 1.2. Models of autophagic elimination of invading pathogens. Intracellular virus proteins are recognized by autophagy receptors and recruited to autophagosomes by interaction between the receptors and LC3. Both bacteria within phagosomes and bacteria that have escaped from phagosomes can be degraded through autophagy. Bacteria residing in a phagosome can be engulfed by a phagophore; after completion of sequestration, the resulting autophagosome then fuses with a lysosome forming an autophagolysosome. (Note that we strongly recommend that this term be reserved to describe the compartment that results from the fusion of lysosomes with autophagosomes containing phagosomes, and not for the compartments that result from the fusion of other autophagosomes with lysosomes, which are termed autolysosomes.) Some bacteria are able to damage the phagosomal membrane and escape into the host cell cytoplasm. These cytosolic bacteria are polyubiquitinated and recognized by autophagy receptor proteins, directing their delivery to phagophores.

With regard to cytosolic bacteria, a major problem/challenge is that these microbes need to be specifically recognized and distinguished from other "self" endomembranes, including their endosymbiotic descendants, the mitochondria. Starvation-induced autophagy is usually nonselective, but there are also selective types of autophagy. Recent studies of selective autophagy reveal a common cargo–ligand–receptor–scaffold model (Mijaljica et al., 2012). A receptor protein recognizes ligands on cargoes and at the same time binds the scaffold protein of the autophagy machinery, selectively targeting cargoes into the autophagy pathway. Specific receptors have been identified that recognize intracellular bacteria during xenophagy, including SQSTM1/p62 (sequestome 1), NBR1 (neighbor of BRCA1 gene 1), CALCOCO2/NDP52 (calcium binding and coiled-coil domain 2) and OPTN (optineurin) (Kraft et al., 2010; Thurston et al., 2009; Wild et al., 2011; Zheng et al., 2009). Usually, cytosolic intracellular bacteria are coated with polyubiquitin, and these receptors are able to simultaneously bind the ubiquitinated bacteria and LC3, linking the cargo with the autophagy machinery. In this way, intracellular bacteria are specifically targeted for degradation.

Despite the utility of xenophagy in degrading intracellular bacteria, certain pathogens have been successful in developing strategies for evading autophagy. One example of such evasion is seen with *Listeria monocytogenes* (Birmingham et al., 2007). After infection of its host macrophages, a population of *L. monocytogenes* damages phagosomes and is released into the cytosol, where they will ultimately be recognized by autophagy. However, the expression of the virulence factor ActA triggers host cell actin polymerization. This provides the bacteria with actin-based motility, which allows cell-to-cell spread and avoidance of autophagic degradation.

Autophagic elimination of viruses The cargo of xenophagy is not restricted to protozoan parasites and bacteria; autophagy can also capture invading viruses. In general, the mechanism involved in the recognition of viruses and their sequestration by phagophores is conceptually similar to that of cytosolic bacteria (Figure 1.2). For example, after Sindbis virus infects the mouse central nervous system, SQSTM1 interacts with Sindbis virus capsid proteins, mediating their further degradation through autophagy (Orvedahl et al., 2010). This action significantly reduces virally-induced cell death.

Similar to bacteria, many viruses also act to inhibit autophagy to confer virulence. First, numerous viruses can either inhibit antiviral signaling pathways that induce autophagy or they can activate an autophagy inhibitory pathway. EIF2AK2/PKR (eukaryotic translation initiation factor 2-alpha kinase 2) is an interferon-inducible double-stranded RNA sensor that mediates overall downregulation of translation in host cells via phosphorylation of EIF2A (eukaryotic translation initiation factor 2A, 65 kDa). This signaling pathway also positively regulates virus-induced autophagy (Levine and Deretic, 2007). Viruses develop multiple strategies to block the EIF2AK2 pathway. For example, during infection herpes simplex virus type 1 (HSV-1) expresses the US11 protein to antagonize EIF2AK2-mediated phosphorylation of EIF2A by binding to the kinase, thus preventing autophagy induction (Lussignol et al., 2013). As discussed above, MTOR signaling is a negative regulator of autophagy. Upon infecting dendritic cells, human immunodeficiency virus-1 (HIV-1) downregulates autophagy by inducing MTOR and RPS6KB/p70 S6 kinase (ribosomal protein S6 kinase, 70 kDa) activation, thus promoting viral proliferation in host cells (Blanchet et al., 2010).

In addition, a virulence factor may also directly target the autophagy machinery to negatively regulate autophagy. For example, the HSV-1 protein ICP34.5 binds BECN1 to block autophagy, possibly through inhibiting PIK3C3 kinase activity (Orvedahl et al., 2007).

Another example of viral evasion of autophagy is seen with viral homologs of CFLAR/FLIP (CASP8 and FADD-like apoptosis regulator) encoded by Kaposi's sarcoma-associated herpesvirus, herpesvirus saimiri, and molluscum contagiosum virus that can directly interact with ATG3 (Lee et al., 2009), the E2-like enzyme mediating conjugation of LC3 with PE. The interaction between viral CFLAR proteins and ATG3 prevents the latter from binding to LC3, thus limiting autophagosome biogenesis.

Moreover, some viruses induce autophagy but block autophagic degradation, facilitating viral replication in host cells. After infection of human hepatoma cells, hepatitis B virus X protein binds to class III PtdIns3K to enhance its activity, thus promoting autophagy initiation (Sir et al., 2010). However, this induction of autophagy is not accompanied with increased autophagic protein degradation. In fact, induction of an autophagic response enhances viral DNA replication, whereas knocking down ATG7 significantly reduces the hepatitis B virus DNA level in infected host cells (Sir et al., 2010). Thus, by inducing autophagosome formation but blocking autophagosome clearance and degradation, the viruses establish a replicative niche within this compartment.

To summarize, xenophagy acts to protect host cells by direct elimination of invading pathogens; however, intracellular pathogens have also developed multiple strategies for evading autophagy to confer pathogenicity. Our discussion of this topic only provides general information about xenophagy, and several chapters in this book will go into much greater detail about autophagy's roles in defense against bacterial and viral infection.

1.3.2 Autophagy and cryptides

Another example of autophagy's role in innate immunity is the involvement of autophagy in generating cryptides (neoantimicrobial peptides) from cytosolic proteins that act against intracellular microbes (Ponpuak and Deretic, 2011). As mentioned above, the bacteria *M. tuberculosis* can actively survive in phagosomes in host cells via inhibiting phagosomal maturation. Aside from direct elimination of those bacteria residing within phagosomes (Figure 1.2), autophagy also facilitates killing the bacteria through delivery of cryptides into the mycobacteria-containing phagosomes.

In *M. tuberculosis*-infected cells, the autophagy receptor protein SQSTM1/p62 captures cytosolic microbicidal proteins such as FAU (a fusion of a ubiquitin-like protein with RPS30) in autophagosomes, mediating their proteolytic degradation into smaller peptides with antimycobacterial activity (i.e., cryptides), which are then conveyed to mycobacteria-containing phagosomes (Ponpuak et al., 2010). Extracts from phagosomes purified from wild-type cells induced for autophagy show a substantial killing capacity for *M. tuberculosis in vitro*, but not those from SQSTM1 knockdown cells (Ponpuak et al., 2010). These results support the idea that autophagic delivery of cryptides to bacteria-containing phagosomes facilitates the control of microbial infection.

1.3.3 Autophagy and pattern recognition receptors (PRRs)

As part of the innate immune system, pattern recognition receptors (PRRs) recognize foreign microbial pathogen-associated molecular patterns (PAMPs) to trigger immune signaling cascades to defend against invading pathogens (Takeuchi and Akira, 2010). Recent studies implicate autophagy's role in delivery of PAMPs to PRRs. One of the first lines of evidence came from a study with a negative-strand RNA virus, vesicular stomatitis virus (VSV) (Lee et al., 2007). In plasmacytoid dendritic cells (pDCs), endosomal TLR7 detects viral nucleic acids and turns on a downstream signaling cascade to mediate robust

IFNA (interferon, alpha) production. However, pharmacological inhibition of autophagy in VSV-infected pDCs diminishes viral recognition by TLR7 and IFNA production. Moreover, intact autophagy machinery is required for this process; ATG5-deficient pDCs express significantly less IFNA and IL12 (interleukin 12 (natural killer cell stimulatory factor, cytotoxic lymphocyte maturation factor)) upon VSV infection (Lee et al., 2007). Thus, it is suggested that cytosolic viral nucleic acids are delivered to endosomal TLRs through the autophagy pathway, initiating a downstream innate immune response against the pathogens.

Moreover, PRRs can act as intracellular sensors for autophagy activation. For example, in primary human macrophages, lipopolysaccharide induces autophagy in a TLR4 signaling-dependent manner (Xu et al., 2007). In addition, cytosolic NOD-like receptors, NOD1 and NOD2 (nucleotide-binding oligomerization domain containing 1 and 2), control bacterial infection by activating autophagy (Travassos et al., 2010). NOD1 and NOD2 detect peptidoglycan and recruit ATG16L1 to the plasma membrane to initiate autophagy, blocking bacterial entry. This process may be relevant to the pathogenesis of Crohn's disease, which will be discussed later in this chapter.

1.3.4 Autophagy and MHC antigen presentation

Autophagy not only plays important roles in innate immunity but is also involved in adaptive immunity. The innate immune system generally or nonspecifically detects foreign antigens, whereas the adaptive immune system initiates antigen-specific responses through a process called antigen presentation. By this process, the adaptive immune system distinguishes the host cell-expressed "self" antigens versus pathogen-expressed "non-self" or foreign antigens. After foreign antigens loaded on to major histocompatibility complex molecules are recognized by a T cell receptor on immature T lymphocytes, the latter mature and become activated to fight against pathogens. There are two types of MHC molecules: class I and class II, which present antigens at the cell surface to $CD8^+$ and $CD4^+$ T cells, respectively (Neefjes et al., 2011). Emerging evidence has indicated that autophagy plays a role in both MHC class I and class II antigen presentations.

MHC class I antigen presentation In the process of MHC class I antigen presentation, endogenous antigens such as viral proteins synthesized by infected host cells are degraded in the cytoplasm by proteasomes and are then translocated to the endoplasmic reticulum, where they are loaded on to MHC class I molecules; this process involves autophagy. For example, at 6–8 h postinfection of macrophages with HSV-1, $CD8^+$ T cell activation induced by MHC class I processing of glycoprotein B (gB) peptide is dependent on autophagy (English et al., 2009). Either pharmacological inhibition of autophagy by 3-MA or genetically knocking down ATG5 leads to significantly decreased activation of gB-specific $CD8^+$ T cells. Also, macrophages infected with an HSV-1 mutant lacking ICP34.5 stimulate gB-specific $CD8^+$ T cells more efficiently than those infected with wild-type HSV-1, further suggesting that autophagy promotes MHC class I antigen loading.

MHC class II antigen presentation Autophagy is required for exogenous HIV-1-derived antigen presentation to MHC class II molecules (Blanchet et al., 2010). Compared to control cells, HIV-1-infected dendritic cells in which LC3A and LC3B are knocked down by siRNA show a strikingly decreased efficiency in presenting HIV-1-derived exogenous antigen to $CD4^+$ T cells. Similarly, treatment of DCs with 3-MA causes decreased antigen-mediated $CD4^+$ T cell responses. These results support the idea that autophagy facilitates exogenous antigen loading on MHC class II molecules.

Although long-standing dogma suggested that only exogenous antigens are loaded on MHC class II molecules, it is now known that cytosolic (endogenous) proteins can also be presented via MHC class II molecules through autophagy, including tumor-related antigens, bacterial peptides, and viral proteins (Dengjel et al., 2005; Dorfel et al., 2005; Irla et al., 2010; Paludan et al., 2005). For example, endogenous Epstein–Barr virus nuclear antigen 1 (EBNA1) is directed to lysosomes for antigen processing through autophagy, and subsequently loaded on MHC class II molecules (Paludan et al., 2005). Inhibition of lysosomal acidification causes accumulation of EBNA1 in autophagosomes. Genetic inhibition of autophagy by knocking down ATG12 leads to downregulation of MHC class II-restricted $CD4^+$ T cell recognition of EBNA1. Moreover, mice with dendritic cell-conditional deletion of *Atg5* show diminished $CD4^+$ T cell priming after HSV-1 or *Listeria monocytogenes* infection (Lee et al., 2010). The *Atg5*-deficient DCs are defective in processing and presenting phagocytosed antigens to MHC class II. These lines of evidence confirm the indispensible role of autophagy in mediating the presentation of cytosolic antigens on MHC class II molecules.

1.3.5 Autophagy regulation by immune signaling molecules

We have discussed much about how autophagy regulates innate and adaptive immunity, but what about the converse—do immune signaling molecules regulate autophagy? The answer is "yes." Immune signaling molecules that positively regulate autophagy include EIF2AK2, IFNG (interferon, gamma), TNF, CD40, and PRRs such as TLRs and NOD-like receptors (Andrade et al., 2006; Cooney et al., 2010; Tallóczy et al., 2002; Travassos et al., 2010; Xu et al., 2007). In contrast, autophagy is inhibited by NFKB (nuclear factor of kappa light polypeptide gene enhancer in B cells) and T helper 2 cytokines such as IL4 (interleukin 4) and IL13 (Levine and Deretic, 2007). Even though for most cases the molecular mechanisms of autophagy regulation by immune signaling molecules are still not clear, there are examples of physical interactions between these molecules and the autophagy machinery that may be relevant. For example, as discussed above, NOD-like receptors NOD1 and NOD2 recruit ATG16L1 to the plasma membrane to block bacterial entry (Travassos et al., 2010). Nonetheless, future studies are still needed to shed more light on the mechanisms through which immune signaling molecules regulate autophagy.

1.3.6 Autophagy, inflammation, and autoimmunity

Autophagy's newly identified role in clearance of inflammasomes strongly implicates the importance of autophagy in inflammation (Shi et al., 2012). Inflammasomes are molecular platforms containing NOD-like receptors. They are activated upon cellular infection and trigger CASP1 (caspase 1, apoptosis-related cysteine peptidase) activation and the maturation of proinflammatory cytokines such as IL1B (interleukin 1, beta) to engage innate immune defenses (Schroder and Tschopp, 2010). Induction of AIM2 (absent in melanoma 2) and NLRP3 (NLR family, pyrin domain containing 3) inflammasomes by cognate stimuli triggers autophagy, and colocalization of autophagosomes and inflammasomes have been observed. Mechanistically, inflammasomes undergo Lys63-linked polyubiquitination and recruit SQSTM1, facilitating delivery of inflammasomes to autophagosomes (Shi et al., 2012).

In addition, autophagy is linked with removal of apoptotic cell debris, which is vital for tissue inflammation prevention. During programmed cell death, both *Atg5*- and *Becn1*-deficient mouse embryos have impaired clearance of apoptotic cells and increased

inflammation in tissues (Qu et al., 2007). Interestingly, deficient clearance of apoptotic cells is also observed in systemic lupus erythematosus autoimmune disease patients, suggesting a possible role of autophagy in this disease (Grossmayer et al., 2005).

Autophagy is also relevant to another autoimmune disease, Crohn disease. Several genome-wide association studies identified three Crohn disease susceptibility genes, *IRGM* (encoding immunity-related GTPase family, M), *NOD2* and *ATG16L1* (Hampe et al., 2007; Levine et al., 2011; Rioux et al., 2007), three genes that are involved in autophagy. First, the IRGM protein is required for INFG-induced autophagy and facilitates autophagic degradation of mycobacteria in macrophages (Singh et al., 2006). Second, as discussed above, NOD1 and NOD2 act as bacterial sensors to induce autophagy (Travassos et al., 2010). NOD2 is also required for MHC class II antigen presentation of bacterial peptides (Cooney et al., 2010). Third, dendritic cells carrying the Crohn disease susceptibility variant of *ATG16L1* (T300A) are defective in presenting bacterial antigen to $CD4^+$ T cells (Cooney et al., 2010). This variant also shows defects in mediating autophagy against *Salmonella Typhimurium* (Kuballa et al., 2008). However, despite a strong implication of a defect in autophagy being associated with Crohn disease, the exact molecular mechanisms are still not known. Nonetheless, further studies on autophagy and Crohn disease may enable us to develop promising therapeutic strategies for the disease.

1.4 CONCLUSION

The evolutionarily conserved lysosome-based degradation of intracellular components, autophagy, is now emerging as an indispensable player in infection and immunity. In this chapter, we introduced the morphology, molecular machinery, and physiological roles of autophagy. We also highlighted recent advances concerning the cross-talk between autophagy and innate and adaptive immune pathways. A selective type of autophagy, xenophagy, acts to defend the host cell by elimination of intracellular microbes, although these microbes also develop multiple strategies to antagonize autophagy-related host defense mechanisms. There is also interplay between autophagy and the TLR and NOD-like receptor pathways to collectively fight against invading pathogens. Autophagy is not only involved in innate immune responses but also plays a role in adaptive immunity by promoting microbial antigen processing and MHC–antigen presentation to T cells. Moreover, autophagy is relevant to inflammation and autoimmune diseases. A better understanding of the molecular mechanisms that underlie autophagy and immune signaling pathways may facilitate insights into many infectious, inflammation, and autoimmune diseases and ultimately promote the discovery of novel therapeutic targets for clinical treatment of these diseases.

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