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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.

Contents

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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) activation by inflammatory caspases, 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 (e.g., influenza A virus [IAV] infection), activation of biochemical markers from all three PCD pathways is observed [22–29], suggesting a united modality of death, conceptualized as PANoptosis. **PANoptosis** 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 three PCD pathways alone. The PANoptosome provides a molecular scaffold for contemporaneous engagement of key molecules from pyroptosis, apoptosis and/or necroptosis [22–37]. Given that PANoptosis has been observed during infections with various viral, bacterial and fungal pathogens as well as in autoinflammatory diseases, cytokine storm and cancer, it has significant pathophysiological relevance [38]. The characterization of PANoptosis was also central to molecularly understanding cytokine storm. **Cytokine storm** is defined as a life-threatening condition caused by excessive production of cytokines mediated by inflammatory cell death, PANoptosis [39]. In addition to pathogenic and inflammatory disease triggers, cytokines themselves can also induce PANoptosis [29].

In this review, we will provide a brief historical overview of the discovery of the genetically defined regulatory pathways involved in pyroptosis, apoptosis and necroptosis, and describe the intricate crosstalk between and among these three PCD pathways that has led to the conceptualization of PANoptosis. We will further discuss the current literature and experimental evidence supporting PANoptosis and propose a general model for PANoptosome activation and assembly in various conditions based on the known molecules and their sub-domains involved in protein–protein interactions. Finally, the importance of the molecular components in the PANoptosome in infectious, autoinflammatory and other diseases will be reviewed, and remaining questions will be discussed to provide future directions for research in the area of cell death.

2. Historic definitions of apoptosis, pyroptosis and necroptosis

Apoptosis was the first PCD to be described and was morphologically characterized in 1972 (Fig. 1) [2]. It was not until 1986 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 allow 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 TNFR-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 paths 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

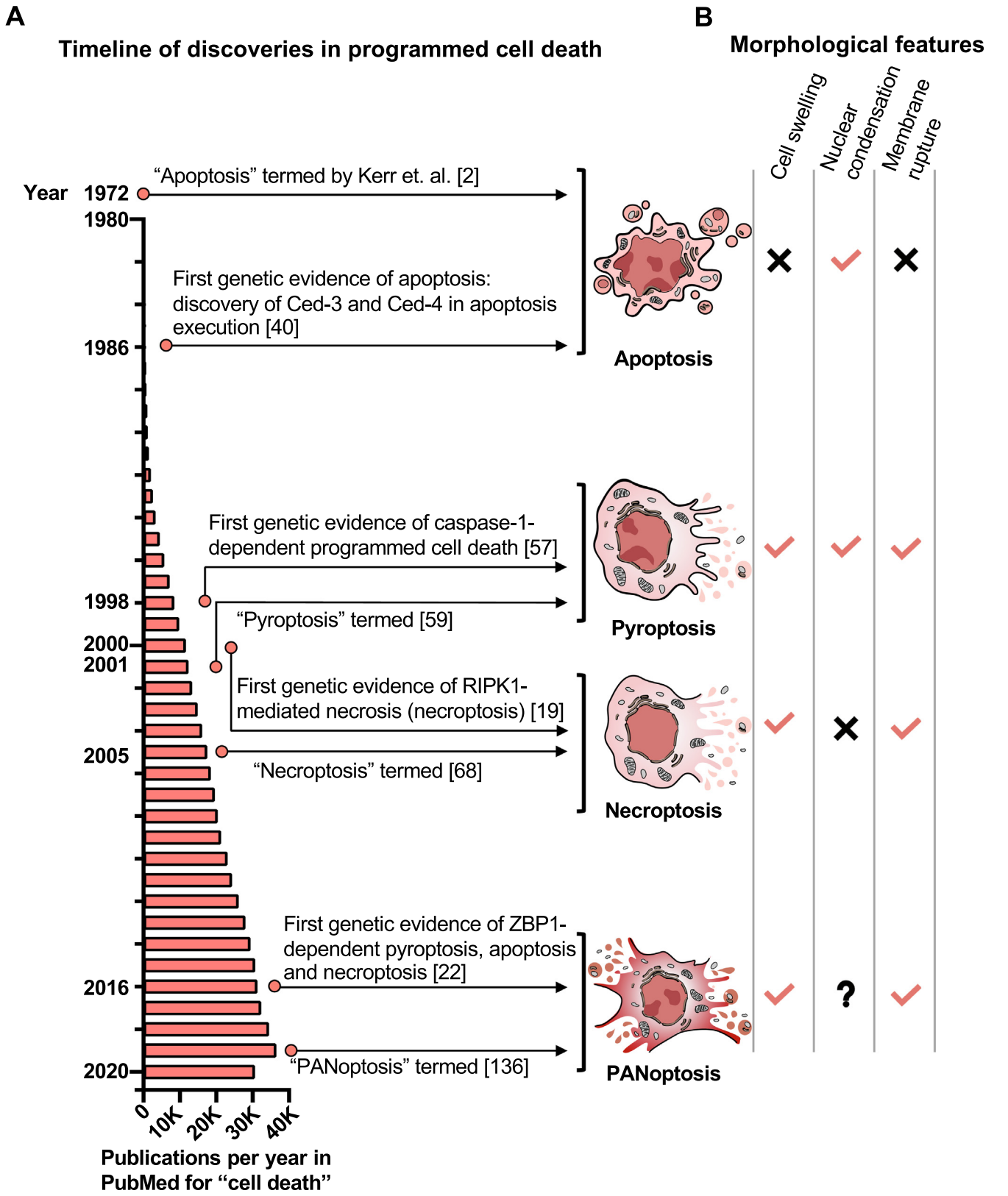


Fig. 1. Research timeline and features of programmed cell death. (A) Key milestones in the discovery and definition of apoptosis, pyroptosis, necroptosis and PANoptosis overlaid on the number of publications per year in PubMed using the search term "Cell death". (B) Morphological features of cells undergoing different forms of programmed cell death.

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, NLRP1b) 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 NLRP1b and NLRC4 can directly interact with CASP1, causing pyroptosis without inducing CASP1 autoprocessing [63,64], although the physiological 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 cytotoxic 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 permeability induced by the bile acid activates intrinsic apoptosis through the APAF1 apoptosome, which can also interact with the pyroptotic 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 CASP11-APAF1 pyroptosome remains elusive.

Overall, these findings suggest that the pyroptotic 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*$^{-/-}$ [109] and enzymatically inactive CASP8 (C362S or C362A) embryos [110,111]. Therefore, either the lack of RIPK1 or

Table 1
Importance of molecules in programmed cell death pathways during development.

Protein	Murine lethality upon deletion	Potential cause of death	Select genetic cross(es) attempted to rescue
RIPK1	Postnatal (immediately after birth)	Massive necroptosis in epidermal cells and apoptosis in intestine [103]	*Ripk3*$^{-/-}$*Tnfr1*$^{-/-}$ (lethality in adulthood due to sepsis) [108] *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ (survive but develop acute lymphoproliferative syndrome [ALPS]) [108,166] *Ripk3*$^{-/-}$*Fadd*$^{-/-}$ (survive but develop ALPS) [108] *Ripk3*$^{-/-}$ (postnatal lethality) [108]
RIPK1 (RHIM deletion)	Postnatal (E18.5, immediately after birth)	Inflammation in both epidermal cells and intestine	*Ripk3*$^{+/-}$ (survive to adulthood) [119] *Ripk3*$^{-/-}$ (survive to adulthood) [119] *Mlkl*$^{-/-}$ (survive to adulthood) [119] *Zbp1*$^{-/-}$ (survive to adulthood) [119] *Zbp1*$^{Z\alpha/Z\alpha}$ (survive to adulthood) [37,259]
RIPK1 (D325A, uncleavable)	Embryonic (E10.5)	Massive apoptosis in yolk sac vasculature	*Ripk1*$^{D138N/D138N}$ (kinase dead RIPK1, survive to birth) [109] *Mlkl*$^{-/-}$*Fadd*$^{-/-}$ (survive but develop ALPS) [109] *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ (survive to birth) [109] *Tnfr1*$^{-/-}$ (survive to birth) [109]
RIPK3 (D161N, kinase dead)	Embryonic (E11.5)	Massive apoptosis in yolk sac vasculature	*Casp8*$^{-/-}$ (survive but develop ALPS) [167] *Ripk1*$^{-/-}$ (survive to birth) [167]
CASP8	Embryonic (E10.5-E11.5)	Hyperemia and heart defect [102]	*Ripk3*$^{-/-}$ (survive but develop ALPS) [106,107] *Ripk1*$^{D138N/D138N}$ (kinase dead RIPK1, lethality delayed to E14) [109] *Mlkl*$^{-/-}$ (survive but develop progressive lymphadenopathy) [260]
CASP8 (C362A or C362S, enzymatically dead)	Embryonic (E10.5-E11.5)	Hyperemia and vasculature defect	*Ripk3*$^{-/-}$ (survive but develop anemia and splenomegaly) [110,111] *Mlkl*$^{-/-}$ (postnatal lethality due to intestinal inflammation) [110,111] *Mlkl*$^{-/-}$*Asc*$^{-/-}$ (survive to adulthood) [110,111] *Mlkl*$^{-/-}$*Casp1*$^{-/-}$ (survive to adulthood) [110,111] *Mlkl*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$ [110] *Ripk3*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$ [110]
FADD	Embryonic (E10.5-E11.5)	Cardiac failure and abdominal hemorrhage [168]	*Ripk1*$^{-/-}$ (postnatal lethality) [169] *Ripk3*$^{-/-}$ (survive but develop ALPS) [170]
FLIP	Embryonic (E10.5-E11.5)	Heart defect [171]	*Ripk3*$^{-/-}$*Fadd*$^{-/-}$ (survive but develop ALPS) [170]
CASP3/7	Postnatal (immediately after birth)	Defective cardiac formation [172]	N/A
TBK1	Embryonic (E14.5)	Liver degeneration	*Ripk1*$^{D138N/D138N}$ (survive to adulthood, fertile) [158] *Ripk3*$^{-/-}$ (low birth rate) [158]
CASP9	Embryonic (after E16.5)	Enlarged and malformed cerebrum [173]	N/A
HOIL-1	Embryonic (E10.5)	Aberrant endothelial cell death	*Tnfr1*$^{-/-}$ (lethality delayed to E16.5) [174] *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ (lethality delayed to E14.5) [174] *Casp8*$^{-/-}$*Ripk3*$^{-/-}$*Ripk1*$^{-/-}$ (viable, runty) [174] *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ (viable, runty) [174]
HOIP	Embryonic (E10.5)	Aberrant endothelial cell death	*Tnfr1*$^{-/-}$ (lethality delayed to E17.5) [175] *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ (viable, runty) [174]
SYK	Postnatal (E18.5, immediately after birth)	Extensive hemorrhaging [176]	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].

3.4. Molecular interactions between components of pyroptosis, apoptosis and necroptosis – The emergence of PANoptosis

As described above, the complexity of crosstalk between each duo of PCD pathways suggests the existence of a dynamic molecular interaction network instead of insulated pathways steering the cell death event. These data led to the emergence of the concept of PANoptosis to define a unique, physiologically relevant, inflammatory PCD activated by specific triggers and with molecular characteristics of pyroptosis, apoptosis and/or necroptosis that cannot be accounted for by any of these PCD pathways alone. The most well-established example of PANoptosis was first shown with direct biochemical evidence in the context of viral infection in 2016 with the identification of ZBP1 as a sensor for influenza A virus (IAV) and a master regulator of cell death during IAV infection [22] (Table 2). Macrophages infected with IAV undergo PANoptotic cell death characterized by activation of CASP1, CASP8, CASP3 and phosphorylation of MLKL, the essential molecular events of pyroptosis, apoptosis and necroptosis [22,27]. Deletion of ZBP1 is sufficient to suppress PANoptosis fully, whereas loss of individual components of the typical PCD pathways fails to rescue the cell death [22,27]. In subsequent studies, ZBP1 was also shown to unleash RIPK3-mediated necroptosis during development when the RHIM domain of RIPK1 is disrupted [118,119]. Additionally, mutation of the RIPK1 RHIM (*Ripk1*^{mRHIM}) results in lethality in mice due to loss of its interaction with RIPK3. These mice can be rescued with further deletion of either ZBP1 or the Zα2 domain of ZBP1, inactivation of RIPK3 kinase function, mutation of the RHIM domain of RIPK3, or deletion of RIPK3 or MLKL to disable the necroptotic pathway (Table 1) [37,118,119]. These observations suggest that the PANoptotic function of ZBP1 is trigger-dependent. IAV activation of ZBP1 through its Zα domain [120,121] may serve as the specific upstream event that initiates the activation of the molecular machinery for PANoptosis execution [37].

Beyond IAV infection, evidence for PANoptosis has also been shown in sterile inflammation. Studies with mice carrying enzymatically inactive CASP8 (*Casp8*$^{C362S/C362S}$ or *Casp8*$^{C362A/C362A}$) demonstrate that the CASP8-RIPK1 platform shared by apoptosis and necroptosis is genetically associated with ASC, the adaptor protein of inflammasomes. The DED domain of CASP8 in cells from mice with enzymatically inactive CASP8 interacts with ASC, triggering pyroptosis and severe inflammation in the intestine [110,111]. Unlike the inflammation observed in epidermal cells in these mutant mice, which can be rescued by deleting necroptotic machinery such as RIPK3 and MLKL, the intestinal inflammation is only rescued by combined deletion of necroptosis and the pyroptotic proteins CASP1 or ASC [110,111]. Thus, *Casp8*$^{C362S/C362S}$*Mlkl*$^{-/-}$*Casp1*$^{-/-}$ mice, which are deficient in extrinsic apoptotic, necroptotic and pyroptotic pathways, are viable (Table 1). This *in vivo* genetic evidence further indicates the molecular crosstalk between these three PCD pathways through PANoptosis.

In addition to the examples discussed above, PANoptosis has been observed in many different conditions, such as during both genetic- and *Yersinia* YopJ-mediated inhibition of TAK1 [25,35,36], other bacterial, viral and fungal infections [23,24,26], cytokine storm [29] and cancer [28]. The activation of PANoptosis under such diverse conditions suggests an intricate regulation of this process. This regulation is achieved through the PANoptosome, a multiprotein complex that serves as a platform for engagement of key molecules from pyroptosis, apoptosis and/or necroptosis.

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.

Table 2
Cell death pathway activation by selected viral, bacterial and fungal pathogens [22–27,145,150,177–245].

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 (MA104) 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]	Macrophage: pMLKL [23]
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 Epithelial (HT-29): necroptosis inhibited [195]
Vaccinia virus (dsDNA)	Macrophage: CASP1 activation [196]	Monocytic cell (THP-1): PARP1, CASP3/9 cleavage (MVA strain) [197]	T cell (Jurkat): Sensitized to TNF-α–induced necroptosis [198]
Encephalomyo-carditis virus (ssRNA)	Macrophage: CASP1 activation [186]	Fibroblast (BHK-21): No apoptosis; inhibited by viral protein 2A [199] Macrophage: CASP3 activation [184]	Macrophage: pMLKL (LPS-primed, z-VAD treated) [186]
Dengue virus (ssRNA)	Monocyte: CASP1 activation and IL-1β release [200]	Monocytic cell (U937): CASP8/9 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: AIM2-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 (A549): CASP6/8/9 activation (R6x strain) [211] Macrophage: No/low CASP3/7 activation [209]	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: NLRC4-dependent CASP1 activation [216]	Epithelial cell (HeLa): CASP3/9 activation [217]	NA
Shigella flexneri	Macrophages: NLRC4-dependent CASP1 activation [218]	Macrophages: Morphology and DNA fragmentation, CASP3 activation [219, 220] Epithelial cell (HT29): CASP3/8 activation inhibited [145]	Epithelial cell: No necroptosis; bacterial degradation of RIPKs [145]
Mycobacterium tuberculosis	Macrophages: NLRP3 inflammasome activation [221, 222]; AIM2 inflammasome activation (rBCG strain) [223]	Macrophage: No apoptosis; undetectable CASP3/8 activation [224]	Macrophage: No necroptosis; undetectable pMLKL; MLKL deletion or RIPK1 inhibitor do not inhibit cell death [224]

Mycobacterium bovis	Macrophage: CASP1 activation and IL-1β release [225]	Macrophage: CASP3/9/12 activation [226]	N/A
Porphyromonas gingivalis	Macrophage (THP-1): CASP1 activation and IL-1β release [227]	Epithelial cells: CASP3 activation and DNA fragmentation [228]	Macrophage (THP-1): pMLKL [229]
Legionella pneumophila	Macrophage: NAIP5/NLRC4-mediated CASP1 activation, cell death and IL-1β release [230]	Human PBMCs: CASP3 activation [231]	N/A
***Salmonella enterica* serovar Typhimurium**	Macrophage: NLRP3- and NLRC4-dependent CASP1 activation [232]	Macrophage: CASP3/8/9 activation [233]	Macrophage: pMLKL [234]
Yersinia pestis	Macrophage: CASP1 activation [25]	Macrophage: CASP3/7 activation [25]	Macrophage: pMLKL [25]
Helicobacter pylori	Macrophage: NLRP3-dependent CASP1 activation and IL-1β release [235]	Epithelial cell (AGS): CASP3/8/9 activation [236]	N/A
Burkholderia pseudomallei	Macrophage: NLRC4-dependent CASP1 activation [237]	Macrophage: CASP3/8/9 activation [237]	N/A
Bacillus anthracis	Macrophage (BMAJ): NLRP1-dependent IL-1β release and cell death [238]	Macrophage: Morphology and DNA fragmentation, CASP3/7 activation [97, 239]	N/A
Fungi			
Candida albicans	Macrophage: NLRP3-dependent CASP1 activation and IL-1β release; CASP1/11 ablation partially rescues cell death [24, 240, 241]	Macrophage and epithelial cell: CASP3/9 activation [24, 242]	Macrophage: *Ripk3* or *Mlkl* deletion partially rescue cell death [243]; pMLKL [24]
Aspergillus fumigatus	Macrophage: NLRP3- and AIM2-dependent CASP1 activation [24, 244]	Macrophage: CASP3/7 activation [24]	Macrophage: pMLKL [24]
Cryptococcus neoformans	Dendritic cell: NLRP3-dependent CASP1 activation [150]	Macrophage: CASP3, PARP1 activation, cytochrome C release [245]	N/A

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.

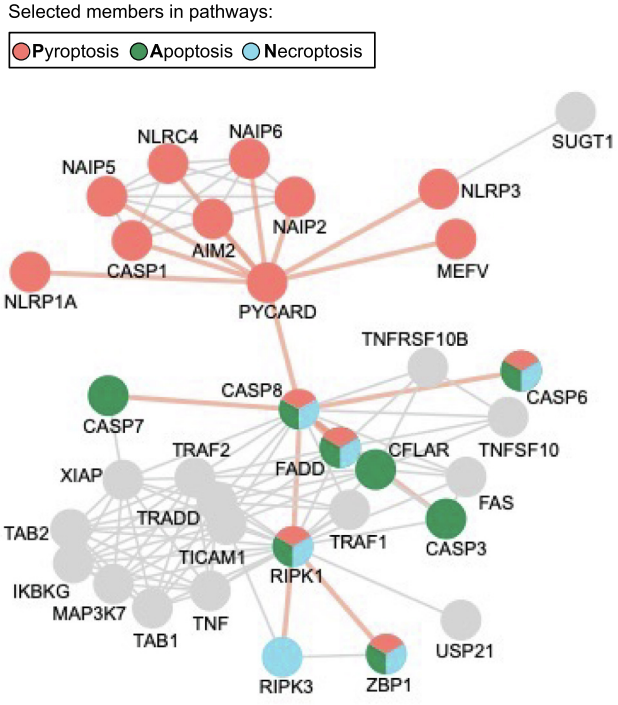


Fig. 2. Interactome analysis of molecules in PCD pathways. A physical network of experimental and database evidence with confidence > 0.7 was retrieved from the STRING database after searching seven proteins (AIM2, PYCARD, MEFV, ZBP1, CASP1, CASP8, RIPK3) with interactor threshold 20. The interaction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

The 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.

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 *Ripk1*^{mRHIM} 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

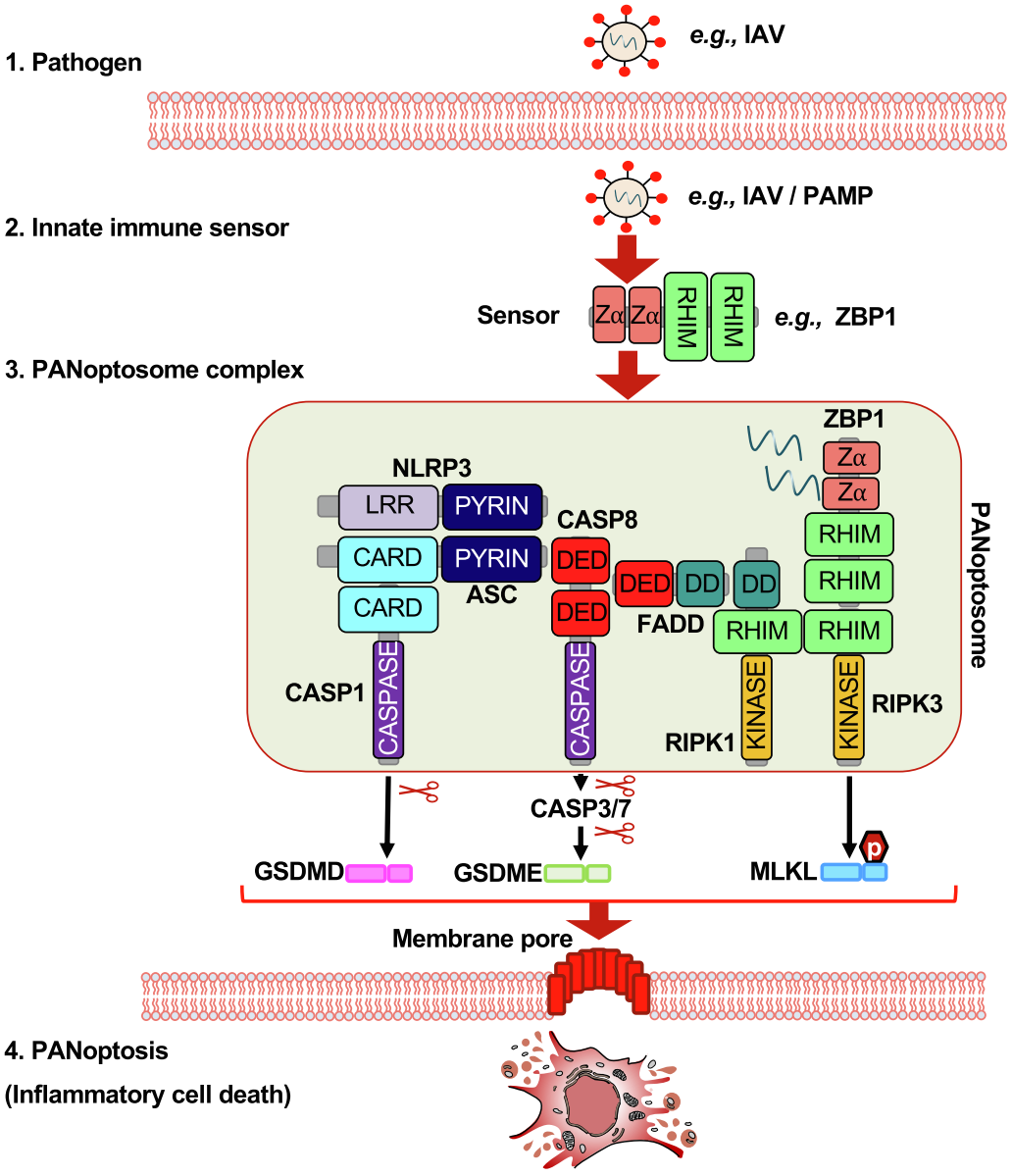
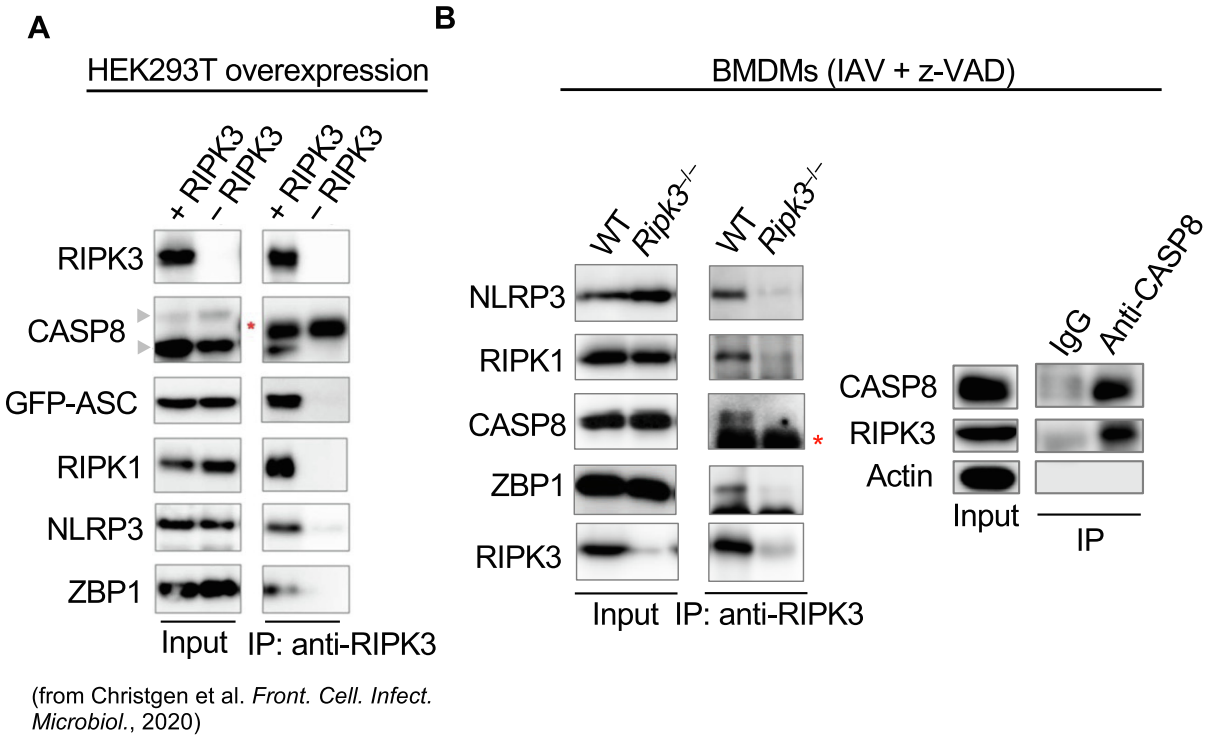


Fig. 3. Model of ZBP1 PANoptosome assembly. 1) Specific trigger (e.g., IAV) is required to initiate PANoptosome formation; 2) specific sensor (e.g., ZBP1) is activated by the trigger; 3) the sensor initiates the assembly of the PANoptosome, which contains molecules required to activate downstream PCD 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.



(from Christgen et al. *Front. Cell. Infect. Microbiol.*, 2020)

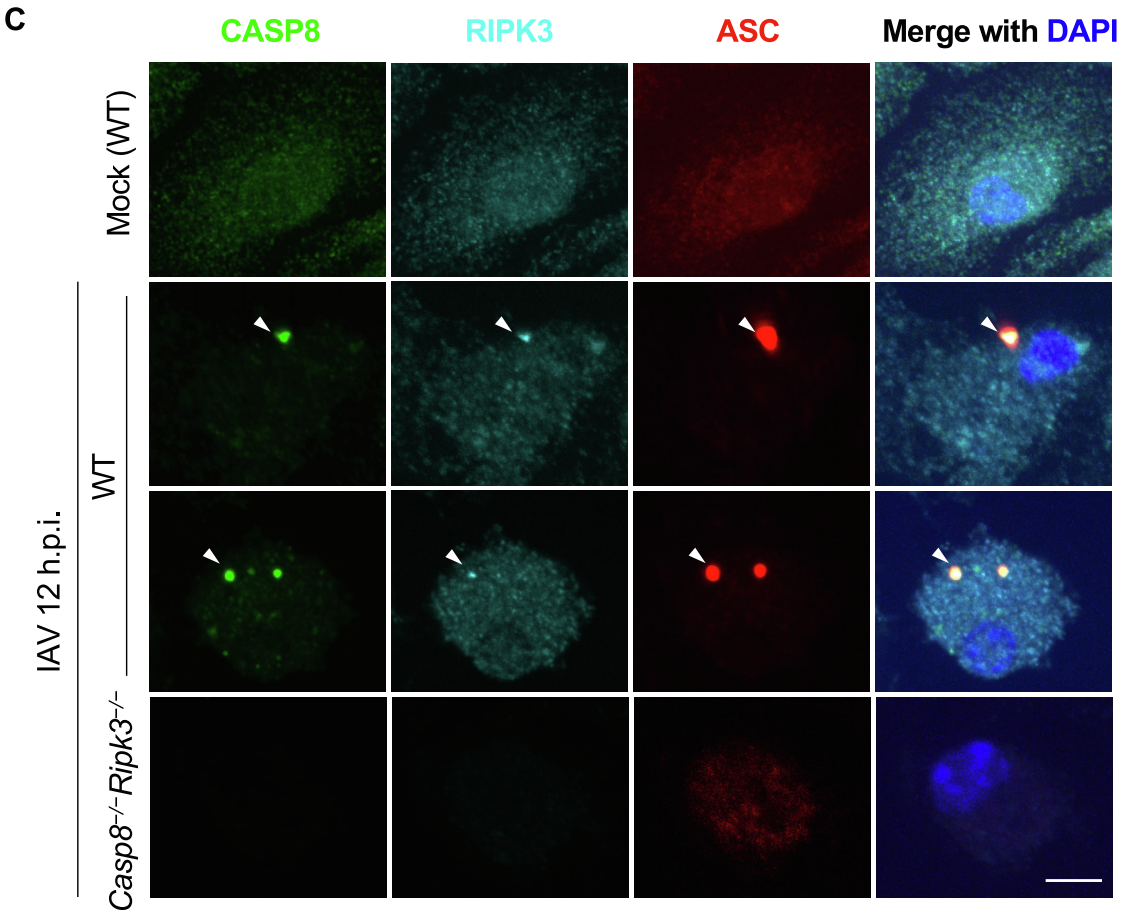


Fig. 4. Experimental evidence of PANoptosome formation. (A) Immunoprecipitation of RIPK3 in HEK293T cells overexpressing PANoptosome components (published data from Christgen, S., et al. Front. Cell. Infect. Microbiol., 2020). (B) Immunoprecipitation of RIPK3 in IAV-infected WT and *Ripk3*$^{-/-}$ BMDMs (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 Zα2 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 RIG-I 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 NLRP3 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 emphasizing 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 Zα2 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-

totic or apoptotic pathways, respectively, fails to influence host survival, while the combination of both decreases host fitness during IAV infection in a viral particle dose-dependent manner [121,151]. Similar results have been found with *Yersinia*; consistent with the importance of the RIPK1-PANoptosome induced by *Yersinia* discussed above *in vitro* [25], RIPK1, CASP8 and FADD, core members of the PANoptosome, are critically involved in host defense against *in vivo* infection [88,152]. Loss of CASP8 or the kinase activity of RIPK1 severely impairs host survival during *Yersinia* infection due to impaired cell death and inflammatory responses [88,152]. As several pathogens are known to activate multiple PCD pathways *in vitro* (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

The first physiological evidence of the functional molecular crosstalk between pyroptosis, apoptosis and necroptosis was found in the *Pstpip2^{cmo}* mouse model of osteomyelitis [33]. Mice carrying a mutation in *Pstpip2* (*Pstpip2*^{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 (*Pstpip2*^{cmo}*Nlrp3*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$ or *Pstpip2*^{cmo}*Casp1*$^{-/-}$*Ripk3*$^{-/-}$*Casp8*$^{-/-}$) 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^{cmo}* 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 [153]. *In vivo*, blocking CS by treating mice with antibodies against TNF and IFN-γ prevents mortality induced by SARS-CoV-2 infection, hemophagocytic lymphohistiocytosis, and LPS shock (sepsis) [29]. Overall, TNF and IFN-γ–mediated PANoptosis provides a molecular basis for CS that is conserved during infection and inflammation.

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 CASP1/11 decreases but does not fully abolish cell death during VSV infection [22,23]. The residual CASP3/7 activation in *Casp8*$^{-/-}$*Ripk3*$^{-/-}$*Casp1/11*$^{-/-}$ 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
Cell death molecules in genetic models of inflammatory diseases.

Bone disruption in *Pstpip2 ^{cmo}* mice model	Bone disruption	Ref
Pstpip2 ^{cmo}	Yes	[246]
Reported genetic crosses		
Pstpip2 ^{cmo} × *Nlrp3$^{-/-}$*	Yes	[247]
Pstpip2 ^{cmo} × *Casp1$^{-/-}$*	Yes	[33]
Pstpip2 ^{cmo} × *Ripk3$^{-/-}$* × *Casp8$^{-/-}$*	Yes	[33,34]
Pstpip2 ^{cmo} × *Ripk3$^{-/-}$* × *Casp8$^{-/-}$* × *Nlrp3$^{-/-}$*	No	
Pstpip2 ^{cmo} × *Ripk3$^{-/-}$* × *Casp8$^{-/-}$* × *Casp1$^{-/-}$*	No	

Skin inflammation in *Sharpin ^{cpdm}* mice model	Skin inflammation	
Sharpin ^{cpdm}	Yes	[248]
Reported genetic crosses		
Sharpin ^{cpdm} × *Nlrp3$^{-/-}$*	Yes (delayed)	[249]
Sharpin ^{cpdm} × *Casp1$^{-/-}$*	Yes (delayed)	[249]
Sharpin ^{cpdm} × *Mlkl$^{-/-}$*	Yes (delayed)	[250]
Sharpin ^{cpdm} × *Bid$^{-/-}$*	Yes (delayed)	
Sharpin ^{cpdm} × *Casp8$^{-/-}$Ripk3$^{-/-}$*	Died before weaning	[250]
Sharpin ^{cpdm} × *Ripk1 $^{K45A/K45A}$* (kinase dead)	No	[251]
Sharpin ^{cpdm} × *Ripk3$^{-/-}$*	Yes (delayed)	
Sharpin ^{cpdm} × *Ripk3$^{-/-}$* × *Fadd $^{E\text{-}KO}$*	No	[252]
Sharpin ^{cpdm} × *Ripk3$^{-/-}$* × *Tradd $^{E\text{-}KO}$*	No	

Footpad inflammation in *Ptpn6 ^{spin}* mice model	Footpad inflammation	
Ptpn6 ^{spin}	Yes	[253]
Reported genetic crosses		
Ptpn6 ^{spin} × *Casp1$^{-/-}$*	Yes	[253]
Ptpn6 ^{spin} × *Nlrp3$^{-/-}$*	Yes	
Ptpn6 ^{spin} × *Ripk3$^{-/-}$*	Yes	
Ptpn6 ^{spin} × *Casp8$^{-/-}$Ripk3$^{-/-}$*	Yes	
Ptpn6 ^{spin} × *Mlkl$^{-/-}$*	Yes	[254]
Ptpn6 ^{spin} × *Ripk3$^{-/-}$* × *Casp8$^{-/-}$* × *Ripk1$^{-/-}$*	No	

Arthritis in *LysM-Cre A20 $^{fl/fl}$* mice model	Arthritis	
LysM-Cre A20 $^{fl/fl}$	Yes	[255]
Reported genetic crosses		
LysM-Cre A20 $^{fl/fl}$ × *Casp1/11$^{-/-}$*	No	[255]
LysM-Cre A20 $^{fl/fl}$ × *Nlrp3$^{-/-}$*	No	
LysM-Cre A20 $^{fl/fl}$ × *Mlkl$^{-/-}$*	No	[256]
LysM-Cre A20 $^{fl/fl}$ × *Ripk3$^{-/-}$*	No	

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 evolutionally 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 *Pstpip2*^{cmo} 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 cytopathy during infection (Table 2). For example, *S. flexneri* inhibits apoptosis in epithelial cells [165] but triggers apoptotic 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, Inflammation, and Pyroptosis. Annu Rev Immunol 2020;38:567–95.
[5] Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol 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):398–414.
[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, Kawane 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.

[10] Fernandes-Alnemri T, Litwack G, Alnemri ES. CPP32, a novel human apoptotic protein with homology to Caenorhabditis elegans cell death protein Ced-3 and mammalian interleukin-1 beta-converting enzyme. J Biol Chem 1994;269 (49):30761–4.
[11] Tewari M et al. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. Cell 1995;81(5):801–9.
[12] Nicholson DW et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 1995;376(6535):37–43.
[13] Stennicke HR et al. Pro-caspase-3 is a major physiologic target of caspase-8. J Biol Chem 1998;273(42):27084–90.
[14] Twiddy D et al. Caspase-7 is directly activated by the approximately 700-kDa apoptosome complex and is released as a stable XIAP-caspase-7 approximately 200-kDa complex. J Biol Chem 2006;281(7):3876–88.
[15] Shi J et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 2015;526(7575):660–5.
[16] Kayagaki N et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature 2015;526(7575):666–71.
[17] Sun L et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 2012;148(1–2):213–27.
[18] Zhao J et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. Proc Natl Acad Sci U S A 2012;109(14):5322–7.
[19] 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):489–95.
[20] 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.
[21] Cai Z et al. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. Nat Cell Biol 2014;16(1):55–65.
[22] Kuriakose T et al. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. Sci Immunol 2016;1(2).
[23] Christgen S et al. Identification of the PANoptosome: a molecular platform triggering pyroptosis, apoptosis, and necroptosis (PANoptosis). Front Cell Infect Microbiol 2020;10:237.
[24] Banoth B et al. ZBP1 promotes fungi-induced inflammasome activation and pyroptosis, apoptosis, and necroptosis (PANoptosis). J Biol Chem 2020;295 (52):18276–83.
[25] Malireddi RKS et al. RIPK1 distinctly regulates yersinia-induced inflammatory cell death, PANoptosis. Immunohorizons 2020;4(12):789–96.
[26] 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.
[27] 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.
[28] Karki R et al. Interferon regulatory factor 1 regulates PANoptosis to prevent colorectal cancer. JCI Insight 2020;5(12).
[29] Karki R et al. Synergism 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.
[30] Lamkanfi M et al. Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. Mol Cell Proteomics 2008;7 (12):2350–63.
[31] Malireddi RK et al. Cutting edge: proteolytic inactivation of poly(ADP-ribose) polymerase 1 by the Nlrp3 and Nlrc4 inflammasomes. J Immunol 2010;185 (6):3127–30.
[32] Gurung P et al. FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. J Immunol 2014;192 (4):1835–46.
[33] Lukens JR et al. Dietary modulation of the microbiome affects autoinflammatory disease. Nature 2014;516(7530):246–9.
[34] Gurung P, Burton A, Kanneganti TD. NLRP3 inflammasome plays a redundant role with caspase 8 to promote IL-1beta-mediated osteomyelitis. Proc Natl Acad Sci U S A 2016;113(16):4452–7.
[35] Malireddi RKS et al. TAK1 restricts spontaneous NLRP3 activation and cell death to control myeloid proliferation. J Exp Med 2018;215 (4):1023–34.
[36] 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).
[37] Kesavardhana S et al. The Zalpha2 domain of ZBP1 is a molecular switch regulating influenza-induced PANoptosis and perinatal lethality during development. J Biol Chem 2020;295(24):8325–30.
[38] Samir P, Malireddi RKS, Kanneganti TD. The PANoptosome: a deadly protein complex driving pyroptosis, apoptosis, and necroptosis (PANoptosis). Front Cell Infect Microbiol 2020;10:238.
[39] Karki R, Kanneganti TD. The 'cytokine storm': molecular mechanisms and therapeutic prospects. Trends Immunol 2021.
[40] Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode C. elegans. Cell 1986;44(6):817–29.
[41] 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.
[42] 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.
[43] Li P et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91(4):479–89.
[44] Tsujimoto Y et al. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 1984;226 (4678):1097–9.
[45] Hengartner MO, Ellis RE, Horvitz HR. Caenorhabditis elegans gene ced-9 protects cells from programmed cell death. Nature 1992;356(6369):494–9.
[46] Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol 2019;20 (3):175–93.
[47] Chinnaiyan AM et al. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995;81 (4):505–12.
[48] Kischkel FC et al. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995;14(22):5579–88.
[49] Boldin MP et al. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. J Biol Chem 1995;270(14):7795–8.
[50] Boldin MP et al. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 1996;85 (6):803–15.
[51] Muzio M et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death–inducing signaling complex. Cell 1996;85(6):817–27.
[52] 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.
[53] Cerretti DP et al. Molecular cloning of the interleukin-1 beta converting enzyme. Science 1992;256(5053):97–100.
[54] 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.
[55] Thornberry NA et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 1992;356(6372):768–74.
[56] Miura M et al. Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the C. elegans cell death gene ced-3. Cell 1993;75(4):653–60.
[57] Hilbi H et al. Shigella-induced apoptosis is dependent on caspase-1 which binds to IpaB. J Biol Chem 1998;273(49):32895–900.
[58] Brennan MA, Cookson BT. Salmonella induces macrophage death by caspase-1-dependent necrosis. Mol Microbiol 2000;38(1):31–40.
[59] Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. Trends Microbiol 2001;9(3):113–4.
[60] Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002;10(2):417–26.
[61] Malik A, Kanneganti TD. Inflammasome activation and assembly at a glance. J Cell Sci 2017;130(23):3955–63.
[62] Fernandes-Alnemri T et al. The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. Cell Death Differ 2007;14(9):1590–604.
[63] Broz P et al. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. Cell Host Microbe 2010;8(6):471–83.
[64] Van Opdenbosch N et al. Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. Nat Commun 2014;5:3209.
[65] Hagar JA et al. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science 2013;341(6151):1250–3.
[66] Kayagaki N et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science 2013;341(6151):1246–9.
[67] Shi J et al. Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 2014;514(7521):187–92.
[68] Degterev A et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 2005;1 (2):112–9.
[69] Degterev A et al. Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol 2008;4(5):313–21.
[70] Takahashi N et al. Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. Cell Death Dis 2012;3:e437.
[71] He S et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 2009;137(6):1100–11.
[72] Zhang DW et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 2009;325 (5938):332–6.
[73] Cho YS et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 2009;137(6):1112–23.
[74] Declercq W, Vanden Berghe T, Vandenabeele P. RIP kinases at the crossroads of cell death and survival. Cell 2009;138(2):229–32.

[75] Kaiser WJ et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. J Biol Chem 2013;288(43):31268–79.
[76] Vanden Berghe T, Hassannia B, Vandenabeele P. An outline of necrosome triggers. Cell Mol Life Sci 2016;73(11–12):2137–52.
[77] Nailwal H, Chan FK. Necroptosis in anti-viral inflammation. Cell Death Differ 2019;26(1):4–13.
[78] Gong Y et al. The role of necroptosis in cancer biology and therapy. Mol Cancer 2019;18(1):100.
[79] Kayagaki N et al. NINJ1 mediates plasma membrane rupture during lytic cell death. Nature 2021;591(7848):131–6.
[80] Snyder AG, Oberst A. The Antisocial Network: Cross Talk Between Cell Death Programs in Host Defense. Annu Rev Immunol 2021.
[81] Doerflinger M et al. Flexible usage and interconnectivity of diverse cell death pathways protect against intracellular infection. Immunity 2020;53(3):533–547 e7.
[82] Schwarzer R et al. FADD and caspase-8 regulate gut homeostasis and inflammation by controlling MLKL- and GSDMD-mediated death of intestinal epithelial cells. Immunity 2020;52(6):978–993 e6.
[83] Tsuchiya K et al. Caspase-1 initiates apoptosis in the absence of gasdermin D. Nat Commun 2019;10(1):2091.
[84] Taabazuing CY, Okondo MC, Bachovchin DA. Pyroptosis and Apoptosis Pathways Engage in Bidirectional Crosstalk in Monocytes and Macrophages. Cell Chem Biol 2017;24(4):507–514 e4.
[85] Man SM et al. Inflammasome activation causes dual recruitment of NLRC4 and NLRP3 to the same macromolecular complex. Proc Natl Acad Sci U S A 2014;111(20):7403–8.
[86] Man SM et al. Salmonella infection induces recruitment of Caspase-8 to the inflammasome to modulate IL-1beta production. J Immunol 2013;191(10):5239–46.
[87] Van Opdenbosch N et al. Caspase-1 engagement and TLR-induced c-FLIP expression suppress ASC/Caspase-8-dependent apoptosis by inflammasome sensors NLRP1b and NLRC4. Cell Rep 2017;21(12):3427–44.
[88] Philip NH et al. Caspase-8 mediates caspase-1 processing and innate immune defense in response to bacterial blockade of NF-kappaB and MAPK signaling. Proc Natl Acad Sci U S A 2014;111(20):7385–90.
[89] Sarhan J et al. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during Yersinia infection. Proc Natl Acad Sci U S A 2018;115(46):E10888–97.
[90] Demarco B et al. Caspase-8-dependent gasdermin D cleavage promotes antimicrobial defense but confers susceptibility to TNF-induced lethality. Sci Adv 2020;6(47).
[91] Orning P et al. Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. Science 2018;362(6418):1064–9.
[92] Pierini R et al. AIM2/ASC triggers caspase-8-dependent apoptosis in Francisella-infected caspase-1-deficient macrophages. Cell Death Differ 2012;19(10):1709–21.
[93] Sagulenko V et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. Cell Death Differ 2013;20(9):1149–60.
[94] Jesenberger V et al. Salmonella-induced caspase-2 activation in macrophages: a novel mechanism in pathogen-mediated apoptosis. J Exp Med 2000;192(7):1035–46.
[95] Roberts TL et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science 2009;323(5917):1057–60.
[96] Puri AW et al. Caspase-1 activity is required to bypass macrophage apoptosis upon Salmonella infection. Nat Chem Biol 2012;8(9):745–7.
[97] de Vasconcelos NM et al. An apoptotic caspase network safeguards cell death induction in pyroptotic macrophages. Cell Rep 2020;32(4):107959.
[98] Place DE CS, Tuladhar S, Vogel P, Malireddi RKS, Kanneganti TD. Hierarchical cell death program disrupts the intracellular niche required for Burkholderia thailandensis pathogenesis. mBio 2021.
[99] Wang Y et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature 2017;547(7661):99–103.
[100] Rogers C et al. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. Nat Commun 2017;8:14128.
[101] Xu W et al. Apaf-1 pyroptosome senses mitochondrial permeability transition. Cell Metab 2021;33(2):424–436 e10.
[102] Varfolomeev EE et al. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. Immunity 1998;9(2):267–76.
[103] Takahashi N et al. RIPK1 ensures intestinal homeostasis by protecting the epithelium against apoptosis. Nature 2014;513(7516):95–9.
[104] Lin Y et al. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 1999;13(19):2514–26.
[105] Feng S et al. Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. Cell Signal 2007;19(10):2056–67.
[106] Kaiser WJ et al. RIP3 mediates the embryonic lethality of caspase-8-deficient mice. Nature 2011;471(7338):368–72.
[107] Oberst A et al. Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 2011;471(7338):363–7.
[108] Dillon CP et al. RIPK1 blocks early postnatal lethality mediated by caspase-8 and RIPK3. Cell 2014;157(5):1189–202.
[109] Newton K et al. Cleavage of RIPK1 by caspase-8 is crucial for limiting apoptosis and necroptosis. Nature 2019;574(7778):428–31.
[110] Newton K et al. Activity of caspase-8 determines plasticity between cell death pathways. Nature 2019;575(7784):679–82.
[111] Fritsch M et al. Caspase-8 is the molecular switch for apoptosis, necroptosis and pyroptosis. Nature 2019;575(7784):683–7.
[112] Li X et al. RIP1-dependent linear and nonlinear recruitments of caspase-8 and RIP3 respectively to necrosome specify distinct cell death outcomes. Protein Cell 2021.
[113] Lalaoui N et al. Mutations that prevent caspase cleavage of RIPK1 cause autoinflammatory disease. Nature 2020;577(7788):103–8.
[114] Chun HJ et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 2002;419(6905):395–9.
[115] Kang S et al. Caspase-8 scaffolding function and MLKL regulate NLRP3 inflammasome activation downstream of TLR3. Nat Commun 2015;6:7515.
[116] Gutierrez KD et al. MLKL Activation triggers NLRP3-mediated processing and release of IL-1beta independently of gasdermin-D. J Immunol 2017;198(5):2156–64.
[117] Conos SA et al. Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner. Proc Natl Acad Sci U S A 2017;114(6):E961–9.
[118] Newton K et al. RIPK1 inhibits ZBP1-driven necroptosis during development. Nature 2016;540(7631):129–33.
[119] Lin J et al. RIPK1 counteracts ZBP1-mediated necroptosis to inhibit inflammation. Nature 2016;540(7631):124–8.
[120] Kesavardhana S et al. ZBP1/DAI ubiquitination and sensing of influenza vRNPs activate programmed cell death. J Exp Med 2017;214(8):2217–29.
[121] Zhang T et al. Influenza virus Z-RNAs induce ZBP1-mediated necroptosis. Cell 2020;180(6):1115–1129 e13.
[122] Szklarczyk D et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47(D1):D607–13.
[123] Lavrik I, Golks A, Krammer PH. Death receptor signaling. J Cell Sci 2005;118(Pt 2):265–7.
[124] Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003;114(2):181–90.
[125] Tenev T et al. The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol Cell 2011;43(3):432–48.
[126] Jang TH et al. Structural study of the RIPoptosome core reveals a helical assembly for kinase recruitment. Biochemistry 2014;53(33):5424–31.
[127] Mompean M et al. The structure of the necrosome RIPK1-RIPK3 core, a human hetero-amyloid signaling complex. Cell 2018;173(5):1244–1253 e10.
[128] Scott FL et al. The Fas-FADD death domain complex structure unravels signalling by receptor clustering. Nature 2009;457(7232):1019–22.
[129] Fu TM et al. Cryo-EM structure of caspase-8 Tandem DED filament reveals assembly and regulation mechanisms of the death-inducing signaling complex. Mol Cell 2016;64(2):236–50.
[130] Lu A et al. Molecular basis of caspase-1 polymerization and its inhibition by a new capping mechanism. Nat Struct Mol Biol 2016;23(5):416–25.
[131] Lu A et al. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell 2014;156(6):1193–206.
[132] Liepinsh E et al. The death-domain fold of the ASC PYRIN domain, presenting a basis for PYRIN/PYRIN recognition. J Mol Biol 2003;332(5):1155–63.
[133] Vajjhala PR et al. The inflammasome adaptor ASC induces procaspase-8 death effector domain filaments. J Biol Chem 2015;290(49):29217–30.
[134] Park HH et al. The death domain superfamily in intracellular signaling of apoptosis and inflammation. Annu Rev Immunol 2007;25:561–86.
[135] Sun X et al. Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. J Biol Chem 2002;277(11):9505–11.
[136] Malireddi RKS, Kesavardhana S, Kanneganti TD. ZBP1 and TAK1: master regulators of NLRP3 inflammasome/pyroptosis, apoptosis, and necroptosis (PAN-optosis). Front Cell Infect Microbiol 2019;9:406.
[137] Muendlein HI et al. ZBP1 promotes LPS-induced cell death and IL-1beta release via RHIM-mediated interactions with RIPK1. Nat Commun 2021;12(1):86.
[138] Rehwinkel J et al. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell 2010;140(3):397–408.
[139] Rajput A et al. RIG-I RNA helicase activation of IRF3 transcription factor is negatively regulated by caspase-8-mediated cleavage of the RIP1 protein. Immunity 2011;34(3):340–51.
[140] Place DE, Lee S, Kanneganti TD. PANoptosis in microbial infection. Curr Opin Microbiol 2021;59:42–9.
[141] Malireddi RKS, Tweedell RE, Kanneganti TD. PANoptosis components, regulation, and implications. Aging (Albany NY) 2020;12(12):11163–4.
[142] Lacey CA, Miao EA. Programmed cell death in the evolutionary race against bacterial virulence factors. Cold Spring Harb Perspect Biol 2020;12(2).
[143] Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. Nat Rev Immunol 2017;17(3):151–64.
[144] Ashida H et al. Cell death and infection: a double-edged sword for host and pathogen survival. J Cell Biol 2011;195(6):931–42.
[145] Ashida H, Sasakawa C, Suzuki T. A unique bacterial tactic to circumvent the cell death crosstalk induced by blockade of caspase-8. EMBO J 2020;39(17):e104469.
[146] Yin X et al. MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells. Cell Rep 2021;34(2):108628.
[147] Li J, Liu Y, Zhang X. Murine coronavirus induces type I interferon in oligodendrocytes through recognition by RIG-I and MDA5. J Virol 2010;84(13):6472–82.
[148] Paquette N et al. Serine/threonine acetylation of TGFbeta-activated kinase (TAK1) by Yersinia pestis YopJ inhibits innate immune signaling. Proc Natl Acad Sci U S A 2012;109(31):12710–5.

[149] Qu Y et al. NLRP3 recruitment by NLRC4 during Salmonella infection. J Exp Med 2016;213(6):877–85.
[150] Chen M et al. Internalized cryptococcus neoformans activates the canonical caspase-1 and the noncanonical caspase-8 Inflammasomes. J Immunol 2015;195(10):4962–72.
[151] Shubina M et al. Necroptosis restricts influenza A virus as a stand-alone cell death mechanism. J Exp Med 2020;217(11).
[152] 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.
[153] Blaise GA et al. Nitric oxide, cell signaling and cell death. Toxicology 2005;208(2):177–92.
[154] Felt SA, Moerdyk-Schauwecker MJ, Grdzelishvili VZ. Induction of apoptosis in pancreatic cancer cells by vesicular stomatitis virus. Virology 2015;474:163–73.
[155] Fricker M et al. Neuronal cell death. Physiol Rev 2018;98(2):813–80.
[156] Friedlander 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.
[157] Krajewska M et al. Neuronal deletion of caspase 8 protects against brain injury in mouse models of controlled cortical impact and kainic acid-induced excitotoxicity. PLoS ONE 2011;6(9):e24341.
[158] Xu D et al. TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. Cell 2018;174(6):1477–1491 e19.
[159] Degterev A, Ofengeim D, Yuan J. Targeting RIPK1 for the treatment of human diseases. Proc Natl Acad Sci USA 2019;116(20):9714–22.
[160] Feldstein AE et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003;125 (2):437–43.
[161] Gautheron J et al. The necroptosis-inducing kinase RIPK3 dampens adipose tissue inflammation and glucose intolerance. Nat Commun 2016;7:11869.
[162] Karunakaran 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.
[163] Sharma BR, Kanneganti TD. NLRP3 inflammasome in cancer and metabolic diseases. Nat Immunol 2021.
[164] Ajibade AA, Wang HY, Wang RF. Cell type-specific function of TAK1 in innate immune signaling. Trends Immunol 2013;34(7):307–16.
[165] Clark CS, Maurelli AT. Shigella flexneri inhibits staurosporine-induced apoptosis in epithelial cells. Infect Immun 2007;75(5):2531–9.
[166] Rickard JA et al. RIPK1 regulates RIPK3-MLKL-driven systemic inflammation and emergency hematopoiesis. Cell 2014;157(5):1175–88.
[167] Newton K et al. Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. Science 2014;343(6177):1357–60.
[168] 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.
[169] Zhang H et al. Functional complementation between FADD and RIP1 in embryos and lymphocytes. Nature 2011;471(7338):373–6.
[170] Dillon CP et al. Survival function of the FADD-CASPASE-8-cFLIP(L) complex. Cell Rep 2012;1(5):401–7.
[171] Yeh WC et al. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 2000;12 (6):633–42.
[172] Lakhani SA et al. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. Science 2006;311(5762):847–51.
[173] Kuida K et al. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 1998;94(3):325–37.
[174] Peltzer N et al. LUBAC is essential for embryogenesis by preventing cell death and enabling haematopoiesis. Nature 2018;557(7703):112–7.
[175] Peltzer N et al. HOIP deficiency causes embryonic lethality by aberrant TNFR1-mediated endothelial cell death. Cell Rep 2014;9(1):153–65.
[176] Turner M et al. Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. Nature 1995;378(6554):298–302.
[177] Zhu S et al. Nlrp9b inflammasome restricts rotavirus infection in intestinal epithelial cells. Nature 2017;546(7660):667–70.
[178] Martin-Latil S et al. Bax is activated during rotavirus-induced apoptosis through the mitochondrial pathway. J Virol 2007;81(9):4457–64.
[179] Liu T et al. NOD-like receptor family, pyrin domain containing 3 (NLRP3) contributes to inflammation, pyroptosis, and mucin production in human airway epithelium on rhinovirus infection. J Allergy Clin Immunol 2019;144 (3):777–787 e9.
[180] Robinson KS et al. Enteroviral 3C protease activates the human NLRP1 inflammasome in airway epithelia. Science 2020;370(6521).
[181] Deszcz L et al. Apoptotic events induced by human rhinovirus infection. J Gen Virol 2005;86(Pt 5):1379–89.
[182] Wark PA et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005;201 (6):937–47.
[183] Lotzerich M et al. Rhinovirus 3C protease suppresses apoptosis and triggers caspase-independent cell death. Cell Death Dis 2018;9(3):272.
[184] Dubois H et al. Nlrp3 inflammasome activation and Gasdermin D-driven pyroptosis are immunopathogenic upon gastrointestinal norovirus infection. PLoS Pathog 2019;15(4):e1007709.
[185] Bok K et al. Apoptosis in murine norovirus-infected RAW264.7 cells is associated with downregulation of survivin. J Virol 2009;83(8):3647–56.
[186] da Costa LS et al. RNA viruses promote activation of the NLRP3 inflammasome through cytopathogenic effect-induced potassium efflux. Cell Death Dis 2019;10(5):346.
[187] Bitzer M et al. Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32). J Virol 1999;73(1):702–8.
[188] Schock SN et al. Induction of necroptotic cell death by viral activation of the RIG-I or STING pathway. Cell Death Differ 2017;24(4):615–25.
[189] Doitsh G et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 2014;505(7484):509–14.
[190] Cicala C et al. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4(+) T cells. Proc Natl Acad Sci U S A 2000;97(3):1178–83.
[191] Kofahi HM et al. Hepatitis C virus infection of cultured human hepatoma cells causes apoptosis and pyroptosis in both infected and bystander cells. Sci Rep 2016;6:37433.
[192] Deng L et al. Hepatitis C virus infection induces apoptosis through a Bax-triggered, mitochondrion-mediated, caspase 3-dependent pathway. J Virol 2008;82(21):10375–85.
[193] Karaba AH et al. Herpes simplex virus type 1 inflammasome activation in proinflammatory human macrophages is dependent on NLRP3, ASC, and caspase-1. PLoS ONE 2020;15(2):e0229570.
[194] Aubert M, O'Toole J, Blaho JA. Induction and prevention of apoptosis in human HEp-2 cells by herpes simplex virus type 1. J Virol 1999;73 (12):10359–70.
[195] Huang Z et al. RIP1/RIP3 binding to HSV-1 ICP6 initiates necroptosis to restrict virus propagation in mice. Cell Host Microbe 2015;17(2):229–42.
[196] Hornung V et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 2009;458(7237):514–8.
[197] Ryerson MR et al. Vaccinia virus encodes a novel inhibitor of apoptosis that associates with the apoptosome. J Virol 2017;91(23).
[198] Chan FK et al. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 2003;278(51):51613–21.
[199] Carocci M et al. Encephalomyocarditis virus 2A protein is required for viral pathogenesis and inhibition of apoptosis. J Virol 2011;85(20):10741–54.
[200] Tan TY, Chu JJH. Dengue virus-infected human monocytes trigger late activation of caspase-1, which mediates pro-inflammatory IL-1beta secretion and pyroptosis. J Gen Virol 2013;94(Pt 10):2215–20.
[201] Klomporn P et al. Dengue infection of monocytic cells activates ER stress pathways, but apoptosis is induced through both extrinsic and intrinsic pathways. Virology 2011;409(2):189–97.
[202] Bedient L et al. Lytic cell death mechanisms in human respiratory syncytial virus-infected macrophages: roles of pyroptosis and necroptosis. Viruses 2020;12(9).
[203] Simpson J et al. Respiratory syncytial virus infection promotes necroptosis and HMGB1 release by airway epithelial cells. Am J Respir Crit Care Med 2020;201(11):1358–71.
[204] Jones JW et al. Absent in melanoma 2 is required for innate immune recognition of Francisella tularensis. Proc Natl Acad Sci USA 2010;107 (21):9771–6.
[205] Lai XH, Sjostedt A. Delineation of the molecular mechanisms of Francisella tularensis-induced apoptosis in murine macrophages. Infect Immun 2003;71 (8):4642–6.
[206] Sauer JD et al. Listeria monocytogenes triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. Cell Host Microbe 2010;7(5):412–9.
[207] Cassidy SK et al. Membrane damage during Listeria monocytogenes infection triggers a caspase-7 dependent cytoprotective response. PLoS Pathog 2012;8 (7):e1002628.
[208] Zwaferink H et al. Stimulation of inducible nitric oxide synthase expression by beta interferon increases necrotic death of macrophages upon Listeria monocytogenes infection. Infect Immun 2008;76(4):1649–56.
[209] Gonzalez-Juarbe N et al. Pore-forming toxins induce macrophage necroptosis during acute bacterial pneumonia. PLoS Pathog 2015;11(12):e1005337.
[210] Fang R et al. Critical roles of ASC inflammasomes in caspase-1 activation and host innate resistance to Streptococcus pneumoniae infection. J Immunol 2011;187(9):4890–9.
[211] Schmeck B et al. Streptococcus pneumoniae-induced caspase 6-dependent apoptosis in lung epithelium. Infect Immun 2004;72(9):4940–7.
[212] Craven RR et al. Staphylococcus aureus alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. PLoS ONE 2009;4(10): e7446.
[213] Winstel V, Schneewind O, Missiakas D. Staphylococcus aureus exploits the host apoptotic pathway to persist during infection. mBio 2019;10(6).
[214] Schaale K et al. Strain- and host species-specific inflammasome activation, IL-1beta release, and cell death in macrophages infected with uropathogenic Escherichia coli. Mucosal Immunol 2016;9(1):124–36.
[215] Klumpp DJ et al. Uropathogenic Escherichia coli induces extrinsic and intrinsic cascades to initiate urothelial apoptosis. Infect Immun 2006;74 (9):5106–13.
[216] Franchi L et al. Critical role for Ipaf in Pseudomonas aeruginosa-induced caspase-1 activation. Eur J Immunol 2007;37(11):3030–9.
[217] Wood SJ et al. Pseudomonas aeruginosa ExoT induces mitochondrial apoptosis in target host cells in a manner that depends on Its GTPase-activating protein (GAP) domain activity. J Biol Chem 2015;290 (48):29063–73.

[218] Suzuki T et al. Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. PLoS Pathog 2007;3(8):e111.
[219] Zychlinsky A, Prevost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. Nature 1992;358(6382):167–9.
[220] Arizmendi O, Picking WD, Picking WL. Macrophage Apoptosis Triggered by IpaD from Shigella flexneri. Infect Immun 2016;84(6):1857–65.
[221] Beckwith KS et al. Plasma membrane damage causes NLRP3 activation and pyroptosis during Mycobacterium tuberculosis infection. Nat Commun 2020;11(1):2270.
[222] Qu Z et al. Mycobacterial EST12 activates a RACK1-NLRP3-gasdermin D pyroptosis-IL-1beta immune pathway. Sci Adv 2020;6(43).
[223] 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.
[224] Stutz MD et al. Necroptotic signaling is primed in Mycobacterium tuberculosis-infected macrophages, but its pathophysiological consequence in disease is restricted. Cell Death Differ 2018;25(5):951–65.
[225] 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.
[226] Cui Y et al. Mycobacterium bovis induces endoplasmic reticulum stress mediated-apoptosis by activating IRF3 in a murine macrophage cell line. Front Cell Infect Microbiol 2016;6:182.
[227] Park E et al. Activation of NLRP3 and AIM2 inflammasomes by Porphyromonas gingivalis infection. Infect Immun 2014;82(1):112–23.
[228] Stathopoulou PG et al. Porphyromonas gingivalis induce apoptosis in human gingival epithelial cells through a gingipain-dependent mechanism. BMC Microbiol 2009;9:107.
[229] Ke X et al. Manipulation of necroptosis by Porphyromonas gingivalis in periodontitis development. Mol Immunol 2016;77:8–13.
[230] Goncalves 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.
[231] Neumeister B et al. Legionella pneumophila induces apoptosis via the mitochondrial death pathway. Microbiology (Reading) 2002;148(Pt 11):3639–50.
[232] 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.
[233] Yu C et al. Salmonella enterica serovar Typhimurium sseK3 induces apoptosis and enhances glycolysis in macrophages. BMC Microbiol 2020;20(1):151.
[234] Hos NJ et al. Type I interferon enhances necroptosis of Salmonella Typhimurium-infected macrophages by impairing antioxidative stress responses. J Cell Biol 2017;216(12):4107–21.
[235] 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.
[236] 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.
[237] Bast A et al. Caspase-1-dependent and -independent cell death pathways in Burkholderia pseudomallei infection of macrophages. PLoS Pathog 2014;10 (3):e1003986.
[238] Levinsohn JL et al. Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog 2012;8(3):e1002638.
[239] Park JM et al. Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. Science 2002;297(5589):2048–51.
[240] Rogiers O et al. Candidalysin crucially contributes to Nlrp3 inflammasome activation by candida albicans hyphae. mBio 2019;10(1).
[241] Uwamahoro N et al. The pathogen Candida albicans hijacks pyroptosis for escape from macrophages. mBio 2014;5(2):e00003–e14.
[242] Villar CC et al. Induction of apoptosis in oral epithelial cells by Candida albicans. Mol Oral Microbiol 2012;27(6):436–48.
[243] Cao M et al. Dectin-1-induced RIPK1 and RIPK3 activation protects host against Candida albicans infection. Cell Death Differ 2019;26(12):2622–36.
[244] Briard B et al. Fungal ligands released by innate immune effectors promote inflammasome activation during Aspergillus fumigatus infection. Nat Microbiol 2019;4(2):316–27.
[245] Coelho C et al. Macrophage mitochondrial and stress response to ingestion of Cryptococcus neoformans. J Immunol 2015;194(5):2345–57.
[246] Ferguson PJ et al. A missense mutation in pstpip2 is associated with the murine autoinflammatory disorder chronic multifocal osteomyelitis. Bone 2006;38(1):41–7.
[247] Cassel SL et al. Inflammasome-independent IL-1beta mediates autoinflammatory disease in Pstpip2-deficient mice. Proc Natl Acad Sci U S A 2014;111(3):1072–7.
[248] HogenEsch H et al. A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. Am J Pathol 1993;143(3):972–82.
[249] Douglas T et al. The inflammatory caspases-1 and -11 mediate the pathogenesis of dermatitis in sharpin-deficient mice. J Immunol 2015;195(5):2365–73.
[250] Rickard JA et al. TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. Elife 2014;3.
[251] Berger SB et al. Cutting edge: RIP1 kinase activity is dispensable for normal development but is a key regulator of inflammation in SHARPIN-deficient mice. J Immunol 2014;192(12):5476–80.
[252] Kumari S et al. Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. Elife 2014;3.
[253] Lukens JR et al. RIP1-driven autoinflammation targets IL-1alpha independently of inflammasomes and RIP3. Nature 2013;498(7453):224–7.
[254] Gurung P et al. Tyrosine kinase SYK licenses MyD88 Adaptor protein to instigate IL-1alpha-mediated inflammatory disease. Immunity 2017;46 (4):635–48.
[255] Vande Walle L et al. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature 2014;512(7512):69–73.
[256] Polykratis A et al. A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. Nat Cell Biol 2019;21(6):731–42.
[257] Karki Rajendra, Kanneganti Thirumala-Devi. The 'cytokine storm': molecular mechanisms and therapeutic prospects. Trends in Immunology 2021;42 (8):681–705.
[258] Malireddi R K Subbarao, Karki Rajendra, Sundaram Balamurugan, Kancharana Balabhaskararao, Lee SangJoon, Samir Parimal, et al. Inflammatory cell death, PANoptosis, mediated by cytokines in diverse cancer lineages inhibits tumor growth. Immunohorizons 2021;5(7):568–80. https://doi.org/10.4049/immunohorizons.2100059.
[259] Jiao H. Z-nucleic-acid sensing triggers ZBP1-dependent necroptosis and inflammation. Nature 2020;580(7803):391–5.
[260] Alvarez-Diaz S. The pseudokinase MLKL and the kinase RIPK3 have distinct roles in autoimmune disease caused by loss of death-receptor-induced apoptosis. Immunity 2016;45(3):513–26.