ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Control of Cell Death in Health and Disease

Nobuhiko Kayagaki,[1] Joshua D. Webster,[2] and Kim Newton[1]

[1]Physiological Chemistry Department, Genentech, South San Francisco, California, USA; email: knewton@gene.com

[2]Pathology Department, Genentech, South San Francisco, California, USA

Annu. Rev. Pathol. Mech. Dis. 2024. 19:157–80

First published as a Review in Advance on October 3, 2023

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathmechdis-051022-014433

Keywords

apoptosis, pyroptosis, necroptosis, inflammation, NINJ1

Abstract

Apoptosis, necroptosis, and pyroptosis are genetically programmed cell death mechanisms that eliminate obsolete, damaged, infected, and self-reactive cells. Apoptosis fragments cells in a manner that limits immune cell activation, whereas the lytic death programs of necroptosis and pyroptosis release proinflammatory intracellular contents. Apoptosis fine-tunes tissue architecture during mammalian development, promotes tissue homeostasis, and is crucial for averting cancer and autoimmunity. All three cell death mechanisms are deployed to thwart the spread of pathogens. Disabling regulators of cell death signaling in mice has revealed how excessive cell death can fuel acute or chronic inflammation. Here we review strategies for modulating cell death in the context of disease. For example, BCL-2 inhibitor venetoclax, an inducer of apoptosis, is approved for the treatment of certain hematologic malignancies. By contrast, inhibition of RIPK1, NLRP3, GSDMD, or NINJ1 to limit proinflammatory cell death and/or the release of large proinflammatory molecules from dying cells may benefit patients with inflammatory diseases.

INTRODUCTION

Cell death is a fundamental biological process that is essential for the development, growth, and persistence of an organism. It contributes to tissue remodeling and the removal of excess cells during development. It then maintains homeostasis by removing cells that are constantly being replaced (for example, intestinal enterocytes and hematopoietic cells) or that pose a danger because they are infected, damaged, or autoreactive. There are three genetically encoded cell death programs: apoptosis, necroptosis, and pyroptosis. These cell death programs are engaged by specific situations and stimuli, and they play distinct roles in modulating the immune response secondary to cell death. For instance, apoptosis is associated with mechanisms that minimize the immune response, whereas necroptosis and pyroptosis release proinflammatory molecules that help amplify the immune response to noxious stimuli, such as infections. Just as cell death is important in the development and normal physiology of an organism, dysregulation of cell death pathways is equally important in disease pathogenesis. Inadequate cell death can lead to cancer, persistent infections, or autoimmunity, whereas excessive cell death is associated with degenerative and inflammatory diseases. Understanding how the cell death machinery is regulated, and when and where it is engaged during disease, is imperative for identifying opportunities for therapeutic intervention. For a discussion of ferroptosis, an alternative mode of cell death that is tightly linked to cell metabolism and does not utilize dedicated cell death genes, we refer the reader elsewhere (1).

INTRINSIC APOPTOSIS

The intrinsic or mitochondrial apoptosis pathway is activated by various stresses, including DNA damage, oncogene activation, and growth factor deprivation. These stresses alter the balance between the proapoptotic and prosurvival members of the BCL-2 (B cell lymphoma 2) protein family, all of which bear one or more BCL-2 homology (BH) domains. Transcriptional or post-translational mechanisms unleash the proapoptotic BH3-only proteins (BAD, BID, BIK, BIM, BMF, HRK, NOXA, and PUMA), which bind to and neutralize their prosurvival relatives (BCL-2, BCL-W, BCL-XL, BCL2-A1/BFL-1, and MCL-1). The proapoptotic effectors BAX and BAK, left unchecked by the prosurvival proteins, form oligomers that permeabilize the mitochondrial outer membrane (**Figure 1**). Some BH3-only proteins, including BIM, BID, and PUMA, neutralize all of the prosurvival proteins, while others, including BAD and NOXA, selectively target a subset. Some BH3-only proteins, including BIM, may also activate BAX and BAK directly (2).

Permeabilized mitochondria release proapoptotic factors, including cytochrome *c* and DIABLO (also called SMAC). Cytochrome *c* interacts with APAF1 to form the apoptosome complex that activates the protease caspase-9, while DIABLO antagonizes inhibitor of apoptosis proteins (IAPs), including XIAP, an inhibitor of caspase-3, -7, and -9 (3). A proteolytic cascade ensues, with caspase-9 cleaving and activating caspase-3 and -7. Hundreds of proteins are cleaved as the cell is dismantled into membrane-enveloped fragments termed apoptotic bodies (4). While these proteolytic cleavage events help break down cellular components, they also function to suppress the immune response. For example, substrates of caspase-3 include inhibitor of caspase-activated deoxyribonuclease (ICAD), scramblase Xkr8, cytoplasmic DNA sensor cyclic GMP–AMP synthase (cGAS), and transcription factor IRF3. Cleavage of cGAS and IRF3 prevents mitochondrial DNA that is released into the cytoplasm by BAX and BAK from activating type I interferon production (5). Cleavage of ICAD also limits activation of DNA sensors because it releases the DNase CAD to degrade chromosomal DNA. Cleavage of scramblase Xkr8 promotes phosphatidylserine exposure on the surface of apoptotic cells, facilitating their recognition, engulfment, and digestion by phagocytes. The rapid clearance of apoptotic cells by phagocytes prevents the release of proinflammatory intracellular contents (6). Hence, apoptosis is often referred to as immunologically silent.

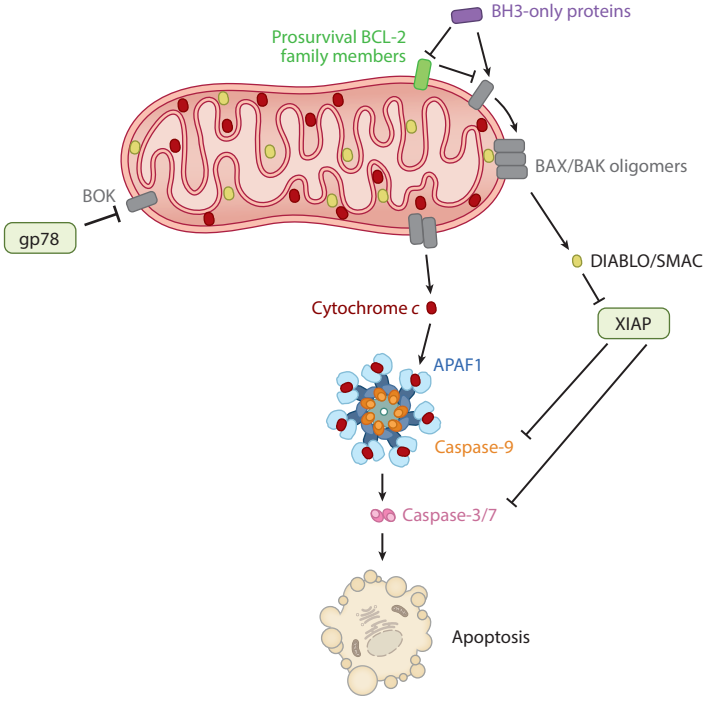

Figure 1

Intrinsic apoptosis. The proapoptotic effectors BAX and BAK are bound and inhibited by prosurvival BCL-2 family members, whereas BOK is kept in check by ubiquitin ligase gp78. BH3-only proteins upregulated through transcriptional or posttranslational mechanisms neutralize the prosurvival proteins or directly activate the proapoptotic effectors. Activated proapoptotic effectors permeabilize the outer mitochondrial membrane and release cytochrome *c*, prompting oligomerization of APAF1 and assembly of the apoptosome complex that activates caspase-9. Permeabilized mitochondria also release DIABLO/SMAC, which binds to XIAP and prevents it from inhibiting caspase-9 plus downstream caspase-3 and -7.

INTRINSIC APOPTOSIS AND MAMMALIAN DEVELOPMENT

Apoptotic cells have a characteristic appearance in tissue sections. They are often observed individually or as localized aggregates, exhibiting condensation of the nucleus (pyknosis) and cytoplasm and/or cellular fragmentation into apoptotic bodies. Occurring focally in the developing embryo, apoptosis has long been thought to shape tissues such as the digits and palate, or to eliminate unnecessary ones such as the Müllerian duct in males (4). (The Müllerian duct gives rise to the female reproductive tract.) Cleaved caspase-3 immunohistochemistry performed on developing mouse embryos highlights multifocal aggregates of apoptotic cells in many tissues. Studies with gene knockout mice lacking both BAX and BAK ($Bax^{-/-}Bak^{-/-}$) have demonstrated the role of intrinsic apoptosis in development. $Bax^{-/-}Bak^{-/-}$ mice retain interdigital webbing on their paws, have an excess of neurons and hematopoietic cells, and display aortic arch and midline fusion defects, and females have an imperforate vagina (7, 8). Most pups die within days of being born but a significant number survive to adulthood.

Intriguingly, defects in $Bax^{-/-}Bak^{-/-}$ pups are more severe and occur earlier in $Bax^{-/-}Bak^{-/-}Bok^{-/-}$ embryos (8). Some of the expanded cell populations in $Bax^{-/-}Bak^{-/-}Bok^{-/-}$ mice are even larger in $Bax^{-/-}Bak^{-/-}Bok^{-/-}Bid^{-/-}$ embryos (9). $Bax^{-/-}Bak^{-/-}Bok^{-/-}Bid^{-/-}$ embryos also exhibit novel urogenital tract defects. Thus, all four of these BCL-2 family members

may act as proapoptotic effectors during development. Although very few triple or quadruple knockout mice survive to weaning, the individual knockouts develop normally. BOK has a similar structure to BAK (8) but is held in check by ubiquitin-dependent proteasomal degradation instigated by the E3 ubiquitin ligase gp78, rather than by prosurvival BCL-2 proteins (10). BID is a BH3-only protein that is unleashed by proteolytic cleavage, but it also has BAX-like properties because it can release cytochrome *c* and kill cells in the absence of BAX, BAK, and BOK (9, 11).

Some healthy human and mouse tissues, including the brain, heart, and kidney, are susceptible to apoptosis induced by cytotoxic chemotherapy or ionizing radiation during development but then become refractory with age, which is likely partially associated with their changes in proliferative activity. Mechanistically, downregulation of transcription factor MYC in adult tissues appears to contribute to reduced expression of BAX, BIM, and BID (12).

INTRINSIC APOPTOSIS AND CANCER

The intrinsic apoptosis pathway is a key mechanism for organisms to remove damaged cells, including transformed cells with genomic alterations. As such, impaired intrinsic apoptosis contributes to both cancer development and the resistance of cancer cells to cytotoxic therapies. The oncogenic potential of *BCL2* (B cell lymphoma 2) was first suggested when the t(14;18) chromosomal translocation found in some human follicular lymphomas was shown to place *BCL2* under the control of the immunoglobulin heavy-chain gene enhancer *Eμ*, resulting in BCL-2 overexpression. Enforced expression of *BCL2* was subsequently shown to protect cells from apoptosis induced by a range of stresses, including growth factor deprivation or uncontrolled expression of *MYC*. *BCL2* was confirmed as a proto-oncogene when an *Eμ-BCL2* transgene was shown to accelerate the development of lymphoid tumors in *Eμ-MYC* transgenic mice (2).

Other prosurvival members of the BCL-2 family have also been implicated in tumor development. For example, translocations involving *BCL2A1* and *MYC* were identified in a human diffuse large B cell lymphoma, a pairing that may functionally mimic the combined *BCL2* and *MYC* alterations that are more common in lymphoma (13). Amplification of *BCL2A1*, encoding BFL-1, is prevalent in human melanoma and is linked to poor responses to inhibitors of BRAF signaling (14). Human tumors often overexpress MCL-1 or BCL-XL. Although many tumors exhibit amplification of *MCL1* or *BCL2L1* (encoding BCL-XL) (2), posttranslational mechanisms also regulate MCL-1 abundance. E3 ubiquitin ligases, including HUWE1, SCF^{FBW7}, and MARCH5, target MCL-1 for proteasomal degradation, whereas deubiquitinating enzymes, including DUB3, USP9X, and USP13, stabilize MCL-1 (15). Therefore, alterations impacting MCL-1 protein stability may contribute to tumor development or sensitivity to cytotoxic therapies.

BH3 MIMETICS AS ANTICANCER AGENTS

A detailed understanding of intrinsic apoptosis and the reliance of some tumors on a specific prosurvival protein, such as BCL-2 in chronic lymphocytic leukemia (CLL), prompted the development of BH3 mimetics as potential cancer therapeutics. These small molecules mimic BH3-only proteins by inhibiting one or more prosurvival proteins, resulting in BAX- and BAK-dependent cell death (16). Normal cells often express multiple prosurvival BCL-2 family members, but complete loss of a single prosurvival protein can be deleterious in some cell types. For example, studies of gene knockout mice have indicated that platelets depend on BCL-XL for their survival, and diverse cell types, including hematopoietic progenitors, cardiomyocytes, and intestinal epithelial cells, rely on MCL-1. Clinical trials of navitoclax, a BH3 mimetic that inhibits both BCL-XL and BCL-2, showed that thrombocytopenia is a dose-limiting toxicity when targeting BCL-XL (16). Efforts to avoid the on-target thrombocytopenia of navitoclax yielded venetoclax,

a BH3 mimetic that spares platelets by selectively inhibiting BCL-2 (17). Venetoclax is currently approved for the treatment of relapsed or refractory CLL, and acute myeloid leukemia in elderly patients, but continues to be evaluated in the clinic in other diseases and in combination with other standard-of-care anticancer therapies (16).

Several MCL-1 inhibitors have entered clinical trials (16), but it remains to be seen if anticancer activity can be achieved without intolerable damage to the healthy tissues that require MCL-1 for their viability. Less effort has been devoted to developing inhibitors of BFL-1 or BCL-W. Elevated expression of BFL-1 in *MYC* and *BCL2* double-hit lymphomas treated with venetoclax has been identified as a drug resistance mechanism (18). In a similar vein, *Bcl2a1* was a top hit in a genome-wide CRISPR activation screen for genes protecting a double-hit mouse lymphoma line from venetoclax-induced death (19). The benefit of targeting both BFL-1 and BCL-2 in patients, however, is uncertain because resistant tumors can be polyclonal and harbor multiple, independent resistance mechanisms.

INTRINSIC APOPTOSIS AND HOST DEFENSE

Intrinsic apoptosis, alongside the other cell death programs, contributes to host defense by removing infected cells and curtailing pathogen replication. For example, BAX/BAK-dependent apoptosis of neutrophils facilitates elimination of the intracellular bacterium *Mycobacterium tuberculosis* (20). Intrinsic apoptosis is also an important antiviral defense. Many viruses have acquired BCL-2 homologs or structural mimics to evade intrinsic apoptosis, including Epstein–Barr virus, Kaposi's sarcoma–associated herpesvirus, adenovirus, myxoma virus, cytomegalovirus, and vaccinia virus (21). The Epstein–Barr virus BCL-2 homolog BHRF1 is expressed in some latently infected Burkitt lymphomas and can enhance MYC-induced lymphomagenesis in mice, prompting the suggestion that BHRF1 inhibitors be considered as a potential therapy for BHRF1-expressing cancers (22).

One way that natural killer (NK) cells and cytotoxic T cells can kill infected or malignant cells is by releasing the pore-forming protein perforin and the protease granzyme B. Perforin pores deliver granzyme B into the target cell, where it either cleaves and activates caspase-3 and -7 directly or cleaves BID and activates the caspases indirectly via the intrinsic pathway. The ratio of cytotoxic cells to their target cells, and therefore the size of the payload delivered to each target cell, may determine whether the intrinsic pathway is required for robust caspase activation. For example, tumor cell apoptosis induced by human NK cells was shown to require BAX and BAK only when the tumor cells outnumbered the NK cells (23). Another recent study identified a unique role of caspase-7 in promoting target cell apoptosis. Thought to exit target cells through perforin pores, caspase-7 can cleave and activate extracellular acid sphingomyelinase to promote membrane repair. The removal of perforin pores from the plasma membrane is proposed to prevent lytic cell death and allow execution of the less inflammatory apoptotic program (24). Intrinsic apoptosis is also important after target cells are cleared, serving to cull expanded T cell populations and restore lymphoid homeostasis (25, 26).

EXTRINSIC APOPTOSIS AND NECROPTOSIS

The extrinsic apoptosis pathway is an alternative route to activation of caspase-3 and -7 that is mediated by caspase-8 (**Figure 2**). It is instigated by death ligands that bind to death receptors on the cell surface. Death ligand and death receptor pairings include FAS ligand (FASL) with FAS, tumor necrosis factor (TNF) or lymphotoxin-α with TNF receptor 1 (TNF-R1), and TRAIL with TRAIL-R1 or TRAIL-R2 (note that mice have only one TRAIL receptor). Despite being named death receptors, these receptors do not exclusively signal cell death. Many cells respond

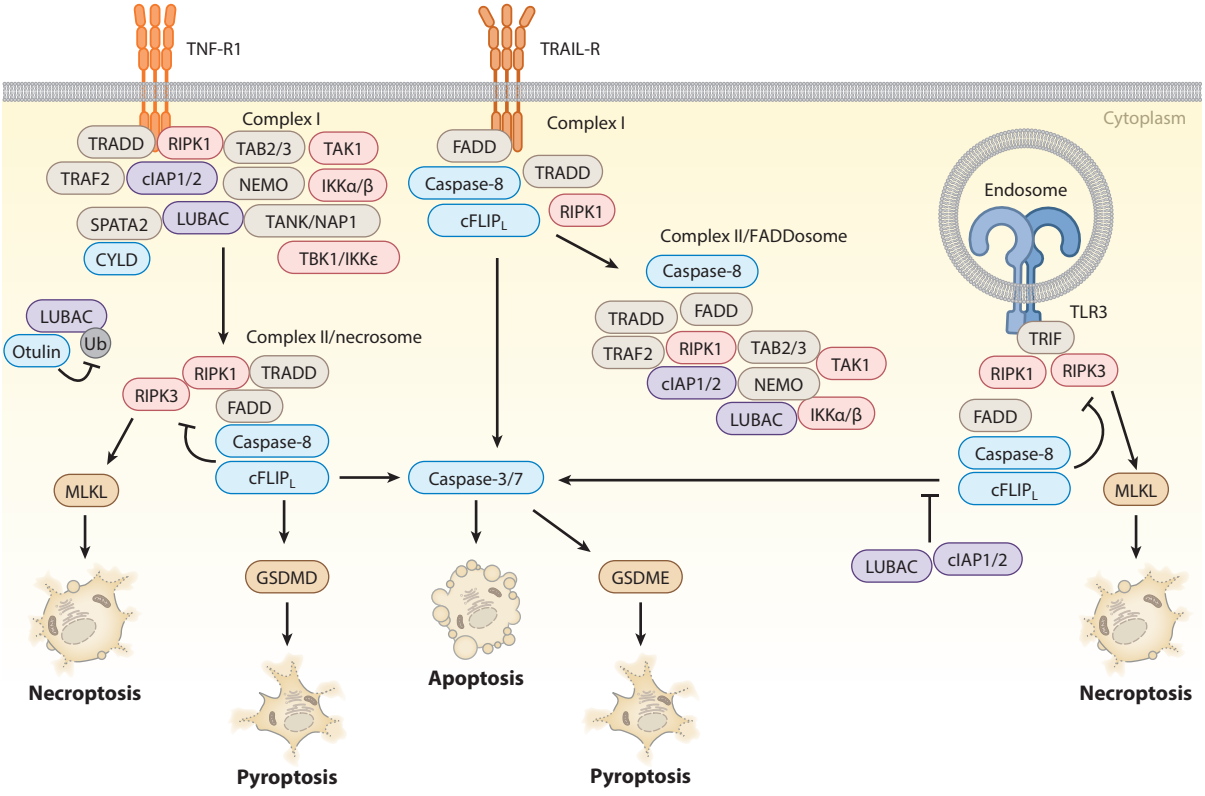

Figure 2

Extrinsic apoptosis and necroptosis. TNF-R1 can signal apoptosis upon perturbation of TNF-R1-associated complex I, whose outputs include MAPK and NF-κB activation (not shown). A secondary death-inducing complex activates caspase-8, which in turn cleaves and activates caspase-3 and -7. In certain contexts, caspase-8 may cleave GSDMD, or caspase-3 may cleave GSDME, to trigger pyroptosis. If the activity of caspase-8 is compromised, then RIPK1 can engage the related kinase RIPK3 to activate MLKL-dependent necroptosis. TRAIL receptors (and FAS; not shown) also form two complexes but in the reverse order. The receptor-associated complex I signals cell death, whereas the secondary FADDosome complex mediates MAPK and NF-κB activation. TLR3 (and TLR4; not shown) can signal cell death using the adaptor protein TRIF to recruit many of the signaling components utilized by TNF-R1. The TRIF-containing complexes are less defined biochemically, so fewer components are illustrated. Adaptors are colored light brown, ubiquitin ligases purple, kinases red, and proteases blue. Abbreviation: Ub, ubiquitin. Figure adapted from Reference 27 with permission from AAAS.

to activation of TNF-R1 not by dying but by activating NF-κB and upregulating the expression of proinflammatory genes. In these cells, other perturbations are required for TNF-induced cell death (described in the section titled TNF-R1 Signaling).

Other types of receptors, besides death receptors, can engage components of the extrinsic apoptosis pathway. Toll-like receptor 3 (TLR3), which senses double-stranded RNA within endosomes, can induce apoptosis in some cells using a signaling mechanism very similar to that of TNF-R1 (28, 29). Death receptors, TLR3, and TLR4 can also induce a caspase-independent and lytic form of cell death called necroptosis (**Figure 2**). This cell death mechanism is an important host defense strategy against pathogens that inhibit caspase-8, the apical caspase in extrinsic apoptosis. Indeed, necroptosis is unleashed only when caspase-8 is inhibited. Highlighting the role that this death program plays in host defense, certain poxviruses, murine cytomegalovirus, and herpes simplex virus express proteins that inhibit necroptosis signaling (30–32). The contribution of necroptosis to sterile inflammation and pathology is less clear. ZBP1, an intracellular sensor of

Z-form nucleic acids made by some viruses or endogenous retroviral elements, can also engage components of the extrinsic apoptosis and necroptosis pathways to kill cells (33–35).

FAS AND TRAIL RECEPTOR SIGNALING

FAS, TRAIL-R1, and TRAIL-R2, like all death receptors, have a cytoplasmic homotypic protein-protein interaction motif called the death domain (DD). Upon ligand-induced receptor oligomerization, the DD of FAS or the TRAIL receptors recruits the DD-containing adaptor protein FADD, which in turn uses its death effector domain (DED) to recruit the zymogen form of caspase-8. Tandem DEDs within the prodomain of caspase-8 drive the assembly of helical DED filaments that incorporate caspase-8, caspase-10 (absent in mice), and the pseudoprotease cFLIP (36, 37).

Caspase-8 is active either as a homodimer or as a heterodimer with the longer isoform of cFLIP ($cFLIP_L$) (38, 39). Autoprocessing between the large and small catalytic subunits of caspase-8 facilitates rearrangements that stabilize the active site (40), but this cleavage is less crucial in heterodimers (39). Therefore, heterodimers may enhance caspase-8 activation initially by cleaving homodimers. Accordingly, TRAIL-R2-induced processing of caspase-8 is slower in cFLIP-deficient cells (41). Active caspase-8 signals apoptosis by cleaving and activating caspase-3 and -7, but in some cells, including mouse hepatocytes, caspase-8 must also cleave BID. Activation of the intrinsic pathway is needed to overcome XIAP-mediated inhibition of caspase-3 and -7 (3).

$cFLIP_S$ and $cFLIP_R$ are shorter isoforms of cFLIP that largely consist of the two DEDs. $cFLIP_S$ inhibits extrinsic apoptosis by perturbing the structure of the helical DED filaments that support caspase-8 dimerization (37). An abundance of $cFLIP_L$ also prevents apoptosis, probably by limiting recruitment of caspase-8 to the death signaling complex (42). Caspase-10 remains something of an enigma. The endogenous protein can activate apoptosis in some circumstances (43) but has been described as an inhibitor of FAS-induced apoptosis (44).

Analyses of TRAIL-R2 signaling have shown that a secondary signaling complex can form after the death signaling complex (45). This secondary complex, containing caspase-8, FADD, RIPK1, TRAF2, and NEMO, mediates activation of mitogen-activated protein kinase (MAPK) and NF-κB-dependent gene transcription. Subsequent studies confirmed that FADD and caspase-8 nucleate a TRAIL receptor signaling complex, now termed the FADDosome, but clarified that caspase-8 serves as a scaffold and does not have to be enzymatically active (46, 47). The adaptor protein TRADD, ubiquitin ligases cIAP1 and cIAP2, kinase TAK1, and ubiquitin-binding protein A20 also regulate the output of the FADDosome. The function of each of these proteins is best characterized during TNF-R1 signaling, which is described in the next section. Given that FAS can signal proinflammatory gene expression in some cells, it probably elicits a similar FADDosome to the TRAIL receptors (48, 49).

Assembly of the FADDosome in the presence of a pan-caspase inhibitor can trigger necroptosis if cells express RIPK3 and MLKL (50, 51). FASL- or TRAIL-induced necroptosis requires the kinase activity of RIPK1, which mediates RIPK1 autophosphorylation (50). No other substrates of RIPK1 have been identified to date. RIPK1 uses its RIP homotypic interaction motif (RHIM) to recruit the RHIM-containing RIPK3, and oligomerization of RIPK3 promotes its autophosphorylation and activation (50). The pseudokinase MLKL is phosphorylated by RIPK3, prompting MLKL oligomerization and translocation to the plasma membrane, where it causes cell lysis (51).

FASL and TRAIL contribute to the clearance of virus-infected cells, but they also play an important role in lymphoid homeostasis (25, 26, 52). Loss-of-function mutations in *FASLG* (encoding FASL) or *FAS* cause autoimmune lymphoproliferative syndrome (ALPS), a disease characterized by the aberrant survival of rare, unconventional B and T cells (53, 54). Curiously, FADD

or caspase-8 deficiency, which disables all extrinsic apoptosis, affects mice and humans differently. Mice lacking either FADD or catalytically active caspase-8 die during embryogenesis from unchecked RIPK1-, RIPK3-, and MLKL-dependent necroptosis, particularly in endothelial cells (50). Necroptosis stems from caspase-8 not cleaving and inactivating RIPK1 (55–57). In humans, however, FADD or caspase-8 deficiency causes ALPS, recurrent infections, and very early onset inflammatory bowel disease (58–60). Immunodeficiency may stem from caspase-8 not cleaving and inactivating N4BP1, a protein that suppresses the expression of a subset of proinflammatory cytokines and chemokines (61). The contribution of aberrant necroptosis to the human disease is unclear. Caspase-8 cleavage of RIPK1 must limit inflammation in some manner, because humans that have heterozygous *RIPK1* mutations that eliminate the caspase-8 cleavage site exhibit a periodic fever syndrome (56, 57).

TNF-R1 SIGNALING

TNF-R1 also signals via two sequential complexes, but in the reverse order to FAS or the TRAIL receptors. The receptor-associated complex I promotes gene expression, whereas secondary, cytoplasmic complexes signal cell death (62). Complex I assembly begins with the DD in oligomerized TNF-R1 recruiting the DD-containing proteins TRADD and RIPK1. TRADD recruits TRAF2, which associates with the ubiquitin ligases cIAP1 and cIAP2. RIPK1 and other components of complex I are ubiquitinated by cIAP1 and cIAP2. K63-linked polyubiquitin chains recruit TAK1 and the linear ubiquitin chain assembly complex (LUBAC) comprising HOIP (also called RNF31), HOIL-1 (also called RBCK1), and SHARPIN. LUBAC modifies TNF-R1, TRADD, RIPK1, and K63-linked polyubiquitin within complex I with M1-linked polyubiquitin, which enhances recruitment of the canonical IκB kinase (IKK) complex comprising NEMO (also called IKKγ), IKKα, and IKKβ. Together, TAK1 and IKK drive the NF-κB, ERK, JNK, and p38 MAPK pathways that induce the expression of proinflammatory genes. LUBAC and NEMO also recruit the kinases TBK1 and IKKε to complex I (62, 63).

A secondary, death-inducing complex forms when either TRADD or RIPK1 moves into the cytoplasm to interact with FADD and caspase-8. Interactions between RIPK1, FADD, and caspase-8 are dependent on the kinase activity of RIPK1, which is first detected in complex I in the form of RIPK1 autophosphorylation. The DD of RIPK1 mediates dimerization of the kinase for autophosphorylation in *trans*. Precisely how RIPK1 autophosphorylation facilitates interactions with FADD is unclear. In contrast to its role in cell death signaling, the kinase activity of RIPK1 is dispensable for TNF-induced MAPK and NF-κB signaling (50). If there is robust activation of caspase-8 within the death signaling complex, then the cell typically dies by apoptosis. In some cells, however, caspase-8 cleavage of GSDMD (64, 65), or caspase-3 cleavage of GSDME (66), elicits a proinflammatory form of cell death called pyroptosis (detailed in the section titled Pyroptosis, below). If caspase-8 is lost or inhibited, then RIPK1 within the death signaling complex engages RIPK3, resulting in MLKL-dependent necroptosis. Note that the RIPK3 scaffold can also contribute to TNF-induced apoptosis in some settings (55, 56, 67). Hence, only MLKL deficiency is suited to determining the contribution of necroptosis to a biological process. Curiously, some organisms, including birds, encode MLKL but not RIPK3. Whether other kinases can activate MLKL in these organisms is unclear.

Many cells, including primary mouse fibroblasts and macrophages, are not killed by treatment with TNF alone. Several mechanisms appear to hold TNF-R1-induced cell death in check. cFLIP, which can be upregulated by NF-κB signaling, limits caspase-8 activation by TRADD and FADD, and low-level caspase-8 activation by RIPK1 and FADD is self-limiting because caspase-8 cleaves RIPK1 and disrupts the death signaling complex. Accordingly, mutations that destroy the

caspase-8 cleavage site in RIPK1 sensitize cells to TNF-induced caspase-8-dependent cell death (55–57). Caspase-8 can also cleave and inactivate CYLD, the enzyme that deubiquitinates RIPK1 and promotes its transition to the cytoplasmic death signaling complex (15). The ubiquitin binding protein A20 suppresses TNF-induced cell death, at least in part, by limiting CYLD access to M1-linked polyubiquitin. The ubiquitin binding protein ABIN1 appears to work in concert with A20 to suppress RIPK1 activation and TNF-induced cell death (15).

Kinases activated by complex I, including IKK, TBK1, and MK2, constitute another brake on TNF-induced cell death (50). They limit activation of RIPK1 by phosphorylating distinct sites on RIPK1. NF-κB signaling downstream of complex I can also limit cell death by inducing prosurvival genes that suppress TNF-induced cell death. *Xiap*, *Tnfaip3* (encoding A20), *Birc2* and *Birc3* (encoding cIAP1 and cIAP2), and *Cflar* (encoding cFLIP) are known NF-κB target genes (50). Perturbations that compromise complex I disable these prosurvival mechanisms. Consequently, cells lacking cIAP1 and cIAP2, NEMO, TAK1, TBK1, LUBAC, or OTULIN are sensitized to TNF-induced cell death. OTULIN is a deubiquitinating enzyme that counters LUBAC autoubiquitination and thereby preserves LUBAC ubiquitin ligase activity (15). Mutation of ubiquitination site Lys376 in mouse RIPK1 also destabilizes complex I and promotes TNF-induced cell death (68–70). Lysosomal targeting of death signaling complexes by autophagy-related 9A (ATG9A) was recently identified as another mechanism limiting TNF-induced cell death (71).

CELL DEATH AS A DRIVER OF INFLAMMATION

Studies using gene knockout mice or blocking antibodies have shown that TNF and TNF-R1 signaling is required for efficient clearance of certain pathogens (20) but also contributes to pathology in chronic inflammatory diseases (62). Indeed, TNF inhibitors are an important part of the medical armamentarium against a range of inflammatory disorders, including inflammatory bowel disease and rheumatoid arthritis. Although TNF-R1 signaling upregulates the expression of proinflammatory genes, genetic studies in mice have illustrated how aberrant TNF-R1-induced cell death can also elicit inflammation. For example, $Sharpin^{Cpdm}$ mutant mice develop chronic proliferative dermatitis that is dependent on TNF-R1, CYLD, FADD, caspase-8, and the kinase activity of RIPK1 (50, 72). Mice lacking *Nemo* in intestinal epithelial cells develop colitis that is dependent on TNF-R1, FADD, and the kinase activity of RIPK1 (73). Thus, in both the *Sharpin* and *Nemo* mutant mice, inflammation coincides with cleaved caspase-3 immunolabeling, and both features are prevented by inhibiting the cell death arm of TNF-R1 signaling. These studies indicate that excessive TNF-R1-induced caspase-8-dependent cell death can drive skin or intestinal inflammation despite impaired NF-κB signaling. Widespread cell death, even apoptosis, might be proinflammatory in these barrier tissues because it creates points of entry for microbes.

Aberrant necroptosis is also proinflammatory. For example, necroptosis of caspase-8-deficient mouse intestinal epithelial cells elicits colitis, but in this model, death signals emanate from either TNF-R1 or ZBP1 (74). In other mouse models, multiple death receptors induce cell death and inflammation. For example, HOIL-1 deficiency in mouse keratinocytes causes a lethal dermatitis that stems from caspase-8-dependent cell death downstream of TNF-R1, FAS, and TRAIL-R, plus a still-to-be-determined death inducer (75). TLR3 and TLR4 are both candidate death inducers because they signal through TRIF, an adaptor with a RHIM that can recruit RIPK1 or RIPK3 (50) (**Figure 2**). TLR3 and TLR4 agonists, like TNF, often upregulate proinflammatory genes without inducing cell death. However, both TLRs trigger caspase-8-dependent cleavage of N4BP1, indicating that they must activate caspase-8 to some extent (61). LUBAC deficiency, or combined cIAP1 and cIAP2 deficiency, skews TLR3 signaling toward robust caspase-8 activation and cell death (29, 76).

HOIP, HOIL-1, OTULIN, and TBK1 suppress extrinsic apoptosis in both mouse and human cells; therefore, increased cell death may contribute to the autoinflammatory syndromes associated with their loss in humans (15, 77). Inflammation caused by TBK1 or OTULIN loss is ameliorated by TNF inhibition, consistent with aberrant TNF signaling fueling these diseases. OTULIN deficiency has been successfully treated with hematopoietic stem cell transplantation (78), suggesting that immune cell dysfunction drives disease. In contrast to humans, mice lacking HOIL-1, HOIP, OTULIN or TBK1 die from aberrant cell death during embryogenesis (50).

Although the catalytic activity of RIPK1 promotes necroptosis and some forms of extrinsic apoptosis, genetic studies indicate that the RIPK1 scaffold plays an important role in limiting inflammation. Humans lacking RIPK1 exhibit lymphopenia, recurrent infections, arthritis, and very early onset inflammatory bowel disease (79, 80). Aberrant cell death may contribute to the disease because patient fibroblasts are abnormally sensitive to TNF- or TLR3-induced cell death (79). Immune cell dysfunction is implicated because hematopoietic stem cell transplantation was beneficial in one patient (79). Mice lacking RIPK1 die soon after birth and have more widespread inflammation, owing to unchecked caspase-8-dependent cell death as well as ZBP1- or TRIF-induced necroptosis. By contrast, mice expressing catalytically inactive RIPK1 are viable, indicating that the kinase activity of RIPK1 is dispensable for its prosurvival function. The RIPK1 RHIM promotes cell survival because mice expressing RHIM mutant RIPK1 die at birth from necroptosis induced by ZBP1 and, to a lesser extent, TRIF (50). Precisely how the RHIM of RIPK1 inhibits ZBP1- or TRIF-induced necroptosis is unclear, as biochemical evidence of a sequestration mechanism has proven elusive. It has been suggested that RIPK1 suppresses caspase-8-dependent cell death by limiting interactions between TRADD and FADD (81).

TARGETING RIPK1 IN INFLAMMATORY DISEASES

Several companies have developed inhibitors of the kinase activity of RIPK1 with the goal of reducing proinflammatory cell death in disease settings. This strategy aims to break the cycle of cell death and inflammation that is thought to drive disease progression. Inhibiting the catalytic activity of RIPK1 has also been shown to limit the expression of *Ifnb* and other genes in the unique setting of caspase-8 inactivation and necroptosis deficiency (82, 83). Thus, although cell death is probably the normal output of signaling complexes containing active RIPK1, it is not the only output, and this second layer of RIPK1-mediated immune defense, namely driving proinflammatory gene expression, may combat pathogens that inhibit both caspase-8 and MLKL. For example, some poxviruses encode homologs of $cFLIP_S$ and MLKL that inhibit caspase-8-dependent cell death and necroptosis, respectively (32). In this regard, it would be interesting to see if RIPK1 inhibition altered immune responses against viruses bearing these death inhibitory molecules.

Inhibiting RIPK1 has been shown to ameliorate disease in preclinical models of ischemia-reperfusion injury, stroke, retinitis pigmentosa, Alzheimer's disease, multiple sclerosis, atherosclerosis, arthritis, inflammatory bowel disease, acetaminophen hepatotoxicity, and chronic obstructive pulmonary disease (50, 84, 85). Although clinical trials of the RIPK1 inhibitor GSK2982772 did not reveal a benefit in rheumatoid arthritis or ulcerative colitis (ClinicalTrials.gov studies NCT02858492 and NCT02903966), other RIPK1 inhibitors continue to be trialed in cutaneous lupus erythematosus (ClinicalTrials.gov study NCT04781816) and amyotrophic lateral sclerosis (ClinicalTrials.gov study NCT05237284).

PYROPTOSIS

The term pyroptosis was coined to describe the lytic death of macrophages infected with *Salmonella* bacteria, but now it refers to all cell death elicited by gasdermin pores in the plasma

membrane (GSDMA, GSDMB, GSDMC, GSDMD, GSDME, and GSDMF in humans) (86–88). Pyroptosis has been observed in diverse cell types, including hematopoietic, endothelial, and epithelial cells. The dying cells often swell and then burst owing to plasma membrane rupture (PMR). Permeabilization of the plasma membrane releases proinflammatory intracellular molecules that are collectively referred to as damage-associated molecular patterns (DAMPs). Neighboring cells sense these DAMPs and trigger antipathogen innate immune responses. Studies with *Gsdmd*-deficient mice have demonstrated that GSDMD-dependent pyroptosis contributes to the clearance of intracellular bacterial pathogens, including *Salmonella* and *Burkholderia* (89, 90). However, some pathogens have evolved strategies to evade this host defense mechanism. For example, *Shigella* bacteria inject the E3 ubiquitin ligase IpaH7.8 into cells to ubiquitinate GSDMD and GSDMB, which then target the gasdermins for proteasomal degradation (91, 92).

Pyroptosis is triggered by multiple upstream mechanisms that all converge on proteolytic cleavage of a gasdermin to liberate the N-terminal pore-forming domain (PFD) from the C-terminal inhibitory domain (**Figure 3**). Cytoplasmic inflammasome complexes, which are assembled in response to invading pathogens or sterile insults, are well-characterized inducers of pyroptosis (88). Canonical inflammasomes activate caspase-1, whereas the noncanonical inflammasome activates caspase-4 or its paralog caspase-5 (the human counterparts of mouse caspase-11). Caspase-1, -4, -5, and -11 each cleave GSDMD to release the PFD that triggers

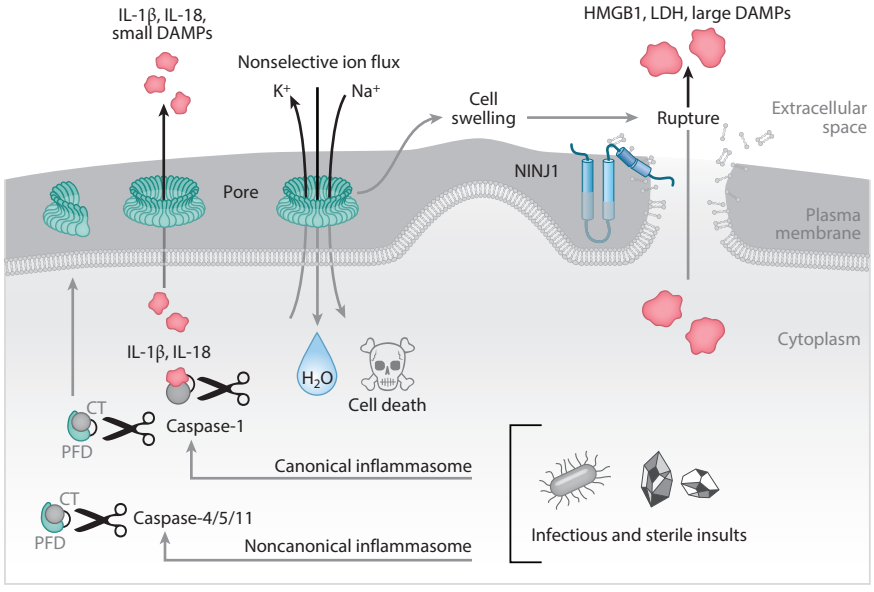

Figure 3

GSDMD-dependent pyroptosis. Infections and sterile perturbations are sensed by cytoplasmic inflammasome sensors that activate caspase-1 (canonical inflammasome) or caspase-4 and -5 (human counterparts of mouse caspase-11). GSDMD cleavage by these caspases separates the PFD of GSDMD from its self-inhibitory CT domain. The PFD translocates to the plasma membrane, where it undergoes dramatic conformational changes, membrane insertion, and oligomerization into a ring-shaped pore. The pore releases IL-1β and small DAMPs and causes nonselective ion flux that induces cell death (pyroptosis). Water influx causes cell swelling, and NINJ1 mediates plasma membrane rupture, releasing LDH (a biomarker for the rupture) and large DAMPs, including HMGB1. Abbreviations: CT, C-terminal; DAMP, damage-associated molecular pattern; IL-1β, interleukin-1β; LDH, lactate dehydrogenase; NINJ1, nerve injury–induced protein 1; PFD, pore-forming domain. Figure adapted from Reference 27 with permission from AAAS.

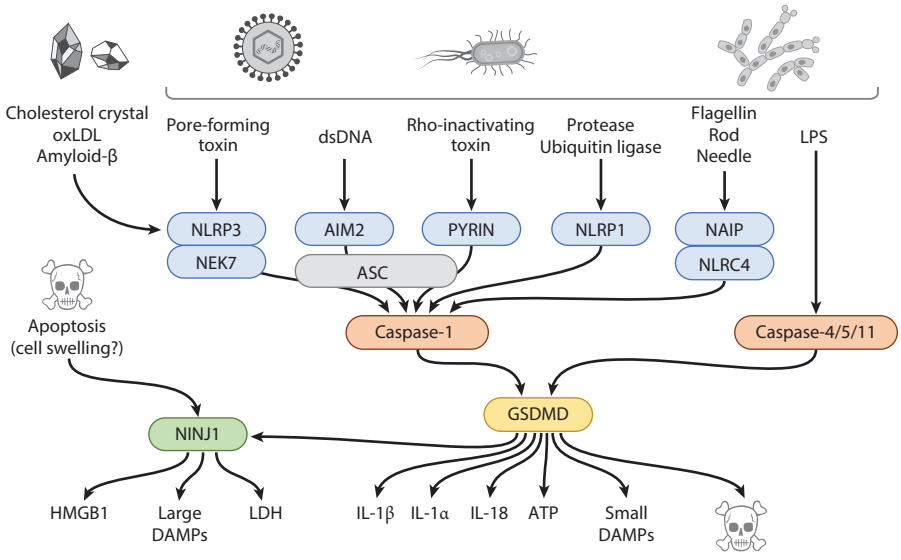

Figure 4

The pyroptosis landscape. Microbes and sterile insults produce unique molecular patterns or cellular perturbations that are detected by cytoplasmic inflammasome sensors such as NLRP3. The sensors activate caspase-1, -4, -5, or -11, which cleave GSDMD to trigger GSDMD pore formation. GSDMD pores in the plasma membrane cause cell death and release small proinflammatory molecules, including IL-1β, IL-1α, and ATP. GSDMD also triggers NINJ1-mediated plasma membrane rupture that releases large DAMPs, including HMGB1. Various inducers of apoptosis and necrosis also engage NINJ1-dependent plasma membrane rupture. Abbreviations: ATP, adenosine triphosphate; DAMP, damage-associated molecular pattern; dsDNA, double-stranded DNA; IL, interleukin; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NINJ1, nerve injury–induced protein 1; oxLDL, oxidized low-density lipoprotein. Figure adapted from Reference 27 with permission from AAAS.

pyroptosis. Caspase-1 also cleaves pro-interleukin-1β (pro-IL-1β) and pro-IL-18 to yield biologically active IL-1β and IL-18, respectively. IL-1β is a potent pyrogen, so its release during pyroptosis makes this a highly proinflammatory form of cell death. Note that activation of the noncanonical inflammasome can also elicit mature IL-1β because cellular perturbations associated with GSDMD pore formation can trigger the NLRP3 inflammasome to activate caspase-1 (88).

Canonical inflammasome complexes are nucleated by proteins that directly or indirectly sense pathogen-associated molecular patterns (PAMPs), DAMPs, or cytoplasmic perturbations. Different sensors respond to different stimuli. For example, nucleotide-binding domain leucine-rich repeat–containing protein 1b (NLRP1b) is activated by the *Bacillus anthracis* lethal factor protease; absent in melanoma 2 (AIM2) recognizes cytoplasmic DNA; PYRIN detects inactivation of Rho GTPase activity by bacterial toxins; and NLRP3 responds to diverse stimuli, including extracellular ATP, potassium ionophore nigericin, bacterial pore-forming toxin listeriolysin O, and gout-associated urate crystals (**Figure 4**). Conformational changes in the canonical inflammasome sensors prompt the assembly of higher order complexes that incorporate the adaptor protein ASC and caspase-1. Noncanonical caspase-4, -5, and -11 are activated by cytoplasmic bacterial lipopolysaccharide (LPS) (88).

Once GSDMD is cleaved, the PFD binds to plasma membrane phospholipids and undergoes a major conformational change, creating a membrane-spanning, four-stranded, antiparallel β sheet. The membrane-bound PFD oligomerizes to form a ring-shaped pore, which in liposomes

has 33-fold symmetry and an inner diameter of approximately 22 nm (93). In cells, GSDMD pores cause nonselective ion flux and collapse electrochemical gradients, resulting in cellular demise. Other proteases that can cleave GSDMD and release the pyroptosis-inducing PFD include caspase-8, neutrophil elastase, cathepsin G, and Zika virus NS2B3 protease (87).

GSDMD pores formed after inflammasome activation release the leaderless cytokines IL-1β and IL-18, as well as many other small DAMPs, including IL-1α and IL-33 (**Figure 3**). Unlike IL-1β and IL-18, IL-1α and IL-33 do not strictly require proteolytic maturation for their cytokine activities (94). Some studies have suggested that live cells can release IL-1β through sublytic GSDMD pore formation (95, 96), but this remains controversial. Single-cell time-lapse microscopy has suggested that IL-1β is released only from dying cells (97, 98).

NINJ1-DEPENDENT PLASMA MEMBRANE RUPTURE

Pyroptotic cells rapidly undergo secondary PMR that releases large intracellular molecules, including lactate dehydrogenase (LDH), a commonly used marker of cell rupture, and HMGB1, a DAMP that binds to DNA in living cells (**Figure 3**). For many years, PMR was considered a passive osmolysis event. That view changed after PMR during pyroptosis was shown to be dependent on nerve injury–induced protein 1 (NINJ1) (99). Cells lacking NINJ1 die with a distinctive ballooned morphology and fail to release many large intracellular molecules, including LDH and HMGB1. As a genetically programmed postdeath event, NINJ1-dependent PMR may have evolved as a host defense mechanism that alerts the immune system to infection. Consistent with this idea, *Ninj1* deficiency in mice confers susceptibility to *Citrobacter rodentium* and *Yersinia pseudotuberculosis* bacterial infections (99, 100).

NINJ1 is a 16-kDa integral membrane protein expressed on the cell surface. It is monomeric or dimeric in live cells but forms oligomers in pyroptotic cells (99). Cryoelectron microscopy revealed that human NINJ1 can oligomerize to form long filaments. These structures might promote PMR by capping the edges of membranes (101). The precise events triggering oligomerization of NINJ1 in cells still have to be defined. The N terminus of NINJ1 has been suggested to mediate cell-to-cell adhesion (102), but this region of the protein is dispensable for PMR and is poorly conserved across species. Given that *Drosophila* orthologs of mammalian NINJ1 can also induce PMR (99), alternative mechanisms of NINJ1 regulation are anticipated.

NINJ1-dependent PMR is not exclusive to pyroptosis and has also been observed in cultured cells dying by necrosis or apoptosis. However, PMR in cells treated with apoptosis-inducing chemotherapeutic drugs or necrosis-inducing bacterial pore-forming toxins does not rely on gasdermins (99). How pyroptosis, necrosis, and apoptosis trigger activation of NINJ1 is of intense interest. An evolutionarily conserved amphipathic helix in NINJ1 may sense membrane lipid packing defects or membrane curvature change caused by cell swelling. Apoptotic cells could swell after ATP depletion causes the Na^+/K^+-ATPase pump to malfunction. Alternatively, if apoptotic cells express GSDME, then caspase-3 may cleave GSDME to facilitate cell swelling that leads to PMR (66). Of note, NINJ1 is not the only mediator of PMR, because NINJ1-deficient cells undergoing necroptosis still release LDH and other large molecules (99). Oligomerized MLKL may be sufficient to disrupt the plasma membrane and release DAMPS during necroptosis.

THE GASDERMIN FAMILY

With the exception of GSDMF, members of the gasdermin family (GSDMA, GSDMB, GSDMC, GSDMD, and GSDME) have a PFD that is kept in check by a C-terminal inhibitory domain. The mouse genome harbors three *Gsdma* genes (*Gsdma1*, *Gsdma2*, and *Gsdma3*), four

Gsdmc genes (*Gsdmc1*, *Gsdmc2*, *Gsdmc3*, and *Gsdmc4*), and *Gsdmd*, *Gsdme*, and *Gsdmf* (87). Mice lack *Gsdmb*. Evolutionary selection pressures from pathogens might explain the large variety of gasdermin genes. Caspase-3 can cleave GSDME (66) to induce pyroptosis or secondary plasma membrane damage after apoptosis (104). The kinetics of gasdermin cleavage compared with other caspase-3 proapoptotic substrates may vary according to cell type and/or context. The kinetics of substrate cleavage may determine whether a cell dies by pyroptosis or apoptosis (with secondary plasma membrane damage). Efficient efferocytosis of apoptotic cells probably limits the impact of secondary membrane damage in vivo (6).

Gsdme deficiency in mice has been shown to limit chemotherapy-induced tissue damage (66) but at the cost of increased susceptibility to *Yersinia* infection. The latter is attributed to impaired neutrophil pyroptosis (105). GSDME, like caspase-3, can also be cleaved by granzyme B (106). Therefore, tumor cells expressing sufficient GSDME may undergo pyroptosis rather than apoptosis when targeted by NK cells and cytotoxic T cells. The proinflammatory nature of pyroptosis may be beneficial in evoking antitumor acquired immunity, but whether pyroptosis is better than apoptosis or necroptosis at promoting antitumor immunity is harder to assess. Apoptotic cancer cells might promote antitumor activity by releasing proinflammatory ATP through the pannexin-1 channel (107) or by being phagocytosed by professional antigen-presenting cells (108).

Granzyme A targeted to tumor cells by cytotoxic lymphocytes is reported to cleave human GSDMB (109), but whether GSDMB induces pyroptosis is contentious (110). *GSDMB* is expressed throughout the gastrointestinal tract but is often silenced in gastric and esophageal cancers (109), which might indicate that GSDMB loss confers a survival advantage to tumor cells. Curiously, there are at least six splice variants of *GSDMB*, and two different promoters have been identified in the *cis*-regulatory elements of *GSDMB* exons 1a and 1b (111). The expression patterns and pore-forming abilities of these different GSDMB isoforms are ill characterized, but the use of different GSDMB isoforms in different studies might explain their discrepant results.

Human GSDMA and mouse GSDMA1 are expressed in epithelial cells and proteolytically activated by streptococcal pyrogenic exotoxin B (SpeB) (112, 113). In this context, GSDMA acts as an intracellular sensor of microbial proteolysis to induce pyroptosis. SpeB, like granzyme B, is a promiscuous protease that can cleave many other proteins, including IL-1β and immunoglobulin. How SpeB enters cells is unclear. Pores formed by streptolysin O, another streptococcal pyrogenic exotoxin, may allow entry of extracellular SpeB. Alternatively, intracellular *Streptococcus pyogenes* bacteria may be the source of GSDMA-activating SpeB. Consistent with GSDMA-induced pyroptosis being an important host defense mechanism, mice lacking *Gsdma1* (or *Gsdma1*, *Gsdma2*, and *Gsdma3*) are more susceptible to infection with group A *Streptococcus* (112, 113). Excessive activation of GSDMA3-dependent pyroptosis, owing to mutation of the C-terminal inhibitory domain in mouse *Gsdma3*, is associated with epidermal hyperplasia and skin inflammation (114).

Less is known about activation of GSDMC. Caspase-8 can cleave GSDMC to induce pyroptosis in certain tumor cells (115), but it does so by cleaving the GSDMC C terminus, rather than the linker region between the N-terminal PFD and the C-terminal inhibitory domain (115). GSDMC has been implicated in host defense because mice lacking *Gsdmc1*, *Gsdmc2*, *Gsdmc3*, and *Gsdmc4* in intestinal epithelial cells have an attenuated response to helminth infection. GSDMC pores are proposed to release IL-33 from goblet and Paneth cells (116).

Gasdermin-like proteins have been identified in bacteria and fungi. Caspase-like proteases cleave and activate these pore-forming proteins in response to phage or virus infection (117). Therefore, gasdermins probably evolved from an ancient cell death machinery that defends against foreign invaders.

PYROPTOSIS IN INFLAMMATORY DISEASES

Although pyroptosis probably evolved to defend against pathogens, excessive pyroptosis can be deleterious. This point is illustrated in mouse models of sepsis, wherein GSDMD drives lethality in response to high-dose LPS or cecal ligation puncture, with the latter triggering polymicrobial sepsis (118, 119). Precisely how GSDMD contributes to sepsis pathology is ill defined and likely complex. GSDMD pores probably release IL-1β, IL-18, and other DAMPs to cause systemic inflammation, while GSDMD-dependent pyroptosis of endothelial cells may contribute to capillary leakage, tissue edema, and organ malfunction (120). Tissue factor activation by GSDMD pore-dependent Ca^{2+} influx may cause microvascular coagulation during acute sepsis (121). Neutrophil extracellular traps (NETs), which are netlike structures consisting of DNA-histone complexes that can be released from pyroptotic neutrophils (122, 123), may also promote microvascular coagulation during sepsis. However, whether GSDMD is essential for NET release is debated (124).

GSDMD has also been shown to contribute to pathology in mouse models of systemic inflammatory response syndrome, acute respiratory distress syndrome, autoimmune encephalomyelitis, whole-body irradiation, and allergic airway inflammation (87, 125). In humans, the pathogenic potential of pyroptosis is inferred from inherited autoinflammatory diseases that are caused by germ-line gain-of-function mutations in *NLRP3* [cryopyrin-associated periodic syndrome (CAPS)] or *MEFV* [encoding *PYRIN*; familial Mediterranean fever (FMF)]. Patients typically respond to anti-IL-1 therapy, and both IL-1β release and pathologic lesions in mice bearing CAPS- or FMF-associated mutations are ameliorated by *Gsdmd* deficiency (126, 127). Whether pyroptosis releases IL-1β in CAPS and FMF patients remains to be determined. In an NLRP3-driven mouse model of autoinflammation, real-time imaging of single cells rather than the standard LDH measurement was required to detect the rare cells that died spontaneously and released IL-1β (98). Pyroptosis is also implicated in severe coronavirus disease 2019 (COVID-19) because early treatment with the IL-1 receptor antagonist anakinra has reduced mortality in COVID-19 patients (128). The NLRP3 inhibitor MCC950/CRID3 has also been shown to ameliorate disease in mice infected with severe acute respiratory syndrome coronavirus 2 (129). Human *GSDMD* loss-of-function polymorphisms have been discovered, but whether they influence disease is unclear (130).

Pyroptosis induced by caspase-3 cleavage of GSDME has been shown to contribute to inflammation in mice after cytotoxic chemotherapy (66), viral infection (131), or chimeric antigen receptor T cell therapy (132). *GSDME* expression is epigenetically silenced by methylation in many tumor lines, suggesting that GSDME may act as a tumor suppressor. In this regard, it would be interesting to see if *Gsdme* deficiency enhances tumorigenesis in sporadic tumor mouse models. Genome-wide association studies link *GSDMB* polymorphisms to multiple chronic diseases, including inflammatory bowel disease, asthma, and rheumatoid arthritis (87, 103), but further mechanistic insights are needed. Skin lesions in mice infected with group A *Streptococcus* are ameliorated by *Gsdma1* deficiency (112), but perplexingly, these lesions are worse in mice concomitantly lacking *Gsdma1*, *Gsdma2*, and *Gsdma3* (112). More work is needed to resolve the roles of the different GSDMA isoforms.

TARGETING PYROPTOSIS AND PLASMA MEMBRANE RUPTURE IN INFLAMMATORY DISEASES

NLRP3 has been seen as the most attractive drug target among the pyroptosis-inducing inflammasome sensors (133). It responds to diverse endogenous insults, including gout-related urate crystals, atherosclerosis-associated cholesterol crystals and oxidized low-density lipoprotein,

and Alzheimer's disease–pertinent amyloid-β. Precisely how NLRP3 senses these structurally unrelated insults is unclear. Studies with *Nlrp3*-deficient mice have revealed that NLRP3 is an important driver of sterile inflammation in many disease models. Small-molecule inhibitors of NLRP3 have been developed, including DFV890 (Novartis/IFM Therapeutics) and Selnoflast (Roche/Inflazome). Preclinical and clinical trials have focused on CAPS, osteoarthritis, COVID-19, and neuroinflammation. Many of the NLRP3 inhibitors being tested in clinical trials are analogs of MCC950/CRID3, which is widely used as a tool inhibitor but has limited clinical potential owing to off-target hepatotoxicity. Cryoelectron microscopy has revealed that MCC950 binds to NLRP3 at the interface of four NACHT domains and inhibits its ATPase activity, thereby preventing oligomerization of the NLRP3 inflammasome (134).

IL-1β has been shown to contribute to atherosclerosis-related cardiovascular events, particularly in patients with clonal hematopoiesis driven by mutant *TET2* (135). Given that NLRP3 deficiency or treatment with MCC950 is atheroprotective in a mouse model of this disease (136), an inhibitor of NLRP3 may provide a similar benefit to IL-1β blockade. By sparing other pathogen sensors, including AIM2 and NLRP1, inhibitors of NLRP3 may offer a lower risk of infection than IL-1β blockade. Intriguingly, when clonal hematopoiesis is driven by a gain-of-function *Jak2* mutation in atherosclerosis-prone mice, it is AIM2 rather than NLRP3 that contributes to disease severity (137). These insights illustrate the potential for personalized therapies where the appropriate inflammasome inhibitor is selected on the basis of the somatic mutations identified.

Allosteric inhibitors of NIMA-related kinase 7 (NEK7) may be an alternative strategy to inhibit NLRP3. NEK7, independent of its kinase activity, promotes assembly of the NLRP3 inflammasome by binding to the leucine-rich repeats in NLRP3 (134). Accordingly, mouse macrophages lacking either NLRP3 or NEK7 are unresponsive to NLRP3 inflammasome stimuli (138). Although the contribution of NEK7 to NLRP3 inflammasome activity in human myeloid cells is less certain (139), the safety of allosteric NEK7 inhibitor HT-6184 (Halia Therapeutics) is being assessed in humans (ClinicalTrials.gov study NCT05447546).

Targeting GSDMD, with the goal of inhibiting pyroptosis and DAMP release downstream of all the different inflammasomes, may be more effective than targeting either NLRP3 or IL-1β alone in fulminant inflammation (**Figure 4**). A subset of patients with severe sepsis or septic shock have been shown to respond to the IL-1 receptor antagonist anakinra (140); thus, biomarkers of pyroptosis will be needed to identify the patients that might benefit from GSDMD inhibition. Although several small-molecule inhibitors of GSDMD have been reported (87), they all appear to lack either specificity or potency. One example is disulfiram (Antabuse), which also inhibits aldehyde dehydrogenase and, for many years, has been used for the treatment of alcoholism. Disulfiram modifies GSDMD covalently and thereby prevents GSDMD pore formation and pyroptosis (141). Upstream caspases and the transcription factor NF-κB, which regulates the expression of *Casp11*, *Il1b*, and *Nlrp3* expression, are other targets of disulfiram. Therefore, disulfiram is not a tool to implicate GSDMD specifically in biological processes. Other limitations of disulfiram include its short half-life and toxic metabolites. Whether disulfiram can inhibit GSDMD at clinically relevant doses in vivo remains unclear.

Sublytic GSDMD pore formation in cells has been proposed (96), but its physiological and pathological significance is still largely unknown. Cells may be able to repair the membrane damage caused by a small number of pores. Transient GSDMD pore formation might explain how anti-ASC nanobodies can enter cultured cells to limit the release of IL-1β and PMR (142). Mice dosed with anti-ASC nanobodies are resistant to urate crystal–induced gout, but it is unclear if the nanobodies target intracellular ASC. Extracellular ASC complexes released from pyroptotic cells might also be targeted, because these complexes have been shown to activate inflammasome signaling when phagocytosed by neighboring cells. Regardless, transient GSDMD pores might

prove a convenient point of entry for other inhibitory nanobodies and cell impermeable compounds.

NINJ1 is dispensable for genetically programmed cell death, but inhibiting NINJ1-dependent PMR and the release of large DAMPs from dying cells may limit the inflammation that is associated with excessive cell death. *Ninj1* deficiency in mice is reported to ameliorate several diseases, including pulmonary fibrosis (143) and multiple sclerosis (144). A single-nucleotide polymorphism in human *NINJ1* has been linked to low serum LDH (145), which is consistent with the role of NINJ1 in PMR (99), but whether this polymorphism is related to disease is unknown. An intriguing observation is that glycine inhibits NINJ1-mediated PMR, albeit with low efficacy [half-maximal inhibitory concentration (IC_{50}) ~ 1.7 mM] (146, 147). This inhibition does not require known glycine receptors. Given that glycine acts as a cryoprotectant and can limit cell injury in certain contexts, such as ischemia, the role of NINJ1 in these settings warrants further study.

CROSS TALK BETWEEN THE DIFFERENT CELL DEATH PROGRAMS

Although apoptosis, necroptosis, and pyroptosis are often discussed as distinct cell death pathways, there is considerable flexibility in how these pathways are deployed. This functional redundancy ensures that cellular suicide, which is vital to pathogen elimination, is not compromised in times of infection or injury. For example, mouse macrophages infected with *Salmonella* or exposed to inflammasome stimuli can undergo apoptosis when pyroptosis is disabled (148). Biochemical hallmarks of apoptosis, necroptosis, and pyroptosis are detected in populations of cells infected with influenza, herpes simplex virus 1, and *Francisella* (149–151). Genetic experiments in mice also illustrate how alternative death signals are unleashed when the default cell death program is compromised. For example, expression of catalytically inactive caspase-8 in mice to impair extrinsic apoptosis causes lethal necroptosis, but if necroptosis is prevented by *Mlkl* deficiency, then aberrant ASC- and caspase-1-dependent signaling contributes to lethality (152, 153). In another example, mice lacking *Fadd* in intestinal epithelial cells develop ileitis that is driven by necroptosis or GSDMD-dependent pyroptosis (74). The ability of cells to pivot between cellular suicide mechanisms provides several layers of pathogen defense.

CONCLUSIONS AND FUTURE DIRECTIONS

Programmed cell death is essential for normal development and homeostasis but must be tightly regulated to prevent disease. A comprehensive understanding of the signaling mechanisms underlying apoptosis, necroptosis, and pyroptosis has revealed strategies to redress imbalances in cell death during disease. For example, patients with certain blood cancers have been treated successfully with the BCL-2 inhibitor venetoclax. A growing field of investigation is whether proinflammatory cell death can be harnessed to enhance immune responses to cancer (154). Conversely, strategies to limit proinflammatory lytic cell death, including inhibition of NLRP3 or GSDMD in the pyroptosis pathway and inhibition of RIPK1 in the necroptosis pathway, are of interest in modulating inflammatory diseases. Whether targeting NINJ1 to limit the release of proinflammatory DAMPs from dying cells is beneficial in inflammatory diseases warrants further investigation. As cell death plays such a critical role in biology, future studies at the bench and in the clinic will inform both how we can modulate these pathways and the clinical impact of this modulation.

DISCLOSURE STATEMENT

All authors are employees of Genentech and shareholders of Roche stock.

LITERATURE CITED

1. Stockwell BR. 2022. Ferroptosis turns 10: emerging mechanisms, physiological functions, and therapeutic applications. *Cell* 185:2401–21
2. Adams JM, Cory S. 2018. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death Differ*. 25:27–36
3. Jost PJ, Vucic D. 2020. Regulation of cell death and immunity by XIAP. *Cold Spring Harb. Perspect. Biol.* 12:a036426
4. Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26:239–57
5. Ning X, Wang Y, Jing M, Sha M, Lv M, et al. 2019. Apoptotic caspases suppress type I interferon production via the cleavage of cGAS, MAVS, and IRF3. *Mol. Cell* 74:19–31.e7
6. Nagata S. 2018. Apoptosis and clearance of apoptotic cells. *Annu. Rev. Immunol.* 36:489–517
7. Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, et al. 2000. The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. *Mol. Cell* 6:1389–99
8. Ke FFS, Vanyai HK, Cowan AD, Delbridge ARD, Whitehead L, et al. 2018. Embryogenesis and adult life in the absence of intrinsic apoptosis effectors BAX, BAK, and BOK. *Cell* 173:1217–30.e17
9. Ke FS, Holloway S, Uren RT, Wong AW, Little MH, et al. 2022. The BCL-2 family member BID plays a role during embryonic development in addition to its BH3-only protein function by acting in parallel to BAX, BAK and BOK. *EMBO J.* 41:e110300
10. Llambi F, Wang YM, Victor B, Yang M, Schneider DM, et al. 2016. BOK is a non-canonical BCL-2 family effector of apoptosis regulated by ER-associated degradation. *Cell* 165:421–33
11. Flores-Romero H, Hohorst L, John M, Albert MC, King LE, et al. 2022. BCL-2-family protein tBID can act as a BAX-like effector of apoptosis. *EMBO J.* 41:e108690
12. Sarosiek KA, Fraser C, Muthalagu N, Bhola PD, Chang W, et al. 2017. Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. *Cancer Cell* 31:142–56
13. Akasaka T, Kishimori C, Fukutsuka K, Nakagawa M, Takeoka K, et al. 2017. The novel double-hit, t(8;22)(q24;q11)/*MYC-IGL* and t(14;15)(q32;q24)/*IGH-BCL2A1*, in diffuse large B-cell lymphoma. *Cancer Genet.* 214/215:26–31
14. Haq R, Yokoyama S, Hawryluk EB, Jonsson GB, Frederick DT, et al. 2013. *BCL2A1* is a lineage-specific antiapoptotic melanoma oncogene that confers resistance to BRAF inhibition. *PNAS* 110:4321–26
15. Newton K, Gitlin AD. 2022. Deubiquitinases in cell death and inflammation. *Biochem. J.* 479:1103–19
16. Roberts AW, Wei AH, Huang DCS. 2021. BCL2 and MCL1 inhibitors for hematologic malignancies. *Blood* 138:1120–36
17. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, et al. 2013. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* 19:202–8
18. Esteve-Arenys A, Valero JG, Chamorro-Jorganes A, Gonzalez D, Rodriguez V, et al. 2018. The BET bromodomain inhibitor CPI203 overcomes resistance to ABT-199 (venetoclax) by downregulation of BFL-1/A1 in in vitro and in vivo models of $MYC^{+}/BCL2^{+}$ double hit lymphoma. *Oncogene* 37:1830–44
19. Deng Y, Diepstraten ST, Potts MA, Giner G, Trezise S, et al. 2022. Generation of a CRISPR activation mouse that enables modelling of aggressive lymphoma and interrogation of venetoclax resistance. *Nat. Commun.* 13:4739
20. Stutz MD, Allison CC, Ojaimi S, Preston SP, Doerflinger M, et al. 2021. Macrophage and neutrophil death programs differentially confer resistance to tuberculosis. *Immunity* 54:1758–71.e7
21. Kvansakul M, Hinds MG. 2013. Structural biology of the Bcl-2 family and its mimicry by viral proteins. *Cell Death Dis.* 4:e909
22. Fitzsimmons L, Cartlidge R, Chang C, Sejic N, Galbraith LCA, et al. 2020. EBV BCL-2 homologue BHRF1 drives chemoresistance and lymphomagenesis by inhibiting multiple cellular pro-apoptotic proteins. *Cell Death Differ.* 27:1554–68
23. Pan R, Ryan J, Pan D, Wucherpfennig KW, Letai A. 2022. Augmenting NK cell–based immunotherapy by targeting mitochondrial apoptosis. *Cell* 185:1521–38.e18

24. Nozaki K, Maltez VI, Rayamajhi M, Tubbs AL, Mitchell JE, et al. 2022. Caspase-7 activates ASM to repair gasdermin and perforin pores. *Nature* 606:960–67
25. Weant AE, Michalek RD, Khan IU, Holbrook BC, Willingham MC, Grayson JM. 2008. Apoptosis regulators Bim and Fas function concurrently to control autoimmunity and $CD8^+$ T cell contraction. *Immunity* 28:218–30
26. Hughes PD, Belz GT, Fortner KA, Budd RC, Strasser A, Bouillet P. 2008. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. *Immunity* 28:197–205
27. Newton K, Dixit VM, Kayagaki N. 2021. Dying cells fan the flames of inflammation. *Science* 374:1076–80
28. Estornes Y, Toscano F, Virard F, Jacquemin G, Pierrot A, et al. 2012. dsRNA induces apoptosis through an atypical death complex associating TLR3 to caspase-8. *Cell Death Differ*. 19:1482–94
29. Zinngrebe J, Rieser E, Taraborrelli L, Peltzer N, Hartwig T, et al. 2016. LUBAC deficiency perturbs TLR3 signaling to cause immunodeficiency and autoinflammation. *J. Exp. Med.* 213:2671–89
30. Daley-Bauer LP, Roback L, Crosby LN, McCormick AL, Feng Y, et al. 2017. Mouse cytomegalovirus M36 and M45 death suppressors cooperate to prevent inflammation resulting from antiviral programmed cell death pathways. *PNAS* 114:E2786–95
31. Guo H, Omoto S, Harris PA, Finger JN, Bertin J, et al. 2015. Herpes simplex virus suppresses necroptosis in human cells. *Cell Host Microbe* 17:243–51
32. Petrie EJ, Sandow JJ, Lehmann WIL, Liang LY, Coursier D, et al. 2019. Viral MLKL homologs subvert necroptotic cell death by sequestering cellular RIPK3. *Cell Rep*. 28:3309–19.e5
33. Zhang T, Yin C, Boyd DF, Quarato G, Ingram JP, et al. 2020. Influenza virus Z-RNAs induce ZBP1-mediated necroptosis. *Cell* 180:1115–29.e13
34. Wang R, Li H, Wu J, Cai ZY, Li B, et al. 2020. Gut stem cell necroptosis by genome instability triggers bowel inflammation. *Nature* 580:386–90
35. Jiao H, Wachsmuth L, Kumari S, Schwarzer R, Lin J, et al. 2020. Z-nucleic-acid sensing triggers ZBP1-dependent necroptosis and inflammation. *Nature* 580:391–95
36. Fu TM, Li Y, Lu A, Li Z, Vajjhala PR, et al. 2016. Cryo-EM structure of caspase-8 tandem DED filament reveals assembly and regulation mechanisms of the death-inducing signaling complex. *Mol. Cell* 64:236–50
37. Fox JL, Hughes MA, Meng X, Sarnowska NA, Powley IR, et al. 2021. Cryo-EM structural analysis of FADD:caspase-8 complexes defines the catalytic dimer architecture for co-ordinated control of cell fate. *Nat. Commun.* 12:819
38. Boatright KM, Deis C, Denault JB, Sutherlin DP, Salvesen GS. 2004. Activation of caspases-8 and -10 by $FLIP_L$. *Biochem. J.* 382:651–57
39. Yu JW, Jeffrey PD, Shi Y. 2009. Mechanism of procaspase-8 activation by c-$FLIP_L$. *PNAS* 106:8169–74
40. Keller N, Mares J, Zerbe O, Grutter MG. 2009. Structural and biochemical studies on procaspase-8: new insights on initiator caspase activation. *Structure* 17:438–48
41. Humphreys LM, Fox JP, Higgins CA, Majkut J, Sessler T, et al. 2020. A revised model of TRAIL-R2 DISC assembly explains how $FLIP_L$ can inhibit or promote apoptosis. *EMBO Rep*. 21:e49254
42. Hughes MA, Powley IR, Jukes-Jones R, Horn S, Feoktistova M, et al. 2016. Co-operative and hierarchical binding of c-FLIP and caspase-8: A unified model defines how c-FLIP isoforms differentially control cell fate. *Mol. Cell* 61:834–49
43. Tanzer MC, Khan N, Rickard JA, Etemadi N, Lalaoui N, et al. 2017. Combination of IAP antagonist and IFNγ activates novel caspase-10- and RIPK1-dependent cell death pathways. *Cell Death Differ*. 24:481–91
44. Horn S, Hughes MA, Schilling R, Sticht C, Tenev T, et al. 2017. Caspase-10 negatively regulates caspase-8-mediated cell death, switching the response to CD95L in favor of NF-κB activation and cell survival. *Cell Rep.* 19:785–97
45. Varfolomeev E, Maecker H, Sharp D, Lawrence D, Renz M, et al. 2005. Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor–related apoptosis-inducing ligand. *J. Biol. Chem.* 280:40599–608
46. Henry CM, Martin SJ. 2017. Caspase-8 acts in a non-enzymatic role as a scaffold for assembly of a pro-inflammatory "FADDosome" complex upon TRAIL stimulation. *Mol. Cell* 65:715–29.e5

47. Hartwig T, Montinaro A, von Karstedt S, Sevko A, Surinova S, et al. 2017. The TRAIL-induced cancer secretome promotes a tumor-supportive immune microenvironment via CCR2. *Mol. Cell* 65:730–42.e5
48. Kreuz S, Siegmund D, Rumpf JJ, Samel D, Leverkus M, et al. 2004. NFκB activation by Fas is mediated through FADD, caspase-8, and RIP and is inhibited by FLIP. *J. Cell Biol.* 166:369–80
49. Tummers B, Mari L, Guy CS, Heckmann BL, Rodriguez DA, et al. 2020. Caspase-8-dependent inflammatory responses are controlled by its adaptor, FADD, and necroptosis. *Immunity* 52:994–1006.e8
50. Newton K. 2020. Multitasking kinase RIPK1 regulates cell death and inflammation. *Cold Spring Harb. Perspect. Biol.* 12:a036368
51. Murphy JM. 2020. The killer pseudokinase mixed lineage kinase domain-like protein (MLKL). *Cold Spring Harb. Perspect. Biol.* 12:a036376
52. Schuster IS, Sng XYX, Lau CM, Powell DR, Weizman OE, et al. 2023. Infection induces tissue-resident memory NK cells that safeguard tissue health. *Immunity* 56:531–46.e6
53. Butt D, Chan TD, Bourne K, Hermes JR, Nguyen A, et al. 2015. FAS inactivation releases unconventional germinal center B cells that escape antigen control and drive IgE and autoantibody production. *Immunity* 42:890–902
54. Maccari ME, Fuchs S, Kury P, Andrieux G, Volkl S, et al. 2021. A distinct $CD38^{+}CD45RA^{+}$ population of $CD4^{+}$, $CD8^{+}$, and double-negative T cells is controlled by FAS. *J. Exp. Med.* 218:e20192191
55. Newton K, Wickliffe KE, Dugger DL, Maltzman A, Roose-Girma M, et al. 2019. Cleavage of RIPK1 by caspase-8 is crucial for limiting apoptosis and necroptosis. *Nature* 574:428–31
56. Lalaoui N, Boyden SE, Oda H, Wood GM, Stone DL, et al. 2020. Mutations that prevent caspase cleavage of RIPK1 cause autoinflammatory disease. *Nature* 577:103–8
57. Tao P, Sun J, Wu Z, Wang S, Wang J, et al. 2020. A dominant autoinflammatory disease caused by non-cleavable variants of RIPK1. *Nature* 577:109–14
58. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, et al. 2002. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature* 419:395–99
59. Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, et al. 2010. Whole-exome-sequencing-based discovery of human FADD deficiency. *Am. J. Hum. Genet.* 87:873–81
60. Lehle AS, Farin HF, Marquardt B, Michels BE, Magg T, et al. 2019. Intestinal inflammation and dysregulated immunity in patients with inherited caspase-8 deficiency. *Gastroenterology* 156:275–78
61. Gitlin AD, Heger K, Schubert AF, Reja R, Yan D, et al. 2020. Integration of innate immune signalling by caspase-8 cleavage of N4BP1. *Nature* 587:275–80
62. Annibaldi A, Walczak H. 2020. Death receptors and their ligands in inflammatory disease and cancer. *Cold Spring Harb. Perspect. Biol.* 12:a036384
63. Xu D, Jin T, Zhu H, Chen H, Ofengeim D, et al. 2018. TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. *Cell* 174:1477–91.e19
64. Orning P, Weng D, Starheim K, Ratner D, Best Z, et al. 2018. Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* 362:1064–69
65. Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, et al. 2018. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. *PNAS* 115:E10888–97
66. Wang Y, Gao W, Shi X, Ding J, Liu W, et al. 2017. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature* 547:99–103
67. Dondelinger Y, Aguileta MA, Goossens V, Dubuisson C, Grootjans S, et al. 2013. RIPK3 contributes to TNFR1-mediated RIPK1 kinase–dependent apoptosis in conditions of cIAP1/2 depletion or TAK1 kinase inhibition. *Cell Death Differ*. 20:1381–92
68. Tang Y, Tu H, Zhang J, Zhao X, Wang Y, et al. 2019. K63-linked ubiquitination regulates RIPK1 kinase activity to prevent cell death during embryogenesis and inflammation. *Nat. Commun.* 10:4157
69. Zhang X, Zhang H, Xu C, Li X, Li M, et al. 2019. Ubiquitination of RIPK1 suppresses programmed cell death by regulating RIPK1 kinase activation during embryogenesis. *Nat. Commun.* 10:4158
70. Kist M, Komuves LG, Goncharov T, Dugger DL, Yu C, et al. 2021. Impaired RIPK1 ubiquitination sensitizes mice to TNF toxicity and inflammatory cell death. *Cell Death Differ*. 28:985–1000
71. Huyghe J, Priem D, Van Hove L, Gilbert B, Fritsch J, et al. 2022. ATG9A prevents TNF cytotoxicity by an unconventional lysosomal targeting pathway. *Science* 378:1201–7

72. Ang RL, Chan M, Legarda D, Sundberg JP, Sun SC, et al. 2021. Immune dysregulation in SHARPIN-deficient mice is dependent on CYLD-mediated cell death. *PNAS* 118:e2001602118
73. Vlantis K, Wullaert A, Polykratis A, Kondylis V, Dannappel M, et al. 2016. NEMO prevents RIP kinase 1-mediated epithelial cell death and chronic intestinal inflammation by NF-κB-dependent and -independent functions. *Immunity* 44:553–67
74. Schwarzer R, Jiao H, Wachsmuth L, Tresch A, Pasparakis M. 2020. FADD and caspase-8 regulate gut homeostasis and inflammation by controlling MLKL- and GSDMD-mediated death of intestinal epithelial cells. *Immunity* 52:978–93.e6
75. Taraborrelli L, Peltzer N, Montinaro A, Kupka S, Rieser E, et al. 2018. LUBAC prevents lethal dermatitis by inhibiting cell death induced by TNF, TRAIL and CD95L. *Nat. Commun.* 9:3910
76. Feoktistova M, Geserick P, Kellert B, Dimitrova DP, Langlais C, et al. 2011. cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. *Mol. Cell* 43:449–63
77. Taft J, Markson M, Legarda D, Patel R, Chan M, et al. 2021. Human TBK1 deficiency leads to autoinflammation driven by TNF-induced cell death. *Cell* 184:4447–63.e20
78. Damgaard RB, Elliott PR, Swatek KN, Maher ER, Stepensky P, et al. 2019. OTULIN deficiency in ORAS causes cell type–specific LUBAC degradation, dysregulated TNF signalling and cell death. *EMBO Mol. Med.* 11:e9324
79. Cuchet-Lourenco D, Eletto D, Wu C, Plagnol V, Papapietro O, et al. 2018. Biallelic *RIPK1* mutations in humans cause severe immunodeficiency, arthritis, and intestinal inflammation. *Science* 361:810–13
80. Li Y, Fuhrer M, Bahrami E, Socha P, Klaudel-Dreszler M, et al. 2019. Human RIPK1 deficiency causes combined immunodeficiency and inflammatory bowel diseases. *PNAS* 116:970–75
81. Anderton H, Bandala-Sanchez E, Simpson DS, Rickard JA, Ng AP, et al. 2019. RIPK1 prevents TRADD-driven, but TNFR1 independent, apoptosis during development. *Cell Death Differ.* 26:877–89
82. Saleh D, Najjar M, Zelic M, Shah S, Nogusa S, et al. 2017. Kinase activities of RIPK1 and RIPK3 can direct IFN-β synthesis induced by lipopolysaccharide. *J. Immunol.* 198:4435–47
83. Wang Y, Karki R, Mall R, Sharma BR, Kalathur RC, et al. 2022. Molecular mechanism of RIPK1 and caspase-8 in homeostatic type I interferon production and regulation. *Cell Rep.* 41:111434
84. Stark K, Goncharov T, Varfolomeev E, Xie L, Ngu H, et al. 2021. Genetic inactivation of RIP1 kinase activity in rats protects against ischemic brain injury. *Cell Death Dis.* 12:379
85. Van Eeckhoutte HP, Donovan C, Kim RY, Conlon TM, Ansari M, et al. 2022. RIPK1 kinase-dependent inflammation and cell death contribute to the pathogenesis of COPD. *Eur. Respir. J.* 61:2201506
86. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. 2018. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25:486–541
87. Liu X, Xia S, Zhang Z, Wu H, Lieberman J. 2021. Channelling inflammation: gasdermins in physiology and disease. *Nat. Rev. Drug Discov.* 20:384–405
88. Kayagaki N, Dixit VM. 2019. Rescue from a fiery death: a therapeutic endeavor. *Science* 366:688–89
89. Karki R, Lee E, Place D, Samir P, Mavuluri J, et al. 2018. IRF8 regulates transcription of *Naips* for NLRC4 inflammasome activation. *Cell* 173:920–33.e13
90. Wang J, Deobald K, Re F. 2019. Gasdermin D protects from melioidosis through pyroptosis and direct killing of bacteria. *J. Immunol.* 202:3468–73
91. Luchetti G, Roncaioli JL, Chavez RA, Schubert AF, Kofoed EM, et al. 2021. *Shigella* ubiquitin ligase IpaH7.8 targets gasdermin D for degradation to prevent pyroptosis and enable infection. *Cell Host Microbe* 29:1521–30.e10
92. Hansen JM, de Jong MF, Wu Q, Zhang LS, Heisler DB, et al. 2021. Pathogenic ubiquitination of GSDMB inhibits NK cell bactericidal functions. *Cell* 184:3178–91.e18
93. Xia S, Zhang Z, Magupalli VG, Pablo JL, Dong Y, et al. 2021. Gasdermin D pore structure reveals preferential release of mature interleukin-1. *Nature* 593:607–11
94. Afonina IS, Muller C, Martin SJ, Beyaert R. 2015. Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. *Immunity* 42:991–1004
95. Evavold CL, Ruan J, Tan Y, Xia S, Wu H, Kagan JC. 2018. The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. *Immunity* 48:35–44.e6

96. Ruhl S, Broz P. 2022. Regulation of lytic and non-lytic functions of gasdermin pores. *J. Mol. Biol.* 434:167246
97. Liu T, Yamaguchi Y, Shirasaki Y, Shikada K, Yamagishi M, et al. 2014. Single-cell imaging of caspase-1 dynamics reveals an all-or-none inflammasome signaling response. *Cell Rep*. 8:974–82
98. Polykratis A, Martens A, Eren RO, Shirasaki Y, Yamagishi M, et al. 2019. A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. *Nat. Cell Biol.* 21:731–42
99. Kayagaki N, Kornfeld OS, Lee BL, Stowe IB, O'Rourke K, et al. 2021. NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature* 591:131–36
100. Bjanes E, Sillas RG, Matsuda R, Demarco B, Fettrelet T, et al. 2021. Genetic targeting of *Card19* is linked to disrupted NINJ1 expression, impaired cell lysis, and increased susceptibility to *Yersinia* infection. *PLOS Pathog.* 17:e1009967
101. Degen M, Santo JC, Pluhackova K, Cebrero G, Ramos S, et al. 2023. Structural basis of NINJ1-mediated plasma membrane rupture in cell death. *Nature* 618:1065–71
102. Araki T, Milbrandt J. 1996. Ninjurin, a novel adhesion molecule, is induced by nerve injury and promotes axonal growth. *Neuron* 17:353–61
103. Chao KL, Kulakova L, Herzberg O. 2017. Gene polymorphism linked to increased asthma and IBD risk alters gasdermin-B structure, a sulfatide and phosphoinositide binding protein. *PNAS* 114:E1128–37
104. De Schutter E, Ramon J, Pfeuty B, De Tender C, Stremersch S, et al. 2021. Plasma membrane perforation by GSDME during apoptosis-driven secondary necrosis. *Cell. Mol. Life Sci.* 79:19
105. Chen KW, Demarco B, Ramos S, Heilig R, Goris M, et al. 2021. RIPK1 activates distinct gasdermins in macrophages and neutrophils upon pathogen blockade of innate immune signaling. *PNAS* 118:e2101189118
106. Zhang Z, Zhang Y, Xia S, Kong Q, Li S, et al. 2020. Gasdermin E suppresses tumour growth by activating anti-tumour immunity. *Nature* 579:415–20
107. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, et al. 2010. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature* 467:863–67
108. Green DR, Ferguson T, Zitvogel L, Kroemer G. 2009. Immunogenic and tolerogenic cell death. *Nat. Rev. Immunol.* 9:353–63
109. Zhou Z, He H, Wang K, Shi X, Wang Y, et al. 2020. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. *Science* 368:eaaz7548
110. Rana N, Privitera G, Kondolf HC, Bulek K, Lechuga S, et al. 2022. GSDMB is increased in IBD and regulates epithelial restitution/repair independent of pyroptosis. *Cell* 185:283–98.e17
111. Ivanov AI, Rana N, Privitera G, Pizarro TT. 2023. The enigmatic roles of epithelial gasdermin B: recent discoveries and controversies. *Trends Cell Biol.* 33:48–59
112. Deng W, Bai Y, Deng F, Pan Y, Mei S, et al. 2022. Streptococcal pyrogenic exotoxin B cleaves GSDMA and triggers pyroptosis. *Nature* 602:496–502
113. LaRock DL, Johnson AF, Wilde S, Sands JS, Monteiro MP, LaRock CN. 2022. Group A *Streptococcus* induces GSDMA-dependent pyroptosis in keratinocytes. *Nature* 605:527–31
114. Ding J, Wang K, Liu W, She Y, Sun Q, et al. 2016. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535:111–16
115. Hou J, Zhao R, Xia W, Chang CW, You Y, et al. 2020. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nat. Cell Biol.* 22:1264–75
116. Zhao M, Ren K, Xiong X, Xin Y, Zou Y, et al. 2022. Epithelial STAT6 *O*-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and Gasdermin C. *Immunity* 55:623–38.e5
117. Johnson AG, Wein T, Mayer ML, Duncan-Lowey B, Yirmiya E, et al. 2022. Bacterial gasdermins reveal an ancient mechanism of cell death. *Science* 375:221–25
118. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, et al. 2015. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526:666–71
119. Zhang H, Zeng L, Xie M, Liu J, Zhou B, et al. 2020. TMEM173 drives lethal coagulation in sepsis. *Cell Host Microbe* 27:556–70.e6

120. Cheng KT, Xiong S, Ye Z, Hong Z, Di A, et al. 2017. Caspase-11-mediated endothelial pyroptosis underlies endotoxemia-induced lung injury. *J. Clin. Investig.* 127:4124–35
121. Yang X, Cheng X, Tang Y, Qiu X, Wang Y, et al. 2019. Bacterial endotoxin activates the coagulation cascade through gasdermin D–dependent phosphatidylserine exposure. *Immunity* 51:983–96.e6
122. Sollberger G, Choidas A, Burn GL, Habenberger P, Di Lucrezia R, et al. 2018. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci. Immunol.* 3:eaar6689
123. Chen KW, Monteleone M, Boucher D, Sollberger G, Ramnath D, et al. 2018. Noncanonical inflammasome signaling elicits gasdermin D–dependent neutrophil extracellular traps. *Sci. Immunol.* 3:eaar6676
124. Chauhan D, Demon D, Vande Walle L, Paerewijck O, Zecchin A, et al. 2022. GSDMD drives canonical inflammasome-induced neutrophil pyroptosis and is dispensable for NETosis. *EMBO Rep.* 23:e54277
125. Chen W, Chen S, Yan C, Zhang Y, Zhang R, et al. 2022. Allergen protease-activated stress granule assembly and gasdermin D fragmentation control interleukin-33 secretion. *Nat. Immunol.* 23:1021–30
126. Xiao J, Wang C, Yao JC, Alippe Y, Xu C, et al. 2018. Gasdermin D mediates the pathogenesis of neonatal-onset multisystem inflammatory disease in mice. *PLOS Biol.* 16:e3000047
127. Kanneganti A, Malireddi RKS, Saavedra PHV, Vande Walle L, Van Gorp H, et al. 2018. GSDMD is critical for autoinflammatory pathology in a mouse model of familial Mediterranean fever. *J. Exp. Med.* 215:1519–29
128. Kyriazopoulou E, Huet T, Cavalli G, Gori A, Kyprianou M, et al. 2021. Effect of anakinra on mortality in patients with COVID-19: a systematic review and patient-level meta-analysis. *Lancet Rheumatol.* 3:e690–97
129. Sefik E, Qu R, Junqueira C, Kaffe E, Mirza H, et al. 2022. Inflammasome activation in infected macrophages drives COVID-19 pathology. *Nature* 606:585–93
130. Rathkey JK, Xiao TS, Abbott DW. 2020. Human polymorphisms in GSDMD alter the inflammatory response. *J. Biol. Chem.* 295:3228–38
131. Dong S, Shi Y, Dong X, Xiao X, Qi J, et al. 2022. Gasdermin E is required for induction of pyroptosis and severe disease during enterovirus 71 infection. *J. Biol. Chem.* 298:101850
132. Liu Y, Fang Y, Chen X, Wang Z, Liang X, et al. 2020. Gasdermin E–mediated target cell pyroptosis by CAR T cells triggers cytokine release syndrome. *Sci. Immunol.* 5:eaax7969
133. Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. 2018. Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat. Rev. Drug Discov.* 17:588–606
134. Xiao L, Magupalli VG, Wu H. 2023. Cryo-EM structures of the active NLRP3 inflammasome disc. *Nature* 613:595–600
135. Svensson EC, Madar A, Campbell CD, He Y, Sultan M, et al. 2022. *TET2*-driven clonal hematopoiesis and response to canakinumab: an exploratory analysis of the CANTOS randomized clinical trial. *JAMA Cardiol.* 7:521–28
136. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, et al. 2017. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 355:842–47
137. Fidler TP, Xue C, Yalcinkaya M, Hardaway B, Abramowicz S, et al. 2021. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. *Nature* 592:296–301
138. Shi H, Wang Y, Li X, Zhan X, Tang M, et al. 2016. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat. Immunol.* 17:250–58
139. Schmacke NA, O'Duill F, Gaidt MM, Szymanska I, Kamper JM, et al. 2022. IKKβ primes inflammasome formation by recruiting NLRP3 to the *trans*-Golgi network. *Immunity* 55:2271–84.e7
140. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, et al. 2016. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: reanalysis of a prior phase III trial. *Crit. Care Med.* 44:275–81
141. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, et al. 2020. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. *Nat. Immunol.* 21:736–45
142. Bertheloot D, Wanderley CW, Schneider AH, Schiffelers LD, Wuerth JD, et al. 2022. Nanobodies dismantle post-pyroptotic ASC specks and counteract inflammation in vivo. *EMBO Mol. Med.* 14:e15415
143. Choi S, Woo JK, Jang YS, Kang JH, Hwang JI, et al. 2018. Ninjurin1 plays a crucial role in pulmonary fibrosis by promoting interaction between macrophages and alveolar epithelial cells. *Sci. Rep.* 8:17542

144. Ahn BJ, Lee HJ, Shin MW, Choi JH, Jeong JW, Kim KW. 2009. Ninjurin1 is expressed in myeloid cells and mediates endothelium adhesion in the brains of EAE rats. *Biochem. Biophys. Res. Commun.* 387:321–25
145. Kristjansson RP, Oddsson A, Helgason H, Sveinbjornsson G, Arnadottir GA, et al. 2016. Common and rare variants associating with serum levels of creatine kinase and lactate dehydrogenase. *Nat. Commun.* 7:10572
146. Borges JP, Saetra RSR, Volchuk A, Bugge M, Devant P, et al. 2022. Glycine inhibits NINJ1 membrane clustering to suppress plasma membrane rupture in cell death. *eLife* 11:e78609
147. Loomis WP, den Hartigh AB, Cookson BT, Fink SL. 2019. Diverse small molecules prevent macrophage lysis during pyroptosis. *Cell Death Dis.* 10:326
148. Doerflinger M, Deng Y, Whitney P, Salvamoser R, Engel S, et al. 2020. Flexible usage and interconnectivity of diverse cell death pathways protect against intracellular infection. *Immunity* 53:533–47.e7
149. Nogusa S, Thapa RJ, Dillon CP, Liedmann S, Oguin TH 3rd, et al. 2016. RIPK3 activates parallel pathways of MLKL-driven necroptosis and FADD-mediated apoptosis to protect against influenza A virus. *Cell Host Microbe* 20:13–24
150. Kuriakose T, Man SM, Malireddi RK, Karki R, Kesavardhana S, et al. 2016. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Sci. Immunol.* 1:aag2045
151. Lee S, Karki R, Wang Y, Nguyen LN, Kalathur RC, Kanneganti TD. 2021. AIM2 forms a complex with pyrin and ZBP1 to drive PANoptosis and host defence. *Nature* 597:415–19
152. Newton K, Wickliffe KE, Maltzman A, Dugger DL, Reja R, et al. 2019. Activity of caspase-8 determines plasticity between cell death pathways. *Nature* 575:679–82
153. Fritsch M, Gunther SD, Schwarzer R, Albert MC, Schorn F, et al. 2019. Caspase-8 is the molecular switch for apoptosis, necroptosis and pyroptosis. *Nature* 575:683–87
154. Zhang T, Yin C, Fedorov A, Qiao L, Bao H, et al. 2022. ADAR1 masks the cancer immunotherapeutic promise of ZBP1-driven necroptosis. *Nature* 606:594–602