Review

From pyroptosis, apoptosis and necroptosis to PANoptosis: A mechanistic compendium of programmed cell death pathways

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Abstract

Pyroptosis, apoptosis and necroptosis are the most genetically well-defined programmed cell death (PCD) pathways, and they are intricately involved in both homeostasis and disease. Although the identification of key initiators, effectors and executioners in each of these three PCD pathways has historically delineated them as distinct, growing evidence has highlighted extensive crosstalk among them. These observations have led to the establishment of the concept of PANoptosis, defined as an inflammatory PCD pathway regulated by the PANoptosome complex with key features of pyroptosis, apoptosis and/or necroptosis that cannot be accounted for by any of these PCD pathways alone. In this review, we provide a brief overview of the research history of pyroptosis, apoptosis and necroptosis. We then examine the intricate crosstalk among these PCD pathways to discuss the current evidence for PANoptosis. We also detail the molecular evidence for the assembly of the PANoptosome complex, a molecular scaffold for contemporaneous engagement of key molecules from pyroptosis, apoptosis, and/or necroptosis. PANoptosis is now known to be critically involved in many diseases, including infection, sterile inflammation and cancer, and future discovery of novel PANoptotic components will continue to broaden our understanding of the fundamental processes of cell death and inform the development of new therapeutics.

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1. Introduction

Cell death is a conserved phenomenon across prokaryotic and eukaryotic cells. It occurs not only as a spontaneous process in response to physical damage but also through active and genetically programmed pathways during normal development and physiology and in response to pathogens [1]. Based on morphological examinations and the DNA fragmentation status of dead cells, cell death was initially classified as “programmed” apoptosis [2] and “accidental” necrosis [3] in mammalian cells. However, decades of research in the field have conceptually advanced our understanding of cell death as regulated processes [4]. Among all the proposed forms of programmed cell death (PCD), pyroptosis, apoptosis and necroptosis are the most well-defined, with intricate molecular machineries responsible for the initiation, transduction and execution of cell death [1,4]. With the identification of key molecules involved in these PCD pathways, the fundamental functions of PCD have been elucidated through genetic and pharmacological manipulations in a variety of scenarios ranging from normal development to infectious, inflammatory and autoimmune diseases and cancer [5–9]. Molecularly, apoptosis is executed by activation of the executioner caspases, caspase-3 (CASP3) and CASP7, downstream of the initiator caspases CASP8, CASP9 and CASP10 [10–14]. Pyroptosis is driven by plasma membrane pore formation by activated gasdermin family members; the prototype is gasdermin D (GSDMD), which forms the apoptosome with CASP1 and CASP11 (mice) or CASP4/5 (humans) [15,16]. Necroptosis is executed by formation of mixed lineage kinase domain-like pseudokinase (MLKL) pores following MLKL phosphorylation downstream of the receptor-interacting protein kinase 1 (RIPK1) and RIPK3 signaling axis [17–21]. Although the pathways leading to executioner activation in these three forms of PCD have historically been considered independent, there is mounting evidence showing significant crosstalk among the three pathways. Indeed, in a growing number of sterile insults and infectious conditions, caspase-1 (CASP1) and CASP7, downstream of the initiator caspases CASP8, CASP9 and CASP10, play key roles in regulating cell death [22,29], suggesting a united modality of death, conceptualized as PANoptosis. PANoptosis is defined as an inflammatory cell death pathway activated by specific triggers and regulated by the PANoptosome complex that has key features of pyroptosis, apoptosis and/or necroptosis but that cannot be accounted for by any of these three PCD pathways alone. The PANoptosome provides a molecular scaffold for contemporaneous engagement of key molecules from pyroptosis, apoptosis and/or necroptosis but that cannot be accounted for by any of these three PCD pathways alone. The PANoptosome complex has key features of pyroptosis, apoptosis and/or necroptosis that are common to both Fas- and TNF-mediated pathways of apoptosis, in a manner that the first two genetic elements in the apoptosis pathway were identified in Caenorhabditis elegans, ced-3 and ced-4 [40], the homologs of human CASP3 [10,11] and APAF1 [41], respectively. Apoptosis is now known to proceed through both intrinsic and extrinsic pathways. The identification of APAF1 defined the intrinsic, mitochondria-dependent pathway of apoptosis [41]. Mitochondrial damage or disruption leads to the permeabilization of the mitochondrial outer membrane, resulting in the release of a number of molecules, including cytochrome C. Cytosolic cytochrome C is sensed by APAF1, which then forms the apoptosome with CASP9, the initiator caspase of the intrinsic apoptotic pathway [42]. This complex allows the cleavage of the pro form of CASP9 into its mature form through a mechanism coupled to the hydrolysis of ATP. Mature CASP9 is then able to activate downstream effector caspases (e.g., CASP3) [43]. The permeability of mitochondria to the release of cytochrome C is regulated by multiple BCL-2 family proteins, the first of which was cloned in 1984 during the study of B cell lymphoma [44]. Another group later also identified BCL-2 family member ced-9 (BCL-XL homologue), which inhibits apoptosis, in a C. elegans genetic screen [45]. The regulation of apoptosis by BCL-2 family members is critical and has been extensively reviewed elsewhere [46]. Shortly after the pathway of intrinsic apoptosis was described, the extrinsic pathway was also identified. Extrinsic apoptosis is initiated through the engagement of death-inducing receptors Fas and TNF-α receptor (TNFR). The first pro-apoptotic signaling molecules downstream of these death receptors to be identified were FADD (Fas-associated protein with death domain [DD]) [47–49] and CASP8 [50,51]. Homotypic interactions between the DDs are common to both Fas- and TNF-mediated cell death events [52]. FADD interacts with the DD of the receptor and recruits CASP8 through homotypic interactions between their death effector domains (DED) [50,51]. CASP8 is recruited in its pro form and undergoes autoprocessing into mature CASP8 to gain its full proteolytic activity to cleave and activate CASP3 and CASP7. Therefore, CASP9 and CASP8 are the initiator caspases of the intrinsic and extrinsic apoptosis pathways, respectively, and these pathways converge for activation of the same executioner enzymes: CASP3 and CASP7.

The work genetically defining the molecular mechanisms of apoptosis laid the foundation for the characterization of other forms of PCD. Homology was found between nematode apoptotic ced-3 and the mammalian IL-1β converting enzyme (ICE) when ICE was cloned in 1992 [53–55]. ICE was renamed as CASP1 based on this homology and its ability to induce PCD when overexpressed [56]. The physiological function of CASP1 in the induction of cell death is now known to proceed through both intrinsic and extrinsic pathways. The identification of APAF1 defined the intrinsic, mitochondria-dependent pathway of apoptosis [41]. Mitochondrial damage or disruption leads to the permeabilization of the mitochondrial outer membrane, resulting in the release of a number of molecules, including cytochrome C. Cytosolic cytochrome C is sensed by APAF1, which then forms the apoptosome with CASP9, the initiator caspase of the intrinsic apoptotic pathway [42]. This complex allows the cleavage of the pro form of CASP9 into its mature form through a mechanism coupled to the hydrolysis of ATP. Mature CASP9 is then able to activate downstream effector caspases (e.g., CASP3) [43]. The permeability of mitochondria to the release of cytochrome C is regulated by multiple BCL-2 family proteins, the first of which was cloned in 1984 during the study of B cell lymphoma [44]. Another group later also identified BCL-2 family member ced-9 (BCL-XL homologue), which inhibits apoptosis, in a C. elegans genetic screen [45]. The regulation of apoptosis by BCL-2 family members is critical and has been extensively reviewed elsewhere [46]. Shortly after the pathway of intrinsic apoptosis was described, the extrinsic pathway was also identified. Extrinsic apoptosis is initiated through the engagement of death-inducing receptors Fas and TNF-α receptor (TNFR). The first pro-apoptotic signaling molecules downstream of these death receptors to be identified were FADD (Fas-associated protein with death domain [DD]) [47–49] and CASP8 [50,51]. Homotypic interactions between the DDs are common to both Fas- and TNF-mediated cell death events [52]. FADD interacts with the DD of the receptor and recruits CASP8 through homotypic interactions between their death effector domains (DED) [50,51]. CASP8 is recruited in its pro form and undergoes autoprocessing into mature CASP8 to gain its full proteolytic activity to cleave and activate CASP3 and CASP7. Therefore, CASP9 and CASP8 are the initiator caspases of the intrinsic and extrinsic apoptosis pathways, respectively, and these pathways converge for activation of the same executioner enzymes: CASP3 and CASP7.

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death was then discovered in 1998, when Shigella-infected macrophages were found to undergo cell death in a CASP1-dependent manner. This cell death was inhibited in Casp1-deficient peritoneal macrophages [57]. Although CASP1-dependent cell death was initially referred to as apoptosis, given that it is “programmed” [54,57], the inflammatory nature (IL-1β maturation) and
morphological features of CASP1-dependent cell death are distinct from those of the classically defined apoptosis (Fig. 1). These differences led to the introduction of the term pyroptosis [58,59].

CASP1 is activated by the inflammasome [60], a multiprotein complex that typically contains a sensor protein, the adaptor protein ASC and CASP1. Canonical inflammasome sensors generally contain a caspase activation and recruitment domain (CARD) (e.g., NLRC4, NLRP1β) or pyrin domain (PYD) (e.g., NLRP3, AIM2, Pyrin), which are critical to initiate inflammasome assembly [61]. The inflammasome adaptor protein ASC contains both a PYD and CARD and uses homotypic domain interactions to bridge PYD-containing and CARD-containing inflammasome sensors and CARD-containing CASP1, leading to CASP1 cleavage and activation [62]. Alternatively, CARD-containing sensors such as NLRP1β and NLRC4 can directly interact with CASP1, causing pyroptosis without inducing CASP1 autoprocessing [63,64], although the physiologic relevance of ASC-independent interactions remains unclear. CASP1 proteolytic cleavage and activation results in CASP1 cleaving its downstream substrates, including the inflammatory cytokines pro-IL-1β and pro-IL-18 to produce their bioactive forms and the pore-forming molecule GSDMD to facilitate plasma membrane pore formation and pyroptosis. In addition, pyroptosis can also be induced by murine CASP11 or human CASP4/5-mediated non-canonical inflammasome activation in response to intracellular lipopolysaccharide (LPS) sensing [65–67]. CASP11 then cleaves GSDMD to form membrane pores, which facilitate NLRP3 inflammasome and CASP1 activation in a cell-intrinsic manner to induce IL-1β and IL-18 maturation [16]. Therefore, inflammasome-mediated cell death is accompanied by the release of cytokines, including IL-1β and IL-18, conferring the pro-inflammatory nature of pyroptosis [15,16].

In addition to apoptosis and pyroptosis, necroptosis has also been molecularly defined. The first genetic evidence of necroptosis was reported in 2000 [19], T cells stimulated with Fas ligand in the presence of caspase inhibitors died through a FADD/RIPK1-dependent manner with necrotic morphology and without the release of cytochrome C [19]. The term “necroptosis” was proposed a few years later in 2005 with the development of necrostatin-1 [68], an inhibitor of necroptosis which was shown to target RIPK1 [69,70]. In TNF-α-induced necroptosis, the kinase activity of both RIPK1 and RIPK3 is essential for cell death, and RIPK1 and RIPK3 interact to form a necrosome through their RIP homotypic interaction motifs (RHIMs) [71–74]. The necrosome phosphorylates the pseudokinase MLKL, which disrupts the plasma membrane to cause cell death [17,18]. In addition, Toll-like receptors (TLRs) can also initiate necroptosis via RIPK3 and MLKL phosphorylation independent of RIPK1 kinase activity [75,76]. Further studies into the mechanisms of plasma membrane rupture during cell death have found that the surface molecule NINJ1 is important for rupture in necroptosis as well as pyroptosis and apoptosis to mediate the release of large proteins, such as LDH and HMGB1 [79], and future studies will be required to fully elucidate the mechanism of pore formation and plasma membrane rupture. Because the onset of necroptosis is generally associated with the inhibition of caspases, in particular CASP8, necroptosis is thought to be a “fail-safe” mechanism to ensure cell death can still occur during infections or in the presence of oncogenic mutations that abrogate caspase activation [77,78].

3. Crosstalk between pyroptosis, apoptosis and necroptosis

Although pyroptosis, apoptosis and necroptosis were historically discovered and described as distinct, independent pathways, mounting evidence shows that extensive interactions exist among these PCD pathways [22,29–36,80–82]. Clear connections have now been described between each pair of pathways, and there is a growing body of literature mechanistically defining interactions among all three pathways. These findings led to the establishment of the concept of PANoptosis, which is defined as an inflammatory PCD pathway activated by specific triggers and regulated by the PANoptosome complex that has key features of pyroptosis, apoptosis and/or necroptosis but that cannot be accounted for by any of these PCD pathways alone [22–37].

3.1. Molecular interactions between components of pyroptosis and apoptosis

The first genetic evidence for a link between pyroptosis and apoptosis was found in 2008, when it was shown that CASP1 can cleave CASP7 at its canonical aspartic acid activation site in macrophages [30]. Furthermore, activation of CASP7 during Salmonella enterica serovar Typhimurium (Salmonella) infection is abolished by CASP1 deficiency [30]. Inflammasome and CASP1 activation can also result in the cleavage of the apoptotic substrate poly(ADP-ribose) polymerase 1 (PARP1) [31]. Subsequent studies found that pyroptotic CASP1 can also activate the apoptotic pathway by inducing cleavage of apoptotic CASP3 in the absence of GSDMD [83,84]. This type of regulation is physiologically important in cells with low or no GSDMD expression, such as neurons and mast cells, which appear to undergo apoptosis rather than pyroptosis upon inflammasome activation [83].

Another key connection between inflammasome activation/pyroptosis and apoptosis has been centered on the initiator caspase of extrinsic apoptosis, CASP8. In response to infection with Salmonella or Citrobacter rodentium or in response to stimulation by LPS + ATP, B. anthracis lethal toxin challenge, FlaTox stimulation and likely under additional conditions that remain to be characterized, CASP8 is recruited into the inflammasome complex [32,85–87]. While CASP8 is dispensable for the cell death during Salmonella infection, the cell death and CASP1 activation in macrophages after LPS + ATP, C. rodentium or Yersinia stimulation is FADD- and CASP8-dependent, positioning FADD/CASP8 upstream of inflammasome activation under these conditions [32,88]. Mechanistically, in addition to the proteolytic ability of CASP8 to process CASP1 in a recombinant system [32], CASP8 and FADD are also essential for the transcription of Nlrp3 and Il1b [32]. Furthermore, TAK1 inhibition, which can be induced by the Yersinia effector YopJ, initiates cell death characterized by pyroptosis, apoptosis and necroptosis [35,36], and CASP8 can directly activate GSDMD in this context [89–91]. The CASP8-mediated GSDMD cleavage also contributes to NLRP3 inflammasome formation and IL-1β production following TAK1 inhibition, suggesting that CASP8 can act on both initiators and executioners of pyroptosis [91].

In addition to its role in crosstalk with the NLRP3 inflammasome and subsequent pyroptosis, CASP8 has been reported to be activated through interaction with other inflammasomes. Activation of the AIM2 inflammasome by Francisella infection or DNA electroporation in the absence of CASP1 leads to the recruitment and activation of CASP8 through the inflammasome adaptor ASC, resulting in CASP3 activation in macrophages [92,93]. Earlier studies also observed a switch in cell death from pyroptosis to apoptosis in CASP1-deficient cells during Salmonella infection [94]. Later reports found apoptotic caspase activity in wild type cells in response to various pyroptotic stimuli [93,95], arguing that the previously observed switch between pyroptosis and apoptosis is actually the result of the phenotypic characteristics of apoptosis being masked by the more rapid execution of pyroptosis [96,97]. It is likely that the specific execution depends on the trigger or the molecular characteristics of a pathogen [98].

In addition to the crosstalk observed during infection, there are also connections between pyroptosis and apoptosis in response to other stresses. For example, at the executioner level, apoptotic
CASP3 can cleave GSDME, another member of the gasdermin family, to induce pyroptosis in multiple cell types, which causes tissue damage during chemotherapy [99,100]. On the other hand, apoptotic CASP3 can also limit pyroptosis by processing GSDMD at a cleavage site in its cytoplasmic N-terminal, generating an inactive fragment and potentially limiting GSDMD-mediated pore formation [84]. Additional crosstalk is observed during bile acid-induced cell death. Mitochondrial permeabilization induced by the bile acid activates intrinsic apoptosis through the APAF1 apoptosome, which can also interact with the pyrototic CASP11 to initiate the cleavage of CASP3 and drive GSDME-dependent pyroptosis [101]. However, the exact mechanism governing the transition between formation of the CASP9-APAF1 apoptosome and CASP3-APAF1 pyroptosome remains elusive.

Overall, these findings suggest that the pyrototic and apoptotic pathways are closely interconnected and mutually regulated on different levels from pathway initiation to final execution.

3.2. Molecular interactions between components of apoptosis and necroptosis

Since necroptosis was historically identified as a backup cell death pathway that occurs in response to the inhibition of CASP8-dependent apoptosis [19], interactions between apoptosis and necroptosis have been well documented. The central molecules governing the balance between apoptosis and necroptosis are the apoptotic initiator CASP8 and its interactor and substrate RIPK1. Deletion of either of these molecules leads to embryonic or postnatal lethality in mice [102,103] (Table 1). Embryonic lethality of Casp8−/− mice can be rescued by loss of necroptotic pathway effectors Ripk3 or Mlkl, suggesting a predominant role of apoptotic CASP8 in preventing necroptosis during development; indeed, both RIPK1 and RIPK3 are substrates for CASP8 [104–107]. Ripk1 knockout mice die postnatally due to massive necroptosis in epidermal cells and apoptosis in the intestine, indicating that RIPK1 can inhibit apoptosis and necroptosis in a cell type-specific manner [103]. Ripk1 knockout mice can survive when both the apoptotic and necroptotic pathways are inhibited with loss of CASP8 and RIPK3 [108]. Although RIPK1 is involved in the inhibition of both apoptosis and necroptosis, as seen by the extensive cell death observed in Ripk1−/− embryos, RIPK1 itself is paradoxically critical for both types of cell death. Mice carrying an uncleavable RIPK1 (D325A) also undergo embryonic lethality due to abnormally abundant apoptotic and necroptotic cell death, resembling Casp8−/− mice [109] and enzymatically inactive CASP8 (C362S or C362A) embryos [110,111]. Therefore, either the lack of RIPK1 or

| Protein       | Murine lethality upon deletion | Potential cause of death                                                                 | Select genetic cross(es) attempted to rescue                        |
|---------------|--------------------------------|------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| RIPK1         | Postnatal (immediately after birth) | Massive necrosis in epidermal cells and apoptosis in intestine [103]                       | Ripk3−/−Tnfr1−/− (lethality in adulthood due to sepsis) [108]        |
| RIPK1 (RHIM deletion) | Postnatal (E18.5, immediately after birth) | Inflammation in both epidermal cells and intestine                                         | Ripk3−/−Casp8−/− (survive but develop acute lymphoproliferative syndrome [ALPS]) [108,166] |
| RIPK1 (D325A, uncleavable) | Embryonic (E10.5) | Massive necrosis in yolk sac vasculature                                                  | Ripk3−/− (postnatal lethality) [108]                                |
| RIPK3 (D161N, kinase dead) | Embryonic (E11.5) | Massive necrosis in yolk sac vasculature                                                  | Ripk3−/− (survive to birth) [109]                                   |
| CASP8         | Embryonic (E10.5-E11.5) | Hyperemia and heart defect [102]                                                          | Ripk3−/− (survive to birth) [167]                                   |
| CASP8 (C362A or C362S, enzymatically dead) | Embryonic (E10.5-E11.5) | Hyperemia and vasculature defect                                                          | Ripk3−/− (survive to birth) [167]                                   |
| FADD          | Embryonic (E10.5-E11.5) | Cardiac failure and abdominal hemorrhage [168]                                             | Ripk3−/−Fadd−/− (survive but develop ALPS) [170]                    |
| FLIP          | Embryonic (E10.5-E11.5) | Heart defect [171]                                                                       | Ripk3−/− (survive but develop ALPS) [170] N/A                      |
| TBK1          | Embryonic (E14.5) | Liver degeneration                                                                        | Ripk3−/− (survive but develop ALPS) [170] N/A                      |
| CASP9         | Embryonic (after E16.5) | Enlarged and malformed cerebrum [173]                                                     | N/A                                                                 |
| HOIL-1        | Embryonic (E10.5) | Aberrant endothelial cell death                                                            | N/A                                                                 |
| HOIP          | Embryonic (E10.5) | Aberrant endothelial cell death                                                            | N/A                                                                 |
| SYK           | Postnatal (E18.5, immediately after birth)       | Extensive hemorrhaging                                                                   | N/A                                                                 |
the presence of an uncleavable form of RIPK1 induces cell death, indicating that the cleavage of RIPK1 by CASP8 is critical to prevent excessive apoptosis and necroptosis [109]. Mechanistically, this could be achieved by regulating the amount of RIPK1 expressed: one recent study suggested higher RIPK1 expression leads to necroptosis while low or no RIPK1 expression drives cells toward apoptosis [112].

Both genetic and biochemical data support that the CASP8-RIPK1 platform is critical in regulating the cell death between apoptosis and necroptosis (and pyroptosis, as discussed later). The absence of either component or point mutation of critical sites involved in enzymatic cleavage not only leads to lethality in mice (Table 1) but also causes immunodeficiencies in humans [113,114].

3.3. Molecular interactions between components of pyroptosis and necroptosis

Compared with our understanding of crosstalk between components of the apoptotic pathway and those of the pyroptotic and necroptotic pathways, the evidence of interplay between pyroptosis and necroptosis has begun to accumulate more recently. MLKL is involved in TLR3-mediated NLRP3 inflammasome activation; the deletion of MLKL impairs ASC oligomerization in response to the combination of TLR3 ligand poly(I:C) and pan-caspase inhibitor z-VAD [115]. Recent studies show that necroptosis can also trigger NLRP3 inflammasome activation through MLKL pore-mediated potassium efflux in a cell-intrinsic manner in macrophages. In this context, activation of MLKL acts upstream of inflammasome activation, as the inhibition of NLRP3 or CASP1 fails to rescue necroptotic death [116,117].

4. Assembly of the PANoptosome: Interaction network of PANoptosome components

In addition to the experimental evidence that has supported the conceptual development of PANoptosis, visualization of the physical interactions of known molecular components of pyroptosis, apoptosis and necroptosis using STRING database [122] highlights numerous connections between molecules from all three pathways (Fig. 2). The STRING database includes reported physical interactions between proteins, which are used in the network construction. These connections indicate that each pathway is unlikely to be “independent” from the others, supporting the notion of a unified PCD network. Based on the current STRING analysis of the molecular interactions between these proteins, CASP8 is the only node to directly connect the three pathways (Fig. 2), but future work may identify others. Many of the proteins involved in PCD pathways contain domains that can participate in homotypic interactions, which forms the basis for the assembly of multiprotein complexes that are essential in regulating PCD. Several such complexes have been characterized to date for individual PCD pathways, including recent evidence showing that there is an additional complex termed the PANoptosome in PANoptosis.

4.1. Many “X-somes” and PANoptosome

Many different multi-protein complexes have historically been identified and found to be critical for cell death and survival. Formation of these complexes is often driven by homotypic and heterotypic interactions between proteins within key conserved domains, including RHIM, DD, DED, PYD and CARD. Canonical inflammasomes are composed of sensors, such as NLRP3, NLRP1, NLRC4, AIM2 or Pyrin, the adaptor protein ASC and CASP1; these molecules are assembled together through PYD-PYD or CARD-CARD interactions as aforementioned [61,62].

Multiple complexes are involved in the regulation of apoptosis. Intrinsic apoptosis can be induced by the apoptosome, while extrinsic apoptosis is controlled by the death-inducing signaling complex (DISC) and complex-II or the stress-induced ripoptosome.
| Pathogen | Pyroptosis | Apoptosis | Necroptosis |
|----------|------------|-----------|-------------|
| **Virus** |            |           |             |
| Influenza A virus (ssRNA) | Macrophage: CASP1 activation [22] | Macrophage: CASP3/7 activation [22] | Macrophage: pMLKL [27] |
| Murine hepatitis virus (ssRNA) | Macrophage: CASP1 activation [26] | Macrophage: CASP3/7 activation [26] | Macrophage: pMLKL [26] |
| *Rotavirus* (dsRNA) | Epithelial cell: NLRP9b-mediated CASP1 activation [177] | Epithelial cell: CASP8, CASP3 and BAX activation; cytochrome C release [178] | N/A |
| *Rhinovirus* (ssRNA) | Epithelial cell: NLRP3 inflammasome activation [179]; NLRP1 inflammasome activation [180] | Epithelial cell: CASP3/9 activation [181, 182] | HeLa cell: Nec-1 cannot inhibit cell death [183] |
| Murine norovirus (ssRNA) | Macrophage: CASP1, GSDMD activation and IL-1β release [184] | Macrophage: CASP3/9 activation [185] | N/A |
| *Sendai virus* (ssRNA) | Macrophage: CASP1 activation [186] | Fibroblast (CV-1): CASP3/8 activation [187] | Fibroblast (L929): RIPK1-dependent cell death with z-VAD treatment [188] |
| Vesicular stomatitis virus (ssRNA) | Macrophage: CASP1 activation [23, 186] | Macrophage: CASP3/7/8 activation [23] | N/A |
| Human immunodeficiency virus (ssRNA) | T cell: CASP1 activation and IL-1β release [189] | T cell: CASP3 activation [190] | N/A |
| Hepatitis C virus (ssRNA) | Hepatocytic cell line (Huh7.5): CASP1 activation [191] | Hepatocytic cell line (Huh7.5): CASP3 activation [191, 192] | N/A |
| *Herpes simplex virus* (dsDNA) | Monocytic cell (THP-1): CASP1 activation [193] | HEP-2 cell: CASP3, PARP1 cleavage in early time point [194] | Fibroblast (L929): RIPK1/3- and MLKL-dependent cell death |
| *Vaccinia virus* (dsDNA) | Macrophage: CASP1 activation [196] | Monocytic cell (THP-1): PARP1, CASP3/9 cleavage (MVA strain) [197] | T cell (Jurkat): Sensitive to TNF-α–induced necroptosis [198] |
| *Encephalomyocarditis virus* (ssRNA) | Macrophage: CASP1 activation [186] | Fibroblast (BHK-21): No apoptosis; inhibited by viral protein 2A [199] | Macrophage: pMLKL (LPS-primed, z-VAD treated) [186] |
| *Dengue virus* (ssRNA) | Monocyte: CASP1 activation and IL-1β release [200] | Monocytic cell (U937): CASP3/9 activation [201] | N/A |
| Respiratory syncytial virus (ssRNA) | Macrophage: cell death rescue by NLRP3 knockout [202] | Macrophage: CASP3 activation [202] | Epithelial cell: pMLKL [203] |
| **Bacteria** |            |           |             |
| *Francisella tularensis* subsp. novicida | Macrophage: CASP1 activation [204] | Macrophage: CASP3 activation [205] | N/A |
| *Listeria monocytogenes* | Macrophage: AM2-dependent IL-1β production [206] | Macrophage: CASP3/7 activation [207] | Macrophage: cell death not inhibited by z-VAD; pMLKL [208, 209] |
| *Streptococcus pneumoniae* | Macrophage: CASP1 activation [210] | Epithelial cell (AS49): CASP6/8/9 activation (R6x strain) [211] | Macrophage: pMLKL; Ripk3 KO and RIPK1 inhibitor rescue cell death [209] |
| *Staphylococcus aureus* | Monocytic cell: CASP1 activation and IL-1β release [212] | Macrophage: CASP3 activation [213] | Macrophage: Ripk3 KO and RIPK1 inhibitor rescue cell death [209] |
| *Escherichia coli* (UPEC) | Macrophage: NLRP3-dependent CASP1 activation and IL-1β release (CFT073 strain) [214] | Urothelial cells: CASP2/3/8 activation [215] | Macrophage: pMLKL; Ripk1 inhibitor rescues cell death [209] |
| *Pseudomonas aeruginosa* | Macrophage: NLR4-dependent CASP1 activation [216] | Epithelial cell (HeLa): CASP3/9 activation [217] | NA |
| *Shigella flexneri* | Macrophages: NLRP3 inflammasome activation [221, 222]; AIM2 inflammasome activation (rBCG strain) [223] | Macrophages: Morphology and DNA fragmentation, CASP3 activation [219, 220] | Epithelial cell: No necroptosis; bacterial degradation of RIPKs [145] |
| *Mycobacterium tuberculosis* | Macrophages: NLRP3 inflammasome activation [221, 222]; AIM2 inflammasome activation (rBCG strain) [223] | Macrophages: No apoptosis; undetectable CASP3/8 activation [224] | Macrophages: No necroptosis; undetectable pMLKL; MLKL deletion or RIPK1 inhibitor do not inhibit cell death [224] |
The apoptosome is formed when APAF1 senses the release of cytochrome C from mitochondria and initiates interaction with CASP9 through its CARD domain [43]. DISC is assembled by death receptor engagement, where DD-containing death receptors (e.g., Fas) and a DD-containing adaptor protein (e.g., FADD) interact [123,124]. Cytosolic translocation of the membrane-bound complex and recruitment and activation of pro-CASP8 through DED-DED interactions between FADD and pro-CASP8 forms complex-II in the cytosol and leads to the initiation of extrinsic apoptosis [123,124]. The ripoptosome contains similar core members, including CASP8, FADD and RIPK1, but this complex is formed in response to distinct triggers such as genotoxic stress. CASP8 and the kinase activity of RIPK1 are essential for ripoptosome-induced apoptosis and necroptosis, respectively [125].

Necroptosis is induced by the necrosome, which can form when RIPK3 is recruited by RIPK1 through RHIM-RHIM interactions and CASP8 activity is inhibited. In the complex containing inactive CASP8, along with FADD, RIPK1 and RIPK3, phosphorylation of RIPK3 by RIPK1 occurs and leads to further phosphorylation of MLKL to induce necroptosis [76].

The aforementioned death-inducing complexes share many core members such as CASP8, FADD and RIPK1. In addition to the clear overlaps in the components of the ripoptosome and the necrosome, inflammasome activation is also dependent on the presence of CASP8 and FADD in many cases [32]. The homotypic interactions of DD superfamily members or RHIM domains are critical to the formation of each of the cell death complexes described to date [126–132]; additionally, heterotypic interactions have been observed between the DED of CASP8 and the PYD of ASC [110,111,133] and may serve as the mechanistic basis to assemble the PANoptosome.

The activation of PCD pathways by pathogens are color coded as red (positive induction), cyan (no induction/inhibition), orange (cell type-specific induction/inhibition) and white (no information).

*This table offers an overview of cell death in selected infections. We apologize to those whose studies could not be cited here due to space limitations.

| Pathogen | Macrophage | Macrophage | Macrophage |
|----------|------------|------------|------------|
| *Mycobacterium tuberculosis* | CASP1 activation and IL-1β release [226] | CASP1 activation and IL-1β release [226] | N/A |
| *Porphyromonas gingivalis* | NLRP3-dependent CASP1 activation and IL-1β release [227] | Epithelial cell: CASP3 activation and DNA fragmentation [228] | Macrophage (THP-1): pMLKL [229] |
| *Legionella pneumophila* | NAP5/NLRC4-mediated CASP1 activation, cell death and IL-1β release [230] | Human PBMCs: CASP3 activation [231] | N/A |
| *Salmonella enterica serovar Typhimurium* | NLRP3- and NLRC4-dependent CASP1 activation [232] | CASP3/8/9 activation [233] | N/A |
| *Yersinia pestis* | CASP1 activation [25] | CASP3/7 activation [25] | pMLKL [25] |
| *Helicobacter pylori* | NLRP3-dependent CASP1 activation and IL-1β release [235] | Epithelial cell (AGS): CASP3/8/9 activation [236] | N/A |
| *Burkholderia pseudomallei* | NLRC4-dependent CASP1 activation [237] | CASP3/8/9 activation [237] | N/A |
| *Bacillus anthracis* | NLRP1-dependent IL-1β release and cell death [238] | Morphology and DNA fragmentation, CASP3/7 activation [97, 239] | N/A |

**Fungi**

| *Candida albicans* | NLRP3-dependent CASP1 activation and IL-1β release; CASP1/11 ablation partially rescues cell death [24, 240, 241] | CASP1/11 activation partially rescues cell death [24, 242] | Macrophage: Ripk3 or Mlkl deletion partially rescue cell death 243; pMLKL [24] |
| *Aspergillus fumigatus* | NLRP3- and AIM2-dependent CASP1 activation [244] | CASP3/7 activation [24] | Macrophage: pMLKL [24] |
| *Cryptococcus neoformans* | NLRP3-dependent CASP1 activation [150] | CASP3, PARP1 activation, cytochrome C release [245] | N/A |

**Fig. 2.** Interactome analysis of molecules in PCD pathways. A physical network of experimental and database evidence with confidence > 0.7 was retrieved from the STRING database after searching seven proteins (AIM2, PYCARD, MEFV, ZBP1, CASP1, CASP8, RIPK3) with interactor threshold 20. The interaction network was replotted by the igraph package, and historically central members of each PCD pathway are colored.

The activation of PCD pathways by pathogens are color coded as red (positive induction), cyan (no induction/inhibition), orange (cell type-specific induction/inhibition) and white (no information).

*This table offers an overview of cell death in selected infections. We apologize to those whose studies could not be cited here due to space limitations.
4.2. PANoptosome assembly—the prototypical ZBP1 PANoptosome

The homotypic and heterotypic domain interactions between proteins provide the backbone for PANoptosome formation (Fig. 3) [38]. To date, two upstream molecules, ZBP1 and RIPK1, have been identified that can trigger PANoptosome assembly in response to specific stimuli [22,25], but it is likely that several others remain to be characterized. The sensor ZBP1 is critical in mediating cell death in both IAV infection and the developmental defect observed in Ripk1mRHIM mice [22,118]. The current model of ZBP1 PANoptosome assembly during IAV infection is centered on known homotypic/heterotypic interactions within the conserved domains, such as RHIM, DD, DED, PYD and CARD [134,135] (Fig. 3). For example, the homotypic interaction between RHIM domains [135] is critical for necroptosis initiation, while the heterotypic interaction between PYD (ASC) and DED (CASP8) can bring pyroptotic and apoptotic machineries together [93,133]; these molecular interactions are likely to serve as the backbone for PANoptosome formation during IAV infection (Fig. 3). Furthermore, CASP6 potentiates the interaction between ZBP1 and RIPK3 in the ZBP1 PANoptosome [27]. The formation of this molecular scaffold is further supported by reported immunoprecipitation experiments using HEK293T cells expressing key PCD components, where RIPK3 co-immunoprecipitates CASP8, ASC, RIPK1, NLRP3 and ZBP1 (Fig. 4A) [23], and in primary macrophages infected with IAV, where RIPK3 also co-immunoprecipitates ZBP1, CASP8, NLRP3 and RIPK1 (Fig. 4B), the crucial molecules in pyroptotic, apoptotic and necroptotic pathways. Beyond the immunoprecipitation evidence, microscopy also demonstrates colocalization between key members of multiple PCD pathways during IAV infection. The ASC speck, which represents inflammasome activation, can colocalize with both apoptotic and necroptotic proteins, such as CASP8 and RIPK3, in the same cell (Fig. 4C). Therefore, in analogy to the well-classified sensor system in inflammasome activation, ZBP1 represents the prototypical PANoptosome sensor that recognizes IAV [136]. Given that PANoptosis has been observed under a

Fig. 3. Model of ZBP1 PANoptosome assembly. 1) Specific trigger (e.g., IAV) is required to initiate PANoptosome formation; 2) specific sensor (e.g., ZBP1) is activated by the trigger; 3) the sensor initiates the assembly of the PANoptosome, which contains molecules required to activate downstream PCD effectors including gasdermins, CASP3/7 and MLKL; 4) PANoptosis execution by engagement of pyroptotic, apoptotic and necroptotic pathway members resulting in lytic inflammatory cell death.
Fig. 4. Experimental evidence of PANoptosome formation. (A) Immunoprecipitation of RIPK3 in HEK293T cells overexpressing PANoptosome components (published data from Christgen, S., et al. Front. Cell. Infect. Microbiol., 2020). (B) Immunoprecipitation of RIPK3 in IAV-infected WT and Ripk3−/− BMDMs (left) and immunoprecipitation of CASP8 in WT cells showing the interaction among key components of the PANoptosome. (C) Immunofluorescence staining of indicated molecules in IAV-infected BMDMs showing the colocalization of principle components of the PANoptosome. BMDMs infected with IAV (PR8) at MOI = 20 for 12 h and stained with anti-ASC (2EI-7, Millipore), anti-CASP8 (1G12, Enzo) and anti-RIPK3 (B-2, Santa Cruz, pre-conjugated). Scale = 5 μm. Asterisks indicate non-specific bands.
variety of conditions, how the PANoptosomes are formed under these other conditions to regulate PANoptosis remains an area for further investigation.

4.3. PANoptosome assembly—additional sensors and interactors

Our current knowledge of PANoptosome assembly is limited regarding the identity of sensors that initiate complex formation. Similar to inflammasome sensors, the sensors for PANoptosome assembly are likely to contain domains involved in both sensing triggers and interacting with the core members of the PANoptosome. For example, ZBP1 uses its Zx2 domain to sense RNA and its RHIM to interact with RIPK3 (Fig. 3). Many proteins encoded by the human genome that contain domains known to function in pattern recognition, such as leucine-rich repeats and HIN domains, also contain interaction domains (e.g., PYD). These proteins can potentially initiate PANoptosome assembly once they have been activated by specific triggers.

In addition to the ZBP1 PANoptosome, recent work has characterized a PANoptosome that is centered on RIPK1, another RHIM-containing protein. This RIPK1 PANoptosome is formed during Yersinia infection, where the apoptotic and pyroptotic arms require RIPK1, while the necroptotic arm is suppressed by RIPK1 [25], implying that PANoptosomes could differentially regulate the effectors of pyroptosis, apoptosis, and necroptosis. In addition to RIPK1, RIPK3 and ZBP1, the mammalian genome includes one other RHIM-containing protein, TRIF. TRIF also mediates cell death through RHIM-RHIM interactions with RIPK3 following TLR3 activation and caspase inhibition [75]. A recent study reported a cell death complex containing FADD, RIPK1 and CASP8 which is triggered by TRIF signaling (referred to as the “TRIFosome”). This complex is critically involved in the cell death induced by LPS when TAK1 is inhibited [137]. Although the physical presence of TRIF in a complex with other death molecules has not been shown, it is possible that TRIF can participate in PANoptosome formation through its RHIM domain.

Another potential candidate sensor to trigger PANoptosome assembly is RIG-I. Sendai virus (SeV) can induce pyroptosis, apoptosis and necroptosis (Table 2) and RIG-I is the viral sensor to trigger interferon production in this context [138]. During SeV infection, RIG-I can form a complex with CASP8 and RIPK1, core members of the PANoptosome (Fig. 3) [139]. The CARD domain of RIG-I is sufficient to pull down CASP8, and this interaction is likely mediated by CARD-CARD interactions between RIG-I and other CARD-containing members of the PANoptosome. Therefore, it is possible that TRIF is critically involved in SeV-induced PANoptosis, though this requires further study.

Beyond the sensors, additional proteins are also likely to be included in the PANoptosome core through expected homotypic and atypical heterotypic interactions, such as the interaction between PYD (ASC) and DED (CASP8). Several other proteins with CARD, PYD and DED domains exist and should be evaluated for their ability to form homotypic and heterotypic interactions and for their involvement in the PANoptosome. For example, there are a total of 22 and 27 proteins containing PYD in the human and mouse genome, respectively. The PYDs with the most structural similarity to the PYD of ASC are likely to have the highest probability to interact with the DED of CASP8 in a similar manner as the ASC PYD does. Therefore, future study to examine the structure of PYDs from these proteins and their potential to interact with DED (CASP8) and other DEDs in the complex will provide new insight on novel PANoptosome formation. Identifying new sensors and molecules involved in PANoptosome formation will be important for improving treatment strategies in infection, autoimmune diseases and cancer, where PANoptosis has been physiologically implicated [27–29,33,34,140,141] (Graphical abstract).

5. PANoptosis in disease

5.1. PANoptosis in microbial infection

Growing evidence has shown an interplay between PCD pathways and microbial factors, suggesting that these pathways may have played important roles during mammalian co-evolution with microbes [142,143]. Many pathogens carry microbial effectors that can actively enhance cell viability by blocking PCD pathways or by activating proliferation pathways [144,145]. It is likely that host cells have developed complex regulation and interconnection of cell death pathways to overcome the challenges presented by such microbial effectors. While IAV was the first infection found to trigger PANoptosis, many other viruses, as well as bacteria and fungi, also induce the activation of multiple PCDs, providing evidence that a number of infections could activate PANoptosis (Table 2).

For example, the mouse coronavirus murine hepatitis virus (MHV) infects macrophages and activates PANoptosis, characterized by cleavage of CASP1/3/7/8 and GSDMD and phosphorylation of MLKL [26]. Loss of proteins in the NLRC3 inflammasome pathway paradoxically enhances the cell death during infection, leading to increased activation of apoptotic CASP8 and phosphorylation of MLKL. Therefore, the pyroptotic components of the PANoptosome appear to inhibit the CASP8-RIPK3-mediated apoptosis and necroptosis during MHV infection [26]. While the master sensor of MHV that triggers PANoptosis is still unknown, MDA5 is a potential candidate. MDA5 can recognize both MHV and SARS-CoV-2 to induce interferon production [26,146,147], and it contains two CARDs, which may interact with core members of the PANoptosome through CARD-CARD interactions; this possibility requires further study.

A prototypical bacterial pathogen that induces PANoptosis is Yersinia, through the inhibition of TAK1 by its effector YopJ [35,36,148]. Other bacteria, such as Salmonella Typhimurium and Listeria monocytogenes can also induce PANoptosis [23,81]. Salmonella-induced cell death is fully blocked in BMDMs from Casp8-/-Ripk3-/-Casp1/11-/- mice, while deletion of RIPK3 alone or CASP8 and RIPK3 together has almost no effect on the cell death, and deletion of CASP1/11 only partially reduces the cell death [23,81]. Since the cell death induced by Salmonella is also predominantly dependent on the inflammasome sensor NLRC4 [149], these findings suggest an NLRC4 PANoptosome may form during Salmonella infection. As shown in Table 2, many other bacteria can also induce activation of pyroptotic, apoptotic and necroptotic effectors, suggesting that additional bacterial pathogens can induce PANoptosis and further emphasize the crosstalk among these PCD pathways as a common feature during bacterial infection.

Besides viral and bacterial pathogens, fungi can also activate PANoptosis. Both Candida albicans and Aspergillus fumigatus induce PANoptosis in macrophages [24]. ZBP1 plays a role in promoting PANoptosis during fungal infections, and this activity is dependent on its Zx2 domain [24]. Interactions between PCD pathways are also observed in Cryptococcus neoformans-infected cells, where CASP8 is activated at the inflammasome in the absence of CASP1 through interactions with ASC [150]. Given the critical role of CASP8 in PANoptosis to interact with essential components from multiple PCD pathways, it is likely that C. neoformans also induces PANoptosis.

Similar to traditional PCD pathways, PANoptosis is also thought to serve as a host defense mechanism during infection [140]. Single ablation of MLKL or CASP8 autoprocessing, which impair the necrop-
PANoptosis in inflammatory diseases and cancer

The first physiological evidence of the functional molecular crosstalk between pyroptosis, apoptosis and necroptosis was found in the Pstpip2<sup>cmo</sup> mouse model of osteomyelitis [33]. Mice carrying a mutation in Pstpip2 (<sup>Pstpip2</sup>cmo) develop osteomyelitic bone inflammation that is driven by hyper-production of IL-1β and characterized by inflammasome activation and cell death. However, deletion of pyroptotic molecules alone cannot protect these mice from disease. Only the combined deletion of pyroptotic, apoptotic and necroptotic molecules (<sup>Pstpip2</sup>cmo × Nlrp3<sup>-/-</sup> × Casp8<sup>-/-</sup> × Ripk3<sup>-/-</sup> × Casp9<sup>-/-</sup> × MLKL<sup>-/-</sup> × Bid<sup>-/-</sup>) can rescue these mice, implicating PANoptosis in this process [33,34] (Table 3). These observations highlighted the physiological relevance of PANoptosis in autoinflammatory disease. In addition to the Pstpip2<sup>cmo</sup> model, there are several other autoinflammatory models where cell death-mediated pathology or PANoptosis have been implicated (Table 3). However, the molecular details of how many of these mutations lead to cell death and/or the assembly of the PANoptosome complex is still unknown, and more work is required to fully characterize these processes.

In addition to autoinflammation, other forms of inflammation can also lead to cell death and PANoptosis. One key example is cytokine storm (CS), which is defined as a life-threatening condition caused by excessive production of cytokines mediated by inflammatory cell death, PANoptosis [257], and is associated with a number of diseases, including the ongoing COVID-19 pandemic. Increased circulating levels of TNF and IFN-γ are associated with poor outcomes in patients, and these two cytokines synergistically induce PANoptosis characterized by activation of pyroptotic (GSDME), apoptotic (CASP8/3/7) and necroptotic (pMLKL) molecules [29]. Mechanistically, the combination of TNF and IFN-γ induces the JAK/STAT1/IRF1 signaling pathway and nitric oxide (NO) production to trigger CASP8/FADD-mediated PANoptosis [29]. Therefore, NO represents a novel PANoptosis signaling molecule during CS, adding new insight into the complicated function of NO in mediating cell death and inflammatory responses [88,152]. As several pathogens are known to activate multiple PCD pathways in vivo (Table 2), it will be important to evaluate the role of PANoptosis during in vivo infections using these pathogens.

5.2. PANoptosis in inflammatory diseases and cancer

In contrast to its generally negative role during inflammatory conditions, PANoptosis could be beneficial in the context of cancer. Resistance to cell death is one of the hallmarks of cancer, and PANoptosis holds potential to kill cancer cells [258]. Therefore, activation of this pathway could be targeted for therapeutic benefit. In fact, well-known oncolytic viruses, such as vaccinia virus and vesicular stomatitis virus (VSV), are potential inducers of PANoptosis (Table 2). VSV activates many of the same PANoptosis markers observed during MHV and IAV infections [23]. However, combined genetic deletion of CASP8, RIPK3 and CASP11 decreases but does not fully abolish cell death during VSV infection [22,23]. The residual CASP3/7 activation in Casp8<sup>-/-</sup> × Ripk3<sup>-/-</sup> × Casp11<sup>−/−</sup> cells may be a result of activation of the CASP9-mediated pathway of intrinsic apoptosis. This raises the possibility that PANoptosis may contain an arm of intrinsic apoptosis, or that intrinsic apoptosis may be activated downstream of, or in concert with, PANoptosis; a property that is likely to be preferred for the development of therapeutics to kill cancer cells harboring defects in PCD pathways [154].

Beyond the described roles in infection, autoinflammatory disease, inflammation and cancer targeting, the components of the PANoptosome are widely implicated in many other pathophysiological settings where cell death plays critical roles. For example, although the cause of neuronal cell loss during ischemic and neurodegenerative disease is still mysterious [155], the inflammasome [156], CASP8 [157] and RIPK1 [158,159], core components of the PANoptosome, are implicated in neuronal death [155]. Similar involvement of key PANoptosome molecules and pyroptosis, apoptosis and necroptosis are also seen in metabolic diseases [160–163]. Therefore, the physiological function of PANoptosis likely extends widely across the disease spectrum [38,141].

### Table 3

| Bone disruption in Pstpip2<sup>cmo</sup> mice model | Bone disruption | Ref |
|---------------------------------------------------|----------------|-----|
| Pstpip2<sup>cmo</sup> | Yes | [246] |
| **Reported genetic crosses** | | |
| Pstpip2<sup>cmo</sup> × Nlrp3<sup>-/-</sup> | Yes | [247] |
| Pstpip2<sup>cmo</sup> × Casp1<sup>-/-</sup> | Yes | [33] |
| Pstpip2<sup>cmo</sup> × Ripk3<sup>-/-</sup> × Casp8<sup>-/-</sup> | Yes | [33] |
| Pstpip2<sup>cmo</sup> × Ripk3<sup>-/-</sup> × Casp8<sup>-/-</sup> × Nlrp3<sup>-/-</sup> | No | [33,34] |

| Skin inflammation in Sharpin<sup>cpdm</sup> mice model | Skin inflammation | |
|--------------------------------------------------------|-----------------|---|
| Sharpin<sup>cpdm</sup> | Yes | [248] |
| **Reported genetic crosses** | | |
| Sharpin<sup>cpdm</sup> × Nlrp3<sup>-/-</sup> | Yes (delayed) | [249] |
| Sharpin<sup>cpdm</sup> × Casp1<sup>-/-</sup> | Yes (delayed) | [249] |
| Sharpin<sup>cpdm</sup> × Mlkl<sup>-/-</sup> | Yes (delayed) | [249] |
| Sharpin<sup>cpdm</sup> × Bid<sup>-/-</sup> | Yes (delayed) | [250] |
| Sharpin<sup>cpdm</sup> × Casp8<sup>-/-</sup> × Ripk3<sup>-/-</sup> | Died before weaning | [250] |
| Sharpin<sup>cpdm</sup> × Ripk3<sup>-/-</sup> | No | [251] |
| Sharpin<sup>cpdm</sup> × Ripk3<sup>-/-</sup> × Fadd<sup>−/−</sup> | No | [252] |

| Footpad inflammation in Ptpn6<sup>spin</sup> mice model | Footpad inflammation | |
|--------------------------------------------------------|-------------------|---|
| Ptpn6<sup>spin</sup> | Yes | [253] |
| **Reported genetic crosses** | | |
| Ptpn6<sup>spin</sup> × Casp1<sup>-/-</sup> | Yes | [253] |
| Ptpn6<sup>spin</sup> × Nlrp3<sup>-/-</sup> | Yes | [253] |
| Ptpn6<sup>spin</sup> × Ripk3<sup>-/-</sup> | Yes | [253] |
| Ptpn6<sup>spin</sup> × Casp8<sup>-/-</sup> × Ripk3<sup>-/-</sup> | Yes | [254] |

| Arthritis in LysM-Cre A20<sup>R89</sup> mice model | Arthritis | |
|---------------------------------------------------|----------|---|
| LysM-Cre A20<sup>R89</sup> | Yes | [255] |
| **Reported genetic crosses** | | |
| LysM-Cre A20<sup>R89</sup> × Casp1/11<sup>-/-</sup> | No | [255] |
| LysM-Cre A20<sup>R89</sup> × Nlrp3<sup>-/-</sup> | No | [255] |
| LysM-Cre A20<sup>R89</sup> × Mlkl<sup>-/-</sup> | No | [256] |
| LysM-Cre A20<sup>R89</sup> × Ripk3<sup>-/-</sup> | No | [256] |
6. Future perspectives and outstanding questions

Decades of research have elucidated key molecular pathways involved in PCD, revolutionizing our understanding of cell death to show that it is genetically programmed rather than a passive, stochastic event. Although distinct genetic elements were initially implicated to delineate three major, separate PCD pathways - pyroptosis, apoptosis and necroptosis, recent advances have found unanticipated and extensive crosstalk among them. These observations suggest that a universal core complex exists to contain and regulate the cell death machinery in a trigger-dependent manner. Here we have reviewed the current evidence for PCD pathway crosstalk that defines PANoptosis as a unique, physiologically relevant, inflammatory PCD pathway.

In the arms race between hosts and pathogens, cell death pathways are constantly altered and subverted by pathogenic effectors. Therefore, the presence of a cell death pathway with mechanically broader activity will provide a higher chance to eliminate the infected cells as an essential component of innate immune defense. The PANoptotic pathway can be targeted by microbial components during infection, resulting in cell death modalities lacking activation of either pyroptotic, apoptotic or necroptotic effectors (e.g., pyroptosis and apoptosis are activated but necroptosis is inhibited by pathogen proteins during *Shigella flexneri* infection in macrophages). Such plasticity in the PANoptotic pathway is an intrinsic feature potentiated by the PANoptosome, where blockade of a key molecule from pyroptosis, apoptosis or necroptosis individually does not prevent the inflammatory cell death. This provides a possible explanation for why PANoptosis would be evolutionarily selected as a strategy to combat infectious diseases. On the other hand, this plasticity also highlights the importance and need to identify specific upstream sensor molecules (e.g., ZBP1) for therapeutic targeting to prevent cell death in diseases where PANoptosis contributes to the pathogenesis.

The existence of PANoptosis does not deny the presence of cell death through any of the other previously established pathways. In fact, it is well-characterized that cells undergo pyroptotic, apoptotic or necroptotic death alone in response to specific chemical or ligand stimulations (e.g., LPS + ATP induces pyroptosis, staurosporine induces apoptosis and TNF-α + z-VAD induces necroptosis). Furthermore, the genetic ablation or pharmacological inhibition of individual pyroptotic, apoptotic or necroptotic proteins can rescue cell death under those conditions; for example, CASP1 or GSDMD ablation prevents cell death in response to LPS + ATP; pan-caspase inhibition prevents cell death during staurosporine stimulation; and RIPK3 or MLKL ablation prevents cell death in response to TNF-α + z-VAD. In contrast, cells tend to undergo PANoptosis in response to microbial pathogens or under autoinflammatory conditions (e.g., IAV infection or *Pstpipz* mutation). Under these circumstances, inhibiting an individual PCD pathway is not effective to rescue cells from death. Therefore, PANoptosis is distinct from pyroptosis, apoptosis or necroptosis, and is defined as a unique type of immunogenic cell death that can be triggered by specific stimulation.

An important remaining question in the cell death field is: what is the molecular basis of the cellular decision to undergo PANoptosis rather than an individual “classic” PCD pathway? The formation of a PANoptosome appears to be highly trigger-dependent and may be influenced by the presence of multiple pathogen- and damage-associated molecular patterns, as is encountered during disease. The identification of novel triggers and sensors is critical to advance our understanding of this unique form of cell death.

Similarly, if cells undergo limited PANoptosis (i.e. cell death lacking either pyroptosis, apoptosis or necroptosis), how is engagement of other pathways prevented? This is best exemplified by *S. flexneri*-encoded death inhibitors OspC1, OspC3 and OspD3, which inhibit CASP8 activation, prevent GSDMD cleavage and degrade RIPK1/3, respectively [145]. Although wild type *S. flexneri* induce limited cell death in epithelial cells, mutants deficient in each inhibitor extensively activate the corresponding PCD pathway [145]. From this perspective, it will be interesting to identify microbial as well as endogenous inhibitory proteins that interact with and block the function of PANoptosome components.

Furthermore, it is well-known that cell type is an important determinant of the induced PCD pathways. For example, TAK1 activates cell death in fibroblasts and T cells, but it blocks PANoptosis in macrophages and inhibits the proinflammatory function of neutrophils [164]. Similarly, pathogens also exhibit distinct cell type-dependent cytotoxicity during infection (Table 2). For example, *S. flexneri* inhibits apoptosis in epithelial cells [165] but triggers apotoptic and pyroptotic cell death of infected macrophages [57]. Because different cell types are characterized by differential gene expression profiles, including varied expression of PANoptosome components, it will be important to examine the cell type-specific execution of cell death and PANoptosome formation in response to pathogen effectors and other triggers to understand the cell death during in vivo infection and disease. The majority of cell death research to date has been performed with innate immune cells, such as macrophages, and further examination of cell death in other cell types is important to enable new discoveries of cell death pathways and effector functions. Moreover, since different PCD modalities have distinct effector functions, the complex messages delivered by pyroptotic, apoptotic, necroptotic and PANoptotic cells of diverse cell types might be optimal for the local tissue to initiate inflammatory and tissue repair responses to handle the systemic response to sterile and infectious insults.

Overall, PANoptosis has been molecularly characterized and is implicated in a number of diseases. Future work focused on identifying additional PANoptosis-inducing sensors and carefully characterizing PANoptosome components that correspond to various sterile and pathogenic triggers will be critical to leverage our understanding of this pathway therapeutically and identify ways to modulate it and improve patient outcomes.

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References

[1] Galluzzi L et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 2018;25 (3):486–541.
[2] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26(4):239–57.
[3] McConkey DJ. Biochemical determinants of apoptosis and necrosis. Toxicol Lett 1998;99(3):157–68.
[4] Kesavardhana S, Malireddi RKS, Kanneganti TD. Caspases in Cell Death, 2017; 213: 157–68.
[5] Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immuno Rev 2017;277(1):61–75.
[6] Boada-Romero E et al. The clearance of dead cells by efferocytosis. Nat Rev Mol Cell Biol 2020;21(7):395–414.
[7] Opferman JT, Korsmeyer SJ. Apoptosis in the development and maintenance of the immune system. Nat Immunol 2003;4(5):410–5.
[8] Nagata S, Hanayama R, Kawana K. Autoimmunity and the clearance of dead cells. Cell 2010;140(5):619–30.
[9] Koren E, Fuchs Y. Modes of regulated cell death in cancer. Cancer Discov 2021;11(2):245–65.
Tewari M et al. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-Holler N et al. Fas triggers an alternative, caspase-8-independent cell death Varfolomeev EE et al. A potential mechanism of “cross-talk” between the p55
Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. Trends Kayagaki N et al. Caspase-11 cleaves gasdermin D for non-canonical
Sun L et al. Mixed lineage kinase domain-like protein mediates necrosis Fernandes-Alnemri T et al. The pyroptosome: a supramolecular assembly of Degterev A et al. Identification of RIP1 kinase as a specific cellular target of
Hilbi H et al. Shigella-induced apoptosis is dependent on caspase-1 which Karki R et al. Synergism of TNF-alpha and IFN-gamma triggers inflammatory
Takahashi N et al. Necrostatin-1 analogues: critical issues on the specificity, Karki R et al. Interferon regulatory factor 1 regulates PANoptosis to prevent
Hengartner MO, Ellis RE, Horvitz HR. Genetic control of programmed cell death, tissue damage, and mortality in SARS-CoV-2 infection and
Kuriakose T et al. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways.Sci Immuno 2016;1:2)
Christgen S et al. Identification of the PANoptosome: a molecular platform for pyroptotic cell death. Nature 2015;526(7575):660–5,
Kawai N et al. Caspses-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature 2015;526(7575):666–71,
Sun L et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 2012;148(1–2):213–27,
Zhao J et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. Proc Nat Acad Sci USA 2012;109(14):5322–7,
Holler N et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000;1(6):485–90,
Wang H et al. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. Mol Cell 2014;54(1):133–46,
Cai Z et al. Plasma membrane translocation of trimerezized MLKL protein is required for TNF-induced necroptosis. Nat Cell Biol 2014;16(1):55–65,
Kuriakose T et al. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. Sci Immuno 2016;1(2),
Christgen S et al. Identification of the PANoptosome: a molecular platform
Banoth B et al. ZBP1 promotes fungi-induced inflammasome activation and pyroptosis, and necroptosis (PANoptosis). Front Cell Infect Microbiol 2020;10:237,
Baneth B et al. ZBP1 promotes fungi-induced inflammasome activation and pyroptosis, and necroptosis (PANoptosis). J Biol Chem 2014;290(52):18276–83,
Malireddi RKS et al. RIPK1 distinctly regulates yersinia-induced inflammatory cell death (PYCARD/NAIP-mediated inflammasomes). Cell Host Microbe 2020;24(2):127–36,
Zheng M et al. Impaired NLRP3 inflammasome activation/pyroptosis leads to robust inflammatory cell death via caspase-8/RIPK3 during coronavirus infection. J Biol Chem 2020;295(41):14040–52,
Zheng M et al. Caspase-6 is a key regulator of innate immunity, inflammasome activation, and host defense. Cell 2020;181(3):674–687 e13,
Karki R et al. Interferon regulatory factor 1 regulates PANoptosis to prevent colorectal cancer. JCI Insight 2020;5(12),
Karki R et al. Synergistic actions of TNF-alpha and IFN-gamma triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. Cell 2021;184(1):149–168 e17,
Lamkanfi M et al. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. Mol Cell Proteomics 2020;20(12):3799–96,
Malireddi RKS et al. RIPK1 processing by the Nlrp3 and Nlrc4 inflammasomes. J Immunol 2010;185(3):1277–83,
Hilbi H et al. Shigella-induced apoptosis is dependent on caspase-1 which binds to Ipaf. J Biol Chem 1998;273(49):32895–900,
Brennan MA, Cookson BT. Salmonella induces macrophage death by caspase-1. Mol Microbiol 1998;30(2):181–93,
Brennan MA, Cookson BT. Salmonella-induced macrophage death is caspase-1-dependent. Mol Microbiol 2005;2008;20(3):397–409,
Lamkanfi M et al. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. Mol Cell Proteomics 2020;7(3):405–13,
Zou H et al. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997;90(3):405–13,
Kim HE et al. Formation of apoptosome is initiated by cytochrome c-induced dATP hydrolysis and subsequent nucleotide exchange on Apaf-1. Proc Natl Acad Sci U S A 2005;102(49):17545–50,
Li P et al. Cytochrome c binding to Apaf-1 is a critical event in the initiation of apoptosis cascades. Cell 1997;91(4):479–89,
Tsujimoto Y et al. Cloning of the chromosome breakpoint of neoplastic B cells with the (14;18) chromosome translocation. Science 1984;226(4678):1097–9,
Hengartner MO, Ellis RE, Horvitz HR. Caenorhabditis elegans gene-9 protects cells from programmed cell death. Nature 1992;356(6369):494–5,
Gurung P, Burton A, Kanneganti TD. NLRP3 inflammasome plays a redundant role in the pathogen-induced cell death via caspase-1 activation. Cell Death Differ 2007;14(9):1590–52,
Yan J et al. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 1995;81(5):801–9,
Cerretti DP et al. Molecular cloning of the interleukin-1 beta converting enzyme. Science 1992;256(5053):97–100,
Muzio M et al. Flice, a novel FADD-homologous ICE/ced-3-like protein, is required to get the CD95 (Fas/APO-1) death–inducing signalling complex. Cell 1996;85(6):817–27,
Varfolomeev EE et al. A potential mechanism of “cross-talk” between the p55 tumor necrosis factor receptor and Fas/APO1: proteins binding to the death domains of the two receptors also bind to each other. J Exp Med 1996;183(3):1271–5,
Cerretti DP et al. Molecular cloning of the interleukin-1 beta converting enzyme. Science 1992;256(5053):97–100,
Yuan J et al. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 1993;75(4):641–52,
Thornberry NA et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 1992;356(6372):768–74,
Miura M et al. Induction of apoptosis in fibroblasts by H-1 beta-converting enzyme, a mammalian homolog of the C. elegans cell death gene-3. Cell 1993;75(3):653–60,
Fernandes-Alnemri T et al. The pyroptosome: a supramolecular assembly of NLRP3 inflammasomes. J Cell Sci 2017;130(23):3955–65,
Malireddi RKS et al. TAK1 restricts spontaneous NLRP3 activation and cell death to control myeloid proliferation. J Exp Med 2018;215(4):1023–34,
Malireddi RKS et al. Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity-independent pyroptosis, apoptosis, necroptosis, and inflammatory disease. J Exp Med 2020;217(3),
Kesavardhana S et al. The Zalp2a domain of ZBP1 is a molecular switch regulating influenza-inflammasome and perinatal lethality during development. J Biol Chem 2020;295(24):8325–30,
Samir P, Malireddi RKS, Kanneganti TD. The PANNtosome: a deadly protein complex driving diverse inflammatory responses. J Exp Med 2020;219:2023–44,
Malireddi RKS et al. Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity-independent pyroptosis, apoptosis, necroptosis, and inflammatory disease. J Exp Med 2020;217(3),
Kesavardhana S et al. The Zalp2a domain of ZBP1 is a molecular switch regulating influenza-inflammasome and perinatal lethality during development. J Biol Chem 2020;295(24):8325–30,
Qu Y et al. NLRP3 recruitment by NLRK4 during Salmonella infection. J Exp Med 2016;213(6):877–85.

Chen M et al. Internalized cryptoccocus neoformans activates the canonical caspase-1 and the noncanonical caspase-8 Inflammasomes. J Immunol 2015;195(10):4962–72.

Shubina M et al. Necrosis restricts influenza A virus as a stand-alone cell death pathway. Cell Death Dis 2020;11(8):217111.

Peterson LW et al. RIPK1-dependent apoptosis bypasses pathogen blockade of innate signaling to promote immune defense. J Exp Med 2017;214(11):3171–82.

Blaise GA et al. Nitric oxide, cell signaling and cell death. Toxicology 2005;208(2):177–92.

Felt SA, Moerdyk-Schauwecker MJ, Gurdzeliishvili VZ. Induction of apoptosis in pancreatic cancer cells by vesicular stomatitis virus. Virology 2014;474:161–70.

Ficker M et al. Neuronal cell death. Physiol Rev 2018;98(2):813–80.

Fiedlanders RM et al. Expression of a dominant negative mutant of interleukin-1 beta converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. J Exp Med 1997;185(5):933–40.

Krajewska M et al. Neuronal deletion of caspase 8 protects against brain injury in mouse models of controlled cortical impact and kaic acid-induced excitotoxicity. PLoS ONE 2011;6(9):e24341.

Xu D et al. TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. Cell 2018;174(6):1477–1491 e19.

Deterges A, Olenegnin D, Yuan J. Targeting RIPK1 for the treatment of human diseases. Proc Natl Acad Sci USA 2019;116(40):2174–2174.

Feldstein AE et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003;125(5):1437–41.

Garzon-Roque J et al. The necroptosis-inducing kinase RIPK3 dampens adipose tissue inflammation and glucose intolerance. Nat Commun 2016;7:11869.

Karunakanadan D et al. RIPK1 gene variants associate with obesity in humans and can be therapeutically silenced to reduce obesity in mice. Nat Metab 2020;2(10):1113–25.

Sharma BR, Kanneganti TD. NLRP3 inflammasome in cancer and metabolic diseases. Nat Immunol 2021.

Ajbade AA, Wang HY, Wang RF. Cell type-specific function of TAK1 in innate immune signaling. Trends Immunol 2013;34(3):307–16.

Clark CS, Maurelli AT. Shigella flexneri inhibits staurosporine-induced apoptosis in epithelial cells. Infect Immun 2004;72(5):2531–9.

Ricciardi JA et al. RIP1 regulates RIPK3 and the noncanonical caspase-8 Inflammasomes. J Immunol 2014;193:45–31.

Newton K et al. Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. Science 2014;343(6177):1357–60.

Yeh WC et al. FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 1998;279(5358):1954–8.

Yeh H et al. Functional complementation between FADD and RIP1 in embryos and lymphocytes. Nature 2011;471(7338):373–6.

Dillon CP et al. Survival function of the FADD-CASPASE-8-FLIP(L) complex. Cell Rep 2012;1(5):401–7.

Yeh WC et al. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 2000;12(2):437–47.

Zhang H et al. RIPK2 regulates RIPK3 and the noncanonical caspase-8 Inflammasomes. J Immunol 2012;188(9):4365–72.

Lai XH, Sjostedt A. Delineation of the molecular mechanisms of Francisella tularensis-induced apoptosis in murine macrophages. Infect Immun 2003;71(11):6338–42.

Jones JW et al. Absent in melanoma 2 is required for innate immune recognition of Francisella tularensis. Proc Natl Acad Sci USA 2010;107(18):8177–81.

Lai XH, Sjostedt A. Delineation of the molecular mechanisms of Francisella tularensis-induced apoptosis in murine macrophages. Infect Immun 2003;71(11):6338–42.

Yeh WC et al. FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 1998;279(5358):1954–8.

Yeh H et al. Functional complementation between FADD and RIP1 in embryos and lymphocytes. Nature 2011;471(7338):373–6.

Dillon CP et al. Survival function of the FADD-CASPASE-8-FLIP(L) complex. Cell Rep 2012;1(5):401–7.

Yeh WC et al. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 2000;12(2):437–47.
Zychlinsky A, Prevost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. Nature 1992;358(6382):167–9.

Arizmendi O, Picking WD, Picking WL. Macrophage Apoptosis Triggered by IpaD from Shigella flexneri. Infect Immun 2016;84(6):1857–65.

Beckwith KS et al. Plasma membrane damage causes NLRP3 activation and pyroptosis during Mycobacterium tuberculosis infection. Nat Commun 2020;11(1):2270.

Qu Z et al. Mycobacterial EST12 activates a BACK1-NLRP3-gasdermin D pyroptosis–IL-1beta immune pathway. Sci Adv 2020;6(63).

Saiga H et al. The recombinant BCG deltaurec::hly vaccine targets the AIM2 inflammasome to induce autophagy and inflammation. J Infect Dis 2015;211(11):1831–41.

Stutz MD et al. Necrotic signaling is primed in Mycobacterium tuberculosis-infected macrophages, but its pathophysiological consequence in disease is restricted. Cell Death Differ 2018;25(5):551–65.

Yang Y et al. The AIM2 inflammasome is involved in macrophage activation during infection with virulent Mycobacterium bovis strain. J Infect Dis 2013;208(11):1849–58.

Cui Y et al. Mycobacterium bovis induces endoplasmic reticulum stress-mediated-apoptosis by activating BIF1 in a murine macrophage cell line. Front Cell Infect Microbiol 2016;6:182.

Park E et al. Activation of NLRP3 and AIM2 inflammasomes by Porphyromonas gingivalis infection. Infect Immun 2014;82(1):112–23.

Stathopoulos PG et al. Porphyromonas gingivalis induce apoptosis in human gingival epithelial cells through a gingipain-dependent mechanism. BMC Microbiol 2009;9:107.

Ke X et al. Manipulation of necroptosis by Porphyromonas gingivalis in periodontitis development. Mol Immunol 2014;67:8–13.

Gonzalves AV et al. Gasdermin-D and caspase-7 are the key caspase-1/8 substrates downstream of the NAIP5/NLRC4 inflammasome required for restriction of legionella pneumophila. PLoS Pathog 2019;15(6):e1007886.

Neumeister B et al. Legionella pneumophila induces apoptosis via the mitochondrion death pathway. Microbiology (Reading) 2002;148(Pt 11):3639–50.

Broz P et al. Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against Salmonella. J Exp Med 2010;207(8):1745–55.

Yu C et al. Salmonella enterica serovar Typhimurium sseK3 induces apoptosis and enhances glycolysis in macrophages. BMC Microbiol 2020;20(1):151.

Hos NJ et al. Type I interferon enhances necroptosis of Salmonella Typhimurium-infected macrophages by impairing antiooxidative stress responses. J Cell Biol 2017;216(12):4107–21.

Semper RP et al. Helicobacter pylori-induced IL-1beta secretion in innate immune cells is regulated by the NLRP3 inflammasome and requires the cag pathogenicity island. J Immunol 2014;193(7):3566–76.

Shibayama K et al. Apoptotic signaling pathway activated by Helicobacter pylori infection and increase of apoptosis-inducing activity under serum-starved conditions. Infect Immun 2001;69(5):3181–9.

Bast A et al. Caspase-1-dependent and -independent cell death pathways in Burkholderia pseudomallei infection of macrophages. PLoS Pathog 2014;10(3):e1003986.

Levinsohn JL et al. Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog 2012;8(3):e1002638.