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Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases

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SUMMARY

Cell death is a fundamental biological phenomenon that is essential for the survival and development of an organism. Emerging evidence also indicate that cell death contributes to immune defense against infectious diseases. Pyroptosis is a form of inflammatory programmed cell death pathway activated by human and mouse caspase-1, human caspase-4 and caspase-5, or mouse caspase-11. These inflammatory caspases are used by the host to control bacterial, viral, fungal or protozoan pathogens. Pyroptosis requires cleavage and activation of the pore-forming effector protein gasdermin D by inflammatory caspases. Physical rupture of the cell causes release of the pro-inflammatory cytokines IL-1 β and IL-18, alarmins and endogenous danger-associated molecular patterns, signifying the inflammatory potential of pyroptosis. Here, we describe the central role of inflammatory caspases and pyroptosis in mediating immunity to infection and clearance of pathogens.

Keywords

Bacteria; caspase-1; caspase-4; caspase-5; caspase-11; cell death; gasdermin D; infection; inflammasomes; inflammation; inflammatory caspases; interferons; lysis; lytic; necrosis; necroptosis; pores; pyroptosis; viruses

INTRODUCTION

Programmed cell death pathways, including apoptosis, pyroptosis and necroptosis, are regulated by unique sets of host proteins that coordinate a variety of biological outcomes (1–6). Both apoptosis and pyroptosis are executed by caspases. Apoptosis is mediated by apoptotic caspases, which include caspase-2, caspase-3, caspase-6, caspase-7, caspase-8 and caspase-9 (7). Humans also express the apoptotic caspase family member caspase-10. Although apoptosis has generally been considered an immunologically silent process, emerging evidence indicates that apoptosis can be inflammatory when induced under certain conditions and has roles in the host defense against infection (3, 8, 9).

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In contrast to apoptosis, pyroptosis is a form of necrotic and inflammatory programmed cell death induced by inflammatory caspases (6). The requirement of inflammatory caspases in executing pyroptosis distinguishes it from another necrotic and inflammatory form of programmed cell death called necroptosis (1, 10, 11), which is executed independently of caspases (Figure 1). Pyroptosis was initially observed in macrophages infected with *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) or *Shigella flexneri* and was thought to be apoptosis (12, 13). A study in 1998 found that cell death induced by *S. flexneri* was abolished in macrophages lacking the gene encoding caspase-1 [also known as interleukin-1 β -converting enzyme (ICE)] (13). A similar study in 1999 also reported caspase-1-dependent cell death in macrophages infected with *S. Typhimurium* (14). These studies together provided important genetic evidence to pinpoint a role for caspase-1 in bacteria-induced cell death. Since previous studies have shown that caspase-1 can mediate proteolytic cleavage of the pro-inflammatory precursor cytokines pro-IL-1 β and pro-IL-18 (15–18), the term pyroptosis was subsequently proposed in 2001 to ascribe the inflammatory nature to caspase-1-dependent cell death (19). The definition of pyroptosis has now been broadened to encompass cell death executed by most inflammatory caspases, namely human caspase-4, human caspase-5, and mouse caspase-11 (3).

With the exception of caspase-12, all inflammatory caspases are activated within an inflammasome (20–22). An inflammasome is a macromolecular protein complex composed of inflammasome-initiating sensors (NLRP1, NLRP3, NLRC4, AIM2 or pyrin) and inflammatory caspases, in the presence or absence of the inflammasome adaptor protein ASC (20, 23). Activation of inflammasome-associated inflammatory caspases drives cleavage of the pro-pyroptotic factor gasdermin D (24–26), generating an N-terminal fragment that oligomerizes to form pores on the host cell membrane and cause the lytic demise of the cell (27–31).

The field of inflammatory caspases has somewhat been confounded by studies showing that previously generated caspase-1-deficient mouse lines also lack caspase-11 (32, 33). In 1995, two groups each generated a caspase-1-deficient mouse line (called “ICE^{-/-}” or “Casp1^{-/-}”) using embryonic stem cells of the 129 background (34, 35). It was later revealed that these mouse lines carry a 129-associated inactivating passenger mutation in the *caspase-11* locus (32). The close proximity of the *caspase-1* and *caspase-11* loci prevented segregation of these two proteins despite extensive backcrossing to the C57BL/6 background, essentially rendering these mice deficient in both caspase-1 and caspase-11 (32). In this review, we will refer previously-generated “ICE^{-/-}” or “Casp1^{-/-}” strains as *Casp1^{-/-}Casp11^{-/-}* (also known as *Casp1^{-/-}Casp11^{129mt/129mt}*) mice (32). Generation of new mutant mouse strains specifically lacking caspase-1 has been useful in propelling studies aiming to refine the biological functions of inflammatory caspases in health and disease (36–38). Here, we provide an overview on the functions and mechanisms of inflammatory caspases and pyroptosis in host defense against pathogens.

Molecular basis of pyroptosis and inflammasome activation

Pyroptosis is regulated via a caspase-1-dependent or caspase-1-independent mechanism (Figure 2). Caspase-1-independent pyroptosis is executed by human caspase-4, human

caspase-5 or mouse caspase-11 (39). The morphological characteristics of caspase-1-dependent and caspase-1-independent pyroptosis are similar. Both are characterized by cell swelling, positivity for Annexin V and TUNEL staining, chromatin condensation and absence of DNA laddering (40–43). The mitochondria of pyroptotic cells also tend to lose membrane potential (42, 44). The terminal event is represented by rupture of the cell membrane, causing release of cytoplasmic contents of the cell, including pro-inflammatory cytokines, endogenous ligands, alarmins and other danger-associated molecular patterns (34, 35, 45–50).

Both caspase-1-dependent and caspase-1-independent pyroptosis lead to the release of IL-1 β and IL-18 – inflammatory hallmarks associated with inflammasome activation. IL-1 β is a potent inducer of inflammation, vasodilation, and immune cell extravasation, but also has roles in shaping adaptive immune responses (51). IL-18 promotes interferon (IFN)- γ production in T_H1 cells, NK cells and cytotoxic T cells, enhances the development of T_H2 cells, and promotes local inflammation (52). The alarmins IL-1 α and HMGB1 as well as other endogenous host molecules such as nuclear and mitochondrial DNA are also released by pyroptotic cells (34, 35, 47–50). Further studies have revealed that pyroptotic cells release ASC specks into the extracellular milieu to propagate inflammasome activation (53, 54). These signals together serve as triggers to initiate, amplify and perpetuate inflammation.

While caspase-1-dependent or caspase-1-independent pyroptosis are morphologically similar, there are notable differences. Caspase-1 is activated by inflammasome-initiating sensors on recognition of pathogen-associated molecular patterns or danger-associated molecular patterns (Figure 2). This mode of activation is called canonical inflammasome activation. The mouse NLRP1b and rat NLRP1 inflammasome sensors are activated following their cleavage by a lethal factor released by the Gram-positive bacterium *Bacillus anthracis* (55, 56). NLRP3 is activated by diverse pathogen-associated molecular patterns and danger-associated molecular patterns (57–59), facilitated by the kinase NEK7 (60–62). NLRP4 is activated by NAIP proteins; a set of NLRs which bind directly to flagellin or the inner rod or needle proteins of the Type III secretion system of bacteria (63–71). AIM2 is activated following direct binding to cytoplasmic dsDNA (72–75). Pyrin responds to bacterial toxin-induced modifications of Rho GTPases and requires microtubule assembly for its activation (76–82). Ultimately, these inflammasome sensors initiate the assembly of a caspase-1-containing inflammasome, licensing caspase-1 to directly cleave the precursor cytokines pro-IL-1 β and pro-IL-18 (15–17, 83–87).

Caspase-1-independent pyroptosis is activated by non-canonical activation of the inflammasome (Figure 2). In this case, caspase-4, caspase-5 and caspase-11 are the apical activators, and, via their CARD domains directly recognize LPS from Gram-negative bacteria in the host cytoplasm (88–90). Caspase-4 has been suggested to be able to cleave pro-IL-1 β and pro-IL-18 (91, 92), a finding which requires further confirmation. Caspase-11 cannot directly cleave pro-IL-1 β and pro-IL-18 (93), but is able to induce pyroptosis independently of caspase-1.

Gasdermin D as a common executor of pyroptosis

All inflammasome-associated caspases directly cleave a 53-kDa substrate called gasdermin D (24–26). Cleavage of gasdermin D by these caspases generates a 31-kDa N-terminal fragment which initiates pyroptosis and a 22-kDa C-terminal fragment which has unknown functions (24, 25, 94). Further mechanistic studies revealed that the N-terminal fragment of gasdermin D associates with the inner leaflet of the cell membrane where it assembles into pores of 10–33 nm in diameter (Figure 2) (24–31). The N-terminal fragment of gasdermin D also drives activation of the NLRP3-dependent caspase-1 inflammasome (24, 25, 37), possibly requiring potassium efflux caused by gasdermin D-induced membrane pores (95). Gasdermin D can also damage and lyse bacteria directly, including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus megaterium* protoplasts (27, 29). The proposed mechanism is that the N-terminal domain of gasdermin D binds to cardiolipin (a lipid found on the bacterial cell membrane) and oligomerize to form pores on the bacterial cell membrane (27, 29).

The physiological role of gasdermin D has only been examined in a mouse model of endotoxemia. Similar to mice lacking caspase-11, mice lacking gasdermin D are remarkably resistant to LPS-induced endotoxemia compared to wild-type mice (25). However, whether secretion of IL-1 β and IL-18 in this model is dependent on gasdermin D remains unclear and further work is required to examine this issue.

Although gasdermin D induces pyroptosis, prolonged activation of the canonical inflammasome pathway by LPS plus ATP or flagellin leads to caspase-1-dependent and gasdermin D-independent pyroptosis (25). This finding suggests that other undefined caspase-1 substrates must also contribute to caspase-1-dependent pyroptosis (25). It is noteworthy to highlight that other members of the gasdermin family can also induce cell death, including mouse gasdermin A3, human gasdermin A, human gasdermin B, human gasdermin C and human and mouse DFNA5 (27, 96–98). Whether these proteins have a role in inflammasome signaling, pyroptosis or in host defense against pathogens remains to be determined.

Physiological roles of caspase-1 and caspase-11 during bacterial infection

Inflammatory caspases protect the host against a variety of bacterial pathogens (Tables 1 and 2). Because previously generated caspase-1-deficient mouse lines also lack caspase-11, the relative contribution of caspase-1 and caspase-11 in mouse models of bacterial infection requires further investigation. In addition, activation of inflammatory caspases leads to pyroptosis and secretion of IL-1 β and IL-18, both of these functions could provide protection against pathogens.

The differential contribution of inflammatory caspases and their substrates is best characterized in a murine model of salmonellosis. Earlier studies found that both IL-1 β and IL-18 were important for the control of oral *S. Typhimurium* infection in the intestine, while IL-18 also controlled the infection at systemic sites (99, 100). The importance of inflammatory caspases in driving protection against *S. Typhimurium* infection is supported by studies showing that *Casp1*^{-/-} *Casp11*^{-/-} mice harbor increased bacterial load and that

these mice succumb to infection more rapidly than wild-type mice (99, 101–103). The relative contribution between the two caspases has since been examined. In order to study the role of caspase-1 in a mouse model, Kayagaki and colleagues microinjected a bacterial artificial chromosome transgene encoding caspase-11 into *Casp1^{-/-}Casp11^{Tg}* mouse embryos to re-establish caspase-11 expression in this strain, generating a new strain known as *Casp1^{-/-}Casp11^{Tg}* (32). A subsequent study has shown that following orogastric infection with *S. Typhimurium* *Casp1^{-/-}Casp11^{Tg}* mice harbor more bacteria in the systemic organs than *Casp1^{-/-}Casp11^{-/-}* mice, and both strains have higher bacterial burden than wild-type mice (102). However, a similar number of bacteria was found between wild-type and *Casp11^{-/-}* mice (102). These data would suggest that caspase-1, but not caspase-11, confers protection to *S. Typhimurium* infection. Moreover, caspase-11 is deleterious to the host in the absence of caspase-1.

A role for caspase-11 in *Salmonella* infection was revealed using a genetically engineered strain of *S. Typhimurium* (*sifA*), which is unable to maintain integrity of the pathogen-containing vacuole and is susceptible to aberrant entry into the host cytoplasm. Caspase-11, but not canonical inflammasomes or IL-1 β and IL-18, was shown to mediate clearance of *S. Typhimurium* *sifA* in vivo (104). Additional studies have found that caspase-11 is required for the secretion of IL-18 in the intestinal tissue in response to *S. Typhimurium* infection and for controlling bacterial numbers in the cecum (92).

The importance of pyroptosis could also be partially inferred by studies showing that *S. Typhimurium* lacking both of its flagellin genes, *fliC* and *fljB*, has reduced capacity to induce inflammation and is attenuated in vivo, possibly because of its lack of motility and/or impaired activation of the flagellin-sensing NLRC4-caspase-1 inflammasome or TLR5 (105–108). A strain of *S. Typhimurium* which overexpresses flagellin due to lack of YdiV, the transcriptional repressor of the flagellin-encoding gene *fliC*, causes excessive pyroptosis in macrophages (109). This strain induces increased levels of IL-1 β and TNF in the serum and fails to colonize the tissue in mice (109).

Pyroptosis releases intracellular bacteria residing within macrophages, including *S. Typhimurium*, *Legionella pneumophila* and *Burkholderia thailandensis* (110). These newly released bacteria can be phagocytosed and killed by neutrophils via a mechanism dependent on the production of reactive oxygen species but independent of IL-1 β and IL-18 (110). Other studies have also reported that pyroptosis can clear bacterial infection even in the absence of IL-1 α , IL-1 β and IL-18 (92, 110, 111). More recent work proposed that viable bacteria can remain trapped within the cellular debris of pyroptotic macrophages called pore-induced intracellular traps (112). These pore-induced intracellular traps are efferocytosed and cleared by neutrophils (112). A more holistic view would be that both unbound and trapped bacteria are released by pyroptotic cells, both of which can be taken up by phagocytic cells in the tissue. An advantage of uptake of pyroptosis-released bacteria by neutrophils is that these host cells are relatively resistant to pyroptosis in response to *S. Typhimurium* infection and other inflammasome activators (113), indicating that neutrophils would be a suitable phagocytic cell type to clear residual bacteria in the tissue. Neutrophils can undergo pyroptosis in mice lacking the NADPH oxidase NOX2 infected with

Pseudomonas aeruginosa (114), suggesting that pyroptosis can be activated to compensate for deficiency of another major antimicrobial pathway.

Another mechanism by which pyroptosis might clear bacteria is through extrusion of infected cells from the tissue. Enterocytes infected with *S. Typhimurium* undergo activation of the caspase-1 or caspase-11 inflammasome, resulting in physical extrusion of infected enterocytes from the intestine (92, 111). In addition to cell-type-specific roles for pyroptosis, organ-specific functions for pyroptosis have also been reported. The NLRC4-caspase-1 inflammasome is essential in mediating host protection to the ubiquitous environmental bacterium *Chromobacterium violaceum* (115). In this context, pyroptosis, but not IL-1 β and IL-18 mediates bacterial clearance in the spleen, whereas both pyroptosis and IL-18-dependent NK cell responses are required in the liver (115). Caspase-1 can also act upstream of caspase-11 in the host defense against bacterial infection. Caspase-1 induces the production of IL-18, which then triggers the production of IFN- γ to prime caspase-11-mediated responses to clear *B. thailandensis* infection in mice (116). These data collectively indicate that pyroptosis and inflammatory cell death is generally protective against bacterial infection. In some cases, excessive inflammasome activation or pyroptosis is detrimental to the host. Unchecked inflammasome activation can either drive immunopathology in response to infection by *Pseudomonas aeruginosa* (117, 118) or impair the generation of CD8⁺ T-cell-mediated immunity to *Listeria monocytogenes* (119).

Caspase-4 and caspase-5 in bacterial infection

The caspase-11 homologs, human caspase-4 and caspase-5, also recognize LPS, induce activation of caspase-1, mediate cleavage of gasdermin D, and drive pyroptosis (24, 25, 90). Although both caspase-4 and caspase-5 have been shown to bind to LPS, the function of these inflammatory caspases is likely to be cell-type-specific and determined by the type of activators encountered by the cell. For example, caspase-4, but not caspase-5, was reported to drive cell death and IL-1 β production in the human monocytic THP-1 cell line in response to LPS transfection (120, 121). Mouse bone-marrow-derived macrophages engineered to express human caspase-4 respond to LPS stimulation and release IL-1 β and IL-18 (122). A further study has demonstrated that caspase-4 necessitated IL-1 α release and cell death in primary human macrophages infected with *S. Typhimurium*, *L. pneumophila* or *Yersinia pseudotuberculosis*; however, caspase-4 was not required for IL-1 β release in this setting (123). Other studies have found non-redundant functions between caspase-4 and caspase-5, with both caspases required for IL-1 β secretion in human monocytes stimulated with LPS or in the THP-1 cell line infected with *S. Typhimurium* (120, 124).

Caspase-4 or caspase-5 can restrict the replication of *L. pneumophila* in human macrophages (125) and of *S. Typhimurium* in human colonic epithelial cells (92). The importance of these caspases is reflected by the presence of bacterial-encoded virulence factors that counteract the effect of these caspases. The effector protein NleF of enteropathogenic *E. coli* can bind the catalytic domain of caspase-4 and inhibit caspase-4-dependent IL-18 secretion in the human intestinal cell line Caco-2 (126). Enteropathogenic *E. coli* and enterohemorrhagic *E. coli* and their mouse relative *C. rodentium* encode effector proteins called NleB (127, 128)

and NleH (129), which bind to components of the cell death pathway to suppress apoptosis and/or necroptosis.

To study the role of human caspase-4 in a mouse model, a transgene encoding human caspase-4 was introduced into *Casp11*^{-/-} mice. Unlike *Casp11*^{-/-} mice, *Casp11*^{-/-} mice expressing human caspase-4 are protected from a lethal *B. thailandensis* infection (116). In an endotoxemia model, *Casp11*^{-/-} mice are normally resistant to LPS-induced lethality; however, *Casp11*^{-/-} mice expressing human caspase-4 are susceptible to LPS-induced lethality (122). These studies highlight the functional complementarity between human and mouse inflammatory caspases in infection and immunity.

Inflammatory caspases are required for cell-autonomous immunity to bacteria

Inflammatory caspases control antimicrobial cellular functions beyond pyroptosis and IL-1 β and IL-18 release. Caspase-1 activation regulates phagosome maturation during both Gram-negative and Gram-positive bacterial infection (130, 131). Caspase-1 promotes fusion between vacuoles containing *L. pneumophila* and lysosomes (130). A further study has shown that caspase-1 regulates the pH of phagosomes containing Gram-positive bacteria such as *Staphylococcus aureus*, resulting in enhanced killing of the internalized pathogen in macrophages (131). Caspase-1 activation also reduces cellular stiffness to prevent excessive uptake of *S. Typhimurium* in macrophages, allowing the cell to control bacterial burden autonomously (132). A further study has shown that the catalytic activity of caspase-1 and caspase-11 is essential for dampening the intracellular growth of *S. Typhimurium* *sifA* (133). In response to infection by *L. pneumophila*, caspase-11 regulates actin polymerization via cofilin to promote fusion between the vacuole containing *L. pneumophila* and lysosomes (125). The cell-autonomous functions of inflammatory caspases clearly expand their mechanistic repertoire beyond pyroptosis and cytokine production in anti-bacterial host defense.

Physiological roles of pyroptosis in viral, fungal and protozoan infection

The ability of inflammasome sensors to recognize a wide array of pathogen-associated molecular patterns suggests that inflammatory caspases are crucial for host defense against pathogens from virtually all domains of life. For example, both DNA and RNA viruses can activate the inflammasome and induce pyroptosis (134, 135). However, a deleterious role of pyroptosis in human immunodeficiency virus (HIV) infection has been reported. The DNA sensor IFI16 recognizes cytosolic viral DNA intermediates produced during HIV-1 infection in human macrophages or CD4⁺ T cells (136, 137). IFI16-mediated recognition of HIV-1 curtails virus replication in human macrophages (136), whereas this response drives pyroptosis in CD4⁺ T cells in lymphoid tissues via cell-to-cell transmission of HIV-1 (137–139). This phenomenon has been suggested to accelerate depletion of CD4⁺ T cells and progression to AIDS in humans (137, 138). Peripheral blood-derived CD4⁺ T cells do not undergo pyroptosis in response to HIV-1 infection owing to reduced levels of IFI16 expression and HIV-1 reverse transcripts in these cells (140). When co-cultured with lymphoid-derived CD4⁺ T cells, peripheral blood-derived CD4⁺ T cells become sensitized to HIV-1-induced pyroptosis (140), suggesting that pyroptosis is transmissible between

different subsets of cells. Uninfected liver cells can also undergo caspase-1-dependent pyroptosis following infection of bystander cells with hepatitis C virus (HCV) (141).

Studies in mice have revealed the importance of caspase-1 and caspase-11 against infection by influenza A virus and West Nile virus (Table 3) (142). The influenza A virus activates the NLRP3 inflammasome (143–146). Indeed, *Casp1^{-/-}Casp11^{-/-}* mice are more susceptible to infection by influenza A virus (143, 144, 147), and produce less IL-1 β and IL-18 in the lungs, and have diminished lung functions and increased viral titers compared to wild-type mice (143, 147). However, *Casp1^{-/-}Casp11^{-/-}* mice are as resistant as Mx1 congenic mice on the C57BL/6 background after infection with influenza A virus but the absence of Casp1 and Casp11 provides protection from lethality in *Tlr7^{-/-}Mavs^{-/-}* mice (148). Individual roles for caspase-1 and caspase-11 have not been deciphered in vivo. Influenza A viruses and pathogens other than Gram-negative bacteria do not carry LPS, the pathogen-associated molecular pattern that activates caspase-11. However, it is still too early to presume that caspase-11 has no role in the host defense against microbial agents other than Gram-negative bacteria in vivo. Indeed, a role for both caspase-1 and caspase-11 has been observed in host defense against the fungal pathogen *Aspergillus fumigatus*, a pathogen which do not carry LPS (36, 149). *A. fumigatus* activates the NLRP3 inflammasome in human THP-1 cells and both the AIM2 and NLRP3 inflammasomes in mouse bone-marrow-derived dendritic cells and the lung tissue (149, 150). This caspase-1-dependent response is crucial for the generation of protective cytokines IL-1 β and IL-18 in a mouse model of aspergillosis (149). Interestingly, *Casp11^{-/-}* mice are also more susceptible to *A. fumigatus*-induced mortality compared with wild-type, but succumb to infection with a delayed kinetics compared with mice lacking caspase-1 or both caspase-1 and caspase-11 (36). How caspase-11 is activated or conferred protection during *A. fumigatus* infection is not known. It is possible that activation of caspase-11 might induce actin-mediated phagosomal killing in order to control *A. fumigatus* dissemination in vivo (6, 125, 151). Caspase-1-dependent release of IL-18 induces production of IFN- γ , which might provide a priming signal for caspase-11 to control aspergillosis in vivo (149). A recent study has suggested that in addition to LPS, caspase-11 recognizes host-derived oxidized phospholipids, a form of danger-associated molecular pattern that can induce IL-1 β release in dendritic cells without triggering pyroptosis (152). Other than *A. fumigatus*, increased susceptibility of *Casp1^{-/-}Casp11^{-/-}* mice to the fungal pathogens *Candida albicans* and *Paracoccidioides brasiliensis*, compared with wild-type mice has also been reported (Table 4) (153–156). In macrophages, pyroptosis induced by *C. albicans* requires the development of fungal hyphae and/or neutralization of the phagosome (157, 158). However, the precise physiological role of pyroptosis in fungal infection has not been investigated.

The biological relevance of inflammatory caspases extends to studies on protozoan parasites, but the specific contribution of pyroptosis is unknown (Table 5). Opposing roles of inflammatory caspases can be observed during leishmaniasis, which appears to depend on the background of the mouse strains and the species of protozoa involved.

Casp1^{-/-}Casp11^{-/-} mice on the C57BL/6 background infected with *Leishmania amazonensis* develop lesions of larger size and harbor increased parasite burden in ear, lymph node and spleen compared with wild-type mice (159). *Casp1^{-/-}Casp11^{-/-}* mice on

the BALB/c background infected with *Leishmania major* have less footpad swelling and parasite burden owing to increased IFN- γ and reduced IL-4 and IL-5 production (160).

Casp1^{-/-}Casp11^{-/-} mice are also susceptible to infection by the protozoa *Toxoplasma gondii* and *Trypanosoma cruzi*, a phenotype associated with reduced IL-1 β or IL-18 production (161–163). A further study demonstrates that in response to infection with *T. gondii*, mice lacking caspase-11 alone have reduced levels of local and systemic cytokines during the acute phase, whereas inflammation is elevated in the brain during the chronic phase of infection (164). Further investigations are required to unveil individual functions of inflammatory caspases in protozoan infections.

IFN signaling and IFN-inducible proteins as critical regulators of pyroptosis and inflammasome activation

IFN signaling is a central modulator of pyroptosis induced by pathogens (165). Extracellular LPS from Gram-negative bacteria is recognized by TLR4, initiating a signaling cascade requiring the TLR adaptor TRIF (102, 166–169). TRIF is crucial in inducing the production of type I IFNs. Type I IFNs bind the heterodimeric type I IFN receptor composed of the subunits IFNAR1 and IFNAR2, and drive transcription of hundreds of IFN-stimulated genes (102, 167). Type I IFN signaling also induces caspase-11, an essential component that activates the non-canonical inflammasome pathway and pyroptosis. Reactive oxygen species and/or the Cpb1-C3-C3aR complement pathway can upregulate the expression of caspase-11 by operating downstream of the TLR4 and type I IFN signaling pathway (170, 171).

The IFN-inducible network of proteins include IFN-inducible GTPases, such as the 47-kD immunity-related GTPases (IRGs) and the 65-kD guanylate-binding proteins (GBPs) (165). GBPs rupture the pathogen-containing vacuole encasing Gram-negative bacteria, allowing the bacteria and their LPS to enter the cytoplasm for sensing by caspase-11 (Figure 3) (172–174). Once the bacteria are in the cytoplasm, IRGB10 further disrupts the structural integrity of the bacteria, enhancing liberation of LPS in the cytoplasm and accessibility by caspase-11 (175). Delivery of LPS into the cytoplasm can also be achieved by bacterial outer membrane vesicles (176), but whether IFN signaling is involved in cytoplasmic entry of bacterial outer membrane vesicles has not been investigated.

Activation of pyroptosis induced by the AIM2 inflammasome in response to *Francisella novicida* infection also requires type I IFN signaling (177–183). This pathway is initiated by the DNA sensor cGAS upon detection of *F. novicida*, inducing STING-dependent production of type I IFNs (177, 183, 184). Type I IFNs potentiate expression of GBPs and IRGs via the transcription factor IRF1 (175, 177). GBP2, GBP5 and IRGB10 co-operate synergistically to rupture *F. novicida* that have entered the cytoplasm, resulting in the exposure of *F. novicida* DNA for sensing by AIM2 (175, 177, 178).

In addition to bacterial infection, type I IFN signaling contributes to cell death induced by influenza A virus. Type I IFN signalling mediates upregulation of the innate immune sensor ZBP1 (also known as DLM-1 or DAI) (146). ZBP1 recognizes the influenza A virus nucleoprotein and RNA polymerase subunit PB1, and mediates activation of the NLRP3 inflammasome, necroptosis and apoptosis via the kinase RIPK3, caspase-8 and FADD (146).

These findings were recently confirmed by another group (185, 186), providing further evidence to underscore the importance of the type I IFN–ZBP1 axis in driving influenza virus-induced cell death (Figure 3).

In addition to type I IFN signaling, IFN- γ can prime caspase-11 expression and activation of the inflammasome in macrophages (172, 178). A further study has shown that IFN- γ rather than type I IFNs is necessary to drive induction of caspase-11 responses in mice infected with *B. thailandensis* (116). IFN- γ is partially required for the upregulation of caspase-11 protein in the colon tissue of mice during DSS-induced colitis (187). These studies collectively highlight a role for both type I and type II IFN signaling pathways in the activation of inflammasomes and pyroptosis.

Conclusions and future perspectives

Host cells are often invaded by intracellular pathogens that are capable of replicating in the pathogen-containing vacuoles or in the cytoplasm. Cell death directly removes these replicative niches exploited by the pathogens. Infectious agents released from dying cells are exposed to extracellular immune defense and are often taken up by other immune cells for killing. In addition, pyroptosis releases cytoplasmic contents from the dying host cells, thereby providing potent signals to initiate an inflammatory cascade. Local inflammation leads to recruitment and priming of immune cells, ultimately contributing to clearance of pathogen from the host.

Our understanding of the molecular mechanisms governing activation and execution of pyroptosis has evolved substantially over the years. Issues associated with the loss of caspase-11 in previously generated caspase-1–deficient mouse strains are being addressed with newly generated single knockout mouse strains (36–38), providing the scientific community new genetic tools to redefine the biological functions of caspase-1 and caspase-11. The identification of gasdermin D as a substrate of inflammatory caspases and a pore-forming protein opens up new avenues by which the function of pyroptosis can be specifically dissected in health and disease. The susceptibility profile of mice lacking gasdermin D or other gasdermin proteins to infectious agents have not been investigated. Direct comparison between mice lacking gasdermin D, mice lacking caspase-1 and/or caspase-11 and mice lacking IL-1 β and IL-18 in response to a range of infectious agents would unequivocally reveal the unique and overlapping contributions of these components in the anti-microbial host defense. Given gasdermin D is a terminal effector of pyroptosis and is thought to be a major pathway in clearing intracellular pathogens, it is reasonable to speculate that mice lacking gasdermin D would be susceptible to a range of infectious agents, phenocopying the susceptibility profile seen in mice lacking caspase-1 and/or caspase-11. Further investigations into the relationship between inflammatory caspases and pyroptosis in innate immunity will uncover novel signaling components that can be targeted and translated to prevent or treat infections in human patients.

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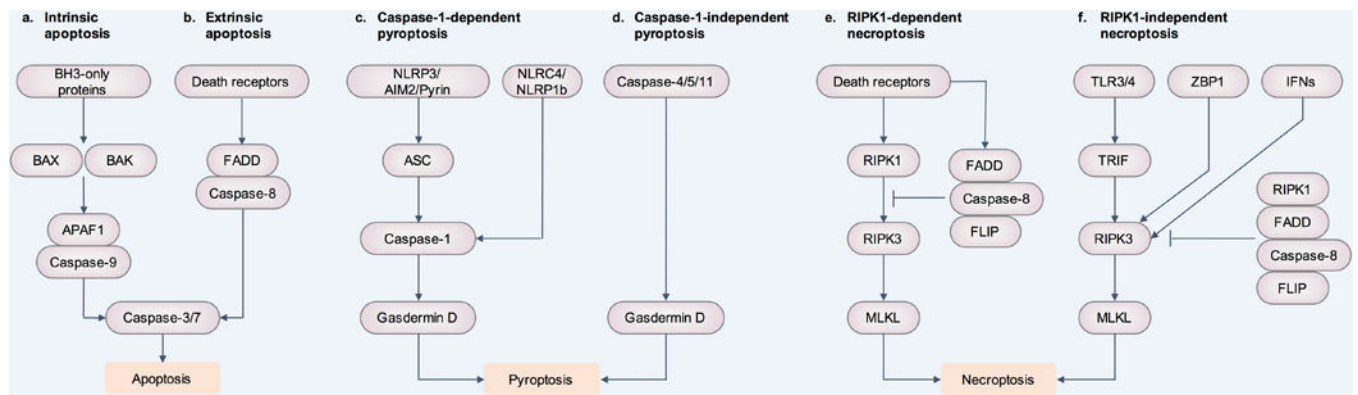


Figure 1. Programmed cell death pathways are regulated by different molecular components (a and b) Apoptosis can be activated via intrinsic and extrinsic pathways. (a) Intrinsic apoptosis requires BCL-2 homology domain 3 (BH3)-only proteins, which engages BAX and BAK activation. This leads to apoptosome assembly via APAF1 and caspase-9, resulting in the activation of the effectors caspase-3 and caspase-7, and apoptosis. (b) Extrinsic apoptosis requires death receptors to induce dimerization of caspase-8 or caspase-10 through the adaptor protein FADD. Active caspase-8 and caspase-10 cleave and activate caspase-3 and caspase-7, leading to apoptosis. (c and d) Pyroptosis can be induced via caspase-1, human caspase-4 and caspase-5, or mouse caspase-11. (c) The inflammasome sensors NLRP3, AIM2 and Pyrin require the inflammasome adaptor protein ASC in order to form a caspase-1-containing inflammasome complex. The inflammasome sensors NLRC4 and NLRP1b can directly bind to caspase-1 without ASC. Caspase-1 mediates cleavage of the substrate gasdermin D, generating an N-terminal fragment of gasdermin D that induces pyroptosis. (d) Human caspase-4 and caspase-5 or mouse caspase-11 directly cleave gasdermin D to induce pyroptosis. (e and f) Necroptosis can be induced via RIPK1 or independently of RIPK1. (e) Death receptors induce activation of RIPK1, RIPK3 and MLKL, leading to necroptosis. FADD, caspase-8, and the caspase-8 paralogue FLIP form a complex to inhibit RIPK1-dependent necroptosis. (f) TLR3 and TLR4 can directly recruit and activate RIPK3 via the adaptor protein TRIF, independently of RIPK1. In addition, ZBP1 (also known as DLM-1 and DAI) can bind and activate RIPK3. Interferons (IFNs) can also activate RIPK3. In this context, RIPK1, along with FADD, caspase-8, and FLIP inhibit RIPK3-dependent necroptosis.

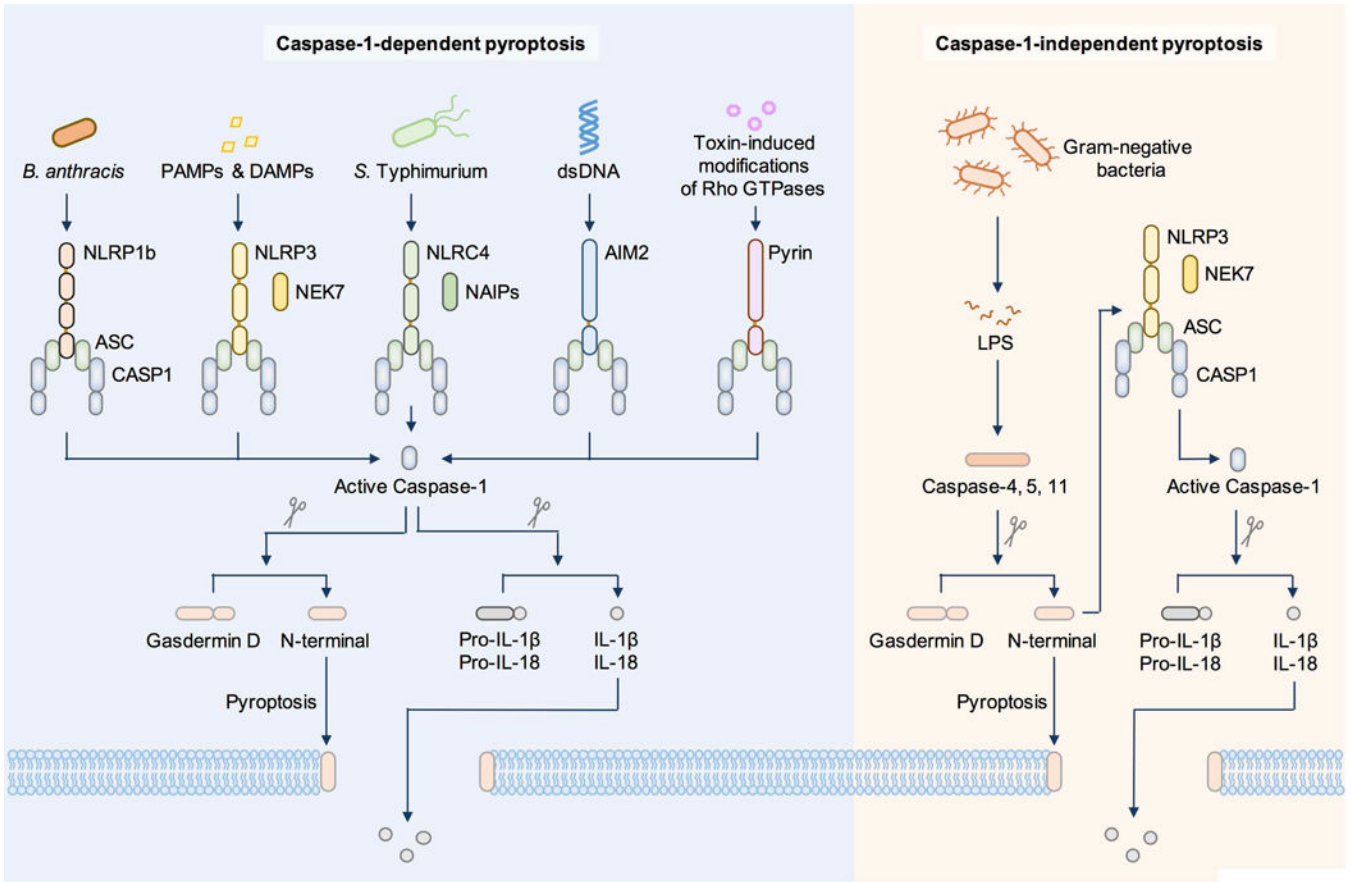


Figure 2. Molecular basis of caspase-1-dependent pyroptosis and caspase-1-independent pyroptosis

Caspase-1-dependent pyroptosis requires activation of the canonical inflammasomes. In this pathway, pathogen-associated molecular patterns or danger-associated molecular patterns activate their respective inflammasome sensors, including NLRP1b, NLRP3, NLRC4, AIM2 or Pyrin. Activation of the NLRP3 and NLRC4 inflammasomes requires the kinase NEK7 and ligand-binding NAIP proteins, respectively. Inflammasome sensors trigger recruitment of the inflammasome adaptor ASC and the cysteine protease caspase-1 into the same macromolecular complex. Caspase-1 directly cleaves gasdermin D and the precursor cytokines pro-IL-1 β and pro-IL-18, initiating pyroptosis and maturation of IL-1 β and IL-18, respectively. The 31-kDa cleaved N-terminal portion of gasdermin D forms pores on the host cell membrane to mediate the release of cytoplasmic contents. Caspase-1-independent pyroptosis requires activation of the non-canonical inflammasome. In this pathway, cytosolic LPS from Gram-negative bacteria is recognized by either caspase-4 or caspase-5 in human cells or by caspase-11 in mouse cells. These inflammatory caspases directly cleave gasdermin D and initiate pyroptosis. The N-terminal fragment also activates the NLRP3 inflammasome and caspase-1-dependent maturation of IL-1 β and IL-18.

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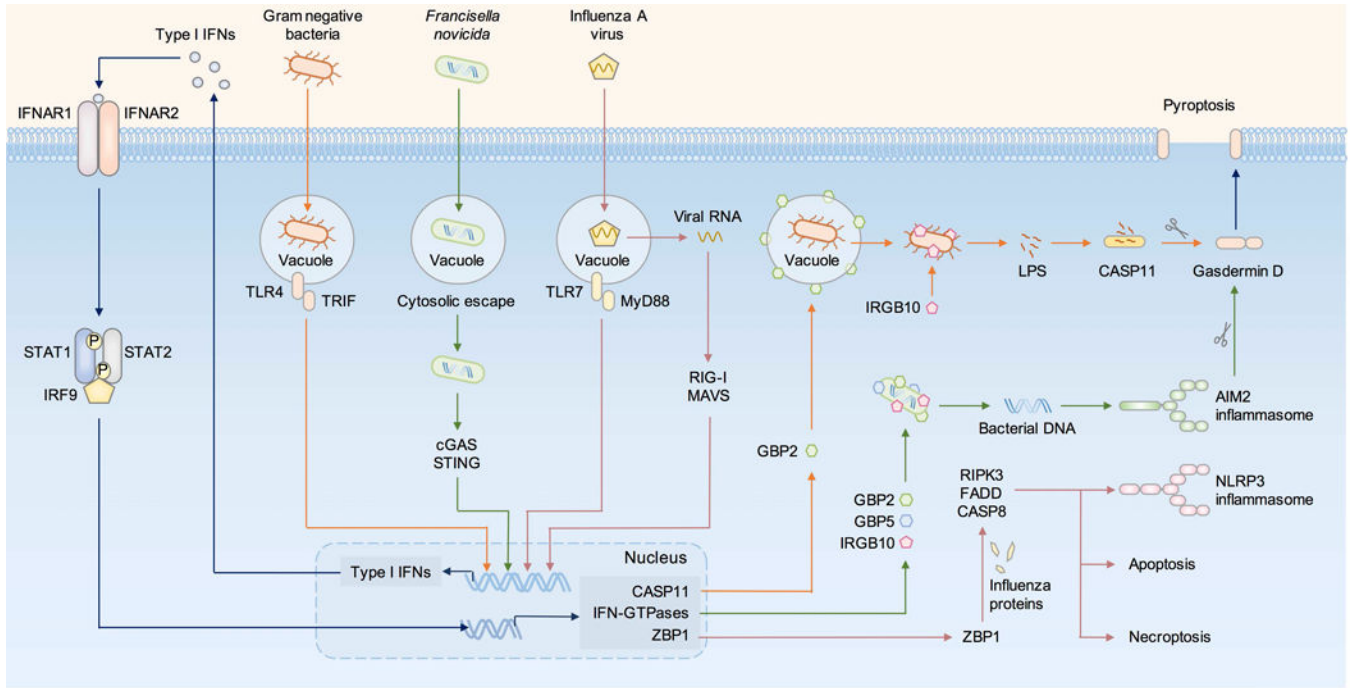


Figure 3. Type I IFN signaling regulates pathogen-induced inflammasome activation and pyroptosis

LPS from Gram-negative bacteria is recognized by TLR4, inducing TRIF-dependent type I IFN production. *Francisella novicida* is a cytosolic bacterium which is recognized by the DNA sensor cGAS, inducing STING-dependent type I IFN production. RNA from influenza A virus triggers the RNA sensors TLR7 via the adaptor MyD88 and RIG-I via the adaptor MAVS, both inducing production of type I IFNs. The type I IFN signaling pathway is activated via the transcription factors STAT1, STAT2 and IRF9, leading to upregulation of caspase-11, IFN-inducible GTPases, including guanylate-binding proteins (GBPs) and Immunity-related GTPases (IRGs), and other IFN-inducible proteins including the sensor ZBP1. GBP2 ruptures the vacuole containing Gram-negative bacteria, mediating the liberation of LPS into the cytoplasm for recognition by caspase-11. IRGB10 further disrupts Gram-negative bacteria to increase LPS accessibility for detection by caspase-11. GBP2, GBP5 and IRGB10 directly target the bacterial membrane of cytosolic-dwelling *F. novicida*, exposing its DNA for sensing by AIM2. Activation of the caspase-11-NLRP3 inflammasome in response to Gram-negative bacteria and activation of the AIM2 inflammasome in response to *F. novicida* lead to pyroptosis via gasdermin D. ZBP1 recognizes proteins from the influenza A virus and induce pyroptosis, necroptosis, and apoptosis via RIPK3, FADD and caspase-8.

Table 1

The role of caspase-1 and caspase-11 in response to Gram-negative bacteria in mice.

Mouse	Bacteria	Phenotype compared to wild-type mice	
<i>Casp1</i> ^{-/-} <i>Casp11</i> ^{-/-}	<i>Acinetobacter baumannii</i>	Decreased IL-1 β and IL-6 in the BALF, decreased lung pathology (188).	
	<i>Burkholderia cepacia</i>	No significant survival difference (115).	
	<i>Burkholderia pseudomallei</i>	Reduced survival, increased bacterial burden in the spleen, lung and liver, decreased IL-1 β , IL-18 and IFN- γ in BALF (104, 189, 190).	
	<i>Burkholderia thailandensis</i>	Reduced survival (104, 115, 116), increased bacterial burden in the spleen, liver and MLN (116).	
	<i>Citrobacter rodentium</i>	Increased body weight loss, bacterial burden in feces, and adaptive immune responses, shorter colon length, elevated pro-inflammatory cytokines, decreased IL-18 (191).	
	<i>Chromobacterium violaceum</i>	Reduced survival, increased bacterial burden in the liver and spleen, increased macroscopic lesions and extensive neutrophil infiltration in the liver (115).	
	<i>Ehrlichia (Ixodes ovatus ehrlichia)</i>	No difference in survival, but higher bacterial burden in the liver (192).	
	<i>Francisella novicida</i>	Increased pathogen burden in the liver, lung and spleen, reduced survival and IL-18 production (177, 178, 193).	
	<i>Francisella philomiragia</i>	No survival difference (115).	
	<i>Francisella tularemia</i>	Reduced survival and increased bacterial burden in lungs (194).	
	<i>Legionella bozemanii</i>	Increased bacterial burden in the lungs (195).	
	<i>Legionella gratiana</i>	Increased bacterial burden in the lungs (196).	
	<i>Legionella micdadei</i>	Increased bacterial burden in the lungs (195).	
	<i>Legionella pneumophila</i>	Increased bacterial burden in the lungs (195, 196).	
	<i>Legionella rubrilucens</i>	Increased bacterial burden in the lungs (195).	
	<i>Salmonella</i> Typhimurium	Increased pathogen burden in the liver, spleen, MLNs, and Peyer's patches, and reduced survival, IL-18 production and bacterial uptake by neutrophils (99, 101–103, 197), increased bacterial burden in colonic mucosa but no significant difference of bacterial burden in the MLN and cecum (111), decreased IL-1 β and IL-18 in the cecal tissue (92).	
	<i>Shigella flexneri</i>	Reduced survival and increased bacterial burden in the lungs (198).	
	<i>Vibrio vulnificus</i>	Reduced survival (199).	
	<i>Yersinia pestis</i>	Increased survival and decreased symptoms of respiratory distress (200).	
	<i>Yersinia pseudotuberculosis</i>	No difference in survival with WT <i>Yersinia</i> but reduced survival and increased bacterial burden in the spleen with <i>YopM</i> (201, 202).	
LPS endotoxemia	Increased survival, reduced IL-1 β and IL-18 production (32, 35, 47, 99, 122, 203).		
<i>Casp1</i> ^{-/-} (also known as <i>Casp1</i> ^{Null})	<i>Francisella novicida</i>	Reduced survival and IL-18 production (175).	
	<i>Casp1</i> ^{-/-} <i>Casp11</i> ^{Tg*}	<i>Legionella gratiana</i>	Increased bacterial burden in the lungs (196).
		<i>Legionella pneumophila</i>	Increased bacterial burden in the lungs (196, 204).
		<i>Salmonella</i> Typhimurium	Increased pathogen burden in the liver, spleen and MLNs and reduced bacterial uptake by neutrophils (102).
LPS endotoxemia	No significant difference in survival, but reduced IL-1 β and IL-18 production (32).		
<i>Casp11</i> ^{-/-}	<i>Burkholderia pseudomallei</i>	Reduced survival (104).	
	<i>Burkholderia thailandensis</i>	Reduced survival (104, 116), increased bacterial burden in the spleen, liver and MLN (116).	

Mouse	Bacteria	Phenotype compared to wild-type mice
	<i>Legionella gratiana</i>	No difference in bacterial burden in the lungs (196).
	<i>Legionella pneumophila</i>	No difference in bacterial burden in the lungs (196).
	<i>Listeria monocytogenes</i>	No difference in survival, pathogen burden in the blood, liver, and spleen (205).
	<i>Salmonella</i> Typhimurium	Increased pathogen burden in the cecum and lumen (92), but not in the liver, spleen and MLNs (102). Reduced IL-18 production in the cecal tissue (92), no significant difference in bacterial burden in the colonic mucosa (111).
	LPS endotoxemia	Increased survival, reduced IL-1 β and IL-18 production (32, 88, 89, 205, 206).

* *Casp1*^{-/-} *Casp11*^{-/-} mouse embryos microinjected with a bacterial artificial chromosome transgene encoding caspase-11, such that the mouse strain expresses caspase-11 to mimic a “*Casp1*^{-/-}” mouse strain (32).

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Table 2

The role of caspase-1 and caspase-11 in response to Gram-positive and other non-Gram-negative bacteria in mice.

Mouse	Bacteria	Phenotype compared to wild-type mice
Gram-positive bacteria		
<i>Casp1^{-/-} Casp11^{-/-}</i>	<i>Bacillus anthracis</i>	Reduced survival and decreased IL-1 β in the serum (207).
	<i>Listeria monocytogenes</i>	Reduced survival, increased bacterial burden in the liver and spleen, decreased IL-18 and IFN- γ in the serum (208).
	<i>Staphylococcus aureus</i>	Reduced survival but no significant changes in bacterial burden (209).
	<i>Streptococcus agalactiae</i> (Group B <i>Streptococcus</i>)	Reduced survival and increased bacterial burden in the kidneys and blood (210).
	<i>Streptococcus pneumoniae</i>	No difference in survival and in bacterial burdens in the lungs and blood (211).
Gram-variable bacteria		
<i>Casp1^{-/-} Casp11^{-/-}</i>	<i>Mycobacterium tuberculosis</i>	No difference in survival and bacterial burden in the lungs, liver and spleen (212). No difference in survival during the acute phase of infection (213).

Table 3

The role of caspase-1 and caspase-11 in response to viral infection in mice.

Mouse	Virus	Phenotype compared to wild-type mice
<i>Casp1^{-/-}Casp11^{-/-}</i>	Encephalomyocarditis virus	No difference in survival (214).
	Influenza A virus	Reduced survival (143, 144, 147), decreased IL-1 β , IL-18, TNF, IL-6, KC, MIP-2 in the BALF, decreased neutrophils and monocytic dendritic cells in the BALF, diminished respiratory function (143), decreased IFN- γ producing CD4 ⁺ and CD8 ⁺ T cells, reduced nasal IgA, increased pulmonary viral titer (147). No difference in survival and body weight change in Mx1 sufficient host (148).
	Murine gamma-herpesvirus 68 (MHV68)	No difference in viral burden in the lungs (215).
	Vesicular stomatitis virus	No difference in survival (214).
	West Nile virus	Reduced survival (142).

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Table 4

The role of caspase-1 and caspase-11 in response to fungal infection in mice.

Mouse	Fungus	Phenotype compared to wild-type mice
<i>Casp1^{-/-}Casp11^{-/-}</i>	<i>Aspergillus fumigatus</i>	Increased lung damage and hemorrhage, fungal dissemination, and reduced survival (36, 149).
	<i>Candida albicans</i>	Reduced survival and increased fungal burden in the kidneys, diminished T _H 1/T _H 17 responses (153–155).
	<i>Paracoccidioides brasiliensis</i>	Reduced survival, increased lung damage and hemorrhage, and fungal dissemination (156).
<i>Casp1^{-/-}</i> (also known as <i>Casp1^{Null}</i>)	<i>Aspergillus fumigatus</i>	Reduced survival (36).
<i>Casp11^{-/-}</i>	<i>Aspergillus fumigatus</i>	Reduced survival (36).

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Table 5

The role of caspase-1 and caspase-11 in response to protozoan infection in mice.

Mouse	Protozoan	Phenotype compared to wild-type mice
<i>Casp1^{-/-}Casp11^{-/-}</i>	<i>Leishmania amazonensis</i>	Increased lesion size and parasite burden in ear, lymphnode and spleen (159).
	<i>Leishmania major</i>	Decreased footpad swelling, decreased pathology score and parasite burden in the footpads, decreased IL-1 β and IL-18 in the footpads, decreased IL-4, IL-5 and increased IFN- γ (160), decreased infiltration of CD11b ⁺ and PMN cells in the ear (216).
	<i>Plasmodium berghei</i>	No difference in survival when infected with sporozoites or iRBCs and no difference in parasitemia level (217, 218).
	<i>Toxoplasma gondii</i>	Reduced survival and decreased IL-1 β and IL-18 in the serum (161).
	<i>Trypanosoma cruzi</i>	Reduced survival, higher parasitism in the heart, spleen and blood, higher heart injury, decreased IL-1 β (162, 163), decreased production of NO from splenocytes (163).
<i>Casp11^{-/-}</i>	<i>Toxoplasma gondii</i>	Increased survival, decreased clinical score, altered immune responses, increased brain cysts and neuroinflammation during late stages of disease (164).