Minireview

Crosstalk between Autophagy and Inflammasomes

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A variety of cellular stresses activate the autophagy pathway, which is fundamentally important in protection against injurious stimuli. Defects in the autophagy process are associated with a variety of human diseases, including inflammatory and metabolic diseases. The inflammasomes are emerging as key signaling platforms directing the maturation and secretion of interleukin-1 family cytokines in response to pathogenic and sterile stimuli. Recent studies have identified the critical role of inflammasome activation in host defense and inflammation. Delineation of the relationship between autophagy and inflammasome activation is now being greatly facilitated by the use of mice models of autophagy gene deficiency and clinical studies. We surveyed the recent research regarding the contribution of autophagy to the control of inflammation, in particular the association between autophagy and inflammasomes. Understanding the mechanisms by which autophagy balances inflammation might facilitate the development of autophagy-based therapeutic modalities for infectious and inflammatory diseases.

INTRODUCTION

Autophagy, the conserved intracellular catabolic pathway, is becoming recognized as a key process for maintaining intracellular homeostasis. Autophagy plays a crucial role in cell-autonomous protection and the activation of innate signaling in response to numerous pathogens invading the host (Deretic, 2011; Lee, 2009). Inflammation is part of the response to tissue damage and infection, through which various inflammatory mediators coordinate host defense and repair (Perry et al., 2007). However, overactivated inflammatory responses can lead to damage and pathogenesis. Therefore, inflammation must be controlled tightly at various levels when activating the host immune defense against pathogens, while concurrently preventing damage to the host. There is increasing evidence that autophagy is also involved in the fine control of inflammatory responses to prevent potential damage and pathogenic stimuli (Deretic, 2012a).

An inflammasome is a molecular platform composed of nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) proteins. It is required for the activation of caspase-1 and subsequent maturation of the proinflammatory cytokines inter-

leukin 1 β (IL-1 β) and IL-18. The inflammasomes in immune cells are fundamental in the innate defense against microbes. However, aberrant activation of inflammasomes is involved in the development and pathogenesis of autoimmune and autoinflammatory diseases (Davis et al., 2011). Recent studies have revealed a link between autophagy and inflammasome activation, and its potential roles in disease progression (Rodgers et al., 2013; Wang et al., 2013). This review discusses the recent evidence for mutual mechanisms regulating autophagy and inflammasome activation.

OVERVIEW OF AUTOPHAGY PATHWAY ACTIVATION

Cellular homeostasis is important to all aspects of life. Autophagy is an essential intracellular process involved in the bulk degradation and recycling of long-lived protein aggregates and damaged organelles to maintain cellular homeostasis under various stresses. Autophagy (we discuss macroautophagy here) activation is a multistage process that is divided into the formation of a membrane phagophore, a preautophagosomal structure (and its elongation into double-membrane autophagosomal structures), and finally fusion with late endosomes and lysosomes, the maturation step (Hamasaki and Yoshimori, 2010; Mizushima et al., 2011; Parkhitko et al., 2013). A double lipid bilayer membrane-bound autophagosome structure is synthesized in the cytoplasm during metabolic stress and initiated by the assembly of autophagy-related gene (Atg) proteins through sequential organization (Hamasaki and Yoshimori, 2010). Recent studies by Yoshimori's group have revealed that newly synthesized autophagic isolation membranes are formed at endoplasmic reticulum (ER)-mitochondria contact sites in mammalian cells (Hamasaki et al., 2013). During the initiation process, Atgs play central roles as a ubiquitin-like protein conjugation system that mediates protein lipidation and participation in autophagy-specific protein kinase complexes (Ichimura et al., 2000; Kamada et al., 2000; Mizushima et al., 1998). Interestingly, in this initiation step, the pre-autophagosome/autophagosome marker ATG14 is recruited to ER-mitochondria contact sites through binding with ER-resident soluble NSF attachment protein receptor (SNARE) protein syntaxin 17 (STX17), and co-localizes in the mitochondria-associated ER membrane fraction (Hamasaki et al., 2013).

In the presence of sufficient nutrients, the mammalian target of rapamycin complex 1 (mTORC1) inhibits autophagy by sup-

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pressing the ULK1 kinase activity (ULK1 is the mammalian ortholog of the yeast Atg1). The ULK1 complex is composed of ULK1 Ser/Thr protein kinase, Atg13, and FIP200 (FIP200 is a 200-kDa FAK-family interacting protein and the mammalian homolog of the yeast Atg17) and is essential in the initiation of autophagy (Mehrpour et al., 2010). The other essential component inducing the nucleation and assembly of the initial phagophore membrane is the B-cell lymphoma (Bcl)-2 interacting coiled-coil protein (Beclin) 1: class III phosphatidylinositol 3-kinase (PI3K) complex. Formation of the Beclin 1: class III PI3K (hVps34) complex is regulated by the interaction of Beclin 1 with antiapoptotic proteins of the Bcl-2 family that block the induction of autophagy (Levine et al., 2008).

The two core conjugation systems for the autophagosomal double-membrane structure consist of the Atg12 conjugation and microtubule-associated protein light chain 3 (LC3) lipidation systems, which are required for expansion and closure of the autophagosomal membrane structure. The Atg12-Atg5-Atg16L1 complex is formed by Atg7 activation of Atg12, which then associates with Atg16 and leads to the induction of LC3-I conjugation to phosphatidylethanolamine (PE) to generate LC3-II. The LC3-PE complex is attached to the outer and inner autophagosomal membranes and is a useful marker of autophagosome formation (Kraft and Martens, 2012). In addition, LC3 protein is required for selective autophagosome formation *via* its association with autophagic adaptors, including p62 and NBR1, which contain a special LC3-interacting region (LIR) motif that binds to LC3 (Kraft and Martens, 2012; Weidberg et al., 2011). Recent studies have also emphasized the role of SNARE proteins in autophagosome biogenesis in yeast cells (Nair et al., 2011) and the homotypic fusion of Atg16L precursors, a key event in autophagosome formation, in mammalian cells (Moreau et al., 2011).

In the maturation step of the autophagy process, the autophagosomes fuse with lysosomes to form autophagolysosomes, in which the cytoplasmic cargo is degraded. Autophagosomes can fuse with late endosomes called the multivesicular body (MVB), and form intermediate vesicular amphisomes, which then fuse with lysosomes (Fader et al., 2008). Among the numerous members of the GTPase family, the function of Rab7 in the late maturation of autophagosomes *via* fusion with lysosomes has been studied extensively (Hytinen et al., 2013). Rap7 governs the trafficking and change in early endosomes into late endosomes, and their ultimate fusion with lysosomes (Hytinen et al., 2013). Furthermore, the UVRAG-class C Vps complex enhances Rab7 GTPase activity and accelerates autophagosome fusion with late endosomes/lysosomes. This is distinct from the autophagy initiation step: UVRAG-Beclin1-mediated autophagosome formation (Liang et al., 2008). Recent studies have identified the SNARE machinery, including vesicle-associated membrane protein (VAMP) 3, VAMP7, and VAMP9, as key molecules involved in the fusion step (Fader et al., 2009; Furuta et al., 2010). Moreover, it was recently shown that the autophagosomal SNARE syntaxin 17, which is found in the outer membrane of completed autophagosomes, is required for autophagosome-lysosome fusion *via* an interaction with SNAP29 and another SNARE: VAMP8 (Itakura et al., 2012). Figure 1 presents a schematic overview of the autophagy activation steps.

INFLAMMASOMES: OVERVIEW, STRUCTURE, AND ROLE

An inflammasome is a multiprotein complex that activates caspase-1, triggering the maturation and release of important

proinflammatory cytokines from the IL-1 family: IL-1 β , and IL-18. Studies have determined the molecular structure of inflammasomes and the mechanisms of inflammasome activation (Cassel et al., 2009; Franchi et al., 2012; Lamkanfi and Dixit, 2009; Schroder and Tschopp, 2010; Shaw et al., 2011; Vladimer et al., 2013). The core component of the inflammasome is an intracellular sensor, the Nod-like receptor (NLR), which mediates the recognition of and responses to microbial and danger signals (Davis et al., 2011; Vladimer et al., 2013). Humans and mice possess 23 and 34 reported NLRs, respectively (Kanneganti, 2010). Several NLRs participate in inflammasome assembly, including the nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin-domain containing proteins (NLRP) 1, NLRP3, NLR family caspase-recruitment domain (CARD)-containing protein 4 (NLRC4 or IPAF), NLRP6, and NLRP12 (Bergsbaken et al., 2009; Kanneganti, 2010). These NLRs sense different endogenous and exogenous stimuli and activate caspase-1. Most NLRs consist of three domains: a C-terminal leucine-rich repeat domain; a central nucleotide-binding oligomerization (NOD or NACHT) domain; and a variable N-terminal effector domain containing a CARD, pyrin domain (PYD), or baculovirus inhibitor of apoptosis repeat domain (Franchi et al., 2012). In addition, pyrin and HIN domain (PYHIN) proteins, including absent in melanoma (AIM) 2 and interferon-gamma inducible protein-16 (IFI16), drive the assembly of NLR-independent inflammasomes (Dowling and O'Neill, 2012; Yin et al., 2013).

Inflammasome complexes recognize microbial or endogenous products released from damaged cells and trigger the release of the mature proinflammatory cytokines IL-1 β and IL-18 (Franchi et al., 2012; Rathinam et al., 2012). Aberrant production of the IL-1 family cytokines is associated with tissue damage and chronic inflammation, so the activation of inflammasomes should be tightly regulated (Lamkanfi et al., 2011). Although the detailed mechanisms of inflammasome regulation are beyond the scope of this review, we emphasize the importance of the roles of mitochondria and autophagy in regulating inflammasome activation.

Of the inflammasomes, the best-characterized is the NLRP3 inflammasome, which is a key platform orchestrating the inflammatory responses to metabolic stresses and innate immune sensors (Franchi et al., 2012; Menu and Vince, 2011). The NLRP3 inflammasome is involved in many complicated multigenic diseases and metabolic disorders, including type 2 diabetes, gout, obesity, and atherosclerosis (Doherty et al., 2011; Menu and Vince, 2011). This review briefly discusses the mechanisms by which NLRP3 inflammasomes are assembled on a molecular basis.

NLRP3 INFLAMMASOMES: MECHANISM OF ACTIVATION

The NLRP3 inflammasome can be activated by numerous microbial (e.g., muramyl dipeptide, polyinosinic-polycytidylic acid, lipopolysaccharides, microbial lipopeptide, and the antiviral imidazoquinolines R837 and R848) and endogenous (e.g., uric acid, cholesterol and hydroxyapatite crystals, amyloid deposits, and fatty acids) stimuli [reviewed in (Franchi et al., 2012; Rathinam et al., 2012)]. The two-signal hypothesis is used to explain NLRP3 inflammasome activation. The first signal, a priming signal, is provided by nuclear factor kappa light-chain enhancer of activated B cells (NF- κ B) activators and is a prerequisite for inflammasome activation *via* the induction of Nlrp3 expression in macrophages (Bauernfeind et al., 2009). The second signal activates assembly of the NLRP3 inflammasome

Fig. 1. Signaling mechanisms proposed for the mammalian autophagy pathway. Numerous molecular events are needed to activate the autophagy pathway, including initiation, nucleation, elongation, autophagosome maturation, and cargo degradation. 1) Initiation and nucleation: an autophagic isolation membrane is generated from several sources, such as the ER membrane, mitochondria (MT), and the plasma membrane (PM) derived from Atg16L1 precursors. Homotypic fusion of the Atg16L1 precursor is regulated by the SNARE protein VAMP7 together with partner SNAREs; this is an essential event in the early phases of autophagy. 2) Elongation: expansion of the autophagic membrane is required for the conjugation system to form phagophore structures. (a) Atg 16 non-covalently conjugates with the Atg5 and Atg12 covalent complex, which functions as an E3-like enzyme; (b) the Atg5/12/16 complex activates the conversion of LC3 into LC3. 3) Maturation and cargo degradation: the autophagosome fuses with the late endosomal/multivesicular body to make intermediates called amphisomes, which ultimately results in formation of the autolysosome via fusion with the lysosome. The autophagosomal SNARE Syntaxin 17 interacts with the endosomal/lysosomal SNARE VAMP8 via SNAP-29, which results in autophagosome-late endosome/lysosome fusion. Formation of the amphisome and autolysosome is dependent on Rab7 GTPase activation by UVRAG-class C complex.

directly and involves host-derived adenosine triphosphate (ATP) or uric acid crystals, certain toxins of bacterial origin, or particulate matter (Fig. 2) (Chen and Nunez, 2010).

Several distinct pathways have been identified and these operate together to induce activation of the NLRP3 inflammasome depending on the triggering agents (Bauernfeind et al., 2011; Franchi et al., 2012; Latz, 2010; Leemans et al., 2011; Martinon et al., 2009). It has been suggested that changes in the intracellular K^+ and Na^+ concentrations are involved in activation of the NLRP3 inflammasome after stimulation with various toxins or ionophores such as nigericin (Franchi et al., 2012). In support of this, activation of the NLRP3 inflammasome is blocked by high concentrations of extracellular K^+ , which block K^+ efflux

from cells (Pétrilli et al., 2007), although this is not specific to the NLRP3 inflammasome (Bauernfeind et al., 2011). Recent studies have suggested that the reduction of the intracellular K^+ concentration is the only common mechanism induced by NLRP3 agonists, including bacterial toxins and particulate matter (Muñoz-Planillo et al., 2013). The other mechanism involves the formation of a large pore in the cell membrane, such as the opening of the hemichannel pannexin-1 upon stimulation with extracellular ATP-gated P2X₇R (Pelegrin and Surprenant, 2006), although a recent study reported that pannexin-1 was dispensable in NLRP3 inflammasome activation in response to a variety of stimuli (Qu et al., 2011).

In addition, particulate matter can activate the NLRP3 in-

Fig. 2. A schematic overview of Nlrp3 inflammasome activation. Extracellular and intracellular pathogen-associated molecular patterns (PAMPs) are recognized by toll-like receptors (TLRs, first signal), which results in the transcription of pro-IL-1β and pro-IL-18 cytokines via NF-κB signaling. NF-κB also activates Nlrp3 expression, which might be a factor limiting inflammasome assembly. NLRP3 activation is induced by K⁺ efflux, reactive oxygen species (ROS) production, and lysosome destabilization (second signal). The activation of P2X7R by ATP and stimulation by numerous toxins facilitates the K⁺ efflux via the formation of membrane pores. The phagocytosis of specific pathogenic microbes or particulate matter results in lysosomal rupture, which leads to the release cathepsin B into the cytoplasm. ROS is also the main trigger activating the NLRP3 inflammasome. The assembly of the NLRP3 inflammasome complex, including the adaptor molecule ASC and pro-caspase-1, leads to caspase-1 activation, which results in the cleavage pro-IL-1β and pro-IL-18 into their mature forms, which lead to the secretion of cytokines.

flammasome, including uric acid, cholesterol crystals, silica, aluminum salts, asbestos, amyloid deposits, and fatty-acids, which leads to the “frustrated phagocytosis” of crystals or leakage of lysosomal proteases via damage caused by the ingested materials (Bauernfeind et al., 2011; Dostert et al., 2008; Halle et al., 2008; Hornung et al., 2008). Moreover, reactive oxygen species (ROS) generated by nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase upon the phagocytosis of particles are involved in NLRP3 inflammasome activation (Dostert et al., 2008), although several studies have shown that patients with chronic granulomatous disease show normal activation of caspase-1 and maturation of IL-1β in their macrophages (Meissner et al., 2010b; van de Veerdonk et al., 2010). Nevertheless, redox signaling is critical to NLRP3 inflamma-

some activation, because thioredoxin (TRX)-interacting protein (TXNIP) interacts with NLRP3, and is essential for activating the NLRP3 inflammasome and secretion of IL-1 β ; it also contributes to insulin resistance and the pathogenesis of metabolic diseases (Schroder et al., 2010; Zhou et al., 2010). It is now clear that ROS generated by mitochondrial damage and oxidized mitochondrial DNA (mtDNA) are required for NLRP3 inflammasome activation (Nakahira et al., 2011; Zhou et al., 2011), as described below in detail.

FUNCTIONAL LINK BETWEEN AUTOPHAGY AND INFLAMMASOME

Autophagy is now recognized as a key adaptive response, and defective autophagy is a potential cause of numerous pathological responses, including neurodegeneration, myopathies, metabolic disturbance, autoimmunity, and excessive inflammation (Féüs et al., 2011; Jones et al., 2013). Studies have found a mutual relationship between autophagy and inflammasomes: 1) autophagy negatively regulates inflammasome activation; 2) autophagy induction is dependent on the presence of specific inflammasome sensors; 3) inflammasomes are ultimately degraded by autophagosomes via the selective autophagic receptor p62; and 4) autophagy plays a role in the biogenesis and secretion of the proinflammatory cytokine IL-1 β (Deretic, 2012b; Dupont et al., 2011; Levine et al., 2011; Shi et al., 2012; Zhou et al., 2011).

Several lines of evidence show that autophagy contributes to the inhibition of inflammasomes and excessive inflammation. Earlier studies showed that Atg16L1-deficient macrophages significantly enhanced the secretion of IL-1 β and IL-18 in response to lipopolysaccharide, via Toll/IL-1 receptor domain-containing adaptor inducing IFN- β (TRIF)-dependent activation of caspase-1 (Saitoh et al., 2008). In addition, mice with hypomorphic ATG16L1 protein expression and Crohn's disease patients carrying the ATG16L1 risk allele showed abnormalities in the granule exocytosis pathway of Paneth cells, and intestinal injury responses (Cadwell et al., 2008). During *Shigella flexneri* infection, the activation of autophagy triggered by bacteria-induced vacuolar membrane remnants is required for dampening the inflammatory response (Dupont et al., 2009). In the model of mutant superoxide dismutase (SOD) 1-induced neuroinflammation, autophagy activation as a stress response to the accumulated cytosolic SOD1 has a beneficial role in the inhibition of inflammation and IL-1 β release (Meissner et al., 2010a). Autophagy is also required to control the processing and secretion of IL-1 β , IL-18, and IL-23 (Harris et al., 2011; Peral de Castro et al., 2012). Blockade of autophagy augments the secretion of IL-23 in macrophages and dendritic cells after stimulation with specific toll-like receptor ligands, and this depends on IL-1R signaling (Peral de Castro et al., 2012). Many studies have indicated that the autophagic machinery plays a critical role in the negative control of the induction and activation of inflammatory immune responses. In addition, Ipaf, an NLR family member, and caspase-1 negatively regulate *Shigella*-induced autophagy in macrophages (Suzuki et al., 2007), suggesting a negative regulatory role of some inflammasomes in autophagy activation during bacterial infection.

Shi et al. (2012) revealed that autophagy induction depends on the presence of specific inflammasome sensors in macrophages, such as NLRP3 and AIM2 inflammasomes. However, autophagosomes can sequester inflammasome components, including AIM2, NLRP3, and ASC, for selective degradation (Shi et al., 2012), although caspase-1 does not co-localize with

the autophagosomal marker LC3. Moreover, toll-like receptor stimulation leads to the specific sequestration of pro-IL-1 β into autophagosomes in macrophages. The activation of autophagy results in the degradation of pro-IL-1 β , thereby blocking the secretion of mature IL-1 β , whereas the inhibition of autophagy promotes the processing and secretion of IL-1 β (Harris et al., 2011). Moreover, autophagy contributes to the unconventional secretion of IL-1 β via an export pathway that depends on Atg5 in mammalian cells, suggesting a role for autophagy in the positive regulation of inflammasome activation (Dupont et al., 2011). These studies suggest that autophagy controls inflammasome activation at various levels, i.e., by activating certain inflammasomes, facilitating the secretion of IL-1 β , and targeting inflammasome components for lysosomal degradation.

CONCLUSION

Autophagy and inflammasome activation are two fundamental cellular responses to a variety of stresses. The former leads to an effective lysosomal degradation pathway for the elimination of large protein aggregates and damaged organelles, whereas the latter allows the molecular platform to activate caspase-1 and secrete IL-1 β when triggered by dangerous stimuli. Evidence suggests that autophagy and inflammasomes mediate dynamic crosstalk between the two systems to maintain homeostasis during stress conditions inside cells. Then, what is the significance of autophagy activation in response to dangerous stimuli? First, autophagy activation contributes to host defense through the clearance of damaged organelles or invasive pathogens. Second, it is also required for the fine regulation of inflammatory responses induced by microbial or danger molecules. However, many questions remain regarding the molecular mechanisms by which autophagy controls inflammasome activation by multiple agonists in diverse situations. Mitochondrial quality control accounts for one mechanism for the autophagic regulation of inflammasome activation. Future studies will clarify the exact mechanisms by which autophagy plays an essential role fine-tuning inflammasome activation. This will provide a basis for manipulating the autophagy and inflammasome pathways to treat various inflammatory and degenerative diseases.

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