Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection

Youssef Aachoui[1], **Vitaliya Sagulenko**[2], **Edward A Miao**[1,*], and **Katryn J. Stacey**[2,*]

[1]Department of Microbiology and Immunology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

[2]The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Qld 4072, Australia

Abstract

Cell death is an effective strategy to limit intracellular infections. Canonical inflammasomes, including NLRP3, NLRC4, and AIM2, recruit and activate caspase-1 in response to a range of microbial stimuli and endogenous danger signals. Caspase-1 then promotes the secretion of IL-1β and IL-18 and a rapid form of lytic programmed cell death termed pyroptosis. A second inflammatory caspase, mouse caspase-11, mediates pyroptotic death through an unknown non-canonical inflammasome system in response to cytosolic bacteria. In addition, recent work shows that inflammasomes can also recruit procaspase-8, initiating apoptosis. The induction of multiple pathways of cell death has probably evolved to counteract microbial evasion of cell death pathways.

Introduction

Whilst viral infection is necessarily intracellular, many bacteria also find a replicative or survival niche within cells, evading immune cell attack. In such cases, death of the infected cell can benefit the host by cutting short the replicative cycle, and releasing the invader for killing by neutrophils [1] Alternatively, cell death could be co-opted by pathogens to deplete immune cells, or to release replicated organisms, enhancing pathogen spread. An emerging mechanism for microbe-induced cell death involves formation of inflammasome complexes. Inflammasomes are well known as the route to activation of caspase-1, which cleaves the precursors of the inflammatory cytokines IL-1β and IL-18 [2]. These cytokines are of great importance in clearance of a number of infections. The induction of cell death by inflammasomes is less studied, but analysis in fish suggests that the inflammasome system evolved as part of a cell death program, and that cytokine processing was a later development [3]. In this review we examine the pathways of inflammasome-induced cell death, and what is known of the contribution of this process to host defense. The role of non-inflammasome cell death pathways in bacterial infection has been reviewed elsewhere [4].

Correspondence to: Edward Miao, emiao@med.unc.edu, 919-966-6773. Katryn Stacey, katryn.stacey@uq.edu.au.
*These authors contributed equally

Caspases in apoptosis and pyroptosis

For many years programmed cell death was synonymous with apoptosis. Apoptosis is described as "immunologically silent", as cell membrane integrity is normally maintained until cells are engulfed by phagocytes (Fig. 1). A lack of inflammatory consequence of apoptosis is important during development and homeostasis. Apoptosis can also be induced by infection, and in that case, immunologically silent death is undesirable and unlikely, since the dying cell contains microbial products that should activate the engulfing macrophage. Cell death by necrosis, involving cell swelling and lysis, was long seen as the passive response to overwhelming environmental stress or injury, independent of signaling pathways. However, programmed necrosis ("necroptosis"), involving RIP1/RIP3 signaling has now been described, and is reviewed elsewhere [5]. Pyroptosis is another form of programmed lytic cell death, frequently induced by infection. Pyroptotic death elicits inflammation due to release of cytosolic contents such as ATP, HMGB1 and IL-1α, and is commonly accompanied by processing of inflammatory cytokines IL-1β and IL-18 (Fig. 1) [1,2].

Both pyroptosis and apoptosis involve activation of members of the caspase family of proteases. Pyroptosis is established as inflammasome-dependent cell death, executed following activation of inflammatory caspases (caspase-1 or mouse caspase-11). In contrast, apoptotic initiator caspases (caspases-2, -8 and -9) subsequently cleave effector caspases (caspases-3, -6 and -7). Caspases then cleave target proteins to trigger programmed pyroptotic or apoptotic cell death. The critical step in commitment to both pathways is the recruitment of initiator caspases into a protein complex where they are activated by dimerization, generally followed by intermolecular cleavage. Caspases-1, -2, -8 and -9 are each recruited into unique activating complexes via their N terminal pro-domain of the death-fold family (either a caspase recruitment domain (CARD) or death effector domain (DED)). For example, the caspase-1 and -9 activating platforms are the inflammasome and apoptosome, respectively [6].

Inflammasome induction of pyroptosis

Inflammasomes activating caspase-1 are initiated either by a Nod-like receptor (NLRP1, 3, 6, 7, 12, NLRC4), AIM2, or Pyrin, all of which contain a CARD or pyrin domain (PYD) (Fig. 2) [2,7]. While NLRP3 responds to a wide array of agonists [2], NLRC4 is specific for cytosolic bacterial flagellin and type III secretion system (T3SS) components [8–11], and AIM2 is specific for cytosolic DNA [12–15]. Many inflammasomes recruit the ASC adaptor, composed of a CARD and a PYD [2], via homotypic interactions. Additional ASC molecules are incorporated via CARD-CARD and PYD-PYD interactions, until all ASC is collected into a single focus (Fig. 2). Attraction of procaspase-1 into the ASC focus via CARD-CARD interaction results in its dimerization and proximity-induced autoproteolytic processing into p10 and p20 subunits. This processed and catalytically active caspase-1 cleaves pro-IL-1β and pro-IL-18.

NLRC4 contains a CARD that can recruit procaspase-1 directly, although caspase-1 activation for IL-1β processing also requires ASC. However NLRC4-induced pyroptosis requires neither the characteristic ASC focus, nor the cleavage of procaspase-1 into p10 and p20 subunits. In the absence of ASC, a spatially diffuse, unprocessed, but catalytically active caspase-1 induces pyroptosis but not cytokine processing [16,17]. A diffuse localization for pyroptotic caspase-1 may facilitate cleavage of the unknown substrates responsible for pyroptosis. It is not known whether there is a similar paradigm of spatially and proteolytically distinct caspase-1 downstream of PYD inflammasomes (such as NLRP3 or AIM2) that only signal through ASC.

Pyroptosis occurs after activation of either caspase-1 or caspase-11, depending on the stimulus. The upstream platform(s) that recruits and activates caspase-11 remains to be defined. The catalytic targets of these caspases which lead to pyroptosis are also unknown, although the catalytic site of caspase-1 is essential as cells expressing only catalytically inactive caspase-1 are resistant to pyroptosis [16].

Protective role of caspase-1-dependent pyroptosis *in vivo*

Many pathogens have evolved strategies to resist the antimicrobial activities of macrophages and to replicate within them, effectively sequestering the bacteria away from extracellular immune defenses. Such pathogens have often not evolved mechanisms to evade extracellular defenses. Pyroptosis attacks this Achilles heel by lysing the infected cell and exposing pathogens to extracellular defenses, for example transferring them to neutrophils, which have significantly greater antimicrobial activities. This was first shown using modified *S. typhimurium* and *L. monocytogenes* strains [18–20]. In these cases, while $IL1b^{-/-}IL18^{-/-}$ mice cleared the infection, $Casp1^{-/-}Casp11^{-/-}$ failed to do so. This suggests, but does not prove, a role for pyroptosis. Because mice specifically competent for IL-1β and IL-18 secretion but lacking pyroptosis do not exist, several pieces of ancillary data are needed to support pyroptosis as the active process (Table 1). There is also evidence that *Francisella novicida*, *Burkholderia thailandensis*, and *Burkholderia pseudomallei* can be cleared fully or in part by pyroptosis [21–23] (Table 1). Some of these analyses become complex; for example during *B. pseudomallei* infection, IL-18 and pyroptosis are protective, while IL-1β has detrimental effects [21]. Normally, IL-1β recruits neutrophils that clear bacteria to the benefit of the host, however, excessive neutrophil influx may result in tissue damage to the detriment of the host. In many of these infections, the relative roles of cytokines and pyroptosis may depend on the dose and/or route of infection, and it is likely that in natural infections IL-1β, IL-18, and pyroptosis will work additively or collectively to mediate protection.

Non-canonical inflammasome-dependent pyroptosis in defense against bacteria

Murine caspase-11 induces pyroptosis independent of caspase-1, but also promotes processing of IL-1β through the NLRP3-ASC-caspase-1 pathway [24]. It remains unclear whether human caspase-4 or -5 are functionally analogous/equivalent to murine caspase-11. Kayagaki *et al.* hypothesized that caspase-11 would be recruited to an oligomerized platform similar to known caspase-1-activating inflammasomes. To differentiate these two pathways, Kayagaki defined "canonical inflammasomes" as those that activate caspase-1 and "non-canonical inflammasomes" as the hypothetical platform(s) for caspase-11 activation [24]. Caspase-11 expression is primed through a TLR4-TRIF-IFN-β-STAT1 pathway [25–29], or an IFN-γ-STAT1 pathway [22,28,30]. Caspase-11 is activated by prolonged exposure to the cholera toxin B subunit or many Gram-negative bacteria *in vitro* [24–27,31]. However, caspase-11 was not shown to benefit the host during *in vivo Citrobacter* or *Salmonella* infection [25,26]. In contrast, we recently showed that rapid caspase-11 activation in response to cytosolic bacteria is protective *in vivo* [22].

Burkholderia pseudomallei is a highly virulent pathogen that causes melioidosis, and is classified as a potential biologic weapon. *B. thailandensis* is a closely related species expressing similar virulence factors, but is avirulent [32]. We showed that *Burkholderia* triggers caspase-11 activation within four hours post infection, and that this was dependent upon their ability to lyse the phagosome and enter the cytosol. The absence of caspase-11 causes mice to be acutely susceptible to both *B. pseudomallei* and *B. thailandensis* [22]. Interestingly, while *B. thailandensis* is avirulent, in the absence of caspase-11 this bacterium

becomes lethal. Similarly, caspase-11 initiated pyroptosis after aberrant cytosolic release of naturally vacuolar bacteria. Mutation of a specific T3SS effector, *sifA* in *S. typhimurium* causes rupture of the *Salmonella*-containing vacuole, and a similar phenotype is described for *L. pneumophila sdhA* mutants. This exit from the vacuole results in caspase-11 detection and played an important role in the clearance of *S. typhimurium ΔsifA in vivo* [22].

Can the protective roles of caspase-11 against cytosolic bacteria be attributed to pyroptosis, or do IL-1β and IL-18 play a role? For *S. typhimurium ΔsifA* infection, IL-1β and IL-18 play no role [22]. The clearance of *B. thailandensis* was partially dependent upon IL-1β/IL-18, but only after intranasal infection [22]. We previously showed that the clearance of bacteria after pyroptosis is mediated by neutrophils through generation of reactive oxygen [18]. Indeed, NADPH oxidase deficient $Ncf1^{-/-}$ (p47^{phox}) mice were defective for clearance of *S. typhimurium ΔsifA* [22]. These results suggest pyroptosis as the mechanism of clearance of cytosolic bacteria (Table 1).

Inflammasome-dependent cell death in defense against viruses

A wide range of DNA and RNA viruses activate inflammasome pathways (reviewed in [33]). Inflammasome-dependent cell death *in vitro* has been observed for both West Nile virus [34] and adenovirus [35], but the contribution of this to antiviral defense *in vivo* remains to be established.

Detrimental effects of pyroptosis *in vivo*

If too many host cells undergo pyroptosis, detrimental effects may occur due to the release of inflammatory cytosolic mediators or the depletion of host cells. *In vivo* caspase-11 can be activated by triggers other than cytosol-localized bacteria, including LPS, hypoxia, and ER stress [24,36–38]. LPS-induced septic shock has been shown to require caspase-11 [24,36], suggesting that overwhelming infection may promote pathological caspase-11 activation. The role of caspase-11 in septic shock was not explained by IL-1β and IL-18 production, suggesting a role for pyroptosis [39]. It is still unclear what events downstream of caspase-11 cause lethality in sepsis, but HMGB1 release has been implicated [40]. Identification of signals activating caspase-11 and the subsequent downstream events will clarify the dual role of caspase-11 in host protection and immunopathology.

In the setting of unresolved bacterial infections that trigger pyroptosis, such as *S. typhimurium* FliC^{ON} in an NADPH oxidase-deficient mouse, lysis and depletion of macrophages in the spleen by pyroptosis causes breakdown of normal splenic architecture [18]. Similarly, constitutively active *Nlrp1a* germline mutations cause pyroptosis in the hematopoietic progenitor cells, resulting in basal myelosuppression [41].

Inflammasome induction of apoptosis

Recent work showed that inflammasomes trigger apoptosis in addition to pyroptosis. Initially, AIM2 was observed to activate caspase-3 in parallel with caspase-1 [15]. Subsequently, AIM2 recognition of DNA released by the cytosolic bacterium *Francisella novicida* [42], the NLRP3 response to the bacterial pore-forming toxin nigericin [43], and ASC-dependent recognition of *Legionella* and *Salmonella* [44,45] have been shown to elicit apoptotic caspase activation. Although wild type cells rapidly undergo pyroptosis with inflammasome stimuli, caspase-1 knockout cells were observed to undergo apoptosis, with a slower timecourse [42,43,45]. Although it was suggested that caspase-1 inhibits the inflammasome-dependent activation of apoptotic caspases by *Salmonella* [45], apoptotic responses can be readily observed in wild type cells responding to AIM2 or NLRP3 stimuli [43]. We found that the balance between apoptosis and pyroptosis in wild type cells

depended on the dose of stimulus applied, with apoptosis predominating at low doses [43]. Thus the type of cell death occurring in infections may depend on the magnitude of the microbial burden.

AIM2 and NLRP3 inflammasome-dependent apoptosis requires caspase-8, which is recruited to the inflammasome via a novel heterotypic interaction between its DED domains and the PYD of ASC [42,43,46]. An apoptotic pathway involving ASC and caspase-8 is consistent with literature from the cancer field, although signaling pathways upstream of ASC relevant to cancer cells have not been established [47]. ASC was initially identified and characterized as an apoptosis-associated protein forming dense perinuclear specks in a leukemia cell line upon treatment with chemotherapeutic drugs [48].

An increased susceptibility of $Asc^{-/-}$ mice to infection in comparison to $Casp1^{-/-}$ mice would suggest a physiologic function of the ASC-caspase-8 pathway *in vivo*, and there are some potential leads in the literature. ASC-dependent apoptosis limited *F. novicida* replication *in vitro*, and *in vivo* $Asc^{-/-}$ mice defective in their IFN-γ production in comparison to $Casp1^{-/-}$ mice [42]. Initially, indirect comparisons suggested that $Asc^{-/-}$ mice were more susceptible to *in vivo F. novicida* infection than $Casp1^{-/-}$ mice [23]. However upon later direct comparison, they were found to succumb with identical kinetics [42], indicating that in this case ASC-dependent apoptosis does not confer a survival benefit *in vivo*, although different doses or routes of infection should be examined. $Asc^{-/-}$ mice have also been suggested to be more susceptible than $Casp1^{-/-}$ mice to *Mycobacterium tuberculosis* infection [49], although this is disputed [50]. Inflammasome-induced apoptosis may be particularly important for combating pathogens that directly inhibit caspase-1; the Yersinia T3SS effector YopM directly inhibits caspase-1 via pseudo-substrate binding to the catalytic site [51]. Caspase-8 may escape YopM inhibition and could serve as a backup system for inflammasome signaling.

Conclusion

Initiation of cell death pathways is a fundamental and evolutionarily ancient defense against infection [52,53]. The presence of pathogen-encoded inhibitors of inflammasome pathways provides evidence for an important role for the inflammasome *in vivo* [33,51,54]. Inflammasome-dependent cell death and cytokine production are likely to work together to promote clearance, but in certain infections, one or the other may have a dominant individual contribution. Caspase-11-induced pyroptosis, and Inflammasome-induced apoptosis are newly appreciated, and their roles in a range of infections remain to be tested. Wherever $Asc^{-/-}$ mice have a more severe defect in host defense than $Casp1^{-/-}$ mice, a role for ASC-dependent apoptosis should be considered. There is good evidence that pyroptosis can benefit the host during infection, but it may be detrimental during overwhelming infection or sepsis. Some pathogens may trigger inflammasome-induced cell death as a virulence strategy to remove host immune cells or promote pathogen spread..

Acknowledgments

References

1. Miao EA, Rajan JV, Aderem A. Caspase-1-induced pyroptotic cell death. Immunol Rev. 2011; 243:206–214. [PubMed: 21884178]

2. Moltke Von J, Ayres JS, Kofoed EM, Chavarría-Smith J, Vance RE. Recognition of Bacteria by Inflammasomes. Annu Rev Immunol. 201210.1146/annurev-immunol-032712-095944

3. Angosto D, López-Castejón G, López-Muñoz A, Sepulcre MP, Arizcun M, Meseguer J, Mulero V. Evolution of inflammasome functions in vertebrates: Inflammasome and caspase-1 trigger fish macrophage cell death but are dispensable for the processing of IL-1β. Innate Immunity. 2012; 18:815–824. [PubMed: 22456941]

4. Ashida H, Mimuro H, Ogawa M, Kobayashi T, Sanada T, Kim M, Sasakawa C. Cell death and infection: a double-edged sword for host and pathogen survival. The Journal of Cell Biology. 2011; 195:931–942. [PubMed: 22123830]

5. Vanlangenakker N, Vanden Berghe T, Vandenabeele P. Many stimuli pull the necrotic trigger, an overview. Cell Death Differ. 2011; 19:75–86. [PubMed: 22075985]

6. Mace PD, Riedl SJ. Molecular cell death platforms and assemblies. Current Opinion in Cell Biology. 2010; 22:828–836. [PubMed: 20817427]

7. Chae JJ, Cho Y-H, Lee G-S, Cheng J, Liu PP, Feigenbaum L, Katz SI, Kastner DL. Gain-of-Function Pyrin Mutations Induce NLRP3 Protein-Independent Interleukin-1β Activation and Severe Autoinflammation in Mice. Immunity. 2011; 34:755–768. [PubMed: 21600797]

8. Miao EA, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, Aderem A. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. Nature Immunology. 2006; 7:569–575. [PubMed: 16648853]

9. Miao EA, Mao DP, Yudkovsky N, Bonneau R, Lorang CG, Warren SE, Leaf IA, Aderem A. From the Cover: Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. Proceedings of the National Academy of Sciences. 2010; 107:3076–3080.

10. Zhao Y, Yang J, Shi J, Gong Y-N, Lu Q, Xu H, Liu L, Shao F. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 2011; 477:596–600. [PubMed: 21918512]

11. Kofoed EM, Vance RE. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. Nature. 2011; 477:592–595. [PubMed: 21874021]

12. Fernandes-Alnemri T, Yu J-W, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature. 2009; 458:509–513. [PubMed: 19158676]

13. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature. 2009; 458:514–518. [PubMed: 19158675]

14. Bürckstümmer T, Baumann C, Blüml S, Dixit E, Dürnberger G, Jahn H, Planyavsky M, Bilban M, Colinge J, Bennett KL, et al. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nature Immunology. 2009; 10:266–272. [PubMed: 19158679]

15. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, Hardy LL, Garceau V, Sweet MJ, Ross IL, et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science. 2009; 323:1057–1060. [PubMed: 19131592]

16••. Broz P, Moltke Von J, Jones JW, Vance RE, Monack DM. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. Cell Host Microbe. 2010; 8:471–483. This study showed that caspase-1-dependent pyroptosis and cytokine processing can occur in spatially distinct locations. Cytokine processing occurs at the ASC focus, while pyroptosis is triggered by catalytically active, but diffuse and unprocessed caspase-1. [PubMed: 21147462]

17. Case CL, Roy CR. Asc Modulates the Function of NLRC4 in Response to Infection of Macrophages by Legionella pneumophila. mBio. 2011; 2:e00117–11–e00117–11. [PubMed: 21771913]

18••. Miao EA, Leaf IA, Treuting PM, Mao DP, Dors M, Sarkar A, Warren SE, Wewers MD, Aderem A. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nature Immunology. 2010; 11:1136–1142. First demonstration that pyroptosis is a critical innate immune defense mechanism against intracellular bacteria. [PubMed: 21057511]

19. Warren SE, Duong H, Mao DP, Armstrong A, Rajan J, Miao EA, Aderem A. Generation of a Listeria vaccine strain by enhanced caspase-1 activation. Eur J Immunol. 2011; 41:1934–1940. [PubMed: 21538346]

20. Sauer J-D, Pereyre S, Archer KA, Burke TP, Hanson B, Lauer P, Portnoy DA. Listeria monocytogenes engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. Proceedings of the National Academy of Sciences. 2011; 108:12419–12424.

21•. Ceballos-Olvera I, Sahoo M, Miller MA, Barrio LD, Re F. Inflammasome-dependent Pyroptosis and IL-18 Protect against Burkholderia pseudomallei Lung Infection while IL-1β Is Deleterious. PLoS Pathog. 2011; 7:e1002452. Demonstration of the complexity of the interplay between IL-1β, IL-18, and pyroptosis. In the case of *B. pseudomallei*, IL-1β is detrimental to the host, while IL-18 and pyroptosis are protective. [PubMed: 22241982]

22••. Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, Zak DE, Tan MH, Cotter PA, Vance RE, Aderem A, et al. Caspase-11 Protects Against Bacteria That Escape the Vacuole. Science. 2013; 339:975–978. First demonstration that caspase-11 detects and protects against cytosolic bacteria. [PubMed: 23348507]

23. Mariathasan S, Weiss DS, Dixit VM, Monack DM. Innate immunity against Francisella tularensis is dependent on the ASC/caspase-1 axis. J Exp Med. 2005; 202:1043–1049. [PubMed: 16230474]

24••. Kayagaki N, Warming S, Lamkanfi M, Walle LV, Louie S, Dong J, Newton K, Qu Y, Liu J, Heldens S, et al. Non-canonical inflammasome activation targets caspase-11. Nature. 2011; 479:117–121. This paper redirected the inflammasome field, revealing that caspase-1 deficient mice also lacked caspase-11, and revealed a distinct role of caspase-11 in pyroptosis and cytokine secretion. [PubMed: 22002608]

25. Gurung P, Malireddi RKS, Anand PK, Demon D, Walle LV, Liu Z, Vogel P, Lamkanfi M, Kanneganti T-D. Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-β (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens. Journal of Biological Chemistry. 2012; 287:34474–34483. [PubMed: 22898816]

26. Broz P, Ruby T, Belhocine K, Bouley DM, Kayagaki N, Dixit VM, Monack DM. Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. Nature. 2012; 490:288–291. [PubMed: 22895188]

27. Rathinam VAK, Vanaja SK, Waggoner L, Sokolovska A, Becker C, Stuart LM, Leong JM, Fitzgerald KA. TRIF Licenses Caspase-11-Dependent NLRP3 Inflammasome Activation by Gram-Negative Bacteria. Cell. 2012; 150:606–619. [PubMed: 22819539]

28. Schauvliege R, Vanrobaeys J, Schotte P, Beyaert R. Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1. J Biol Chem. 2002; 277:41624–41630. [PubMed: 12198138]

29. Case CL, Kohler LJ, Lima JB, Strowig T, de Zoete MR, Flavell RA, Zamboni DS, Roy CR. Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to Legionella pneumophila. Proceedings of the National Academy of Sciences. 2013; 110:1851–1856.

30. Hur J, Kim SY, Kim H, Cha S, Lee MS, Suk K. Induction of caspase-11 by inflammatory stimuli in rat astrocytes: lipopolysaccharide induction through p38 mitogen-activated protein kinase pathway. FEBS Lett. 2001; 507:157–162. [PubMed: 11684090]

31. Akhter A, Caution K, Abu Khweek A, Tazi M, Abdulrahman BA, Abdelaziz DHA, Voss OH, Doseff AI, Hassan H, Azad AK, et al. Caspase-11 promotes the fusion of phagosomes harboring pathogenic bacteria with lysosomes by modulating actin polymerization. Immunity. 2012; 37:35–47. [PubMed: 22658523]

32. Wiersinga WJ, Van Der Poll T, White NJ, Day NP, Peacock SJ. Melioidosis: insights into the pathogenicity of Burkholderia pseudomallei. Nat Rev Micro. 2006; 4:272–282.

33. Gram AM, Frenkel J, Ressing ME. Inflammasomes and viruses: cellular defence versus viral offence. Journal of General Virology. 2012; 93:2063–2075. [PubMed: 22739062]

34. Wu M-F, Chen S-T, Yang A-H, Lin W-W, Lin Y-L, Chen N-J, Tsai I-S, Li L, Hsieh S-L. CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages. Blood. 2013; 121:95–106. [PubMed: 23152543]

35. Barlan AU, Griffin TM, McGuire KA, Wiethoff CM. Adenovirus membrane penetration activates the NLRP3 inflammasome. Journal of Virology. 2011; 85:146–155. [PubMed: 20980503]

36. Wang S, Miura M, Jung YK, Zhu H, Li E, Yuan J. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. Cell. 1998; 92:501–509. [PubMed: 9491891]

37. Kim N-G, Lee H, Son E, Kwon O-Y, Park J-Y, Park J-H, Cho GJ, Choi WS, Suk K. Hypoxic induction of caspase-11/caspase-1/interleukin-1beta in brain microglia. Brain Res Mol Brain Res. 2003; 114:107–114. [PubMed: 12829320]

38. Endo M, Mori M, Akira S, Gotoh T. C/EBP homologous protein (CHOP) is crucial for the induction of caspase-11 and the pathogenesis of lipopolysaccharide-induced inflammation. J Immunol. 2006; 176:6245–6253. [PubMed: 16670335]

39. Sarkar A, Hall MW, Exline M, Hart J, Knatz N, Gatson NT, Wewers MD. Caspase-1 regulates Escherichia coli sepsis and splenic B cell apoptosis independently of interleukin-1beta and interleukin-18. American Journal of Respiratory and Critical Care Medicine. 2006; 174:1003–1010. [PubMed: 16908867]

40•. Lamkanfi M, Sarkar A, Vande Walle L, Vitari AC, Amer AO, Wewers MD, Tracey KJ, Kanneganti T-D, Dixit VM. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. The Journal of Immunology. 2010; 185:4385–4392. Antibodies against HMGB1 protected mice from LPS challenge. Together with the data from Kayagaki et al. [28] this paper suggests that release of cytosolic danger molecules by pyroptosis causes mortality during septic shock. [PubMed: 20802146]

41. Masters SL, Gerlic M, Metcalf D, Preston S, Pellegrini M, O'Donnell JA, McArthur K, Baldwin TM, Chevrier S, Nowell CJ, et al. NLRP1 Inflammasome Activation Induces Pyroptosis of Hematopoietic Progenitor Cells. Immunity. 2012; 37:1009–1023. [PubMed: 23219391]

42••. Pierini R, Juruj C, Perret M, Jones CL, Mangeot P, Weiss DS, Henry T. AIM2/ASC triggers caspase-8-dependent apoptosis in Francisella-infected caspase-1-deficient macrophages. Cell Death Differ. 2012; 19:1709–1721. Demonstrated that activation of the AIM2-ASC inflammasome can lead to apoptosis in addition to pyroptosis. [PubMed: 22555457]

43••. Sagulenko V, Thygesen SJ, Sester DP, Idris A, Cridland JA, Vijjhala PR, Roberts TL, Schroder K, Vince JE, Hill JM, et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. Cell Death Differ. 2013 in press Showed caspase-8 dependent apoptosis in response to AIM2 and NLRP3 stimuli, recruitment of procaspase-8 to the ASC focus, and stimulus dose dependent switch between inflammasome-induced apoptosis and pyroptosis.

44. Abdelaziz DHA, Gavrilin MA, Akhter A, Caution K, Kotrange S, Khweek AA, Abdulrahman BA, Hassan ZA, El-Sharkawi FZ, Bedi SS, et al. Asc-Dependent and Independent Mechanisms Contribute to Restriction of Legionella Pneumophila Infection in Murine Macrophages. Front Microbiol. 2011:2. [PubMed: 21687401]

45. Puri AW, Broz P, Shen A, Monack DM, Bogyo M. Caspase-1 activity is required to bypass macrophage apoptosis upon Salmonella infection. Nat Chem Biol. 2012; 8:745–747. [PubMed: 22797665]

46. Masumoto J, Dowds TA, Schaner P, Chen FF, Ogura Y, Li M, Zhu L, Katsuyama T, Sagara J, Taniguchi S, et al. ASC is an activating adaptor for NF-kappa B and caspase-8-dependent apoptosis. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. 2003; 303:69–73. [PubMed: 12646168]

47. Motani K, Kawase K, Imamura R, Kinoshita T, Kushiyama H, Suda T. Activation of ASC induces apoptosis or necrosis, depending on the cell type, and causes tumor eradication. Cancer Sci. 2010; 101:1822–1827. [PubMed: 20500518]

48. Masumoto J, Taniguchi S, Ayukawa K, Sarvotham H, Kishino T, Niikawa N, Hidaka E, Katsuyama T, Higuchi T, Sagara J. ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic leukemia HL-60 cells. J Biol Chem. 1999; 274:33835–33838. [PubMed: 10567338]

49. McElvania Tekippe E, Allen IC, Hulseberg PD, Sullivan JT, McCann JR, Sandor M, Braunstein M, Ting JP-Y. Granuloma formation and host defense in chronic Mycobacterium tuberculosis infection requires PYCARD/ASC but not NLRP3 or caspase-1. PLoS ONE. 2010:5.

50. Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Núñez G, et al. Cutting Edge: Caspase-1 Independent IL-1{beta} Production Is Critical for Host Resistance to Mycobacterium tuberculosis and Does Not Require TLR Signaling In Vivo. J Immunol. 201010.4049/jimmunol.0904189

51. LaRock CN, Cookson BT. The Yersinia Virulence Effector YopM Binds Caspase-1 to Arrest Inflammasome Assembly and Processing. Cell Host Microbe. 2012; 12:799–805. [PubMed: 23245324]

52. Galluzzi L, Kepp O, Morselli E, Vitale I, Senovilla L, Pinti M, Zitvogel L, Kroemer G. Viral strategies for the evasion of immunogenic cell death. J Intern Med. 2010; 267:526–542. [PubMed: 20433579]

53. Lamkanfi M, Dixit VM. Manipulation of host cell death pathways during microbial infections. Cell Host Microbe. 2010; 8:44–54. [PubMed: 20638641]

54. Lamkanfi M, Dixit VM. Modulation of inflammasome pathways by bacterial and viral pathogens. The Journal of Immunology. 2011; 187:597–602. [PubMed: 21734079]

55. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Micro. 2009; 7:99–109.

56. Raupach B, Peuschel S-K, Monack DM, Zychlinsky A. Caspase-1-Mediated Activation of Interleukin-1 (IL-1) and IL-18 Contributes to Innate Immune Defenses against Salmonella enterica Serovar Typhimurium Infection. Infect Immun. 2006; 74:4922–4926. [PubMed: 16861683]

57. Tsuji NM, Tsutsui H, Seki E, Kuida K, Okamura H, Nakanishi K, Flavell RA. Roles of caspase-1 in Listeria infection in mice. International Immunology. 2004; 16:335–343. [PubMed: 14734619]

Highlights

- Pyroptosis is a form of programmed cell death that is lytic and pro-inflammatory.

- Pyroptosis occurs after caspase-1 or caspase-11 activation.

- ASC can trigger pyroptosis via caspase-1 and apoptosis via caspase-8

- Pyroptosis protects against vacuolar or cytosolic microbes.

- Caspase-11 protects against infection by cytosolic bacteria.
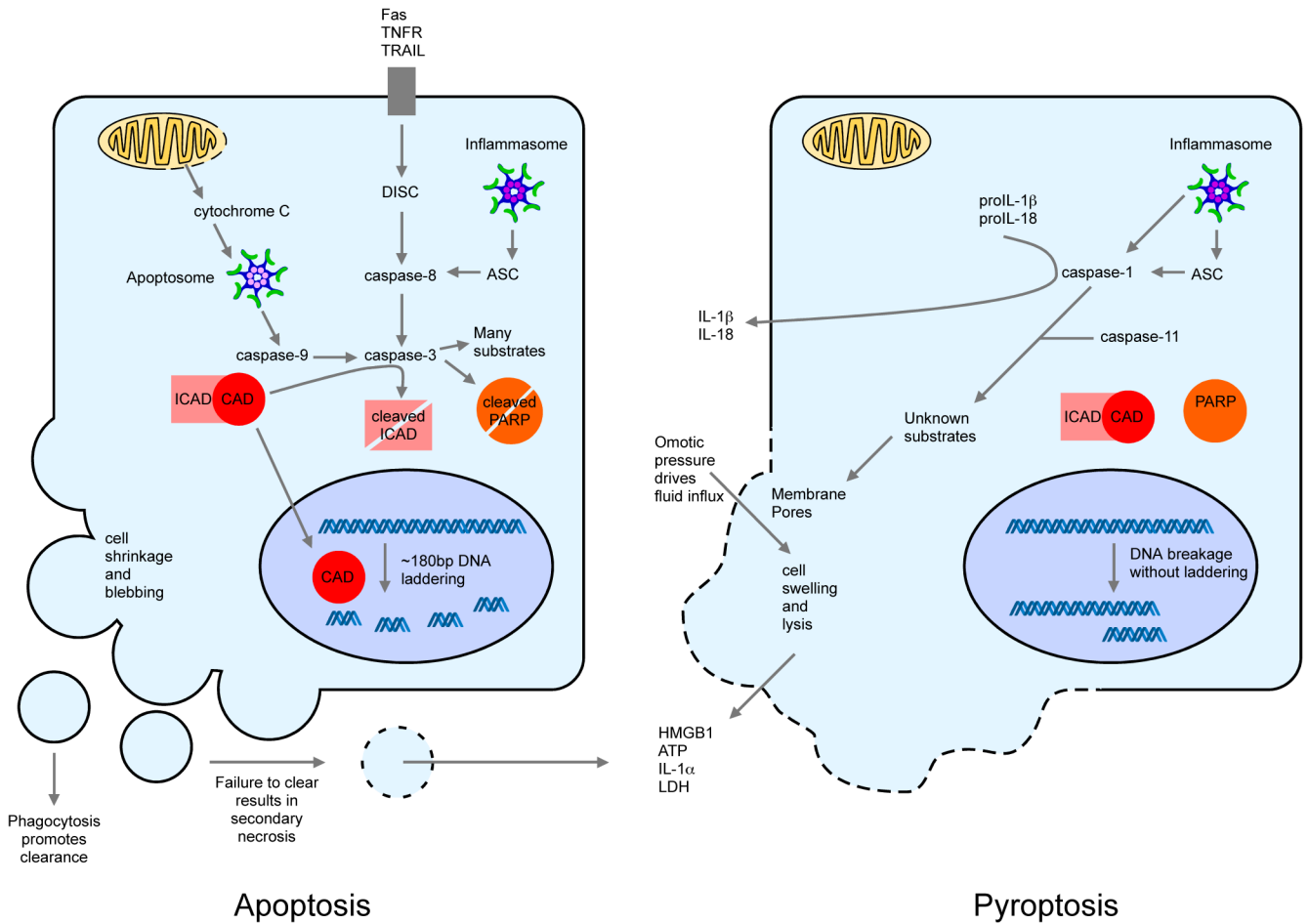
Figure 1. The cellular events during pyroptosis and apoptosis

Both pyroptosis and apoptosis require activation of caspases, caspase-1/11 or caspase-2/3/7/8/9, respectively, but differ morphologically. Pyroptosis is characterized by formation of membrane pores between 1.1 and 2.4 nm in diameter. These pores likely cause dissipation of cellular ionic gradients leading to osmotic water influx, cell swelling, and plasma membrane lysis, releasing the cytosolic content into the extracellular space. Specific markers for this lysis are useful *in vitro*, for example lactate dehydrogenase release is readily detected by enzyme assay. Other cytosolic components released have inflammatory biologic functions, for example HMGB1, IL-1α, and ATP. On the other hand during apoptosis, plasma membrane integrity is maintained and cellular contents are not released. The plasma membrane loses leaflet lipid composition asymmetry as phosphatidyl serine becomes exposed on the outer leaflet, promoting phagocytosis. However, in the absence of phagocytosis, at later timepoints the apoptotic bodies undergo secondary necrosis characterized by rupture of the membrane. Apoptosis is an energy requiring process during which cells shrink and form apoptotic blebs. The DNA repair factor PARP1 is inactivated by cleavage, and nuclear condensation and fragmentation occur. Internucleosomal DNA cleavage, detected as laddering on gel electrophoresis or by TUNEL staining for DNA ends, occurs due to ICAD degradation and activation of CAD (caspase-activated DNase). Weak TUNEL staining is also seen in pyroptosis, but DNA laddering and ICAD degradation are absent [55].
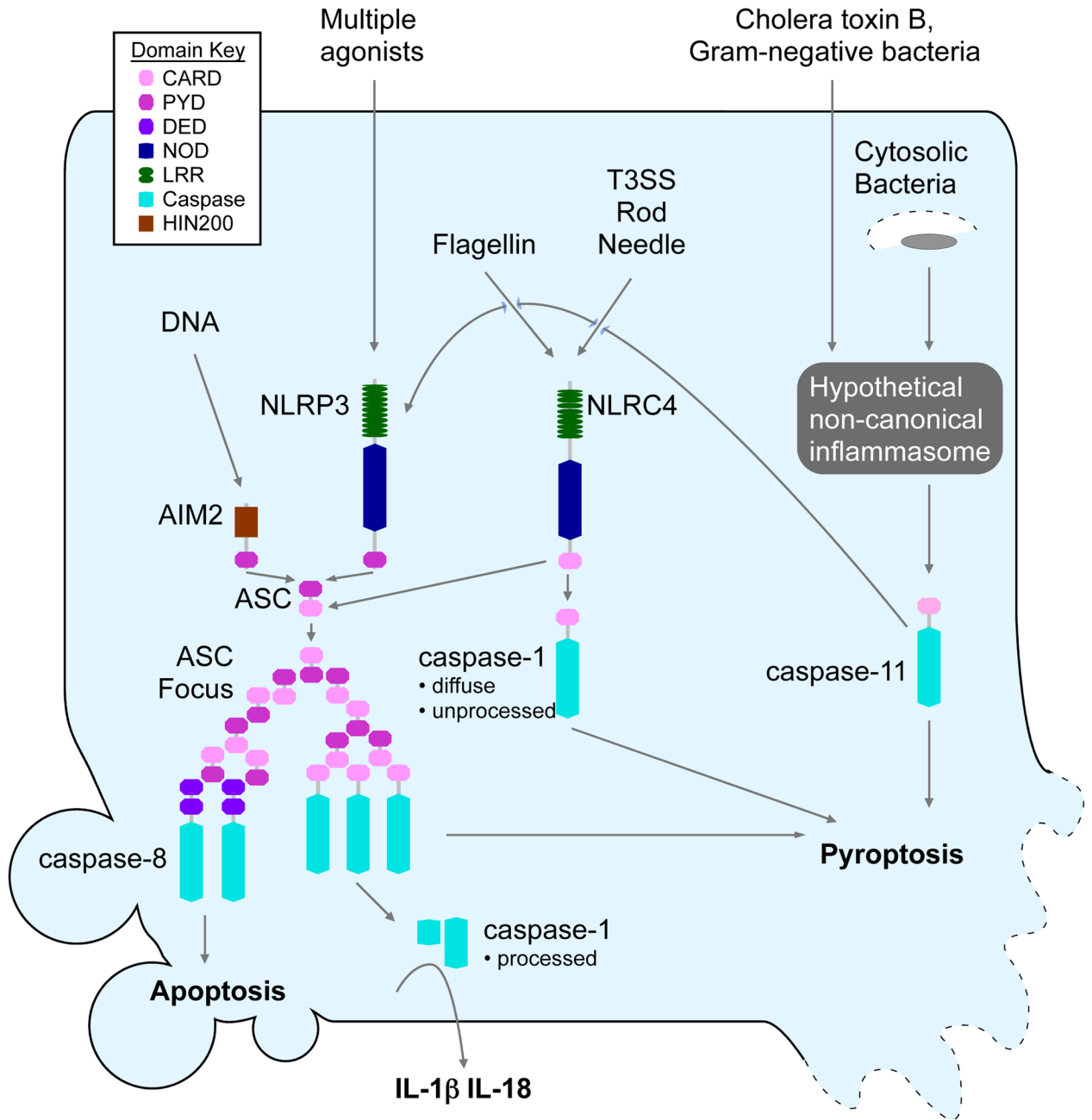
Figure 2. Canonical and non-canonical inflammasome pathways to cell death and cytokine secretion

Canonical inflammasomes are cytosolic platforms that activate caspase-1 in response to pathogen- or danger- associated molecular patterns. Activation of caspase-1 leads to secretion of IL-1β and IL-18, and pyroptosis. Two domain structures for inflammasome initiator proteins are known. 1) NLRs contain a signaling domain (PYD or CARD), a nucleotide binding oligomerization domain (NOD/NACHT), and a leucine rich repeat (LRR) domain. 2) AIM2 contains a PYD signaling domain and a DNA-binding HIN-200 domain. AIM2 detects cytosolic DNA. NLRP3 detects multiple agonists such as extracellular ATP, bacterial pore-forming toxins, monosodium urate crystals, and

cholesterol crystals. NLRC4 detects cytosolic flagellin or T3SS components that have been injected into the cytosol. The NLR or AIM2 signaling domains (PYD or CARD) bind to ASC by homotypic interactions, triggering formation of the ASC focus, which recruits procaspase-1, leading to its activation and processing. The ASC focus also recruits procaspase-8, initiating an apoptotic pathway. NLRC4 can additionally interact directly with caspase-1, resulting only in pyroptosis. The platform(s) that activate caspase-11 in response to cytosolic bacteria remain unknown. Caspase-11 triggers pyroptosis directly, and can also activate the canonical NLRP3-ASC-caspase-1 inflammasome pathway leading to IL-1β and IL-18 processing (denoted by the long curved arrow to NLRP3).

Table 1

Evidence for pyroptotic clearance of bacteria *in vivo*

Evidence	WT	S.tm FliC^ON	ΔsifA	WT	L.mo FlaA^ON	PrgJ^ON	F.no WT	B.th WT	B.ps WT
Bacterial clearance requirements									
Requires caspase-1 or -11	casp1	casp1	casp11	casp1	casp1	casp1	casp1	casp1/11	casp1/11
Fully or partially independent of IL-1β / IL-18	no	yes	yes	-	yes	yes	yes	yes	yes
Independent of ASC (for NLRC4 or caspase-11 detection only)	n/a	yes	yes	n/a	yes	-	n/a	yes	yes
NADPH oxidase or other antimicrobial effector required	yes	yes	yes	yes	-	-	-	yes	-
Evidence that infected cells undergo pyroptosis in vivo									
Caspase dependent pores form *in vivo*	-	yes	-	-	-	-	-	-	-
Intracellular microbes released to the extracellular space	-	yes	-	-	-	-	-	-	-
Microbes are phagocytosed by a new cell type and killed.	-	yes	-	-	-	-	-	-	-
Reference	[56]	[18]	[22]	[57]	[19,20]	[19,20]	[23]	[22]	[21,22]

S.tm = *S. typhimurium*, *L.mo* = *L. monocytogenes*, *F.no* = *F. novicida*, *B.th* = *B. thailandensis*, *B.ps* = *B. pseudomallei*, - = no data or direct comparisons have not been made, n/a = not applicable