MolecularMedicine

Inflammasome and Autophagy Regulation: A Two-way Street

Qian Sun,[1] Jie Fan,[1,2] Timothy R Billiar,[1] and Melanie J Scott[1]

[1]Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States of America; and
[2]Research and Development, Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, United States of America

Inflammation plays a significant role in protecting hosts against pathogens. Inflammation induced by noninfectious endogenous agents can be detrimental and, if excessive, can result in organ and tissue damage. The inflammasome is a major innate immune pathway that can be activated via both exogenous pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs). Inflammasome activation involves formation and oligomerization of a protein complex including a nucleotide oligomerization domain (NOD)-like receptor (NLR), an adaptor protein and pro-caspase-1. This then allows cleavage and activation of caspase-1, followed by downstream cleavage and release of proinflammatory cytokines interleukin (IL)-1β and IL-18 from innate immune cells. Hyperinflammation caused by unrestrained inflammasome activation is linked with multiple inflammatory diseases, including inflammatory bowel disease, Alzheimer's disease and multiple sclerosis. So there is an understandable rush to understand mechanisms that regulate such potent inflammatory pathways. Autophagy has now been identified as a main regulator of inflammasomes. Autophagy is a vital intracellular process involved in cellular homeostasis, recycling and removal of damaged organelles (eg, mitochondria) and intracellular pathogens. Autophagy is regulated by proteins that are important in endosomal/phagosomal pathways, as well as by specific autophagy proteins coded for by autophagy-related genes. Cytosolic components are surrounded and contained by a double-membraned vesicle, which then fuses with lysosomes to enable degradation of the contents. Autophagic removal of intracellular DAMPs, inflammasome components or cytokines can reduce inflammasome activation. Similarly, inflammasomes can regulate the autophagic process, allowing for a two-way mutual regulation of inflammation that may hold the key for treatment of multiple diseases.
Online address: http://www.molmed.org
doi: 10.2119/molmed.2017.00077

INTRODUCTION

Inflammatory innate immune responses are important in host defense against pathogens (1,2). Similar pathways can be activated by sterile exogenous or endogenous agents, but the resulting inflammation can be detrimental through the induction of organ and tissue damage, resulting in inflammatory disease (2,3). One of the main inflammatory pathways leading to disease involves activation of the inflammasome, a multiprotein complex that initiates inflammatory responses to both pathogen and endogenous activators (3). Understandably, a lot of recent research has focused on how inflammasomes are activated and how their activation is regulated, and this has led to a new appreciation of the interregulation of inflammasomes and autophagy. Autophagy is an intracellular process important for cellular homeostasis and recycling of damaged organelles and proteins, as well as destruction of intracellular pathogens (4). In diseases where autophagy is restricted or diminished (eg, inflammatory bowel disease), there is hyperinflammation and hyperactivation of inflammasomes (5,6). Similarly, inflammasome activation can upregulate autophagy in an attempt to protect the host from excessive inflammation (7). This mutual regulation is important to understand, as it applies to multiple disease processes, and in this review we discuss currently available information about the two-way street of regulation that exists between inflammasomes and autophagy.

Activation of Inflammasomes

Inflammasomes are cytosolic multiprotein platforms assembled in response to wide-ranging stimuli, including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (1). A typical inflammasome contains a sensor protein belonging either to the nucleotide oligomerization domain (NOD)-like receptor (NLR) nucleotide-binding domain (NBD) and leucine-rich repeat (LRR)-containing family or the absent in melanoma 2 (AIM2)-like receptor (ALR) family of pattern recognition receptors; an adaptor protein, apoptosis-associated speck-like

Address correspondence to *Melanie J Scott, Department of Surgery Labs, University of Pittsburgh, NW653 MUH, 3459 Fifth Ave, Pittsburgh, PA 15213. Phone: 412-647-5806; Fax: 412-647-5959; E-mail: scottm@upmc.edu.*
Submitted May 2, 2017; Accepted for Publication July 20, 2017;
Published Online (www.molmed.org) July 24, 2017.

Feinstein Institute for Medical Research
Northwell Health™

(ASC) protein, containing a caspase-recruitment domain; and pro-caspase-1 (8). Activation of these inflammasome complexes results in proteolytic cleavage of zymogen pro-caspase-1 into its enzymatically active form, which then leads to maturation of proinflammatory cytokines interleukin (IL)-1β and IL-18 (9).

Four main inflammasomes have been identified: NLR members NOD-, LRR- and pyrin domain–containing 1 (NLRP1); NLRP3; NOD-, LRR- and caspase recruitment domain–containing 4 (NLRC4); and AIM2, a sensor for nucleic acids (Figure 1) (9). NLRP1 and NLRC4 inflammasomes are activated by specific PAMPs, such as muramyl dipeptide and flagellin, respectively (2). The NLRP3 inflammasome is interesting, as it can be activated by a wide range of stimuli that include both pathogenic microorganisms and endogenous mediators, such as reactive oxygen species (ROS), mitochondrial DAMPs and adenosine triphosphate (ATP), as well as by crystalline structures (eg, uric acid crystals) and other fibrillar proteins (eg, β-amyloid fibrils) and environmental irritants (eg, silica, alum) (10). AIM2 responds specifically to double-stranded DNA (dsDNA), which can be

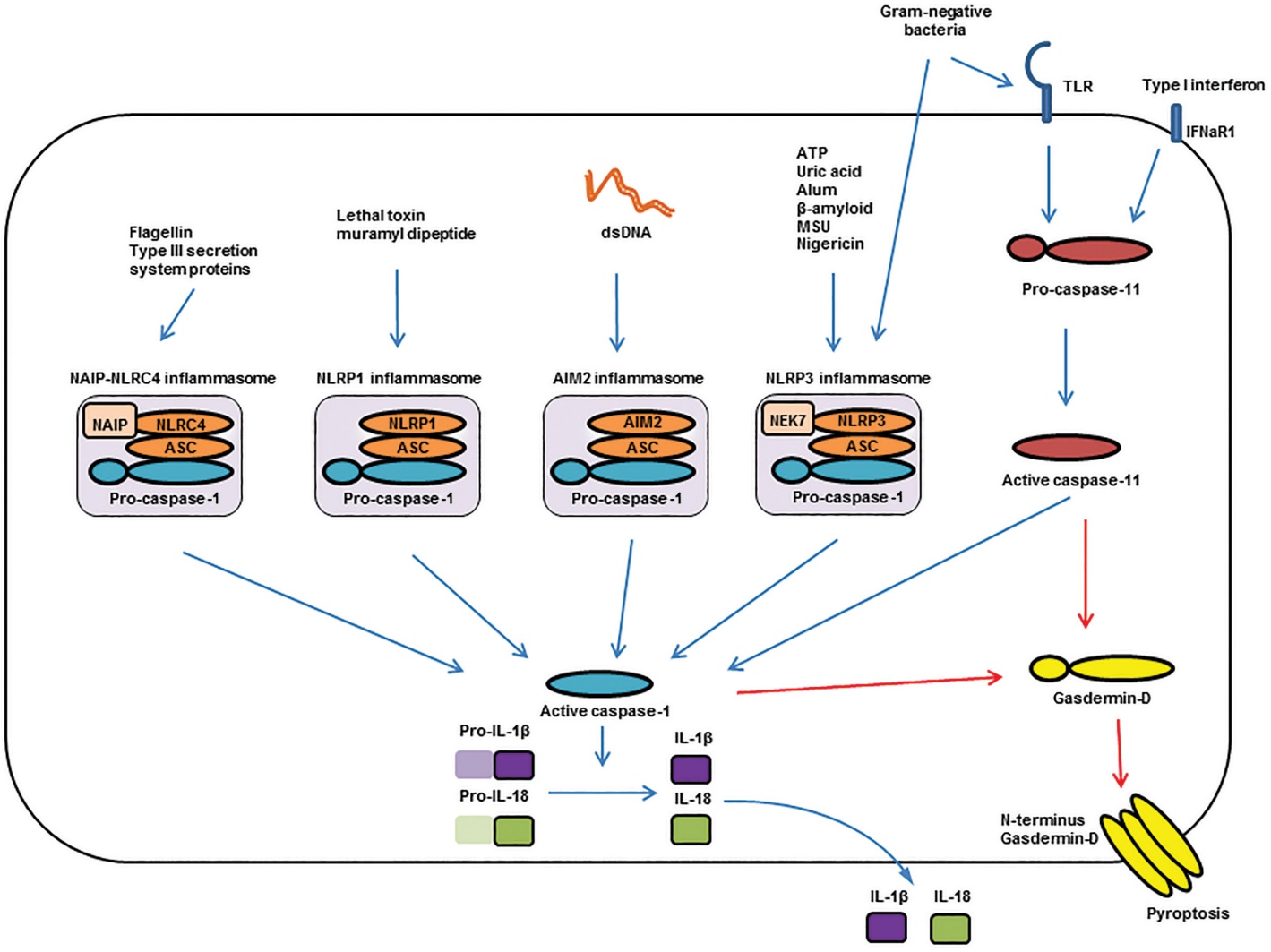

Figure 1. Canonical and noncanonical inflammasomes. A typical inflammasome contains a sensor protein belonging to either the NLR or ALR family of pattern recognition receptors, an adaptor protein ASC and pro-caspase-1. NAIP-NLRC4 is activated by bacterial flagellin and type III secretion system proteins, NLRP1 is activated by lethal toxin and muramyl dipeptide, AIM2 is activated by cytosolic dsDNA and NLRP3 is activated by a variety of activators including ATP, uric acid, alum, β-amyloid, MSU and nigericin. Activation of these inflammasome complexes results in proteolytic cleavage of zymogen pro-caspase-1 into its enzymatically active form, which then leads to maturation of proinflammatory cytokines IL-1β and IL-18. In the noncanonical inflammasome pathway, pro-caspase-11 expression is induced by a variety of Toll-like receptor ligands, or via type I interferon signaling. Caspase-11 is self-oligomerized and activated by cytosolic LPS, which subsequently leads to caspase-1 activation, as well as gasdermin D–mediated pyroptotic cell death in macrophages.

derived either from host (nucleic and mitochondrial dsDNA) or from pathogen (3). Recent work has shown that in most cases, activation of individual NLR inflammasomes is relatively specific, but can only occur in response to such wide-ranging stimuli through direct association with select protein mediators. This explains why evidence of direct interaction between inflammasome activators and NLRs was lacking for such a long time (1,3). NLRC4 is activated after binding of individual neuronal apoptosis inhibitory protein (NAIP) family members, which bind specifically to NLRC4 activators such as flagellin and type III secretion system proteins (11). Similarly, NLRP3 is activated through association with Nek7, which is able to bind in response to activation by multiple NLRP3 activators that cause a drop in intracellular potassium levels (12). Even AIM2, which has been shown to directly bind dsDNA for activation, is able to use high mobility group box 1 (HMGB1) to enhance activation and regulate its activation in response to redox stress through differences in the affinity of HMGB1 binding in different oxidized states (13). In general, however, inflammasome activation is regulated by the levels of activating stimuli, be they endogenous or exogenous, as well as the availability of individual inflammasome components, which are kept at low levels of expression in innate immune responders such as macrophages (3).

Multifaceted Roles for Autophagy

Autophagy is a vital intracellular homeostatic recycling process (4). Cytosolic components, including overproduced or misfolded proteins, cellular organelles (eg, mitochondria) or even pathogenic cytosolic bacteria, are packaged into double-membraned autophagosomes and delivered to lysosomes for degradation and recycling of amino acids (4). The process of autophagy is regulated by a large number of proteins that are also important in endosomal and phagosomal pathways, as well as by specific autophagy proteins coded for by autophagy-related genes (Atg) (4,14). Autophagy plays an important role in multiple biological processes, including cell metabolism, cellular development and differentiation, and degenerative diseases, and also in pathology related to aging (14). The initiation of autophagy in response to cell starvation and the reduced availability of amino acids highlights its role in cell homeostasis and metabolism. Much of this is regulated through the inhibition of mammalian target of rapamycin (mTOR) (15), but autophagy can also be induced by organelle stress and intracellular infection, and can induce both protective and cell death pathways (16).

Autophagy Regulation of Inflammasome Activation

Saitoh *et al.* were the first to show that autophagy can negatively regulate inflammasome activation, and this was followed by multiple studies showing similar effects in a variety of experimental systems (17,18) (Table 1). The initial study showed that loss of autophagy-related 16-like 1 (Atg16L1), a protein essential for initiation of autophagy, results in increased caspase-1 activation, as well as increased production of IL-1β and IL-18 in macrophages after endotoxin treatment (17). Similarly, IL-1β production in macrophages deficient in autophagic protein Atg7, or treated with a chemical inhibitor of autophagy 3-methyladenine (3-MA), is significantly enhanced in response to inflammasome inducers (17). Later studies further indicated that regulation of inflammasome activation by autophagy can occur in multiple ways, through either removal of endogenous inflammasome activators or removal of inflammasomes and their downstream cytokines directly (Figure 2).

Autophagic Removal of Mitochondrial-Derived DAMPs

Autophagy removes damaged organelles such as mitochondria, leading to reduced release of mitochondrial-derived DAMPs and subsequent suppression of inflammasome activation (19–21).

Table 1. Autophagy regulation of inflammasome activation.

Manipulation of Autophagy Pathway	Inflammasome Activator	Downstream Signaling Pathway	Cell Type	References
Loss of Atg16L1	LPS	↑ caspase 1 activation ↑ IL-1β/IL-18 production	Macrophage	(17)
Loss of Atg7	LPS Viral RNA Alum Nigericin	↑ caspase 1 activation ↑ IL-1β production	Macrophage	(17,23,37)
Loss of Atg5	LPS MSU Nigericin	↑ caspase 1 activation ↑ IL-1β production	Macrophage	(19,40)
Loss of beclin1	LPS MSU Nigericin	↑ mtDNA release ↑ caspase 1 activation ↑ IL-1β production	Macrophage	(19,20)
Loss of LC3B	LPS + ATP	↑ caspase 1 activation ↑ IL-1β production	Macrophage	(20)
3-MA	LPS	↑ NLRP3 activation ↑ caspase 1 activation ↑ IL-1β production	Macrophage Dendritic cells	(19,38)
Rapamycin	Poly(dA:dT)	↓ AIM2 activation ↓ caspase 1 activation ↓ IL-1β production	Macrophage Non-parenchymal cells	(31,39)
Starvation	Poly(dA:dT)	↓ AIM2 activation ↓ caspase 1 activation ↓ IL-1β production	Macrophage	(31)

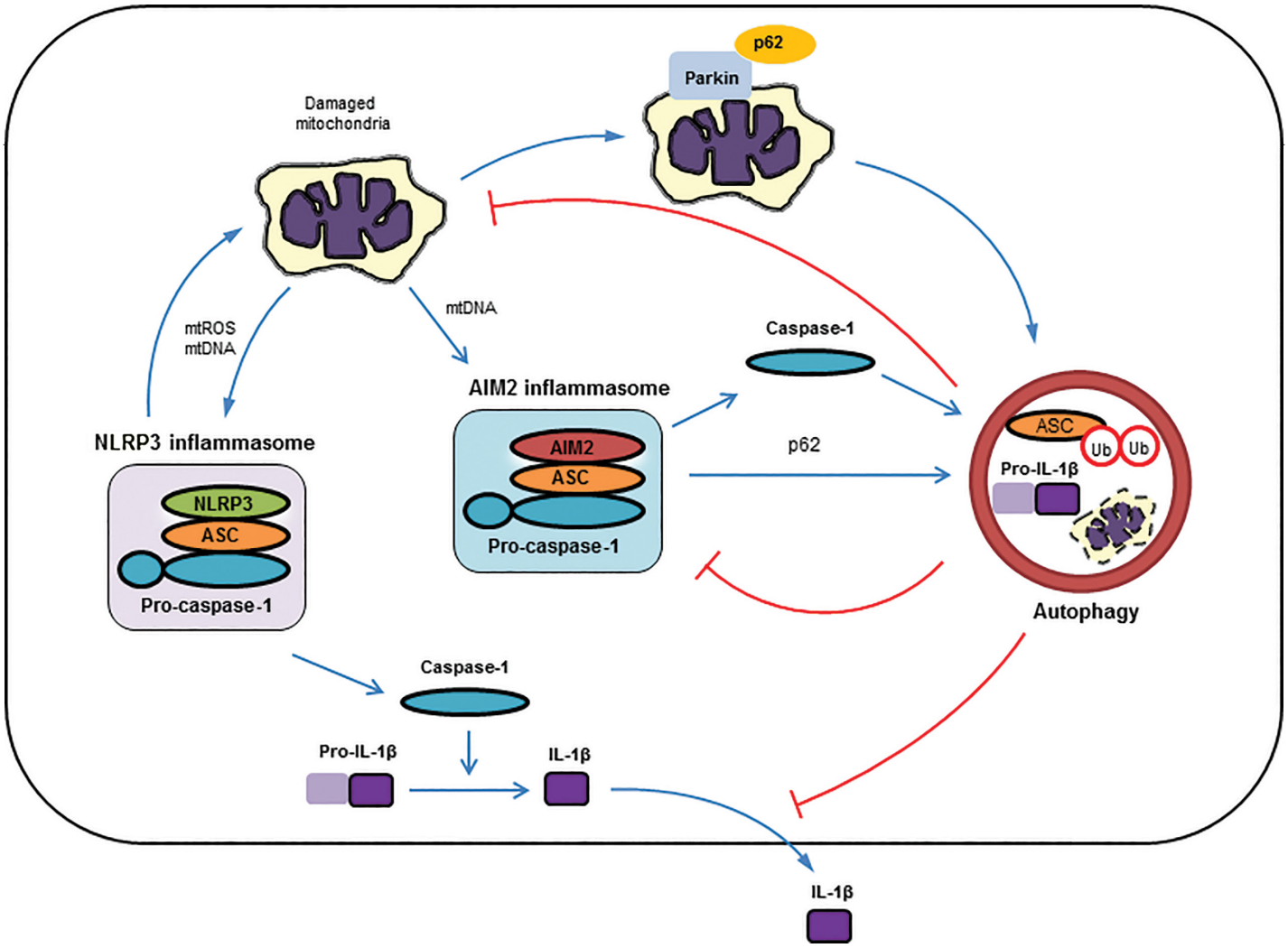

Figure 2. Inflammasome and autophagy interregulation. Autophagy can negatively regulate inflammasome activation by removing mitochondrial-derived DAMPs as well as through selective degradation of inflammasome complexes and mitochondria. Autophagy machinery also directly regulates IL-1β activation, release and signaling. Conversely, inflammasome activation regulates autophagosome formation through direct or indirect interactions.

Zhou *et al.* demonstrated that following pharmacological inhibition of mitochondrial complex I and III, increased mitochondrial ROS production is responsible for NLRP3-mediated caspase-1 activation and IL-1β release in monocytes, which can be further reversed by ROS scavenger (19). Similarly, loss of autophagy results in accumulation of ROS-producing mitochondria, and subsequently enhanced activation of NLRP3 inflammasome in response to NLRP3 agonist ATP, monosodium urate crystals, palmitic acid or influenza A virus (19,20,22,23). Two other studies, by Nakahira *et al.* and Shimada *et al.*, suggest that following the production of mitochondrial ROS and loss of mitochondrial integrity, cytosolic release of mitochondrial DNA (mtDNA) is responsible for activating NLRP3 inflammasome (20,21). Furthermore, oxidized mtDNA was shown to activate NLRP3 inflammasome to a greater extent than regular mtDNA, suggesting that both mitochondrial ROS and mtDNA are important for NLRP3 activation (21). Disruption of autophagy in this process impairs mitochondrial homeostasis and promotes cytosolic translocation of mtDNA, leading to greatly enhanced activation of caspase-1 and downstream cytokine release (20).

It is not yet fully understood how mitochondrial ROS contribute to NLRP3 inflammasome activation and how the process is regulated by autophagy. Several steps remain to be fully elucidated. First, reduction of autophagy by itself does not seem to be sufficient to trigger inflammasome activation in the absence of NLRP3 inducers (19,20). Second, since mitochondrial ROS are normal byproducts of mitochondrial respiration, it is unknown

whether NLRP3 can sense ROS generated from intact mitochondria as a result of increased mitochondrial metabolism. Moreover, ROS are short-lived molecules and can be scavenged by intracellular antioxidants such as glutathione and thioredoxin, so it is possible that the availability of antioxidants is another important regulatory factor (24). Additionally, caspases (including caspase-1) can be post-translationally modified (eg, by S-nitrosylation and ubiquitination) (25–27) in processes that may be influenced by redox status and antioxidant availability. These modifications can affect not only caspase activity (28,29), but also degradation (27) and so reduce cytokine cleavage and release following inflammasome activation. Although the exact mechanism of ROS-mediated NLRP3 inflammasome activation remains unclear, several hypotheses have been tested. The first hypothesis suggests that upon activation of the NLRP3 inflammasome by its agonist nigericin, monosodium urate crystals or alum (aluminum hydroxide and aluminum phosphate adjuvants), NLRP3 translocates to mitochondria and mitochondria-associated endoplasmic reticulum membranes (MAMs), placing it in proximity to newly generated mitochondrial ROS (19). Another hypothesis involves the interaction between thioredoxin-interacting protein (TXNIP) and NLRP3, which has been shown to be crucial for redox stress-mediated inflammasome activation (30). In this scenario, TXNIP translocates to MAMs/mitochondria following NLRP3 activation in a ROS-dependent manner, allowing its potential binding to NLRP3 (19). However the NLRP3 inflammasome is activated, it seems likely that a prolonged presence of damaged and ROS-producing mitochondria is necessary to trigger activation, and by removing dysfunctional mitochondria, autophagy functions to reduce excessive activation of the NLRP3 inflammasome.

Selective Autophagy in Inflammasome Activation

Another mechanism by which autophagy regulates inflammasome activation is through p62-dependent degradation of inflammasome complexes and mitochondria. Upon stimulation of AIM2 and NLRP3 inflammasomes, K63 (Lys 63)-linked polyubiquitination of ASC is triggered, which is then recognized by ubiquitin sensor p62, and ASC is targeted to autophagosomes for degradation (31). Both pharmacological inhibition of autophagy and loss of p62 can greatly increase activation of caspase-1 and inflammatory cytokine production in response to AIM2 inducer poly(dA:dT) in monocytes (31). AIM2 can also undergo ubiquitination and be degraded by p62-dependent selective autophagy in macrophages upon poly(dA:dT) treatment (32). Tripartite motif 11, an E3 ubiquitin ligase, was shown to associate with AIM2 and facilitate its recruitment to p62 for autophagic degradation (32,33). Supporting this notion of ubiquitination of inflammasome resulting in degradation as a form of regulation, TRIM20 was also shown in a separate study to target a group of inflammasome components for autophagic degradation in macrophages, including NLRP3, NLRP1 and pro-caspase-1 in response to IFN-γ stimulation (34). In addition to targeting inflammasome proteins themselves, dysfunctional mitochondria can also be marked with ubiquitin and tagged for autophagic disposal (35). During this process, another E3 ubiquitin, ligase parkin, is essential for the ubiquitination of outer mitochondrial membrane proteins and subsequent selective degradation of damaged mitochondria, or mitophagy (35,36). A recent study by Zhong *et al.* suggested that a parkin-dependent clearance of p62-bound mitochondria can reduce NLRP3 activation and IL-1β release in macrophages (37), providing another mechanism for autophagy-mediated inhibition of inflammasome activation.

Autophagy and IL-1β Signaling

Last but not least, autophagy machinery directly regulates IL-1β activation, release and signaling. Harris *et al.* showed that pro-IL-1β can be sequestered into autophagosomes for degradation following treatment of macrophages with rapamycin, a pharmacological inducer of autophagy that inhibits mTOR (38). This pathway therefore contributes to autophagy-mediated reduction in IL-1β secretion in response to NLRP3 inducers (38). However, two other studies suggest that autophagy is actually required for the unconventional secretion of IL-1β, and reducing autophagy can lead to reduced IL-1β release in response to AIM2 and NLRP3 activators in macrophages (39,40). In one of the studies, Wang *et al.* showed that autophagy-dependent secretion of IL-1β after AIM2 inflammasome activation required microtubule-associated protein end-binding protein 1 (39). Disruption of autophagy by inhibition of AMPK activity was able to block poly(dA:dT)-induced AIM2-mediated IL-1β secretion (39). Dupont *et al.* also demonstrated that induction of autophagy by starvation triggered increased secretion of IL-1β in response to nigericin, an NLRP3 inflammasome activator (40). A similar unconventional secretory pathway also contributes to the extracellular delivery of another inflammasome activated cytokine, IL-18 (40). At present, it is not clear why there is a discrepancy in the results between data showing autophagy inducing and reducing IL-1β release. The regulatory effect of autophagy on IL-1β release is obviously complicated and requires further investigation, and may be dependent on cell type and inducer/activator.

Inflammasome Regulation of Autophagy

Regulation of autophagy by NLRs. NLR inflammasomes are the most well-studied inflammasomes, and include NLRP1, NLRP3 and NLRC4 (previously known as IPAF) (9). Most NLR proteins contain an amino-terminal death-fold domain caspase recruitment domain or pyrin, a central domain nucleotide-binding domain (NACHT, or NAIP, CIITA, HET-E and TP1), as well as LRRs at the carboxy terminus (10). These domains allow NLRs to oligomerize and to interact with other inflammasome proteins with similar domains to form large inflammasome ultrastructures that have a

prion-like appearance and can even be visualized at times by light microscopy (41). Interaction of autophagy proteins with NLR domains has also been reported, and provides a mechanism for direct NLR regulation of autophagy (Figure 2).

Jounai *et al.* recently suggested that a number of inflammasome-forming and non-inflammasome–forming NLRs can interact with beclin1, a protein important in autophagy initiation, via the NACHT domain (42). Among these NLRs, NLRP4 (an NLR not known to form inflammasomes) in particular has a strong affinity to beclin1, and this interaction leads to autophagy inhibition (42). Following group A *Streptococcus* infection, NLRP4 transiently dissociates from beclin1, allowing it to interact with other autophagy proteins to initiate beclin1-mediated autophagy in macrophages (42). Inflammasome-forming NLRP3 has also been shown to negatively regulate autophagy through downregulation of phosphatase and tensin homolog–induced putative kinase 1 expression, an initiator of mitophagy (43). Zhang *et al.* demonstrated that NLRP3-deficient mice were protected from hyperoxia exposure, and this protection was driven by increased phosphatase and tensin homolog–induced putative kinase 1 expression and preservation of mitophagy in lung endothelial cells of these mice (43). In addition to NLRP3, NLRC4 can also inhibit autophagy in infection (44). In response to *Shigella* infection, autophagy was significantly increased in NLRC4-deficient macrophages, and this NLRC4-mediated inhibition was dependent on caspase-1 (44,45), although the exact mechanism of NLRC4/caspase-1 regulation of autophagy remains unknown.

Conversely, there is also evidence to suggest that NLRs can stimulate autophagosome formation. NLRP6, another non-inflammasome–forming NLR, is crucial for autophagy in intestinal epithelial cells (46). Deletion of NLRP6 in mice results in impaired autophagy in the gut, with impaired mucus secretion, which makes the mice highly susceptible to persistent *Citrobacter rodentium* infection (46). Another example is a role for NLRP3 inflammasome in the promotion of autophagy following *Pseudomonas aeruginosa* infection in macrophages (47). In this case, NLRP3-mediated autophagy is important for bacterial clearance in these cells. Autophagy regulation by NLRs therefore seems to differ depending on the cell type under investigation and the conditions leading to autophagy or inflammasome activation.

Regulation of autophagy by ALRs. ALR inflammasomes include AIM2 and IFI16 inflammasomes, which have also been studied in multiple cell types and multiple experimental models. Unlike NLRs, ALRs can directly associate with their ligand, dsDNA, through a HIN200 domain (1). Recognition of microbial DNA by AIM2 and IFI16 triggers production of proinflammatory cytokines IL-1β and IL-18, and this drives host defense responses to a number of intracellular microbes, including *Francisella*, *Listeria*, *Mycobacterium sp.*, mouse cytomegalovirus and Kaposi sarcoma–associated herpesvirus (48,49). On the other hand, host-derived dsDNA can also be recognized by AIM2, such as in a model of whole-body irradiation (50), leading to sustained inflammation and resulting in the development of inflammatory diseases such as dermatitis and psoriasis (48). In addition to the cytokine-maturation role of ALRs, recent studies have begun to implicate ALRs as regulators of cytoprotective autophagy responses.

Shi *et al.* were the first to show that induction of AIM2 inflammasomes with synthetic dsDNA poly(dA:dT) triggers increased autophagosome formation in macrophages (31). Upon activation of AIM2, a Ras-like small G-protein, RalB, is activated, which then induces the assembly of autophagy initiation complexes containing beclin1 (31,51). Interestingly, in this case, induction of autophagy is not dependent on caspase-1 or the adaptor protein ASC, as the phenotype cannot be recapitulated in caspase-1 or ASC-deficient macrophages (31). AIM2-mediated activation of autophagy has now been confirmed in two separate models (13,52). In the first model, AIM2 activation by host-derived dsDNA led to increased autophagic flux in hepatocytes after redox stress induced by hypoxia with reoxygenation (13). Moreover, *in vivo* induction of autophagy by AIM2 inflammasome was protective by inducing removal of ROS-generating mitochondria in the liver (13,53). Similar to the previous study with RalB, beclin1 was an important mediator in autophagy activation mediated by AIM2 (13,53). In contrast, in hepatocytes, AIM2-dependent regulation of autophagy was mediated by caspase-1, suggesting that mechanisms of AIM2-mediated autophagy regulation may differ between immune and nonimmune cell types (53,54). In the second model, Saiga *et al.* demonstrated that following infection of recombinant bacillus Calmette-Guerin, a live attenuated vaccine against tuberculosis, autophagy induction was partially dependent on AIM2 in macrophages (52). Therefore, it seems that activation of AIM2 inflammasome, by either microbial or endogenous self-DNA, triggers host adaptive autophagy responses designed to limit excessive inflammation and restore cellular homeostasis.

Regulation of autophagy by caspase-1. Caspase-1 is a key component of inflammasome and has been reported to regulate the autophagic process through proteolytic cleavage of its substrates (55,56). Yu *et al.* showed that caspase-1 activation can result in cleavage of a key mitophagy regulator, parkin, thereby decreasing mitophagy and increasing the numbers of damaged mitochondria in the cell after AIM2 activator dsDNA (55). These damaged mitochondria have alterations in mitochondrial permeability transition/depolarization, increased mitochondrial ROS production and swelling, and this allows further inflammasome activation and inflammatory pyroptotic cell death in bone marrow–derived macrophages (55). In another study, caspase-1 was demonstrated to cleave Toll/interleukin-1 receptor domain-containing adapter-inducing interferon-β (TRIF), leading to

downregulation of TRIF-mediated autophagy following *Pseudomonas aeruginosa* infection (56). Moreover, expression of a caspase-resistant TRIF mutant significantly increased autophagy in infected macrophages. Therefore, proteolytic cleavage of key autophagic regulatory proteins by caspase-1 can lead to loss of function of these substrates and defective autophagy, particularly in immune cells. However, similar to other aspects of inflammasome-autophagy interregulation, the effects of caspase-1 activation seem to be cell type–specific, and also potentially experimental model or caspase-1 activator–dependent.

Noncanonical activation of inflammasome and autophagy. Recent evidence suggests that caspase-1 activation can occur in response to LPS (endotoxin), a key component of Gram negative bacteria, independent of canonical inflammasomes (57). Instead, caspase-11, an inflammatory caspase closely related to caspase-1, is required to activate NLRP3 inflammasome and caspase-1 in cells exposed to cytosolic LPS. Caspase-11 binds directly to cytosolic LPS, and this triggers its self-oligomerization and activation, which subsequently leads to caspase-1 activation, as well as pyroptotic cell death in macrophages (58) (Figure 1). This pathway has been termed noncanonical activation of inflammasome. Parts of this pathway have now been elucidated and involved the protein gasdermin D, which is a substrate for both caspase-1 and caspase-11. The N-terminus of gasdermin D, once cleaved, can itself oligomerize at cell membranes to form pores through which inflammatory cell death known as pyroptosis can occur (59). The N-terminus has also been shown to associate directly with NLRP3 and results in noncanonical NLRP3-caspase-1 activation (60).

Similar to canonical inflammasome activation, caspase-11 activation is regulated by autophagy. Pharmacological inhibition of autophagy by 3-MA increases noncanonical inflammasome activation in macrophages (61). These results were further confirmed in macrophages deficient in autophagy protein 5, suggesting that autophagy is a negative regulator of caspase-11 activation (61). Although the underlying mechanisms of autophagy regulation of caspase-11 activation remain largely unclear, it is may be that autophagy inhibits activation of caspase-11 by removing excessive mitochondrial ROS, as these were suggested to enhance caspase-11 expression and activation (62). On the other hand, noncanonical inflammasome activation also seems to regulate levels of autophagy machinery expression. A study by Akhter *et al.* describes the ability of caspase-11 to promote fusion of lysosomes with bacteria-containing phagosomes, providing a role for caspase-11 in antibacterial autophagy (63,64). Specifically, upon bacterial infection, caspase-11 promotes dynamic formation of polymerized actin around phagosomes, which is required for phagosome-lysosome fusion and clearance of bacteria (63). These studies suggest that mutual regulation of caspase-11 activation and autophagy is also important for the maintenance of cellular homeostasis and optimized innate immune response to intracellular bacterial pathogens.

CONCLUSION

There is now a large body of published data to suggest that inflammasomes and autophagy mutually regulate each other. In most cases it would appear that this two-way street of regulation provides necessary checks and balances between required host defense inflammatory responses and prevention of excessive inflammation that can lead to organ and tissue damage and inflammatory disease. As we learn more about how both autophagy and inflammasome pathways are individually regulated, it seems we are finding multiple layers of regulation of inflammatory responses. It is very likely that the interaction between such vital pathways can control cell fate, helping cells to survive an inflammatory insult or pushing them toward some form of cell death. Much of the outcome seems to be dependent on the cell type involved, as well as the specific conditions that are inducing inflammation, inflammasome activation and autophagy. This highly variable response surely signifies an ultimately adaptable response that we may be able to manipulate in the future to provide relief for millions of patients suffering from degenerative, inflammatory or tumorigenic disease – or even for the rest of us, who are merely steadily aging.

ACKNOWLEDGMENTS

Work performed for this paper was supported by NIH-R01-GM102146 (to MJS).

DISCLOSURE

The authors declare they have no competing interests as defined by *Molecular Medicine* or other interests that may be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Vanaja SK, Rathinam VA, Fitzgerald KA. (2015) Mechanisms of inflammasome activation: recent advances and novel insights. *Trends Cell. Biol.* 25:308–15.
2. Broz P, Dixit VM. (2016) Inflammasomes: mechanism of assembly, regulation and signalling. *Nat. Rev. Immunol.* 16:407–20.
3. Guo H, Callaway JB, Ting JP. (2015) Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 21:677–87.
4. Glick D, Barth S, Macleod KF. (2010) Autophagy: cellular and molecular mechanisms. *J. Pathol.* 221:3–12.
5. Deretic V, Saitoh T, Akira S. (2013) Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* 13:722–37.
6. Levine B, Mizushima N, Virgin HW. (2011) Autophagy in immunity and inflammation. *Nature.* 469:323–35.
7. Cadwell K. (2016) Crosstalk between autophagy and inflammatory signalling pathways: balancing defence and homeostasis. *Nat. Rev. Immunol.* 16:661–75.
8. Schroder K, Tschopp J. (2010) The inflammasomes. *Cell.* 140:821–32.
9. Latz E, Xiao TS, Stutz A. (2013) Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* 13:397–411.
10. Davis BK, Wen H, Ting JP. (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu. Rev. Immunol.* 29:707–35.
11. Zhao Y, Shao F. (2015) The NAIP-NLRC4 inflammasome in innate immune detection of bacterial flagellin and type III secretion apparatus. *Immunol. Rev.* 265:85–102.
12. He Y, Zeng MY, Yang DH, Metro B, Nunez G. (2016) NEK7 is an essential mediator of NLRP3

activation downstream of potassium efflux. *Nature.* 530:354–7.
13. Sun Q, *et al.* (2017) Redox-dependent regulation of hepatocyte absent in melanoma 2 inflammasome activation in sterile liver injury in mice. *Hepatology.* 65:253–68.
14. Kaur J, Debnath J. (2015) Autophagy at the crossroads of catabolism and anabolism. *Nat. Rev. Mol. Cell Biol.* 16:461–72.
15. Kim YC, Guan KL. (2015) mTOR: a pharmacologic target for autophagy regulation. *J. Clin. Invest.* 125:25–32.
16. Fitzwalter BE, Thorburn A. (2015) Recent insights into cell death and autophagy. *FEBS J.* 282:4279–88.
17. Saitoh T, *et al.* (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature.* 456:264–68.
18. Shibutani ST, Saitoh T, Nowag H, Munz C, Yoshimori T. (2015) Autophagy and autophagy-related proteins in the immune system. *Nat. Immunol.* 16:1014–24.
19. Zhou R, Yazdi AS, Menu P, Tschopp J. (2011) A role for mitochondria in NLRP3 inflammasome activation. *Nature.* 469:221–5.
20. Nakahira K, *et al.* (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12:222–30.
21. Shimada K, *et al.* (2012) Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity.* 36:401–14.
22. Wen H, *et al.* (2011) Fatty acid–induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat. Immunol.* 12:408–15.
23. Lupfer C, *et al.* (2013) Receptor interacting protein kinase 2–mediated mitophagy regulates inflammasome activation during virus infection. *Nat. Immunol.* 14:480–8.
24. Aon MA, et al. (2012) Glutathione/thioredoxin systems modulate mitochondrial H2O2 emission: an experimental-computational study. *J. Gen. Physiol.* 139:479–91.
25. Sengupta R, Billiar TR, Kagan VE, Stoyanovsky DA. (2010) Nitric oxide and thioredoxin type 1 modulate the activity of caspase 8 in HepG2 cells. *Biochem. Biophys. Res. Commun.* 391:1127–30.
26. Sengupta R, Billiar TR, Atkins JL, Kagan VE, Stoyanovsky DA. (2009) Nitric oxide and dihydrolipoic acid modulate the activity of caspase 3 in HepG2 cells. *FEBS Lett.* 583:3525–30.
27. Zamaraev AV, Kopeina GS, Prokhorova EA, Zhivotovsky B, Lavrik IN. (2017) Post-translational Modification of Caspases: The Other Side of Apoptosis Regulation. *Trends Cell. Biol.* 27:322–39.
28. Li J, Billiar TR, Talanian RV, Kim YM. (1997) Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. *Biochem. Biophys. Res. Commun.* 240:419–24.
29. Sengupta R, Billiar TR, Atkins JL, Kagan VE, Stoyanovsky DA. (2009) Nitric oxide and dihydrolipoic acid modulate the activity of caspase 3 in HepG2 cells. *FEBS Lett.* 583:3525–30.
30. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11:136–40.
31. Shi CS, *et al.* Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* 13:255–63.
32. Liu T, *et al.* (2016) TRIM11 Suppresses AIM2 Inflammasome by Degrading AIM2 via p62-Dependent Selective Autophagy. *Cell Rep.* 16:1988–2002.
33. Hatakeyama S. (2011) TRIM proteins and cancer. *Nat. Rev. Cancer.* 11:792–804.
34. Kimura T, et al. (2015) TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. *J. Cell Biol.* 210:973–89.
35. Matsuda N, Tanaka K. (2015) Cell biology: Tagged tags engage disposal. *Nature.* 524:294–5.
36. Youle RJ, Narendra DP. Mechanisms of mitophagy. (2011) *Nat. Rev. Mol. Cell. Biol.* 12:9–14.
37. Zhong Z, *et al.* (2016) NF-kappaB Restricts Inflammasome Activation via Elimination of Damaged Mitochondria. *Cell.* 164:896–910.
38. Harris J, *et al.* (2011) Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. *J. Biol. Chem.* 286:9587–97.
39. Wang LJ, *et al.* (2014) The microtubule-associated protein EB1 links AIM2 inflammasomes with autophagy-dependent secretion. *J. Biol. Chem.* 289:29322–33.
40. Dupont N, et al. (2011) Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. *EMBO J.* 30:4701–11.
41. Lechtenberg BC, Mace PD, Riedl SJ. (2014) Structural mechanisms in NLR inflammasome signaling. *Curr. Opin. Struct. Biol.* 29:17–25.
42. Jounai N, et al. (2011) NLRP4 Negatively Regulates Autophagic Processes through an Association with Beclin1. *J. Immunol.* 186:1646–55.
43. Zhang Y, *et al.* (2014) Endothelial PINK1 mediates the protective effects of NLRP3 deficiency during lethal oxidant injury. *J. Immunol.* 192:5296–304.
44. Suzuki T, *et al.* (2007) Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. *PLoS Pathog.* 3:1082–91.
45. Suzuki T, Nunez G. (2008) A role for Nod-like receptors in autophagy induced by Shigella infection. *Autophagy.* 4:73–5.
46. Wlodarska M, *et al.* (2014) NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell.* 156:1045–59.
47. Deng Q, *et al.* (2015) Pseudomonas aeruginosa Triggers Macrophage Autophagy to Escape Intracellular Killing by Activation of the NLRP3 Inflammasome. *Infect. Immun.* 84:56–66.
48. Man SM, Karki R, Kanneganti TD. (2016) AIM2 inflammasome in infection, cancer, and autoimmunity: Role in DNA sensing, inflammation, and innate immunity. *Eur. J. Immunol.* 46:269–80.
49. Dowling JK, O'Neill LAJ. (2012) Biochemical regulation of the inflammasome. *Crit. Rev. Biochem. Mol. Biol.* 47:424–43.
50. Hu B, *et al.* (2016) The DNA-sensing AIM2 inflammasome controls radiation-induced cell death and tissue injury. *Science.* 354:765–8.
51. Bodemann BO, *et al.* (2011) RalB and the exocyst mediate the cellular starvation response by direct activation of autophagosome assembly. *Cell.* 144:253–67.
52. Saiga H, *et al.* (2015) The Recombinant BCG ΔureC::hly Vaccine Targets the AIM2 Inflammasome to Induce Autophagy and Inflammation. *J. Infect. Dis.* 211:1831–41.
53. Sun Q, et al. (2013) Caspase 1 Activation Is Protective against Hepatocyte Cell Death by Up-regulating Beclin 1 Protein and Mitochondrial Autophagy in the Setting of Redox Stress. *J. Biol. Chem.* 288:15947–58.
54. Sun Q, Scott MJ. (2016) Caspase-1 as a multifunctional inflammatory mediator: noncytokine maturation roles. *J. Leukoc. Biol.* 100:961–7.
55. Yu J, et al. (2014) Inflammasome activation leads to caspase-1–dependent mitochondrial damage and block of mitophagy. *Proc. Natl. Acad. Sci. USA.* 111:15514–19.
56. Jabir MS, *et al.* (2014) Caspase-1 cleavage of the TLR adaptor TRIF inhibits autophagy and beta-interferon production during Pseudomonas aeruginosa infection. *Cell Host Microbe.* 15:214–27.
57. Kayagaki N, *et al.* (2011) Non-canonical inflammasome activation targets caspase-11. *Nature.* 479:117–21.
58. Shi J, *et al.* (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature.* 514:187–92.
59. Sborgi L, *et al.* (2016) GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* 35:1766–78.
60. He WT, *et al.* (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1 beta secretion. *Cell Res.* 25:1285–98.
61. Meunier E, *et al.* (2014) Caspase-11 activation requires lysis of pathogen-containing vacuoles by IFN-induced GTPases. *Nature.* 509:366–70.
62. Lupfer CR, et al. (2014) Reactive oxygen species regulate caspase-11 expression and activation of the non-canonical NLRP3 inflammasome during enteric pathogen infection. *PLoS Pathog.* 10:e1004410.
63. Akhter A, *et al.* (2012) Caspase-11 promotes the fusion of phagosomes harboring pathogenic bacteria with lysosomes by modulating actin polymerization. *Immunity.* 37:35–47.
64. Roberts JS, Yilmaz. (2015) Dangerous Liaisons: Caspase-11 and Reactive Oxygen Species Crosstalk in Pathogen Elimination. *Int. J. Mol. Sci.* 16:23337–54.

Cite this article as: Sun Q, Fan J, Billiar TR, and Scott MJ. (2017) Inflammasome and autophagy regulation: A two-way street *Mol. Med.* 23:188–95.