Review Article

Inflammasomes, Autophagy, and Cell Death: The Trinity of Innate Host Defense against Intracellular Bacteria

Teresa Krakauer (ORCID)

Department of Immunology, Molecular and Translational Sciences Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA

Correspondence should be addressed to Teresa Krakauer; teresa.krakauer.civ@mail.mil

Inflammasome activation is an innate host defense mechanism initiated upon sensing pathogens or danger in the cytosol. Both autophagy and cell death are cell autonomous processes important in development, as well as in host defense against intracellular bacteria. Inflammasome, autophagy, and cell death pathways can be activated by pathogens, pathogen-associated molecular patterns (PAMPs), cell stress, and host-derived damage-associated molecular patterns (DAMPs). Phagocytosis and toll-like receptor (TLR) signaling induce reactive oxygen species (ROS), type I IFN, NFκB activation of proinflammatory cytokines, and the mitogen-activated protein kinase cascade. ROS and IFNγ are also prominent inducers of autophagy. Pathogens, PAMPs, and DAMPs activate TLRs and intracellular inflammasomes, inducing apoptotic and inflammatory caspases in a context-dependent manner to promote various forms of cell death to eliminate pathogens. Common downstream signaling molecules of inflammasomes, autophagy, and cell death pathways interact to initiate appropriate measures against pathogens and determine host survival as well as pathological consequences of infection. The integration of inflammasome activation, autophagy, and cell death is central to pathogen clearance. Various pathogens produce virulence factors to control inflammasomes, subvert autophagy, and modulate host cell death in order to evade host defense. This review highlights the interaction of inflammasomes, autophagy, and host cell death pathways in counteracting *Burkholderia pseudomallei*, the causative agent of melioidosis. Contrasting evasion strategies used by *B. pseudomallei*, *Mycobacterium tuberculosis*, and *Legionella pneumophila* to avoid and dampen these innate immune responses will be discussed.

1. Introduction

Neutrophils and macrophages are professional phagocytes that act together in innate host defense against invading bacteria [1–3]. These cells capture, phagocytose, and engulf bacteria into phagosomes. Phagosome maturation by sequential fusion with endocytic and lysosomal compartments results in the formation of an acidic and oxidative phagolysosome to degrade microbes. Macrophages are long-lived cells, and intracellular entry into these cells allows bacteria to persist and spread to other organs and tissues. Pathogenic microbes manipulate host macrophages to reside and replicate in these cells. Macrophages provide a nutrient-rich environment and shelter microbes from extra-cellular host defense such as complement-mediated lysis or uptake by neutrophils for degradation [4, 5]. Neutrophils have a shorter life span and higher microbicidal activities compared to macrophages, releasing degradative granule proteases and antimicrobial peptides upon activation. At sites of infection, activated macrophages release cytokines and chemokines to recruit neutrophils to infected areas. Once inside the macrophage, intracellular bacteria can live in vacuolar compartments or the cytosol, depending on their virulence factors which help them to evade host defense and replicate in these compartments [5]. Macrophages act as professional antigen-presenting cells in the development of adaptive immune response to pathogens. In addition, macrophages are also responsible for tissue homeostasis by phagocytosis of apoptotic cells and facilitate tissue repair and resolution of inflammation.

In the past few years, the biodefense community has undertaken research on the facultative, gram-negative

bacterium, *B. pseudomallei*, because it is highly pathogenic, and melioidosis is difficult to treat contributing to high mortality in endemic regions [6, 7]. Infection with *B. pseudomallei* results in various outcomes, ranging from acute fatal sepsis to chronic infection with or without clinical symptoms [6]. Prolonged combinational antibiotic therapy is required for treatment and to prevent relapse of melioidosis [7]. The high rate of infection recurrence and the possibility of latent infection with *B. pseudomallei* indicate the bacterium evades host defense mechanisms successfully. *B. pseudomallei* is resistant to the early microbicidal activities of macrophages and neutrophils after phagocytosis and replicates in both cell types *in vitro* [8]. *B. pseudomallei* and its virulence factors counteract and/or evade innate host defense components in phagocytes early to persist intracellularly. This review highlights the cross-regulation of three innate components of host defense: inflammasomes, autophagy, and cell death in counteracting the intracellular bacterium *B. pseudomallei*. The evasion strategies used by pathogenic bacteria such as *Mycobacterium tuberculosis* (*Mtb*), *Legionella pneumophila*, and *B. pseudomallei* to subvert these innate cellular processes will also be discussed.

2. Host Defender Macrophage as an Intracellular Niche for Bacteria

Macrophage phagocytosis of microbes activates NADPH oxidase resulting in reactive oxygen species (ROS) and inducible nitric oxide synthase which generates nitric oxide (NO). ROS and NO are antimicrobial and mediate the killing of microbes upon their engulfment in phagosomes [1]. Innate mediators, type I IFN (IFNI includes IFNα and IFNβ) and type II IFN (IFNγ), are potent activators of NO and ROS [9, 10]. Both types of IFNs also induce the expression of macrophage NADPH oxidase subunits to enhance ROS production [10]. Many intracellular bacteria including *B. pseudomallei* avoid colocalization with ROS and NO by escaping the phagosome. Bacteria use type III, type IV, type VI, or type VII secretion systems to inject diverse effectors to evade these potent antimicrobial molecules and escape various phagosomal compartments inside the macrophage [11, 12]. For example, the type III secretion system (T3SS) effectors of *B. pseudomallei* aid bacterial escape from the phagosome into the cytosol of mouse macrophages, where it replicates [13, 14]. In addition, the extracellular polysaccharide capsule of *B. pseudomallei* reduces macrophage phagocytosis by decreasing complement deposition [15]. The T4SS Icm/Dot apparatus of *L. pneumophila* inhibits phagosomal fusion with lysosomes and recruits endoplasmic reticulum (ER) vesicles to form a specialized vacuole for *Legionella* replication [16]. *Mtb* secretes SapM, a lipid phosphatase that hydrolyzes phosphatidylinositol 3-phosphate to prevent phagosome maturation and survive within the phagosome [17]. Pore-forming toxin, listeriolysin O, mediates the escape of *Listeria monocytogenes* from the phagolysosome and specialized vacuoles into the cytosol [18, 19]. *Francisella tularensis* uses T6SS effectors to escape endosome-like vacuoles and phagosomes to replicate in the cytosol [20]. Thus, pathogenic bacteria have evolved different mechanisms to subvert initial macrophage antimicrobial defense and replicate within these phagocytes [4, 5].

3. Autophagy in Host Defense against Intracellular Bacteria

Bacteria that escape the phagosome and phagosomal bacteria can be targeted for elimination by another innate component of host defense termed xenophagy, a selective form of autophagy [21–25]. Autophagy is an evolutionarily conserved cellular process for sequestration of cytoplasmic protein aggregates, dysfunctional organelles, and microbes for lysosomal degradation [21, 22]. Successful pathogens subvert the autophagic process or evade autophagy to avoid degradation [21–25]. Autophagy is activated by phagocytosis and pattern recognition receptors (PRRs), especially toll-like receptor 4 (TLR4) sensing of bacterial lipopolysaccharide (LPS), and is dependent on ROS generation from phagocytes [26]. In canonical autophagy, phagosomal intracellular microbes are enclosed by a double-membrane autophagosome which then fuses with lysosomes to form an autolysosome. Initiation of canonical autophagy requires ULK1 (Unc-51-like kinase 1), beclin 1, microtubule-associated protein light chain 3 (LC3), phosphatidylinositol 3-kinases, and many autophagy-related proteins (Atgs). LC3 conjugation onto a phagosome promotes the fusion of phagosome with lysosome. The capture of intracellular *B. pseudomallei* relies on a noncanonical autophagic process called LC3-associated phagocytosis (LAP). LAP uses a single membrane phagosome to enclose bacteria, which then fuses with lysosomes for degradation of bacteria [21–25]. Briefly, LAP requires a fewer number of Atg proteins and is highly dependent on NADPH oxidase 2 and phosphatidylinositol 3-phosphate for LC3 conjugation onto a phagosome [23, 26]. The T3SS effector protein BopA facilitates the escape of *B. pseudomallei* from LAP in mouse Raw264.7 cells [27]. BipD, a T3SS translocator protein, also participates in phagosomal escape of *B. pseudomallei* into the cytosol [28]. Importantly, evasion of autophagy improves *B. pseudomallei* survival in mouse macrophages. This is consistent with previous observations that most *B. pseudomallei* escape phagosomal degradation in human macrophages and neutrophils and replicate in the cytosol of these phagocytes [8, 29].

Mtb, the causative agent for tuberculosis, uses multiple mechanisms to subvert autophagy [30]. *Mtb* limits autophagy by preventing phagosomal maturation [31]. *Mtb* also blocks autophagosomal maturation, by inhibition of phagosome acidification. In contrast, *L. pneumophila*, an opportunistic human pathogen, inhibits autophagy directly by secretion of RavZ, a cysteine protease which cleaves phosphatidylethanolamine (PE) from PE-conjugated LC3 and inhibits autophagosome formation [32].

4. Cooperative Sensing of Bacteria by Pattern Recognition Receptors

Macrophages sense pathogens by using pattern recognition receptors (PRRs) to detect pathogen-associated molecular

patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs) induced by microbes and cell damage [33–37]. Cell surface PRRs, which include toll-like receptors (TLRs) and C-type lectin receptors (CLRs), detect bacteria and their secreted products extracellularly. Endosomal TLR3, TLR7, TLR8, and TLR9 sense bacterial and viral nucleic acids, inducing IFNI and proinflammatory cytokines [34–36]. Cytosolic PRRs include nucleotide-binding domain and leucine-rich repeat-containing protein (NLR) family members, absent in melanoma 2- (AIM2-) like receptors (ALRs), retinoic acid-inducible gene I- (RIG-I-) like receptors (RLRs), pyrin and HIN domain-containing family members, and other cytosolic nucleic sensors [38].

4.1. Toll-Like Receptors and Bacterial Ligands.
Extracellular TLR2 and TLR4 detect bacterial lipoproteins and lipopolysaccharide (LPS), respectively, to activate intracellular myeloid differentiation protein 88- (MyD88-) dependent NFκB and induce proinflammatory cytokines including IL1β and TNFα. An additional MyD88-independent TIR-domain-containing adaptor protein-inducing interferon-β (TRIF) pathway is triggered by TLR3 and TLR4 upon interaction with their respective ligands, dsRNA and LPS, to induce IFNβ via TRIF phosphorylation of the transcription factor IFN regulatory factor 3. Cell stress response pathways such as the mitogen-activated protein kinase (MAPK) cascade are also upregulated by various TLRs via MyD88. IL1β and TNFα are generally host protective. IL1β promotes neutrophil recruitment for pathogen clearance whereas TNFα stimulates IFNγ production from natural killer cells and T cells, initiates apoptosis, and activates MAPKs and the production of other cytokines [39]. IFNI has variable cell-type-specific effects and enhances host susceptibility to intracellular bacteria [40]. Tonic IFNI signaling protects against pathogens early in an infection, but sustained IFNI is detrimental during bacterial infection [41]. Some pathogenic bacteria produce virulence factors to downregulate TLR and cytokine signaling. For example, B. pseudomallei secretes a deubiquitinase TssM inside the macrophage to interrupt TLR-mediated NFκB signaling and proinflammatory cytokine induction [42]. TssM mutants are less virulent in mice and exhibit higher inflammation. Yersinia pestis virulence factor YopJ inhibits NFκB and MAPK signaling to dampen inflammation [43]. Virulent Mtb strains induce IFNI to limit IL1β production [44]. Mtb also inhibits IFNI signaling to promote its pathogenicity [45].

LPS, a conserved PAMP present in the outer membranes of gram-negative bacteria, is a potent activator of TLR4 [46]. LPS binds to host LPS-binding protein (LBP) and CD14 which then recruit MD2 and TLR4 on the macrophage cell surface for intracellular signal transduction [47, 48]. LPS consists of a lipid A moiety conjugated to an O-linked polysaccharide. Lipid A, the active moiety of LPS, is usually hexa-acylated, and changes in acylation decrease its ability to activate TLR4 [49]. Pathogenic bacteria modify their cell wall components to avoid detection by TLRs [50]. Y. pestis deacylates lipid A from its hexa- to tetra-acylated forms at human body temperature to avoid detection by human TLR4 as the bacteria transition from the flea to the human

host [51, 52]. F. tularensis also modifies the acylation of lipid A in response to temperature in order to maintain virulence in its host [53]. The tetra-acylated LPS of B. pseudomallei is a weak inducer of innate responses in vitro and in vivo [54]. Recently, hydroxylation was discovered as another modification that renders lipid A of B. pseudomallei less recognizable by TLR4 [55]. Various strains of B. pseudomallei with hydroxylated or tetra-acylated lipid A were found to be less cytotoxic and induce less TNFα in the mouse Raw264.7 cell line.

The cell wall components of Mtb are different from other intracellular bacteria. Cell wall lipomannan, lipoarbinomannan, lipoproteins, and mycolic acid of Mtb are mainly detected by TLR2 and, to a lesser degree, TLR4 [56]. TLR2 signaling activates NFκB and MAPK cascades. TLR2-dependent recognition of Legionella peptidoglycan and lipopeptides has been shown to stimulate a protective response in mice that limits Legionella replication [57, 58]. The cytosolic NOD2 (nucleotide-binding domain and leucine-rich repeat-containing receptor 2) binds to the Mtb cell wall component peptidoglycan and induces IFNI [59].

In addition to TLR4, recent studies reveal activation of a cytosolic noncanonical inflammasome by direct binding of LPS to mouse caspase 11 or human caspase 4 and caspase 5 [60]. Importantly, caspase 11 is required for LPS-induced endotoxemia, whereas TLR4 only primes this response [61, 62]. Mouse caspase 11 also detects B. pseudomallei LPS and induces pyroptosis in macrophages and low levels of IL1β and IL18 by indirect activation of NLRP3 (NLR family pyrin domain-containing protein 3) [62]. TLR5 binds to flagellin and stimulates host response caused by flagellated bacteria, including B. pseudomallei and L. pneumophila [63, 64]. Flagellin is also detected by cytosolic sensors NLRC4 (NLR family CARD-containing protein 4) and NAIPs (NLR family apoptosis inhibitory proteins), which also detect various bacterial T3SS effectors [65].

4.2. Cytosolic Pattern Recognition Receptors Activate Inflammasomes.
Intracellular PRRs patrol cytosolic compartments for pathogens, PAMPs, and DAMPs with NLRs, which serve as platforms for the assembly of multiprotein complexes called inflammasomes [66–69]. NLRs are classified based on their protein structure with the sensor and signaling domain-defining proteins that each NLR interacts with [70]. Most NLRs contain a C-terminal leucine-rich repeat domain which mediates ligand binding, a nucleotide-binding domain, and a N-terminal signaling domain which can be a pyrin domain or a caspase activation and recruitment domain (CARD) [70]. The signaling domain CARD recruits caspase 1 directly whereas pyrin domain receptors (NLRP3 and AIM2) require an additional adaptor ASC (apoptosis-associated speck-like protein containing a carboxy-terminal CARD) for interaction with caspase 1. NLRP3 and AIM2 oligomerize ASC through the interaction between the pyrin domain of ASC and the pyrin domain of NLR. The CARD of ASC then interacts with the CARD of caspase 1, resulting in autoproteolytic activation of caspase 1. Active caspase 1 then cleaves pro-IL1β and pro-IL18 and induces the extracellular release of active IL1β and IL18

and pyroptosis [71]. IL1β and IL18 have host protective functions as IL-1 recruits neutrophils to eliminate bacteria and IL18 induces IFNγ to activate phagocytes.

The triggers for activation of NLRs are different, with NLRP3 being the most complex, as it is activated by a diverse group of stimuli, including bacteria, viruses, fungi, pore-forming toxins hemolysin and nigericin, ATP, uric acid crystals, and peptide aggregates. Other proposed upstream activators of NLRP3 are ROS, mitochondrial DNA, phagosomal or lysosomal rupture, and potassium efflux [72]. Since these stimuli do not have a common structure, it is likely that induced cellular perturbation activates NLRP3. In contrast, NLRC4 and AIM2 inflammasomes recognize defined PAMPs. Bacterial T3SS effectors and flagellin activate NLRC4 after recruiting NAIP coreceptors without ASC [73, 74]. AIM2 recognizes cytosolic dsDNA from vacuolar bacteria that escape to the cytosol and recruits ASC for caspase 1 activation [75]. Necrotic cell DNA can also be detected by AIM2 [76].

Caspase 11, the intracellular sensor of LPS and a noncanonical inflammasome, induces pyroptosis without additional proteins/adaptors and does not process IL1β and IL18 directly [61, 62]. Mouse caspase 1 and caspase 11 cleave the protein gasdermin D (GSDMD) to induce pyroptosis [77, 78]. The N-terminal fragment of GSDMD binds to membrane lipids, then oligomerizes to form pores resulting in cell lysis [79]. Caspase 4 and caspase 5 are human orthologs of murine caspase 11 and detect cytosolic LPS [80–83]. The transcription of caspase 4 and caspase 5 is upregulated by LPS or IFNγ [84]. Caspase 11-deficient mice are highly susceptible to B. pseudomallei [85] confirming that, at least in mice, caspase 11-mediated pyroptosis is host protective by eliminating the replicative niche for this bacterium.

Most in vitro investigations of inflammasome activation using bacterial virulence factors or bacteria mutants lacking these factors were performed with mouse cell lines or bone marrow-derived macrophages. Mouse inflammasomes differ from human inflammasomes, both in ligand specificity and in number and types of inflammasomes. There are three NLRP1 isoforms in mice and only one human NLRP1 with different ligand specificities from mouse NLRP1s [86]. Mouse NLRC4 isoforms can be stimulated by flagellin, T3SS rod, and needle proteins [87]. The only ligand for human NLRC4 is T3SS needle protein [74]. The IFN-induced GTPase proteins, immunity-related GTPase (IRG) and guanylate-binding proteins (GBPs), are critical for modulating macrophage activities including autophagy and inflammasome activation [10]. The human and mouse IFN-induced GTPase proteins are vastly different [88, 89]. Mice have more IRGs and GBPs, each with more diverse functions when compared to human IFN-induced GTPase proteins. These differences create some confusion regarding NLR activation by different PAMPs and their regulation by IFNs in human and mouse macrophages.

Murine NLRC4 does not bind to flagellin or T3SS rod and needle proteins directly, rather it requires NAIPs for binding, which then recruit NLRC4 to assemble a canonical caspase 1-dependent inflammasome in the cytoplasm [74, 87, 90]. B. pseudomallei flagellin binds to NAIP5 and activates NLRC4 in mouse macrophages. In a murine model of respiratory melioidosis, TLR5 and NLRC4 each contributed equally to survival as single-knockout mice were as susceptible as animals deficient in both TLR5 and NLRC4 [91]. Thus, TLR5 and NLRC4 have nonredundant functions in pulmonary melioidosis, although they both bind to flagellin [91]. Interestingly, deficiency of caspase 1 and caspase 11 in this mouse model leads to more severe pulmonary inflammation than deficiency of NLRC4, which demonstrates cooperation between the different types of inflammasomes.

The flagellin of L. pneumophila binds to NAIP5 and oligomerizes with NLRC4 to form an inflammasome in mouse macrophages [73]. NLRC4 interacts with caspase 1 directly through its respective CARD without recruitment of ASC. NLRC4 and NAIP5 also stimulate autophagosome turnover and coordinate pyroptosis with autophagy to prevent excessive cell death in L. pneumophila-infected macrophages [92]. It appears that in unstimulated mouse macrophages from resistant C57B6 mice, NLRC4 associates with autophagic protein beclin 1/Atg6. Upon Legionella infection, PAMPs binding to NLRC4 release beclin 1 to induce autophagy. This autophagic response protects macrophages from caspase 1-mediated pyroptosis. Thus, unlike humans, C57B6 mice resist L. pneumophila by increasing autophagy when inflammasome NLCR4 is activated by flagellin. Considering that autophagy also controls IL1β by targeting IL1β for degradation [93], the upregulation of autophagy after NLRC4 activation by Legionella PAMPs is likely a host feedback mechanism to control bacterial replication. Mtb only activates the NLRP3 inflammasome, likely by potassium depletion after phagocytosis [94]. The production of IL1β by inflammasomes during infection with Mtb is critical for a protective host immune response. In contrast, IFNβ induced after Mtb infection can suppress NLRP3 inflammasome activation. Inflammasome regulation by both IFNβ and IL1β illustrates the opposing role of cytokines in modulating inflammation and cell death.

The T7SS effector ESX-1 of Mtb damages the phagosomal membrane and releases phagosomal mycobacterial products to the cytosol [95]. One such product is bacterial DNA, which is detected by cytosolic DNA sensors and induces IFNI [96]. The induction of IFNI by Mtb is associated with mycobacterial virulence and macrophage necrosis [97]. Another sensor of cytosolic bacterial DNA is cyclic GMP-AMP synthase (cGAS). cGAS binds to endoplasmic reticulum-associated adaptor protein STING (stimulator of IFN genes) which then recruits other IFN-inducing proteins including IRF3 [98]. The cGAS/STING pathway induces IFN, cytokines, and chemokines. Legionella and Mtb DNA can be detected by cGAS/STING, inducing IFNs which can indirectly modulate inflammasomes [98, 99].

5. Phagocyte Cell Death Modes Determine Pathogenesis

Induction of cell death is an effective strategy used by macrophages to clear intracellular pathogens by expelling bacteria from their replicative niche [4, 5]. Paradoxically, successful

intracellular bacteria modulate different forms of cell death in phagocytes: apoptosis, pyroptosis, and necrosis/ necroptosis to evade host defense [100, 101]. The mode of cell death in phagocytes is highly dependent on the bacterial load and secreted bacterial cytotoxins. The Cif (cycle inhibiting factor) homolog in BP (CHBP) effector induces apoptosis in mouse BMDM as it deamidates host ubiquitin (Ub) and an Ub-like protein [102].

Apoptosis is an active programmed process of packaging cell contents for elimination without inducing inflammation and is dependent on sequential proteolytic activation of caspases [103]. Apoptosis has homeostatic functions during development, infection, and the recovery phase of infection, as this form of cell death is noninflammatory [104, 105]. An apoptotic cell packages cellular contents into membrane-bound apoptotic bodies which are cleared later by another phagocyte in a process termed efferocytosis [105]. Apoptotic cells display many recognition receptors for their removal by phagocytes [106]. However, the uptake of apoptotic cells leads to a reduction of antimicrobial activities in the efferocytosing cell [105]. Uncleared apoptotic cells usually become necrotic and release cellular DAMPs to promote tissue injury. In the context of intracellular infection, excess apoptosis may also be detrimental as efferocytosing phagocytes are less antimicrobial and contribute to bacterial expansion and persistence.

The extrinsic or intrinsic pathways of apoptosis are mediated by ligands binding to the TNF receptor superfamily or by mitochondrial damage, respectively [103]. Extrinsic apoptosis is dependent on caspase 8 activation whereas intrinsic apoptosis is dependent on caspase 9. Both pathways are dependent on caspase 3 and caspase 7 as the executioners of apoptosis. The TNF receptor family includes TNFα receptor (TNFR1) and death receptors and activates the extrinsic pathway of apoptosis upon binding to their respective ligands. Induction of apoptosis of infected cells likely includes both the extrinsic and intrinsic pathways. TNFα produced by PAMP activation of TLRs in infected cells triggers the extrinsic pathway. Simultaneously, the intrinsic pathway of apoptosis is activated by mitochondrial ROS and damage induced by intracellular pathogens.

TNFR1 contains a death domain (DD) and can trigger apoptosis, necroptosis, and NFκB-activated prosurvival signals depending on the different signaling complexes formed after binding to TNFα [107]. The primary function of TNFR1 signaling is proinflammatory gene induction, with the cell death pathway as a backup response when infection is unresolved [39]. The TNFR1 signaling complex for NFκB activation is highly dependent on the presence of cellular inhibitors of apoptosis proteins (cIAP1 and cIAP2) [107, 108]. Other DD-containing death receptors include the FAS (CD95) and TNF-related apoptosis-inducing ligand (TRAIL) receptors which are also induced by intracellular bacteria. Ligand binding to FAS and TRAIL receptors induces either apoptosis or necroptosis depending on the activation status of the receptor-interacting serine/threonine-protein kinase 3 (RIPK3) [107]. The death-inducing signaling complexes for apoptosis and necroptosis formed by TNFR1, FAS, and TRAIL receptors are similar, initiating necroptosis when RIPK3 is activated by phosphorylation.

Necrosis is a form of lytic cell death used by some bacteria to disseminate and evade host immune responses. *Mtb* infects macrophages and induces necrosis to avoid the intracellular immune response in macrophages and to disseminate [109]. Virulent *Mtb* strains induce macrophage necrosis and IFNI, whereas avirulent strains do not [99, 109]. *Mtb* replicates in necrotic macrophages, underscoring its persistence even after macrophage demise. Necroptosis is programed necrosis that is induced when caspase 8 activity is blocked [107]. Necroptosis occurs when the homologous serine/threonine kinases, RIPK1 and RIPK3, interact through their RHIM (RIP homotypic interaction motif) resulting in RIPK3 phosphorylation and activation. RIPK3 then phosphorylates and activates the pseudokinase MLKL (mixed lineage kinase domain-like). Activated MLKL induces pore formation in the plasma membrane and cell leakage of DAMPs. A number of recent studies reveal that the apoptotic caspase 8 has many other activities, including suppression of necroptosis by cleavage of RIPK1, RIPK3, and caspase 1; proteolytic processing of IL1β to its mature active form; priming NLRP3; and regulation of cytokine transcriptional responses [110, 111]. The crossover inflammatory activities of caspase 8 indicate that caspase 8 also acts as an inflammasome depending on its catalytic action on caspase 1, IL1β, RIPK1, and RIPK3, as well as the absence or depletion of caspase inhibitors.

Canonical inflammasomes NLRP3, NLRC4, and AIM2 recruit caspase 1 through the CARD of NLR or recruited ASC adaptor. Autoproteolytic activity of caspase 1 results in cleavage of pro-IL1β, pro-IL18, GSDMD, and pyroptosis. Human macrophage THP-1 cell line and mouse peritoneal macrophages infected with *B. pseudomallei* succumb to caspase 1-dependent pyroptosis, presumably via NLRP3 activation [112]. *In vitro*, peritoneal macrophages from caspase 1-deficient mice are resistant to *B. pseudomallei*-induced cytotoxicity [112]. Caspase 11 also promotes pyroptosis but with inefficient cytokine production. Caspase 1 and caspase 11 knockout mice are highly susceptible to *B. pseudomallei*, and both types of mice have higher bacterial burdens [85, 113]. IL1β and the IFNγ-inducing factor IL18 released by canonical inflammasomes are inflammatory and host protective, as IL1β recruits neutrophils to sites of infection and IFNγ activates phagocyte microbicidal activities. In murine melioidosis, multiple T3SS effectors activate NLRC4 to induce caspase 1 and pyroptosis in BMDM [114]. *Legionella* also activates NLRC4, and in resistant C57B6 macrophages, NLRC4 inflammasome activation induces autophagy to dampen inflammation [92]. Neutrophils are unable to undergo pyroptosis and continue to produce IL1β after bacterial infection [115]. Unlike most other bacteria, *B. pseudomallei* replicates in both macrophages and neutrophils [8, 29]. Thus, neutrophils harboring *B. pseudomallei* can be taken up by another phagocyte, either a macrophage or a neutrophil, promoting infection by cell-to-cell spread [116]. Pyroptosis contributes to pathogen clearance in *Legionella* infection by removing the macrophage, their only replicative niche. In contrast, pyroptosis

TABLE 1: Interplay of *Burkholderia pseudomallei* (*Bp*), *Mycobacterium tuberculosis* (*Mtb*), and *Legionella pneumophila* (*Lp*) with autophagy, inflammasomes, and host cell death modes.

	Autophagy	Inflammasomes	Cell death
Bp	T3SS effectors aid bacterial escape to the cytosol [13, 14]. Bacteria evade autophagy [27].	LPS is a weak agonist of TLR4 [54]. Cytosolic LPS activates caspase 11 [85]. Flagellin activates TLR5 [63] and NAIP5/NLRC4 [90, 114].	Caspase 1 and caspase 11 mediate pyroptosis [85, 112, 113]. Caspase 1- and caspase 11-knockout mice are highly susceptible to pyroptosis [85, 113]. T3SS BsaK induces pyroptosis [114].
Mtb	Autophagy restricts *Mtb* growth [30]. SapM prevents phagosome maturation [17]. Viable *Mtb* disrupts phagolysosome maturation [31].	ESX-1 induces lysosomal release of IL1β and IL18 [95]. ESAT-6 activates NLRP3 and ruptures phagosomes [94]. *Mtb* DNA released by ESX-1 activates cGAS/STING/IFNI [96, 99].	Virulent *Mtb* promotes macrophage necrosis via ESX-1 [95, 109]. Phagosome rupture induces necrosis [97]. Virulent *Mtb* induces IFNI to suppress inflammasomes [41] and promotes necrosis [97].
Lp	RavZ inhibits autophagy [32]. T4SS Icm/Dot promotes specialized vacuoles for bacterial replication [16, 23].	Lipopeptides activate TLR2 and limit bacterial replication [57, 58]. Flagellin activates NAIP5/NLRC4 [73] and enhances autophagy [92]. cGAS/STING/IFNI activation targets vacuoles to eliminate bacteria [118].	NAIP5/NLRC4 activation promotes pyroptosis [73]. Coordination of pyroptosis and autophagy by inflammasomes prevents excessive cell death in C57/B6 mice [92].

of *B. pseudomallei*-infected macrophages may not be a beneficial host response as DAMPs released from cell lysis promote tissue necrosis and lower the infection barrier. The continued IL1β release by inflammasomes fuels neutrophil recruitment, which is detrimental, since *B. pseudomallei* replicates in neutrophils. Dampening pyroptosis and necroptosis is central to control tissue injury and host survival.

6. Cross-Regulation of Inflammasome, Autophagy, and Cell Death in Bacterial Infection

Inflammasome activation by cytosolic PAMPs and DAMPs promotes proinflammatory cytokine production and different types of cell death. Macrophage apoptosis limits cells from further cytokine production and restricts bacteria that cannot replicate in other cell types. Macrophage pyroptosis induced by inflammasomes after detecting cytosolic bacteria expels intracellular bacteria from their replicative niche but also releases proinflammatory IL1β and DAMPs. IL1β is generally host protective against bacteria such as *Mtb* and *Legionella* but is inflammatory nonetheless. IL1β recruitment of neutrophils is damaging in *B. pseudomallei* infection, since this bacterium replicates in neutrophils, evades autophagy, and activates inflammasomes. C57B6 macrophages resist *Legionella* infection by increasing autophagy to eliminate bacteria [92]. Cytokines IL1β, TNFα, and IFNs induced by TLRs and inflammasomes via pathogens, PAMPs, and DAMPs modulate inflammasomes, autophagy, and cell death depending on the timing of cytokine induction and cellular context. TNFα can induce different types of cell death depending on the formation of death complexes with caspase 8 and cIAPs as critical modulators of necroptosis. IFNI plays a critical role in cell death as it synergizes with TNFα to induce necroptosis. Basal IFNI is protective against certain pathogens by sustaining key intermediates of signaling molecules that have antimicrobial activities [10, 88, 117]. IFNβ signaling induces caspase 11 and AIM2 expression, leading to enhanced cytosolic sensing of bacterial contamination in the cytosol [10, 118]. In addition, IFN-induced IRGs and GBPs play pivotal roles in antimicrobial defense against intracellular bacteria, especially those that reside in vacuoles. GBPs recognize specific host lipid molecules on pathogen-containing vacuoles and mark them for disruption or delivery to lysosomes. Thus, IFNI promotes clearance of *Legionella* as it disrupts vacuolar compartments where the bacterium localizes. The coordination of pyroptosis and enhanced autophagy by inflammasome activation in *Legionella* infection results in bacterial clearance and survival of C57/B6 mice [92]. Cytosolic ubiquitylated bacteria are also detected by GBPs which mark them for autophagic degradation. Host-intrinsic IFN-sustained GBPs promote NLRP3, caspase 1, and caspase 11 activation. IFNI, IL1β, and TNFα fine-tune inflammasomes, autophagy, and caspase 8-mediated cell death modes to control many aspects of bacterial infections. A summary of the interactions of autophagy, inflammasomes, and host cell death modes with *B. pseudomallei*, *Mtb*, and *Legionella* is presented in Table 1.

7. Conclusions and Perspectives

Autophagy, inflammasome, and cell death are cellular processes fundamental to innate host defense against pathogens. Initiation of autophagy upon phagocytosis ensures pathogen capture and elimination. Inflammation via inflammasome activation after sensing pathogens in the cytosol clearly guards host cells against microbial contamination in the cytosol. TLRs, NLRs, AIM2, and noncanonical inflammasomes caspase 11 and caspase 8 cooperate to ensure a robust immune response by induction of inflammatory mediators to recruit and activate phagocytes to kill pathogens. Pyroptosis of macrophages, induced by caspase 11

and NLRs, expels cytosolic bacteria from their replicative niche. This form of inflammatory cell death has detrimental consequences as macrophage death decreases the very cell that can kill microbes and releases DAMPs that further stimulate inflammation and prevent resolution of disease and pathogen clearance. In *B. pseudomallei* infection, inflammasome activation, pyroptosis, and necroptosis appear to be detrimental due to excessive cell death and collateral damage in vital organs. A better understanding of the cross-regulation of these processes of innate host defense against intracellular bacteria might facilitate target identification for host-directed therapeutics against pathogens.

Disclosure

Conflicts of Interest

The author declares no conflict of interest.

Acknowledgments

References

[1] M. T. Silva, "When two is better than one: macrophages and neutrophils work in concert in innate immunity as complementary and cooperative partners of a myeloid phagocyte system," *Journal of Leukocyte Biology*, vol. 87, no. 1, pp. 93–106, 2010.

[2] S. Gordon, "Phagocytosis: an immunobiologic process," *Immunity*, vol. 44, no. 3, pp. 463–475, 2016.

[3] P. Nordenfelt and H. Tapper, "Phagosome dynamics during phagocytosis by neutrophils," *Journal of Leukocyte Biology*, vol. 90, no. 2, pp. 271–284, 2011.

[4] J. V. Price and R. E. Vance, "The macrophage paradox," *Immunity*, vol. 41, no. 5, pp. 685–693, 2014.

[5] G. Mitchell, C. Chen, and D. A. Portnoy, "Strategies used by bacteria to grow in macrophages," *Microbiology Spectrum*, vol. 4, no. 3, 2016.

[6] W. J. Wiersinga, B. J. Currie, and S. J. Peacock, "Melioidosis," *New England Journal of Medicine*, vol. 367, no. 11, pp. 1035–1044, 2012.

[7] D. Dance, "Treatment and prophylaxis of melioidosis," *International Journal of Antimicrobial Agents*, vol. 43, no. 4, pp. 310–318, 2014.

[8] S. Pruksachartvuthi, N. Aswapokee, and K. Thankerngpol, "Survival of Pseudomonas pseudomallei in human phagocytes," *Journal of Medical Microbiology*, vol. 31, no. 2, pp. 109–114, 1990.

[9] A. H. Ding, C. F. Nathan, and D. J. Stehr, "Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production," *The Journal of Immunology*, vol. 141, pp. 2407–2412, 1988.

[10] J. D. MacMicking, "Interferon-inducible effector mechanisms in cell-autonomous immunity," *Nature Reviews Immunology*, vol. 12, no. 5, pp. 367–382, 2012.

[11] S. Méresse, O. Steele-Mortimer, E. Moreno, M. Desjardins, B. Finlay, and J.-P. Gorvel, "Controlling the maturation of pathogen-containing vacuoles: a matter of life and death," *Nature Cell Biology*, vol. 1, no. 7, pp. E183–E188, 1999.

[12] E. A. Creasey and R. R. Isberg, "Maintenance of vacuole integrity by bacterial pathogens," *Current Opinion in Microbiology*, vol. 17, pp. 46–52, 2014.

[13] M. N. Burtnick, P. J. Brett, V. Nair, J. M. Warawa, D. E. Woods, and F. C. Gherardini, "Burkholderia pseudomallei type III secretion system mutants exhibit delayed vacuolar escape phenotypes in RAW 264.7 murine macrophages," *Infection and Immunity*, vol. 76, no. 7, pp. 2991–3000, 2008.

[14] J. K. Stone, D. DeShazer, P. J. Brett, and M. N. Burtnick, "Melioidosis: molecular aspects of pathogenesis," *Expert Review of Anti-Infective Therapy*, vol. 12, no. 12, pp. 1487–1499, 2014.

[15] S. L. Reckseidler-Zenteno, R. DeVinney, and D. E. Woods, "The capsular polysaccharide of Burkholderia pseudomallei contributes to survival in serum by reducing complement factor C3b deposition," *Infection and Immunity*, vol. 73, no. 2, pp. 1106–1115, 2005.

[16] I. Derre and R. R. Isberg, "Legionella pneumophila replication vacuole formation involves rapid recruitment of proteins of the early secretory system," *Infection and Immunity*, vol. 72, no. 5, pp. 3048–3053, 2004.

[17] I. Vergne, J. Chua, H.-H. Lee, M. Lucas, J. Belisle, and V. Deretic, "Mechanism of phagolysosome biogenesis block by viable Mycobacterium tuberculosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 11, pp. 4033–4038, 2005.

[18] C. L. Birmingham, V. Canadien, N. A. Kaniuk, B. E. Steinberg, D. E. Higgins, and J. H. Brumell, "Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles," *Nature*, vol. 451, no. 7176, pp. 350–354, 2008.

[19] P. Schnupf and D. A. Portnoy, "Listeriolysin O: a phagosome-specific lysin," *Microbes and Infection*, vol. 9, no. 10, pp. 1176–1187, 2007.

[20] J. R. Barker, A. Chong, T. D. Wehrly et al., "The Francisella tularensis pathogenicity island encodes a secretion system that is required for phagosome escape and virulence," *Molecular Microbiology*, vol. 74, no. 6, pp. 1459–1470, 2009.

[21] V. Deretic and B. Levine, "Autophagy, immunity, and microbial adaptations," *Cell Host & Microbe*, vol. 5, no. 6, pp. 527–549, 2009.

[22] C. He and D. J. Klionsky, "Regulation mechanisms and signaling pathways of autophagy," *Annual Review of Genetics*, vol. 43, no. 1, pp. 67–93, 2009.

[23] M. Cemma and J. H. Brumell, "Interactions of pathogenic bacteria with autophagy systems," *Current Biology*, vol. 22, no. 13, pp. R540–R545, 2012.

[24] J. Huang and J. H. Brumell, "Bacteria-autophagy interplay: a battle for survival," *Nature Reviews Microbiology*, vol. 12, no. 2, pp. 101–114, 2014.

[25] A. Bah and I. Vergne, "Macrophage autophagy and bacterial infections," *Frontiers in Immunology*, vol. 8, p. 1483, 2017.

[26] J. Huang, V. Canadien, G. Y. Lam et al., "Activation of anti-bacterial autophagy by NADPH oxidases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 15, pp. 6226–6231, 2009.

[27] L. Gong, M. Cullinane, P. Treerat et al., "The Burkholderia pseudomallei type III secretion system and BopA are required for evasion of LC3-associated phagocytosis," *PLoS One*, vol. 6, no. 3, article e17852, 2011.

[28] V. Muangsombut, S. Suparak, P. Pumirat et al., "Inactivation of Burkholderia pseudomallei bsaQ results in decreased invasion efficiency and delayed escape of bacteria from endocytic vesicles," *Archives of Microbiology*, vol. 190, no. 6, pp. 623–631, 2008.

[29] A. L. Jones, T. J. Beveridge, and D. E. Woods, "Intracellular survival of Burkholderia pseudomallei," *Infection and Immunity*, vol. 64, no. 3, pp. 782–790, 1996.

[30] M. G. Gutierrez, S. S. Master, S. B. Singh, G. A. Taylor, M. I. Colombo, and V. Deretic, "Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages," *Cell*, vol. 119, no. 6, pp. 753–766, 2004.

[31] R. A. Fratti, J. Chua, I. Vergne, and V. Deretic, "Mycobacterium tuberculosis glycosylated phosphatidylinositol causes phagosome maturation arrest," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 9, pp. 5437–5442, 2003.

[32] A. Choy, J. Dancourt, B. Mugo et al., "The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation," *Science*, vol. 338, no. 6110, pp. 1072–1076, 2012.

[33] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[34] A. L. Blasius and B. Beutler, "Intracellular toll-like receptors," *Immunity*, vol. 32, no. 3, pp. 305–315, 2010.

[35] N. Arpaia and G. M. Barton, "The impact of toll-like receptors on bacterial virulence strategies," *Current Opinion in Microbiology*, vol. 16, no. 1, pp. 17–22, 2013.

[36] T. Kawai and S. Akira, "Toll-like receptors and their crosstalk with other innate receptors in infection and immunity," *Immunity*, vol. 34, no. 5, pp. 637–650, 2011.

[37] S. Akira, "TLR signaling," *Current Topics of Microbiology and Immunology*, vol. 311, pp. 1–16, 2006.

[38] P. Broz and D. M. Monack, "Newly described pattern recognition receptors team up against intracellular pathogens," *Nature Reviews Immunology*, vol. 13, no. 8, pp. 551–565, 2013.

[39] T. Krakauer, J. Vilcek, and J. J. Oppenheim, "TNF family cytokines, chemokines and other pro- or anti-inflammatory cytokines," in *Fundamental Immunology*, W. Paul, Ed., pp. 783–819, Raven Press, New York, NY, USA, 4th edition, 1998.

[40] F. McNab, K. Mayer-Barber, A. Sher, A. Wack, and A. O'Garra, "Type I interferons in infectious disease," *Nature Reviews Immunology*, vol. 15, no. 2, pp. 87–103, 2015.

[41] S. A. Stifter and C. G. Feng, "Interfering with immunity: detrimental role of type I IFNs during infection," *Journal of Immunology*, vol. 194, no. 6, pp. 2455–2465, 2015.

[42] K. S. Tan, Y. Chen, Y. C. Lim et al., "Suppression of host innate immune response by Burkholderia pseudomallei through the virulence factor TssM," *Journal of Immunology*, vol. 184, no. 9, pp. 5160–5171, 2010.

[43] C. R. Sweet, J. Conlon, D. T. Golenbock, J. Goguen, and N. Silverman, "YopJ targets TRAF proteins to inhibit TLR-mediated NF-kappaB, MAPK and IRF3 signal transduction," *Cellular Microbiology*, vol. 9, no. 11, pp. 2700–2715, 2007.

[44] A. Novikov, M. Cardone, R. Thompson et al., "Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1β production in human macrophages," *Journal of Immunology*, vol. 187, no. 5, pp. 2540–2547, 2011.

[45] S. Prabhakar, Y. Qiao, Y. Hoshino et al., "Inhibition of response to alpha interferon by Mycobacterium tuberculosis," *Infection and Immunity*, vol. 71, no. 5, pp. 2487–2497, 2003.

[46] C. R. H. Raetz and C. Whitfield, "Lipopolysaccharide endotoxins," *Annual Review of Biochemistry*, vol. 71, no. 1, pp. 635–700, 2002.

[47] Z. Jiang, P. Georgel, X. Du et al., "CD14 is required for MyD88-independent LPS signaling," *Nature Immunology*, vol. 6, no. 6, pp. 565–570, 2005.

[48] B. S. Park, D. H. Song, H. M. Kim, B. S. Choi, H. Lee, and J. O. Lee, "The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex," *Nature*, vol. 458, pp. 191–195, 2009.

[49] H. Takada, S. Kotani, and O. Lüderitz, "Structural requirements of lipid A for endotoxicity and other biological activities," *CRC Critical Reviews in Microbiology*, vol. 16, no. 6, pp. 477–523, 2009.

[50] Y. Li, D. A. Powell, S. A. Shaffer et al., "LPS remodeling is an evolved survival strategy for bacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 22, pp. 8716–8721, 2012.

[51] K. Kawahara, H. Tsukano, H. Watanabe, B. Lindner, and M. Matsuura, "Modification of the structure and activity of lipid A in Yersinia pestis lipopolysaccharide by growth temperature," *Infection and Immunity*, vol. 70, no. 8, pp. 4092–4098, 2002.

[52] M. Matsuura, H. Takahashi, H. Watanabe, S. Saito, and K. Kawahara, "Immunomodulatory effects of Yersinia pestis lipopolysaccharides on human macrophages," *Clinical and Vaccine Immunology*, vol. 17, no. 1, pp. 49–55, 2009.

[53] X. Wang, A. A. Ribeiro, Z. Guan, S. N. Abraham, and C. R. H. Raetz, "Attenuated virulence of a Francisella mutant lacking the lipid A 4'-phosphatase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 10, pp. 4136–4141, 2007.

[54] M. H. Norris, H. P. Schweizer, and A. Tuanyok, "Structural diversity of Burkholderia pseudomallei lipopolysaccharides affects innate immune signaling," *PLoS Neglected Tropical Diseases*, vol. 11, no. 4, article e0005571, 2017.

[55] M. H. Norris, N. Somprasong, H. P. Schweizer, and A. Tuanyok, "Lipid A remodeling is a pathoadaptive mechanism that impacts lipopolysaccharide recognition and intracellular survival of Burkholderia pseudomallei," *Infection and Immunity*, vol. 86, no. 10, 2018.

[56] C. E. Stamm, A. C. Collins, and M. U. Shiloh, "Sensing of Mycobacterium tuberculosis and consequences to both host and bacillus," *Immunological Reviews*, vol. 264, pp. 204–219, 2005.

[57] K. A. Archer and C. R. Roy, "MyD88-dependent responses involving toll-like receptor 2 are important for protection and clearance of Legionella pneumophila in a mouse model of Legionnaires' disease," *Infection and Immunity*, vol. 74, no. 6, pp. 3325–3333, 2006.

[58] T. R. Hawn, K. D. Smith, A. Aderem, and S. J. Skerrett, "Myeloid differentiation primary response gene (88)— and toll-like receptor 2—deficient mice are susceptible to infection with aerosolized Legionella pneumophila," *The Journal of Infectious Diseases*, vol. 193, no. 12, pp. 1693–1702, 2006.

[59] A. K. Pandey, Y. Yang, Z. Jiang et al., "NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to Mycobacterium tuberculosis," *PLoS Pathogens*, vol. 5, no. 7, article e1000500, 2009.

[60] J. Shi, Y. Zhao, Y. Wang et al., "Inflammatory caspases are innate immune receptors for intracellular LPS," *Nature*, vol. 514, no. 7521, pp. 187–192, 2014.

[61] N. Kayagaki, M. T. Wong, I. B. Stowe et al., "Noncanonical inflammasome activation by intracellular LPS independent of TLR4," *Science*, vol. 341, no. 6151, pp. 1246–1249, 2013.

[62] J. A. Hagar, D. A. Powell, Y. Aachoui, R. K. Ernst, and E. A. Miao, "Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock," *Science*, vol. 341, no. 6151, pp. 1250–1253, 2013.

[63] T. E. West, N. Chantratita, W. Chierakul et al., "Impaired TLR5 functionality is associated with survival in melioidosis," *Journal of Immunology*, vol. 190, no. 7, pp. 3373–3379, 2013.

[64] T. R. Hawn, W. R. Berrington, I. A. Smith et al., "Altered inflammatory responses in TLR5-deficient mice infected with Legionella pneumophila," *Journal of Immunology*, vol. 179, no. 10, pp. 6981–6987, 2007.

[65] J. L. Tenthorey, E. M. Kofoed, M. D. Daugherty, H. S. Malik, and R. E. Vance, "Molecular basis for specific recognition of bacterial ligands by NAIP/NLRC4 inflammasomes," *Molecular Cell*, vol. 54, no. 1, pp. 17–29, 2014.

[66] E. Latz, T. S. Xiao, and A. Stutz, "Activation and regulation of the inflammasomes," *Nature Reviews Immunology*, vol. 13, no. 6, pp. 397–411, 2013.

[67] M. H. Shaw, T. Reimer, Y. G. Kim, and G. Nunez, "NOD-like receptors (NLRs): bona fide intracellular microbial sensors," *Current Opinion in Immunology*, vol. 20, no. 4, pp. 377–382, 2008.

[68] D. E. Place and T. D. Kanneganti, "Recent advances in inflammasome biology," *Current Opinion Immunology*, vol. 50, pp. 32–38, 2008.

[69] V. A. K. Rathinam and K. A. Fitzgerald, "Inflammasome complexes: emerging mechanisms and effector functions," *Cell*, vol. 165, no. 4, pp. 792–800, 2016.

[70] J. P.-Y. Ting, R. C. Lovering, E. S. Alnemri et al., "The NLR gene family: a standard nomenclature," *Immunity*, vol. 28, no. 3, pp. 285–287, 2008.

[71] E. A. Miao, J. V. Rajan, and A. Aderem, "Caspase-1-induced pyroptotic cell death," *Immunology Reviews*, vol. 243, no. 1, pp. 206–214, 2011.

[72] R. Muñoz-Planillo, P. Kuffa, G. Martínez-Colón, B. L. Smith, T. M. Rajendiran, and G. Núñez, "K$^+$ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter," *Immunity*, vol. 38, no. 6, pp. 1142–1153, 2013.

[73] Y. Zhao, J. Yang, J. Shi et al., "The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus," *Nature*, vol. 477, no. 7366, pp. 596–600, 2011.

[74] R. E. Vance, "The NAIP/NLRC4 inflammasomes," *Current Opinion in Immunology*, vol. 32, pp. 84–89, 2015.

[75] V. Hornung, A. Ablasser, M. Charrel-Dennis et al., "AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC," *Nature*, vol. 458, no. 7237, pp. 514–518, 2009.

[76] T. Komada, H. Chung, A. Lau et al., "Macrophage uptake of necrotic cell DNA activates the AIM2 inflammasome to regulate a proinflammatory phenotype in CKD," *Journal of the American Society of Nephrology*, vol. 29, no. 4, pp. 1165–1181, 2018.

[77] N. Kayagaki, I. B. Stowe, B. L. Lee et al., "Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling," *Nature*, vol. 526, no. 7575, pp. 666–671, 2015.

[78] J. Shi, Y. Zhao, K. Wang et al., "Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death," *Nature*, vol. 526, no. 7575, pp. 660–665, 2015.

[79] J. Ding, K. Wang, W. Liu et al., "Pore-forming activity and structural autoinhibition of the gasdermin family," *Nature*, vol. 535, no. 7610, pp. 111–116, 2016.

[80] P. J. Baker, D. Boucher, D. Bierschenk et al., "NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5," *European Journal of Immunology*, vol. 45, no. 10, pp. 2918–2926, 2015.

[81] C. N. Casson, J. Yu, V. M. Reyes et al., "Human caspase-4 mediates noncanonical inflammasome activation against gram-negative bacterial pathogens," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 21, pp. 6688–6693, 2015.

[82] J. L. Schmid-Burgk, M. M. Gaidt, T. Schmidt, T. S. Ebert, E. Bartok, and V. Hornung, "Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells," *European Journal of Immunology*, vol. 45, no. 10, pp. 2911–2917, 2015.

[83] E. Viganò, C. E. Diamond, R. Spreafico, A. Balachander, R. M. Sobota, and A. Mortellaro, "Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes," *Nature Communications*, vol. 6, no. 1, pp. 8761–8765, 2015.

[84] X. Y. Lin, M. S. K. Choi, and A. G. Porter, "Expression analysis of the human caspase-1 subfamily reveals specific regulation of the CASP5 gene by lipopolysaccharide and interferon-γ," *Journal of Biological Chemistry*, vol. 275, no. 51, pp. 39920–39926, 2000.

[85] Y. Aachoui, I. A. Leaf, J. A. Hagar et al., "Caspase-11 protects against bacteria that escape the vacuole," *Science*, vol. 339, no. 6122, pp. 975–978, 2013.

[86] J. Chavarría-Smith and R. E. Vance, "The NLRP1 inflammasomes," *Immunology Reviews*, vol. 265, no. 1, pp. 22–34, 2015.

[87] Y. Zhao and F. Shao, "The NAIP-NLRC4 inflammasome in innate immune detection of bacterial flagellin and type III secretion apparatus," *Immunology Reviews*, vol. 265, no. 1, pp. 85–102, 2015.

[88] B.-H. Kim, J. D. Chee, C. J. Bradfield, E. S. Park, P. Kumar, and J. D. MacMicking, "Interferon-induced guanylate-binding proteins in inflammasome activation and host defense," *Nature Immunology*, vol. 17, no. 5, pp. 481–489, 2016.

[89] C. Bekpen, J. P. Hunn, C. Rohde et al., "The interferon-inducible p 47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage," *Genome Biology*, vol. 6, no. 11, p. R92, 2005.

[90] E. A. Miao, D. P. Mao, N. Yudkovsky et al., "Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 7, pp. 3076–3080, 2010.

[91] T. E. West, N. D. Myers, N. Chantratita et al., "NLRC4 and TLR5 each contribute to host defense in respiratory melioidosis," *PLoS Neglected Tropical Diseases*, vol. 8, no. 9, article e3178, 2014.

[92] B. G. Byrne, J.-F. Dubuisson, A. D. Joshi, J. J. Persson, and M. S. Swanson, "Inflammasome components coordinate autophagy and pyroptosis as macrophage responses to infection," *mBio*, vol. 4, no. 1, 2013.

[93] C.-S. Shi, K. Shenderov, N.-N. Huang et al., "Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction," *Nature Immunology*, vol. 13, no. 3, pp. 255–263, 2012.

[94] B. B. Mishra, P. Moura-Alves, A. Sonawane et al., "Mycobacterium tuberculosis protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome," *Cellular Microbiology*, vol. 12, no. 8, pp. 1046–1063, 2010.

[95] A. M. Abdallah, N. C. Gey van Pittius, P. A. DiGiuseppe Champion et al., "Type VII secretion–mycobacteria show the way," *Nature Reviews Microbiology*, vol. 5, no. 11, pp. 883–891, 2007.

[96] P. S. Manzanillo, M. U. Shiloh, D. A. Portnoy, and J. S. Cox, "Mycobacterium tuberculosis activates the DNA-dependent cytosolic surveillance pathway within macrophages," *Cell Host & Microbe*, vol. 11, no. 5, pp. 469–480, 2012.

[97] R. Simeone, A. Bobard, J. Lippmann et al., "Phagosomal rupture by Mycobacterium tuberculosis results in toxicity and host cell death," *PLoS Pathogens*, vol. 8, no. 2, article e1002507, 2012.

[98] T. S. Xiao and K. A. Fitzgerald, "The cGAS-STING pathway for DNA sensing," *Molecular Cell*, vol. 51, no. 2, pp. 135–139, 2013.

[99] R. O. Watson, S. L. Bell, D. A. MacDuff et al., "The cytosolic sensor cGAS detects Mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy," *Cell Host & Microbe*, vol. 17, no. 6, pp. 811–819, 2015.

[100] M. Lamkanfi and V. M. Dixit, "Manipulation of host cell death pathways during microbial infections," *Cell Host & Microbe*, vol. 8, no. 1, pp. 44–54, 2010.

[101] S. L. Fink and B. T. Cookson, "Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells," *Infection and Immunity*, vol. 73, no. 4, pp. 1907–1916, 2005.

[102] Q. Yao, J. Cui, J. Wang et al., "Structural mechanism of ubiquitin and NEDD8 deamidation catalyzed by bacterial effectors that induce macrophage-specific apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 50, pp. 20395–20400, 2012.

[103] A. Strasser, L. O'Connor, and V. M. Dixit, "Apoptosis signaling," *Annual Review of Biochemistry*, vol. 69, no. 1, pp. 217–245, 2000.

[104] C. J. Martin, K. N. Peters, and S. M. Behar, "Macrophages clean up: efferocytosis and microbial control," *Current Opinion in Microbiology*, vol. 17, pp. 17–23, 2014.

[105] P. M. Henson, "Cell removal: efferocytosis," *Annual Review of Cell and Developmental Biology*, vol. 33, no. 1, pp. 127–144, 2017.

[106] S. J. Gardai, D. L. Bratton, C. A. Ogden, and P. M. Henson, "Recognition ligands on apoptotic cells: a perspective," *Journal of Leukocyte Biology*, vol. 79, no. 5, pp. 896–903, 2006.

[107] T. Vanden Berghe, B. Hassannia, and P. Vandenabeele, "An outline of necrosome triggers," *Cellular and Molecular Life Sciences*, vol. 73, no. 11-12, pp. 2137–2152, 2016.

[108] Y. Dondelinger, M. A. Aguileta, V. Goossens et al., "RIPK3 contributes to TNFR1-mediated RIPK1 kinase-dependent apoptosis in conditions of cIAP1/2 depletion or TAK1 kinase inhibition," *Cell Death and Differentiation*, vol. 20, no. 10, pp. 1381–1392, 2013.

[109] M. Chen, H. Gan, and H. G. Remold, "Mechanism of virulence: virulent Mycobacterium tuberculosis strain H37Rv, but not attenuated H37Ra, causes significant mitochondrial inner membrane disruption in macrophages leading to necrosis," *Journal of Immunology*, vol. 176, no. 6, pp. 3707–3716, 2006.

[110] S. M. Man and T.-D. Kanneganti, "Converging roles of caspases in inflammasome activation, cell death and innate immunity," *Nature Reviews Immunology*, vol. 16, no. 1, pp. 7–21, 2016.

[111] R. Feltham, J. E. Vince, and K. E. Lawlor, "Caspase-8: not so silently deadly," *Clinical & Translational Immunology*, vol. 6, no. 1, article e124, 2017.

[112] G. W. Sun, J. Lu, S. Pervaiz, W. P. Cao, and Y. H. Gan, "Caspase-1 dependent macrophage death induced by Burkholderia pseudomallei," *Cellular Microbiology*, vol. 7, no. 10, pp. 1447–1458, 2005.

[113] K. Breitbach, G. W. Sun, J. Kohler et al., "Caspase-1 mediates resistance in murine melioidosis," *Infection and Immunity*, vol. 77, no. 4, pp. 1589–1595, 2009.

[114] A. Bast, K. Krause, I. H. E. Schmidt et al., "Caspase-1-dependent and -independent cell death pathways in Burkholderia pseudomallei infection of macrophages," *PLoS Pathogens*, vol. 10, no. 3, article e1003986, 2014.

[115] K. W. Chen, C. J. Groß, F. V. Sotomayor et al., "The neutrophil NLRC4 inflammasome selectively promotes IL-1β maturation without pyroptosis during acute Salmonella challenge," *Cell Reports*, vol. 8, no. 2, pp. 570–582, 2014.

[116] T. Krakauer, "Living dangerously: Burkholderia pseudomallei modulates phagocyte cell death to survive," *Medical Hypotheses*, vol. 121, pp. 64–69, 2018.

[117] B. C. Liu, J. Sarhan, A. Panda et al., "Constitutive interferon maintains GBP expression required for release of bacterial components upstream of pyroptosis and anti-DNA responses," *Cell Reports*, vol. 24, no. 1, pp. 155–168.e5, 2018.

[118] J. Lippmann, H. C. Müller, J. Naujoks et al., "Dissection of a type I interferon pathway in controlling bacterial intracellular infection in mice," *Cellular Microbiology*, vol. 13, no. 11, pp. 1668–1682, 2011.