Published in final edited form as:

Immunol Cell Biol. 2017 February ; 95(2): 146–151. doi:10.1038/icb.2016.113.

A motive for killing: effector functions of regulated lytic cell death

Meghan Bliss-Moreau^{1,2}, Alyce A Chen^{1,2}, Akshay D'Cruz^{1,2}, and Ben A Croker^{1,2}

¹Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA

²Department of Pediatrics, Harvard Medical School, Boston, MA, USA

Abstract

Immunological responses activated by pathogen recognition come in many guises. The proliferation, differentiation and recruitment of immune cells, and the production of inflammatory cytokines and chemokines are central to lifelong immunity. Cell death serves as a key function in the resolution of innate and adaptive immune responses. It also coordinates cell-intrinsic effector functions to restrict infection. Necrosis was formally considered a passive form of cell death or a consequence of pathogen virulence factor expression, and necrotic tissue is frequently associated with infection. However, there is now emerging evidence that points to a role for regulated forms of necrosis, such as pyroptosis and necroptosis, driving inflammation and shaping the immune response.

INTRODUCTION

There are several types of regulated necrosis, including caspase-1-mediated pyroptosis, caspase-4/11-driven pyroptosis and mixed lineage kinase domain-like/receptor interacting serine/threonine kinase 3 (MLKL/RIPK3)-dependent necroptosis. Each pathway can be distinguished at the genetic, biochemical, and cellular level. However, extricating the functional consequences of controlled cell death from the effects of immunomodulatory cytokines, such as IL-1 α , IL-1 β , IL-18, and IL-33 that can be released as part of these biochemical pathways, continues to be a major challenge *in vivo*. The development of new methods to detect pathway activation will help define the importance of their contribution to disease *in vivo*. In this review, we discuss recent literature demonstrating that regulated forms of apoptotic and nonapoptotic cell death serve as cell-intrinsic effector functions to kill intracellular and extracellular microbes, while also stimulating inflammation via the release of endogenous host molecules called DAMPs (damage-associated molecular patterns or danger-associated molecular patterns).

Correspondence: Dr BA Croker, Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA. ben.croker@childrens.harvard.edu.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CASPASE-1 AND INFLAMMASOME-DEPENDENT PYROPTOSIS

Pyroptosis is a nonapoptotic form of cell death originally defined by the presence of caspase-1 activation.¹ Activation of caspase-4 in humans, or caspase-11 in mice, also leads to death associated with the release of highly inflammatory mediators, consistent with 'pyro'-ptosis (Figure 1).^{2–6} However, the precise cellular, biochemical, and structural features of this process appears to differ from caspase-1-dependent pyroptotic death. Additionally, caspase-4/11 cell death may not rely on a multiprotein cytoplasmic inflammasome complex as originally defined by Martinon and colleagues.^{2–8} Therefore, the relationship between caspase-1-dependent pyroptosis and caspase-4/11-mediated cell death needs further examination.

Pyroptosis elicits a strong inflammatory response, in part due to the processing of pro-IL-1 β and pro-IL-18 by caspase-1 to their bioactive forms.⁹ Defining cell-intrinsic effector functions that promote inflammation, beyond the release of these inflammatory cytokines, has been challenging. Many studies aimed at elucidating the function of caspase-1 have investigated the physiology and immunology of caspase-1-deficient mice. Reports have also examined biochemical readouts of caspase-1 and IL-1 β processing; however, there have been few experiments outlining the effects on cell viability. As a result, we have an incomplete understanding of the biochemistry and cell biology of caspase-1-dependent cell death. From a limited number of studies, we know that caspase-1-dependent cell death can be a distinct process from IL-1 β processing,¹⁰ which may reduce pathogen load,¹¹ and when triggered by non-infectious stimuli such as chemotherapy, has the capacity to kill hematopoietic stem and progenitor cells.¹² Additionally, when activated systemically, the NLRP1a inflammasome can deplete hematopoietic cells, resulting in cytopenia and immunosuppression.¹²

Early studies demonstrate that active caspase-1 can localize on the external surface of the plasma membrane, often in clusters on microvilli, but without signs of membrane disruption or loss of cell viability.¹³ Processed IL-1 β can also be detected on the cell surface of monocytes with intact lipid bilayers and normal nuclear architecture. As illustrated in Singer *et al.*,¹³ the processing of IL-1 β at the plasma membrane occurred in viable monocytes, suggesting that subsequent caspase-1-dependent loss of cell viability may be a late event in the activation of the inflammasome. *Salmonella typhimurium*-induced macrophage pyroptosis involves cell swelling, poly ADP ribose polymerase (PARP) activation, and inhibitor of caspase-activated DNase (ICAD)-independent DNA fragmentation.^{11,14} However, PARP cleavage and DNA cleavage are not required for pyroptosis. Pore size in pyroptotic cells is estimated at 1.1–2.4 nm, but these data are based on an indirect assay using osmoprotectants; of note, this method has the potential to interfere with other processes leading to inflammasome assembly and caspase-1 activation.¹⁴ More work is needed to determine the relationship between caspase-1-dependent death and cytokine processing and the extent to which these events are influenced by the type of inflammasome engaged and the cell type involved.

RECOGNITION OF INTRACELLULAR BACTERIAL INFECTION

The induction of cell death upon intracellular infection is a fundamental component of the innate immune response that is conserved across kingdoms.^{15–17} In plants, the induction of cell death forms a dominant part of the innate immune response. In mammals, we now recognize and will summarize, a number of distinct biochemical pathways that can be activated during infection, leading to the loss of membrane integrity and cell viability.

When Gram-negative bacteria escape the phagosome and enter the cytoplasm, lipopolysaccharides (LPS) trigger activation of caspase-4 in humans and caspase-11 in mice.² Activation of caspase-4/11 can engage the NLRP3 inflammasome to drive caspase-1-dependent processing of IL-1 β and IL-18.^{4,18} Binding of caspase-4 to phosphate groups in the lipid A moiety of LPS occurs via basic residues in the caspase activation and recruitment domain (CARD domain). Introduction of K19E, K52E/R53E/W54A or K62E/K63E/K64E mutations in the CARD domain of caspase-4 abrogated binding of LPS.⁶ A caspase-1 chimeric protein containing the caspase-11 CARD domain sensitized cells to pyroptosis induced by cytoplasmic LPS.⁶ However, a crystal structure has yet to be reported demonstrating this structural interaction. Forms of LPS that fail to induce macrophage pyroptosis also fail to induce oligomerization of caspase-4/11. It remains to be determined if other LPS-binding cofactors participate in presentation of LPS to the inflammatory caspases. While caspase-5 is reported to be a redundant regulator of responses to cytoplasmic LPS,⁶ two subsequent studies using caspase-5-deficient myeloid cell lines failed to confirm a role for caspase-5 in responses to LPS.^{18,19} Thus, while the activation of caspase-4/11 by LPS is unanimously supported by the literature, a role for caspase-5 still remains unclear.

CASPASE-4 AND CASPASE-11 ACTIVATE GASDERMINS TO KILL

Gram-negative bacteria in the cytoplasm cannot be sensed by Toll-like receptors (TLRs), which are restricted to the plasma membrane and endosomes. Outer membrane vesicles (OMV) are actively secreted from Gram-negative bacteria, such as *Shigella* and *Burkholderia*, or by vesiculating bacteria including: *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis* as part of symbiotic and pathogenic interactions with mammalian hosts and other microorganisms.^{20–22} LPS within OMV can be internalized by host cells via clathrin-mediated endocytosis and then released from early endosomes to engage caspase-4/11 in the cytoplasm.²³ Detection of cytoplasmic LPS by caspase-4/11 enables mammalian cells to mount an inflammatory response to this intracellular threat.

Caspase-4/11 activation triggers the oligomerization of gasdermin D (GSDMD) by cleaving human and mouse GSDMD at Asp275 and Asp276, respectively, to release an autoinhibitory C-terminal fragment. The resulting cleavage product (GSDMD-NT) oligomerizes to form 10–16 nm pores containing 16–24 protomers of the p30 fragment.^{24–26} Reducing the concentration of GSDMD reduces the number of pores, but not the pore size. A GSDMD mutation has been described that impairs oligomerization but not recruitment of the protomers to the membrane, thus resulting in a reduction in pore number.²⁵ Caspase-4/11 triggers oligomerization of GSDMD and kills cells without involvement of nucleotide-

binding domain and leucine-rich repeat (NLR) receptors or the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC),² thus it is questionable whether this complex constitutes any form of canonical or non-canonical inflammasome.

Other members of the gasdermin family include GSDMA, GSDMB, GSDMC, DFNA5 and DFNB59 in humans, and GSDMA1–3 and GSDMC1–4 in mice. The N-terminal fragments of GSDMD, GSDMA, and GSDMA3 exhibit pore-forming capacity but only GSDMD appears to be a substrate of caspase-4/11.^{24,25} The stimuli driving activation of other gasdermin family members are not known. Mutation of four basic residues predicted to be in amphipathic α -helices of GSDMD, Arg138, Lys146, Arg152, and Arg154 prevented oligomerization, membrane binding, pore formation and pyroptosis.²⁴

GSDMD-NT binds mono-, bis- and tris-phosphorylated phosphatidylinositols (PtdIns(3)P, PtdIns(4)P, PtdIns(5)P, PtdIns(3,4)P₂, PtdIns(3,5)P₂, PtdIns(4,5)P₂, and PtdIns(3,4,5)P₃), phosphatidic acid, and phosphatidylserine, all of which are usually localized to the inner membrane of mammalian cells.^{24–28} GSDMD-NT binding specificity has also been described for cardiolipin, which is found in bacterial cell membranes.^{24,25,29} In contrast to GSDMD-NT, perforin kills cells from the exterior, consistent with its lipid-binding preference to phosphatidylethanolamine (PE), found on the outer leaflet of the membrane.^{30,31} The inner membrane lipid-binding preference of GSDMD-NT enables specificity of killing. Consequently, surrounding mammalian cells would remain unaffected but the capacity to lyse extracellular bacteria would persist. The lipid composition of the phagosome and endosome membranes makes them a possible target of GSDMD-NT, in addition to the bacteria confined to these vacuoles. Whether extracellular GSDMD-NT can target apoptotic cells with externalized phosphatidylserine has not been addressed. These elegant studies provide evidence for cell-intrinsic effector functions of GSDMD-mediated pyroptosis in the face of intracellular and extracellular bacterial infection. Further work is needed to define the cell types that activate GSDMD, the role of extracellular inhibitors, and the mechanisms controlling its half-life.

CASPASE-1 ACTIVATES GSDMD

Caspase-1 can cleave GSDMD and is hypothesized to form GSDMD pores that control the release of IL-1 β and the induction of pyroptotic death.³² Primary *Gsdmd*^{−/−} or *Gsdmd*^{J05N/J105N} bone marrow-derived macrophages (BMDM) are resistant to pyroptosis and produce less IL-1 β when treated with ATP (NLRP3), poly(dA:dT) (AIM2), or TcdB (pyrin).³² GSDMD deficiency also protects macrophages from the cytopathic effects of nigericin stimulation.³³ However, loss of GSDMD does not fully protect BMDM from cell death. Therefore, it is possible that an alternative pathway such as NLRP3-dependent, caspase-8-mediated signaling may kill GSDMD-deficient BMDM.^{34,35} More studies are required to investigate if GSDMD forms equal numbers and sizes of pores in response to canonical inflammasome stimuli.

It is not yet clear whether NLRP3-dependent and caspase-1-dependent GSDMD cleavage can trigger IL-1 β release without pyroptotic death. However, our current understanding of GSDMD pore formation provides a clear mechanism for IL-1 β and IL-18 secretion from

viable cells and a framework to determine if IL-1 β secretion can be completely uncoupled from subsequent loss of cell viability. For example, neutrophils produce IL-1 β in response to caspase-1 activation but are resistant to caspase-1-induced cell death.³⁶ It remains unknown if neutrophils can also cleave GSDMD or if they respond to cytoplasmic LPS to induce GSDMD pores. Experiments in GSDMD- or caspase-1 deficient neutrophils will be needed to clarify the role of these proteins in neutrophil proinflammatory death.

MLKL: MEMBRANE-DISRUPTING OR PORE-FORMING PROTEIN?

Mixed lineage kinase domain-like (MLKL) mediates nonapoptotic cell death, specifically necroptosis, by membrane disruption. Phosphorylation of MLKL by RIPK3 at S345, S347, and T349 is followed by the translocation of MLKL to the membrane.³⁷ Translocation of MLKL to the membrane itself is not sufficient to cause cell death, therefore suggesting spontaneous assembly of an MLKL-dependent membrane-disrupting complex does not occur. The data suggest that additional events coordinated by MLKL or partner molecules are likely essential for MLKL-dependent cytotoxicity.³⁷ The proposed trimeric or hexameric pore in the membrane is yet to be visualized; however, structural information on the four helical bundle is available.^{38–43}

MLKL binds a similar lipid profile as GSDMD, interacting with PI (3)P, PI(4)P, PI(5)P, PI(4,5)P₂, PI(3,4,5)P₃, PI(3,4)P₂, PE(3,5)P₂ and cardiolipin, but only weakly associates with phosphatidylserine, phosphatidylglycerol, sulfatide, and phosphatidic acid.^{38,40,44} Competitive PIP binding assays using overexpression of the PIP-binding pleckstrin homology (PH) domain-containing proteins phospholipase C δ (PLC δ), which binds PI(4,5)P₂, or Bruton's Tyrosine Kinase (BTK), which binds PI(3,4,5)P₃, abrogated induction of necroptosis by MLKL.³⁸ This overlapping lipid-binding profile of GSDMD and MLKL suggests that MLKL may also have the capacity to lyse intracellular and extracellular bacteria via interactions with cardiolipin in bacterial membranes. Thus, in addition to the ability of necroptotic cells to release DAMPs to induce IL-1 α and IL-33, this mode of cell death may also directly contribute to the killing of pathogens.^{45,46}

NEUTROPHIL EXTRACELLULAR TRAPS: AN EFFECTOR FUNCTION OF MLKL ACTIVATION?

Neutrophil extracellular traps (NETs) are networks of double stranded DNA, histones, and microbicidal proteins released from neutrophils to trap and kill pathogens.^{47,48} NETs can restrict the replication of diverse pathogens including *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Shigella flexneri*, and *Candida albicans*.^{48–52} Systemic release of NETs, or the chronic release and prolonged presence of NETs, has been implicated in the pathogenesis of sepsis, systemic lupus erythematosus,⁵³ rheumatoid arthritis,⁵⁴ diabetes,⁵⁵ acute lung injury,⁵⁶ and thrombosis.^{57–59} Neutrophil necroptosis may contribute to the pathogenesis of some of these infectious and inflammatory diseases. Both necroptosis and NETosis require the inhibition of apoptotic caspases to proceed, and they both result in a loss of plasma membrane integrity and the release of DAMPs. Using uric acid, LPS, and phorbol 12-myristate 13-acetate (PMA) to trigger NETs, two groups provide opposing data on the role of RIPK3 and MLKL in the generation of NETs.^{60,61} The use of

necrostatin-1 in the context of NET generation is also problematic because of off-target effects on indoleamine-2,3-dioxygenase (IDO).⁶² Therefore, additional experiments using alternate stimuli known to specifically engage RIPK3 and MLKL will be needed to determine if NET formation is an effector function of the necroptosis machinery.

CELL DEATH TO ELIMINATE INTRACELLULAR PATHOGENS

In mammals, apoptotic and nonapoptotic cell death pathways have fundamental roles in host-pathogen interactions. To counter the immunoregulatory roles of cell death and the cytokines elicited by regulated cell death, pathogens have evolved numerous mechanisms to suppress these pathways. The Myxoma virus pyrin domain-containing M13L protein inhibits caspase-1 processing and IL-1 β production through an interaction with ASC.⁶³ It is unclear if pyroptosis has an effector role in restricting viral replication given the dual role of M13 in regulating NF κ B and caspase-1-dependent processing of IL-1 β .⁶⁴ However, in cell cultures of rabbit fibroblasts and lymphocytes, loss of M13L suppresses viral titers.⁶⁴ These data suggest that caspase-1-dependent pyroptosis may also have an effector function in the pathway to prevent viral replication and viral dissemination.

Death receptor-induced apoptosis of infected cells is a key antimicrobial defense strategy for enteropathogenic bacteria.^{65,66} Mice deficient in Fas are highly sensitive to *Citrobacter rodentium*, an enteropathogenic-like mouse pathogen. The bacterial type III secretion system of enteropathogenic bacteria can deliver virulence factors to the host cytosol, including proteins that interfere with the formation of the death-inducing signaling complex (DISC). Modification of FADD by the N-acetylglucosamine transferase activity of NleB1 prevented apoptosis of cells, suggesting that bacteria target apoptosis to interfere with this antimicrobial host pathway.^{65,66}

Pseudomonas strains lacking the effector protein ExoU activate the NLRC4 inflammasome and trigger caspase-1-dependent death of BMDM.⁶⁷ *Pseudomonas* strains expressing ExoU can inhibit caspase-1 and drive caspase-1-independent cell death. In these strains, neutrophil elastase, but not NLRC4 or caspase-1, is responsible for IL-1 β production in a mouse corneal model of *Pseudomonas* infection.⁶⁸ Like *Pseudomonas*, *Legionella* is an intracellular pathogen that activates the NLRC4 inflammasome, but when pyroptosis is not activated, it relies on expression of the pro-survival host protein BCL-XL to maintain host cell viability and enable bacterial replication.^{69–71} Antagonists of BCL-XL, known as BH3 mimetics, trigger BAK/BAX-dependent apoptotic cell death. This induction of cell death eliminates intracellular *Legionella* and prevents fatal lung infection in mice.⁷¹ The extent to which apoptosis of *Legionella*-infected cells can amplify the inflammatory response, particularly in comparison with inflammatory forms of cell death such as NLRC4-dependent pyroptosis and necroptosis, remains to be determined.⁷⁰

Yersinia promotes pathogenesis by delivering virulence factors to the cytoplasm via a type III secretion system. The activity of these effector proteins can be recognized by the NLRP3, NLRC4, pyrin and NLRP12 inflammasomes, triggering cytokine processing and cell death. *Yersinia* outer proteins (Yop) YopE and YopT inactivate Rho GTPases, which trigger the formation of the pyrin inflammasome.⁷² YopM recruits ribosomal S6 kinase (RSK) and the

serine/threonine protein kinase C-related kinases (PRK) PRK1 and PRK2 to phosphorylate 14-3-3 and prevent activation of the pyrin inflammasome.^{72,73} Inflammasome activation and pyroptosis are also antagonized by YopK and JopJ.^{74–78} It is therefore possible that gain-of-function mutations in PYRIN in patients with Familial Mediterranean Fever (FMF) or PYRIN-associated autoinflammation with neutrophilic dermatoses (PAAND) have been maintained in the population by conferring protection to *Yersinia* and other pathogens that inactivate the PYRIN inflammasome.^{79–81}

MLKL: A KILLER OR AN ACCOMPLICE?

There are many pathogens known to trigger alternate forms of cell death by impairing proapoptotic caspase function. For example, the cowpox serpin protein cytokine response modifier A (CrmA) inhibits caspase-1 and caspase-8.⁸² Mouse cytomegalovirus (MCMV) can inhibit both apoptosis and necroptosis by expressing viral inhibitor of caspase-8-induced apoptosis (vICA), an inhibitor of Caspase-8, and M45 (viral inhibitor of RIP activation; vIRA), a RHIM domain containing protein that binds to RIPK3.^{83–85} MCMV mutants that are deficient in M45 (M45), or with mutations in the RHIM domain, cannot inhibit RIPK3-dependent necroptosis and are avirulent.⁸⁵ Human cytomegalovirus is even more intriguing because it inhibits necroptosis downstream of RIPK3 activation and MLKL phosphorylation.⁸⁶ This therefore indicates the existence of a signaling network downstream of the MLKL phosphorylation event. This downstream signaling network may contribute to the membrane-disrupting capacity of MLKL or it may alter other cellular responses that contribute to the loss of cell viability.

The vaccinia virus F1L protein shares homology with Bcl-2 and can inhibit apoptosis.^{87,88} F1L also suppresses NLRP1-induced IL-1 β production.⁸⁹ Despite the ability of vaccinia virus to evade several cell death pathways, it fails to avoid the MLKL-dependent necroptosis pathway. This illustrates the benefits associated with the evolution of diverse biochemical pathways, all leading to cell death (Figure 1). Host-regulated cell death pathways therefore contribute to hostpathogen interactions, and they are targets of a diverse range of molecules deployed by pathogens. Pharmacological manipulation of these host cell death pathways may provide new opportunities to treat infection without exerting selective pressure on the pathogen.

SUMMARY

The activation of caspase-1-dependent, caspase-4/11-dependent and RIPK3/MLKL-dependent cell death pathways is inflammatory *in vivo* and thereby acts as an immunomodulatory event. Why these pathways have evolved to counter infection, how they are utilized in different innate immune cells, and whether they function beyond the indirect elicitation of high levels of inflammatory cytokines, has remained largely obscure. The study of how specific cell types have co-opted these pathways to serve key effector functions in resistance to infection, or aberrantly activate the pathway in other disease settings, will reveal new approaches to treat infection, kill cancer cells and provide novel targets for drug discovery.

References

1. Brennan MA, Cookson BT. Salmonella induces macrophage death by caspase-1-dependent necrosis. *Mol Microbiol.* 2000; 38:31–40. [PubMed: 11029688]
2. Kayagaki N, Warming S, Lamkanfi M, Walle LV, Louie S, Dong J, et al. Non-canonical inflammasome activation targets caspase-11. *Nature.* 2011; 479:117–121. [PubMed: 22002608]
3. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science.* 2013; 341:1246–1249. [PubMed: 23887873]
4. Aachoui Y, Leaf IA, Hagar JA, Fontana MF, Campos CG, Zak DE, et al. Caspase-11 protects against bacteria that escape the vacuole. *Science.* 2013; 339:975–978. [PubMed: 23348507]
5. Hagar JA, Powell DA, Aachoui Y, Ernst RK, Miao EA. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. *Science.* 2013; 341:1250–1253. [PubMed: 24031018]
6. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, et al. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature.* 2014; 514:187–192. [PubMed: 25119034]
7. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009; 27:229–265. [PubMed: 19302040]
8. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell.* 2002; 10:417–426. [PubMed: 12191486]
9. Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, et al. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell.* 1995; 80:401–411. [PubMed: 7859282]
10. Vince JE, Wong WW, Gentle I, Lawlor KE, Allam R, O'Reilly L, et al. Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity.* 2012; 36:215–227. [PubMed: 22365665]
11. Fink SL, Cookson BT. Pyroptosis and host cell death responses during Salmonella infection. *Cell Microbiol.* 2007; 9:2562–2570. [PubMed: 17714514]
12. Masters SL, Gerlic M, Metcalf D, Preston S, Pellegrini M, O'Donnell JA, et al. NLRP1 inflammasome activation induces pyroptosis of hematopoietic progenitor cells. *Immunity.* 2012; 37:1009–1023. [PubMed: 23219391]
13. Singer II, Scott S, Chin J, Bayne EK, Limjuco G, Weidner J, et al. The interleukin-1 beta-converting enzyme (ICE) is localized on the external cell surface membranes and in the cytoplasmic ground substance of human monocytes by immuno-electron microscopy. *J Exp Med.* 1995; 182:1447–1459. [PubMed: 7595215]
14. Fink SL, Cookson BT. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell Microbiol.* 2006; 8:1812–1825. [PubMed: 16824040]
15. Moffett P, Farnham G, Peart J, Baulcombe DC. Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. *EMBO J.* 2002; 21:4511–4519. [PubMed: 12198153]
16. Zhang Y, Goritschnig S, Dong X, Li X. A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1-1, constitutive 1. *Plant Cell.* 2003; 15:2636–2646. [PubMed: 14576290]
17. Bendahmane A, Farnham G, Moffett P, Baulcombe DC. Constitutive gain-of-function mutants in a nucleotide binding site-leucine rich repeat protein encoded at the Rx locus of potato. *Plant J.* 2002; 32:195–204. [PubMed: 12383085]
18. Baker PJ, Boucher D, Bierschenk D, Tebartz C, Whitney PG, D'Silva DB, et al. NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. *Eur J Immunol.* 2015; 45:2918–2926. [PubMed: 26173988]
19. Schmid-Burgk JL, Gaidt MM, Schmidt T, Ebert TS, Bartok E, Hornung V. Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells. *Eur J Immunol.* 2015; 45:2911–2917. [PubMed: 26174085]

20. Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host Microbe*. 2012; 12:509–520. [PubMed: 22999859]
21. Kaparakis M, Turnbull L, Carneiro L, Firth S, Coleman HA, Parkington HC, et al. Bacterial membrane vesicles deliver peptidoglycan to NOD1 in epithelial cells. *Cell Microbiol*. 2010; 12:372–385. [PubMed: 19888989]
22. Stephens DS, Edwards KM, Morris F, McGee ZA. Pili and outer membrane appendages on *Neisseria meningitidis* in the cerebrospinal fluid of an infant. *J Infect Dis*. 1982; 146:568. [PubMed: 6126510]
23. Vanaja SK, Russo AJ, Behl B, Banerjee I, Yankova M, Deshmukh SD, et al. Bacterial outer membrane vesicles mediate cytosolic localization of lps and caspase-11 activation. *Cell*. 2016; 165:1106–1119. [PubMed: 27156449]
24. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, et al. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 2016; 535:111–116. [PubMed: 27281216]
25. Aglietti RA, Estevez A, Gupta A, Ramirez MG, Liu PS, Kayagaki N, et al. GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc Natl Acad Sci USA*. 2016; 113:7858–7863. [PubMed: 27339137]
26. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. 2016; 535:153–158. [PubMed: 27383986]
27. Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med*. 1995; 182:1545–1556. [PubMed: 7595224]
28. Montigny C, Lyons J, Champeil P, Nissen P, Lenoir G. On the molecular mechanism of flippase- and scramblase-mediated phospholipid transport. *Biochim Biophys Acta*. 2016; 1861:767–783. [PubMed: 26747647]
29. Tucker AN, White DC. Heterogeneity of phospholipid composition in the bacterial membrane. *J Bacteriol*. 1970; 102:508–513. [PubMed: 4986762]
30. Kamio Y, Nikaido H. Outer membrane of *Salmonella typhimurium*: accessibility of phospholipid head groups to phospholipase c and cyanogen bromide activated dextran in the external medium. *Biochemistry*. 1976; 15:2561–2570. [PubMed: 820368]
31. Schick PK, Kurica KB, Chacko GK. Location of phosphatidylethanolamine and phosphatidylserine in the human platelet plasma membrane. *J Clin Invest*. 1976; 57:1221–1226. [PubMed: 1262468]
32. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*. 2015; 526:666–671. [PubMed: 26375259]
33. Russo HM, Rathkey J, Boyd-Tressler A, Katsnelson MA, Abbott DW, Dubyak GR. Active caspase-1 induces plasma membrane pores that precede pyroptotic lysis and are blocked by lanthanides. *J Immunol*. 2016; 197:1353–1367. [PubMed: 27385778]
34. Allam R, Lawlor KE, Yu EC, Mildenhall AL, Moujalled DM, Lewis RS, et al. Mitochondrial apoptosis is dispensable for NLRP3 inflammasome activation but non-apoptotic caspase-8 is required for inflammasome priming. *EMBO Rep*. 2014; 15:982–990. [PubMed: 24990442]
35. Gurung P, Anand PK, Malireddi RK, Vande Walle L, Van Opdenbosch N, Dillon CP, et al. FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. *J Immunol*. 2014; 192:1835–1846. [PubMed: 24453255]
36. Chen KW, Gross CJ, Sotomayor FV, Stacey KJ, Tschopp J, Sweet MJ, et al. The neutrophil NLR4 inflammasome selectively promotes IL-1 β maturation without pyroptosis during acute *Salmonella* challenge. *Cell Rep*. 2014; 8:570–582. [PubMed: 25043180]
37. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, et al. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity*. 2013; 39:443–453. [PubMed: 24012422]
38. Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep*. 2014; 7:971–981. [PubMed: 24813885]

39. Wang Z, Jiang H, Chen S, Du F, Wang X. The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell*. 2012; 148:228–243. [PubMed: 22265414]
40. Wang H, Sun L, Su L, Rizo J, Liu L, Wang LF, et al. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. *Mol Cell*. 2014; 54:133–146. [PubMed: 24703947]
41. Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*. 2012; 148:213–227. [PubMed: 22265413]
42. Cai Z, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, et al. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nat Cell Biol*. 2014; 16:55–65. [PubMed: 24316671]
43. Hildebrand JM, Tanzer MC, Lucet IS, Young SN, Spall SK, Sharma P, et al. Activation of the pseudokinase MLKL unleashes the four-helix bundle domain to induce membrane localization and necroptotic cell death. *Proc Natl Acad Sci USA*. 2014; 111:15072–15077. [PubMed: 25288762]
44. Su L, Quade B, Wang H, Sun L, Wang X, Rizo J. A plug release mechanism for membrane permeation by MLKL. *Structure*. 2014; 22:1489–1500. [PubMed: 25220470]
45. Rickard JA, O'Donnell JA, Evans JM, Lalaoui N, Poh AR, Rogers T, et al. RIPK1 regulates RIPK3-MLKL-driven systemic inflammation and emergency hematopoiesis. *Cell*. 2014; 157:1175–1188. [PubMed: 24813849]
46. Dillon CP, Weinlich R, Rodriguez DA, Cripps JG, Quarato G, Gurung P, et al. RIPK1 blocks early postnatal lethality mediated by caspase-8 and RIPK3. *Cell*. 2014; 157:1189–1202. [PubMed: 24813850]
47. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007; 176:231–241. [PubMed: 17210947]
48. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004; 303:1532–1535. [PubMed: 15001782]
49. Beiter K, Wartha F, Albiger B, Normark S, Zychlinsky A, Henriques-Normark B. An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps. *Curr Biol*. 2006; 16:401–407. [PubMed: 16488875]
50. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Kockritz-Blickwede M. Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. *J Innate Immun*. 2010; 2:576–586. [PubMed: 20829609]
51. Thammavongsa V, Missiakas DM, Schneewind O. *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death. *Science*. 2013; 342:863–866. [PubMed: 24233725]
52. Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat Immunol*. 2014; 15:1017–1025. [PubMed: 25217981]
53. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci USA*. 2010; 107:9813–9818. [PubMed: 20439745]
54. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Med*. 2013; 5:178ra40.
55. Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med*. 2015; 21:815–819. [PubMed: 26076037]
56. Thomas GM, Carbo C, Curtis BR, Martinod K, Mazo IB, Schatzberg D, et al. Extracellular DNA traps are associated with the pathogenesis of TRALI in humans and mice. *Blood*. 2012; 119:6335–6343. [PubMed: 22596262]
57. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, et al. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. *Proc Natl Acad Sci USA*. 2013; 110:8674–8679. [PubMed: 23650392]

58. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci USA*. 2010; 107:15880–15885. [PubMed: 20798043]
59. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. *Proc Natl Acad Sci USA*. 2012; 109:13076–13081. [PubMed: 22826226]
60. Amini P, Stojkov D, Wang X, Wicki S, Kaufmann T, Wong WW, et al. NET formation can occur independently of RIPK3 and MLKL signaling. *Eur J Immunol*. 2016; 46:178–184. [PubMed: 26549703]
61. Desai J, Kumar SV, Mulay SR, Konrad L, Romoli S, Schauer C, et al. PMA and crystal-induced neutrophil extracellular trap formation involves RIPK1-RIPK3-MLKL signaling. *Eur J Immunol*. 2016; 46:223–229. [PubMed: 26531064]
62. Vandenabeele P, Grootjans S, Callewaert N, Takahashi N. Necrostatin-1 blocks both RIPK1 and IDO: consequences for the study of cell death in experimental disease models. *Cell Death Differ*. 2013; 20:185–187. [PubMed: 23197293]
63. Johnston JB, Barrett JW, Nazarian SH, Goodwin M, Ricciuto D, Wang G, et al. A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity*. 2005; 23:587–598. [PubMed: 16356857]
64. Rahman MM, Mohamed MR, Kim M, Smallwood S, McFadden G. Co-regulation of NF-kappaB and inflammasome-mediated inflammatory responses by myxoma virus pyrin domain-containing protein M013. *PLoS Pathog*. 2009; 5:e1000635. [PubMed: 19851467]
65. Li S, Zhang L, Yao Q, Li L, Dong N, Rong J, et al. Pathogen blocks host death receptor signalling by arginine GlcNAcylation of death domains. *Nature*. 2013; 501:242–246. [PubMed: 23955153]
66. Pearson JS, Giogha C, Ong SY, Kennedy CL, Kelly M, Robinson KS, et al. A type III effector antagonizes death receptor signalling during bacterial gut infection. *Nature*. 2013; 501:247–251. [PubMed: 24025841]
67. Sutterwala FS, Mijares LA, Li L, Ogura Y, Kazmierczak BI, Flavell RA. Immune recognition of *Pseudomonas aeruginosa* mediated by the IPAF/NLRC4 inflammasome. *J Exp Med*. 2007; 204:3235–3245. [PubMed: 18070936]
68. Karmakar M, Sun Y, Hise AG, Rietsch A, Pearlman E. Cutting edge: IL-1beta processing during *Pseudomonas aeruginosa* infection is mediated by neutrophil serine proteases and is independent of NLRC4 and caspase-1. *J Immunol*. 2012; 189:4231–4235. [PubMed: 23024281]
69. Vinzing M, Eitel J, Lippmann J, Hocke AC, Zahlten J, Slevogt H, et al. NAIP and Ipaf control *Legionella pneumophila* replication in human cells. *J Immunol*. 2008; 180:6808–6815. [PubMed: 18453601]
70. Cerqueira DM, Pereira MS, Silva AL, Cunha LD, Zamboni DS. Caspase-1 but not caspase-11 is required for NLRC4-mediated pyroptosis and restriction of infection by flagellated *Legionella* species in mouse macrophages and in vivo. *J Immunol*. 2015; 195:2303–2311. [PubMed: 26232428]
71. Speir M, Lawlor KE, Glaser SP, Abraham G, Chow S, Vogrin A, et al. Eliminating *Legionella* by inhibiting BCL-XL to induce macrophage apoptosis. *Nat Microbiol*. 2016; 1:15034. [PubMed: 27572165]
72. Chung LK, Park YH, Zheng Y, Brodsky IE, Hearing P, Kastner DL, et al. The *Yersinia* virulence factor YopM hijacks host kinases to inhibit type III effector-triggered activation of the Pyrin inflammasome. *Cell Host Microbe*. 2016; 20:1–11. [PubMed: 27414492]
73. McDonald C, Vacratsis PO, Bliska JB, Dixon JE. The *Yersinia* virulence factor YopM forms a novel protein complex with two cellular kinases. *J Biol Chem*. 2003; 278:18514–18523. [PubMed: 12626518]
74. Brodsky IE, Palm NW, Sadanand S, Ryndak MB, Sutterwala FS, Flavell RA, et al. A *Yersinia* effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. *Cell Host Microbe*. 2010; 7:376–387. [PubMed: 20478539]
75. Vladimer GI, Weng D, Paquette SW, Vanaja SK, Rathinam VA, Aune MH, et al. The NLRP12 inflammasome recognizes *Yersinia pestis*. *Immunity*. 2012; 37:96–107. [PubMed: 22840842]

76. LaRock CN, Cookson BT. The *Yersinia* virulence effector YopM binds caspase-1 to arrest inflammasome assembly and processing. *Cell Host Microbe*. 2012; 12:799–805. [PubMed: 23245324]
77. Schoberle TJ, Chung LK, McPhee JB, Bogin B, Bliska JB. Uncovering an important role for YopJ in the inhibition of caspase-1 in activated macrophages and promoting *Yersinia pseudotuberculosis* virulence. *Infect Immun*. 2016; 84:1062–1072. [PubMed: 26810037]
78. Zwack EE, Snyder AG, Wynosky-Dolfi MA, Ruthel G, Philip NH, Marketon MM, et al. Inflammasome activation in response to the *Yersinia* type III secretion system requires hyperinjection of translocon proteins YopB and YopD. *mBio*. 2015; 6:e02095–e02114. [PubMed: 25691590]
79. Masters SL, Lagou V, Jeru I, Baker PJ, Van Eyck L, Parry DA, et al. Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. *Sci Transl Med*. 2016; 8:332ra45.
80. French FMFC. A candidate gene for familial Mediterranean fever. *Nat Genet*. 1997; 17:25–31. [PubMed: 9288094]
81. Consortium IF. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. The International FMF Consortium. *Cell*. 1997; 90:797–807.
82. Zhou Q, Snipas S, Orth K, Muzio M, Dixit VM, Salvesen GS. Target protease specificity of the viral serpin CrmA: analysis of five caspases. *J Biol Chem*. 1997; 272:7797–7800. [PubMed: 9065443]
83. Upton JW, Kaiser WJ, Mocarski ES. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. *Cell Host Microbe*. 2012; 11:290–297. [PubMed: 22423968]
84. Upton JW, Kaiser WJ, Mocarski ES. Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. *J Biol Chem*. 2008; 283:16966–16970. [PubMed: 18442983]
85. Upton JW, Kaiser WJ, Mocarski ES. Virus inhibition of RIP3-dependent necrosis. *Cell Host Microbe*. 2010; 7:302–313. [PubMed: 20413098]
86. Omoto S, Guo H, Talekar GR, Roback L, Kaiser WJ, Mocarski ES. Suppression of RIP3-dependent necroptosis by human cytomegalovirus. *J Biol Chem*. 2015; 290:11635–11648. [PubMed: 25778401]
87. Wasilenko ST, Stewart TL, Meyers AF, Barry M. Vaccinia virus encodes a previously uncharacterized mitochondrial-associated inhibitor of apoptosis. *Proc Natl Acad Sci USA*. 2003; 100:14345–14350. [PubMed: 14610284]
88. Kvansakul M, Yang H, Fairlie WD, Czabotar PE, Fischer SF, Perugini MA, et al. Vaccinia virus anti-apoptotic F1L is a novel Bcl-2-like domain-swapped dimer that binds a highly selective subset of BH3-containing death ligands. *Cell Death Differ*. 2008; 15:1564–1571. [PubMed: 18551131]
89. Gerlic M, Faustin B, Postigo A, Yu EC, Proell M, Gombosuren N, et al. Vaccinia virus F1L protein promotes virulence by inhibiting inflammasome activation. *Proc Natl Acad Sci USA*. 2013; 110:7808–7813. [PubMed: 23603272]

Figure 1.

Immunomodulatory pathways of apoptotic and pyroptotic cell death. A full color version of this figure is available online at the *Immunology and Cell Biology* website.