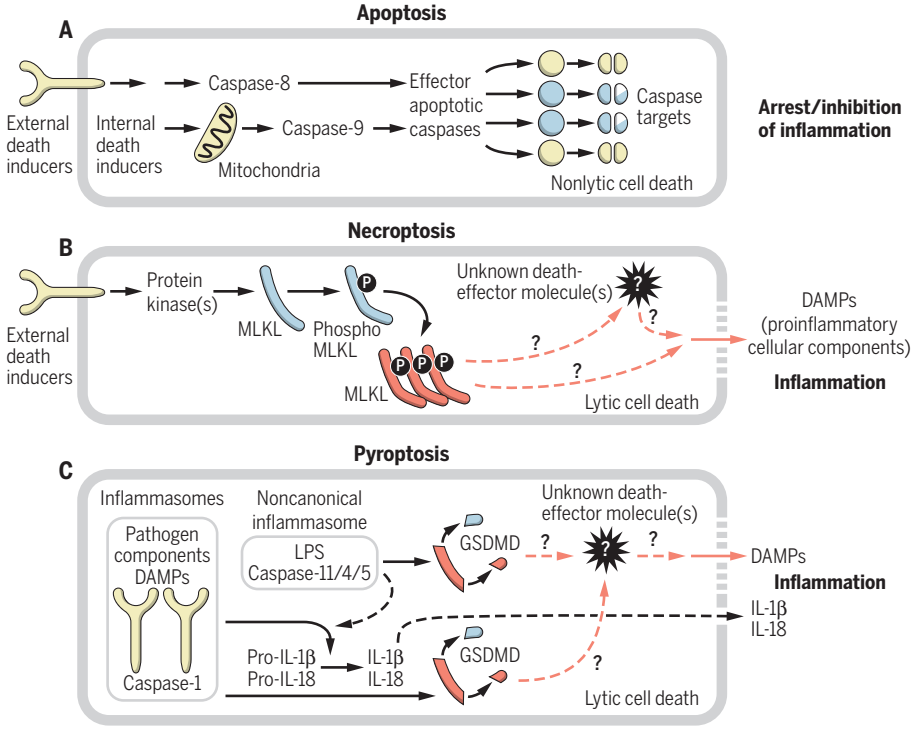
REVIEW SUMMARY

CELL DEATH PATHWAYS

Programmed necrosis in inflammation: Toward identification of the effector molecules

David Wallach,* Tae-Bong Kang, Christopher P. Dillon, Douglas R. Green*

BACKGROUND: Inflammatory lesions often contain dead cells. Cell death in developmental processes characteristically occurs by apoptosis, a form of programmed cell death in which dying cells are phagocytized before undergoing membrane damage. In inflammation, however, cell death is often necrotic, where cellular constituents are released after membrane rupture. The death of cells in inflammation was until recently thought to result from other changes in the inflamed tissue. This view has changed, however, owing to several findings: that necrotic cell death can be induced by biomolecules known to initiate inflammation [such as the cytokine tumor necrosis factor (TNF) or the pathogen component lipopolysaccharide (LPS)]; that it can be dictated in a programmed manner by distinct sets of signaling mechanisms; and that it yields release of some cellular components capable of facilitating inflammation. It now seems probable that necrotic cell death is not always a consequence of inflammation, but is sometimes rather its trigger. To confirm this notion, we need reliable tools for detection of programmed necrosis in vivo. Because programmed necrosis cannot be distinguished morphologically from accidental cell death, its identification in inflamed tissues must be based on its distinctive molecular details.



Effector mechanisms in lytic and nonlytic cell death (**A**) In apoptosis, caspases cleave substrate proteins that orchestrate the cell-death process. (**B**) In necroptosis, protein kinases phosphorylate MLKL, thereby activating it. Phospho-MLKL then causes cell lysis. (**C**) In pyroptosis, other caspases cleave and hence activate gasdermin-D (GSDMD), thus causing death. These caspases also activate cytokines IL-1β and IL-18. In both necroptosis and pyroptosis, the cell membrane ruptures, releasing cellular components that may trigger inflammation.

ADVANCES: We now have quite detailed knowledge of the mechanisms initiating apoptotic cell death and those initiating two forms of programmed necrosis—necroptosis and pyroptosis. Apoptosis is triggered by proteases of the caspase family. Necroptosis is triggered by specific protein kinases, most crucially receptor-interacting protein kinase–3 (RIPK3). Pyroptosis is triggered by caspases distinct from those mediating apoptosis, and whose activation yields proteolytic activation of the inflammatory cytokines interleukin-1β (IL-1β) and IL-18. All of these molecular initiators of death programs, however, also contribute to the initiation of nondeadly cell functions and are thus not specific markers for death. Two proteins were recently found to act further downstream in the signaling pathways leading to programmed necrosis. One, the pseudokinase mixed lineage kinase domain–like protein (MLKL), is crucial for necroptosis. The other, gasdermin-D (GSDMD), after cleavage by the pyroptosis-mediating caspases, is a major player in their induction of death. Mere expression of activated MLKL, or of the N-terminal proteolytic fragment of GSDMD, can trigger necrotic death. The finding that MLKL and GSDMD play roles in necroptosis and pyroptosis raises hopes that we are approaching the identification of molecules that exclusively serve these forms of death.

ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aaf2154

OUTLOOK: What are the parameters that can reliably allow us to define a cause-effect relationship between necrotic death and inflammation—and more generally, to define the causal relationships between any disease and its co-occurring pathogenic events? In the case of cell death, this question boils down to the need to identify molecular events that contribute specifically enough to allow their use as definitive molecular probes. The effectors of death—proteins found to mediate deadly changes in the cell—are likely to have that absolute specificity. Whether MLKL and GSDMD are such death effectors has yet to be established. Some studies suggest that MLKL directly permeabilizes membranes, whereas others suggest that it does not. Regarding GSDMD, there is still no knowledge of the mechanisms by which its N-terminal fragment triggers death. Identifying death-effector molecules will be of immense importance to medicine. Such molecules will not only be the best markers for detecting programmed necrosis, but will also serve as the optimal targets for its pharmaceutical arrest in disease. ■

The list of author affiliations is available in the full article online.
*Corresponding author. E-mail: d.wallach@weizmann.ac.il (D.W.); douglas.green@stjude.org (D.R.G.)
Cite this article as D. Wallach *et al.*, Science 352, aaf2154 (2016). DOI: 10.1126/science.aaf2154

REVIEW

CELL DEATH PATHWAYS

Programmed necrosis in inflammation: Toward identification of the effector molecules

David Wallach,[1]* Tae-Bong Kang,[2] Christopher P. Dillon,[3] Douglas R. Green[3]*

Until recently, programmed cell death was conceived of as a single set of molecular pathways. We now know of several distinct sets of death-inducing mechanisms that lead to differing cell-death processes. In one of them—apoptosis—the dying cell affects others minimally. In contrast, programmed necrotic cell death causes release of immunostimulatory intracellular components after cell-membrane rupture. Defining the in vivo relevance of necrotic death is hampered because the molecules initiating it [such as receptor-interacting protein kinase–1 (RIPK1), RIPK3, or caspase-1] also serve other functions. Proteins that participate in late events in two forms of programmed necrosis [mixed lineage kinase domain–like protein (MLKL) in necroptosis and gasdermin-D in pyroptosis] were recently discovered, bringing us closer to identifying molecules that strictly serve in death mediation, thereby providing probes for better assessing its role in inflammation.

"When I use a word…it means just what I choose it to mean—neither more nor less." This declaration from Lewis Carroll's *Through the Looking Glass* aptly describes a common practice of scientists. By defining terms and deciding what they stand for, they spur interest in testing the validity of their hypotheses; unlike Humpty Dumpty's assertion, however, terms defined by scientists often turn out to mean more than initially conceived.

The term "programmed cell death" has made a huge and persisting impact on cell death research (*1*). However, recent developments in cell death research have altered our view of what this term means. Until recently, programmed cell death was thought to occur only in one form, called "apoptosis." This form is characterized by induced changes that yield death in a way that minimizes its impact on neighboring living cells. Recent findings have revealed, however, that another form of death, "necrosis" or "oncosis," can also be dictated by defined molecular pathways. Necrosis involves release of the dying cell's intracellular components, and the effects of this release on neighboring cells can yield inflammation (*2*).

Based on the findings regarding apoptosis, programmed cell death was initially viewed as a set of mechanisms that act exclusively within the dying cell and serve only to dictate its demise. In programmed necrosis, the end of the plan of action toward death is believed to be not only death induction but also induction of inflammation, another complex programmed process capable of causing death in other ways. Here, we address the challenges faced in the study of programmed necrosis in vivo and the attempts to overcome them by harnessing the knowledge gained ex vivo. While there are various pathways by which necrosis can be "programmed," we focus here on those of necroptosis and pyroptosis. (See glossary in Box 1.)

[1]Department of Biomolecular Sciences, The Weizmann Institute of Science, 76100 Rehovot, Israel. [2]Department of Biotechnology, College of Biomedical and Health Science, Konkuk University, Chung-Ju 380-701, Korea. [3]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.
*Corresponding author. E-mail: d.wallach@weizmann.ac.il (D.W.); douglas.green@stjude.org (D.R.G.)

Possible roles of lytic and nonlytic cell death in inflammation

Inflammation is a multicellular process that destroys pathogens and promotes recovery from injury through a variety of cooperating changes, including cell death (*2*). Initially identified as a pathological phenomenon, cell death is still often described in pathology textbook chapters on tissue damage. Indeed, some of the most harmful consequences of infection and inflammation, including the massive damage to tissues in necrotizing fasciitis ("flesh-eating" syndrome) and the functional arrest of regions in the heart and brain pursuant to infarct and stroke, can be ascribed to massive cell death.

Various manifestations of the pathology of inflammation are known to be mediated by cellular functions that are potentially beneficial, yet cause damage when induced in excess. Similarly, when cell death in inflammation occurs in a restricted manner, its role can be beneficial, as in the elimination of injured or infected cells, which are taken up by phagocytosis as they die (*3*, *4*).

Phagocytic uptake of a dying infected cell, however, might not suffice to eradicate the pathogens that infected it, and might instead have the opposite effect. Being able to survive and replicate within cells, intracellular pathogens may continue to survive in the cells that engulf them and might thus exploit this uptake for their further spread. This is particularly true of pathogens that can withstand destructive activities within professional phagocytes. Indeed, a variety of harmful intracellular pathogens prosper in phagocytic cells, including *Mycobacterium tuberculosis*, *Shigella flexneri*, and others. In order for cell death to assist in destroying such pathogens, these pathogens must permeate through the cell membrane into the extracellular milieu, where they might be unable to grow and/or be exposed to other defensive mechanisms.

Lytic cell death can also contribute to defense in another way. Various intracellular components, once released from dying cells, can trigger inflammatory activities in other cells. By allowing the release of such "damage associated molecular patterns" (DAMPs), lytic cell death may self-amplify or even initiate the inflammatory process.

Thus, depending on the role that cell death might play in inflammation, it should optimally occur in one of two ways. For eliminating intracellular pathogens that resist destruction by intracellular defense mechanisms, death of the cells would preferably involve rupture of their membranes. In contrast, death destined to eliminate pathogens that are easily destroyed within professional phagocytes, or facilitation of repair by phagocytic uptake of malfunctioning cells, would be better served without membrane rupture (nonlytic cell death), thus maximizing removal of the cellular constituents and preventing DAMP-mediated perpetuation of inflammation.

Cell death also plays a role in promoting adaptive immune responses to antigens associated with the dying cells (e.g., produced by an infecting organism or by cancer-associated mutations). This "immunogenic cell death" is distinct from inflammation per se (*5*). Immunogenic cell death involves activation of dendritic cells and a process of "cross-priming," in which molecules in the dying cell are processed and presented on class I major histocompatibility complex (MHC) molecules in the dendritic cell for recognition by $CD8^+$ T lymphocytes (*6*).

Apoptosis—a nonlytic form of death mediated by proteases of the caspase family

The characteristics of apoptotic cell death minimize disturbance of living cells by the dying cells. This cell death program is prevalent in development and tissue remodeling, processes not associated with inflammation. When apoptosis occurs in inflammatory lesions, it might actually serve to terminate the inflammation. In apoptosis, a group of structurally related proteases called "effector" (or "executioner") caspases (in humans, caspase-3, -6, and -7) cleave specific target sites in numerous cellular proteins. These caspases initiate multiple enzymatic and structural changes that lead to the morphological and functional changes characteristic of apoptosis. As expected for a programmed process, the various molecular pathways that are targeted for cleavage by the

Box 1. Glossary of terms.

Apoptosis: a programmed form of nonlytic cell death resulting from the activation of effector caspases via a variety of upstream signals

Caspases: cysteine-dependent aspartate-directed proteases that cleave numerous targets in the cell after activation by upstream signals

Cross-priming: the process of uptake and presentation of antigen on class I MHC by phagocytic cells such as dendritic cells in order to activate CD8 response

Damage associated molecular patterns (DAMPs): a series of intracellular components released upon cellular damage that induce inflammatory activities in other cells

Effector caspase: a caspase activated via an initiator caspase that targets cellular proteins for cleavage and orchestrates the process of apoptosis; also referred to as an executioner caspase

IL-1β: an inflammatory cytokine produced in proform requiring caspase cleavage for maturation and release

Immunogenic cell death: cell death that directly activates the immune response through uptake of the dying cells and presentation of associated antigens to T lymphocytes

Inflammasome: a general term for macromolecular complexes acting as activation platforms for caspase-1, -4, -5, or -11

Inflammation: a complex biological response involving production of cytokines to recruit innate and adaptive immune cells in order to eliminate the initial insult, clear out damaged tissue, and initiate the repair process through a variety of cooperating functional changes

Inflammatory caspases: caspases 1 and 11 in mouse and 1, 4, and 5 in human whose activation leads to the processing of IL-1β

Initiator caspase: a regulatory caspase that requires the formation of a macromolecular complex for activation, and functions to activate effector caspases

Koch postulates: a set of four criteria published by the German physician Robert Koch in 1890 designed to evaluate the causative link between a microbe and disease

Lytic cell death: cell death that results in the acute permeabilization of the plasma membrane

Necroptosis: programmed form of lytic cell death in which RIPK3 activation leads to downstream activation of MLKL and the acute permeabilization of the plasma membrane

Nonlytic cell death: cell death in which cells are packaged for disposal by phagocytes, generally preventing the release of intracellular contents

Phagocytosis: the process of a cell (i.e., macrophages, dendritic cells) ingesting cell fragments or microorganisms, leading to the formation of a vacuole to degrade the engulfed components

Pleiotropy: the phenomenon in which something, such as a protein, plays more than one, often opposing, role in cellular processes; for example, caspase-8 is necessary for death receptor–mediated apoptosis, but restrains necroptosis

Programmed cell death: originally referred specifically to cell death that is "programmed" in development, but now taken to mean any cell death engaged by an active predestined molecular mechanism

PRR: pattern recognition receptor; cellular proteins evolved to specifically bind components of pathogens and to initiate signaling for defense

Pyroptosis: lytic form of cell death characterized by activation of inflammatory caspases and usually associated with secretion of IL-1β and IL-18

effector caspases are evolutionarily conserved (*7*). Some of these molecular pathways serve to safeguard against the release of DAMPs, limiting the initiation of inflammation; other molecular features actively suppress inflammatory activity by leukocytes (*8*).

The ability of a wide range of different inducers to activate the same apoptotic program has been ascribed to the existence of molecular hubs that convey activating signals to the effector caspases through some other members of the caspase family defined as "initiator caspases." In the so-called "intrinsic cell death pathway," effects of intracellular death inducers converge, mainly through the function of members of the Bcl-2 protein family. The latter act to permeabilize the outer membranes of the mitochondria, releasing cytochrome c and other intermembrane space proteins. Cytochrome c triggers the oligomerization of an adapter protein, APAF1, which then binds and thereby activates the initiator caspase, caspase-9 (*9*, *10*).

In the extrinsic cell death pathway, signals emanating from the receptors for tumor necrosis factor (TNF) and from some other membranous receptors converge to oligomerize the adapter protein FADD/MORT1. This in turn binds and thus activates another initiator caspase, caspase-8 (*11*).

In both the intrinsic and the extrinsic cell death pathways, the triggering of apoptotic death occurs by imposed dimerization of the initiator caspases within distinct signaling complexes. Association of the caspases with their adapter proteins in these complexes is mediated by homotypic binding domains encompassing one of four related "death-fold" motifs (*12*). Once activated, caspase-8 and -9 proteolytically process and activate effector caspases.

Necroptosis—a regulated, lytic cell death involving specific kinases

The term "necrosis" (or "oncosis") refers to all forms of death that are characterized by swelling of the cells and their organelles, followed by permeabilization of the cellular membranes. This is the typical pattern of death of cells in response to accidental damage, and also of the two forms of programmed cell death described here—necroptosis and pyroptosis. Whereas the morphologic features of apoptosis, both in vivo and in cell culture, distinguish it clearly from other forms of cell death, there is currently no way to distinguish necroptosis or pyroptosis from accidental necrotic death merely by visual inspection of the cells. Our knowledge of those forms of programmed death and their consequences is therefore largely restricted to information based on identifying the molecules involved, either in vitro or through the use of genetically modified animals in vivo.

The discovery of necroptosis can be traced back to the seminal finding in 1965 that certain leukocyte-produced cytokines, called lymphotoxins, cytotoxins, or TNF, can trigger cell death (*13*, *14*). This death was initially reported to occur by apoptosis. By 1988 it had been clarified, however, that in some cells, TNF also triggers necrotic death (*15*).

The most upstream signaling activity required for induction of necroptosis by a TNF ligand family member is the protein kinase function of receptor-interacting protein kinase–1 (RIPK1) (*16*, *17*) (Fig. 1). Several other biological stimuli trigger necroptosis independently of RIPK1: the interferons (IFNs) exert necroptotic effects that depend on the interferon-induced protein kinase PKR (*18*, *19*). Also, Toll-like receptor 3 (TLR3), a membrane-anchored receptor for viral nucleic acid, induces necroptosis via the adapter protein TRIF (*20*). Some viruses can also trigger necroptosis independently of RIPK1. The cell death response to mouse cytomegalovirus (MCMV) infection, for example, employs the IFN-induced protein DAI (*21*–*23*). Unlike RIPK1, neither TRIF nor DAI possesses protein-kinase or any other enzymatic activity.

The protein kinase RIPK3 acts downstream of RIPK1 in the necroptotic signaling pathway (*24*–*26*). It is activated by associating with RIPK1, TRIF or DAI, with which it associates via a RIP homotypic interacting motif, RHIM. Strong negative regulation of the initiation of signaling for necroptosis is mediated by caspase-8 together with a caspase-like molecule, c-FLIP$_L$ (*16*, *27*, *28*), and by the ubiquitin ligases cIAP1 and cIAP2 (*29*). The last two mediate their effect by facilitating the anchorage of K63-linked polyubiquitin chains to RIPK1, thereby arresting the integration of RIPK1 into the signaling protein complex that initiates necroptosis.

RIPK3 mediates necroptosis by binding mixed lineage kinase domain–like protein (MLKL), a

protein kinase homolog that, despite binding adenosine 5′-triphosphate, is devoid of protein kinase activity (*30*, *31*). RIPK3 phosphorylates MLKL, thereby imposing a conformational change that negates a self-inhibitory effect of the kinase-homologous C-terminal region in MLKL. This allows the N-terminal coiled-coil region to impose oligomerization of the MLKL molecules and subsequent cell lysis (*32*–*34*). Mere expression of the coiled-coil portion of MLKL, or of MLKL mutants in which the coiled-coil region is constitutively exposed, is toxic to cells (*33*, *35*, *36*). MLKL is therefore currently believed to be the major and perhaps the only RIPK3 target that mediates this form of death.

However, RIPK3 also participates in signaling to other processes, including other forms of cell death, independently of MLKL. Kinase-inactive RIPK3 can signal, via RIPK1, to trigger caspase-8–mediated apoptosis (*37*–*39*). Several metabolic enzymes (*24*), as well as PGAM5, a mitochondrial protein with serine-threonine phosphatase activity (*40*), bind RIPK3 and contribute to induction of reactive oxygen species (ROS) and production of inflammatory cytokines. In addition, RIPK3 binds Ca^{2+}-calmodulin–dependent protein kinase (CaMKII) and activates it. Induction of necrotic death of cardiomyocytes by oxidative stress appears to depend on the RIPK3-mediated activation of CaMKII and on the above metabolic enzymes. This death also appears to involve the generation of ROS and the function of cyclophilin-D—a key regulator of the mitochondrial permeability transition (MPT) pore—but not MLKL (*41*). MPT-mediated regulated necrosis is believed to occur by mechanisms distinct from those mediating necroptosis (*42*). In some cells, induction of necroptosis can be suppressed by ROS scavengers (*43*), suggesting a cell-specific requirement for synergism of the two pathways in death induction.

That necroptosis is induced by TNF, a ligand for which almost all cells express receptors, might appear to suggest that this form of death is exerted in all cells. In reality, the situation is the opposite; almost all cells are normally resistant to the death-inducing activities of TNF. The cytotoxic activities of this cytokine are therefore usually assessed either by the use of certain transformed cell lines that are uniquely sensitive to them or in the presence of drugs that sensitize cells to those activities (inhibitors of caspase action, or of the function of the ubiquitin ligases cIAP1 and cIAP2, of RNA synthesis, or of protein synthesis).

This strict dependence of necroptosis on sensitizing mechanisms endows it with selectivity. Infection of cells by certain viruses sensitizes them to the cytotoxic activities of TNF and IFN (*44*, *45*). In the case of several of these viruses and some bacteria, the form of death so enhanced is necroptosis. One molecular effect through which these pathogens facilitate necroptosis is prevention of caspase action (and apoptosis) by caspase-inhibitory proteins that they express (Fig. 1). There are also viruses that facilitate necroptosis through the direct action of viral proteins that mimic the function of signaling proteins in the pathway.

On the basis of these observations, it is believed that necroptotic death has evolved to serve as a second line of defense, to assure that immune-mediated killing of pathogen-infected

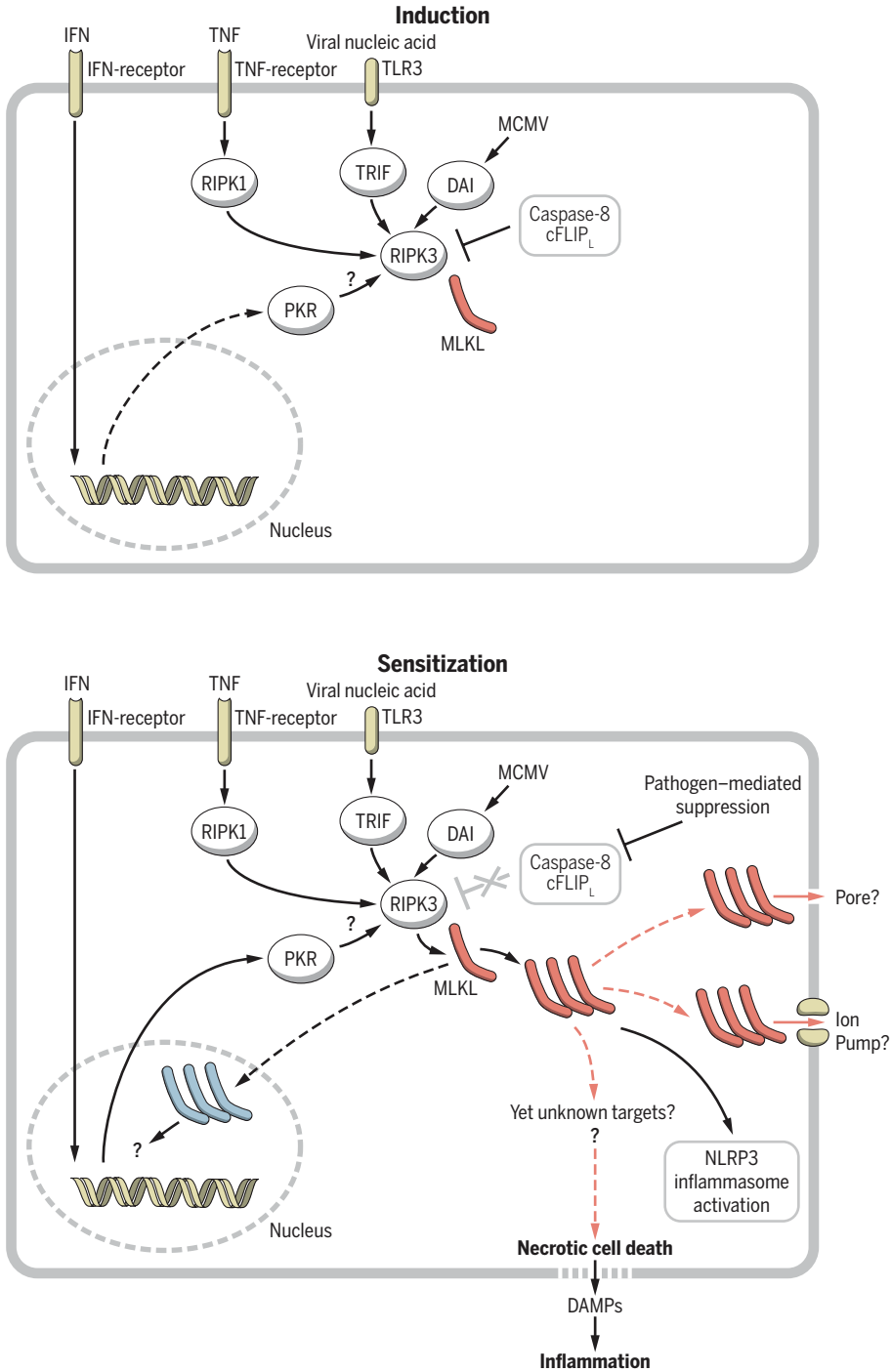


Fig. 1. Initiation of necroptosis: distinct roles of inducing ligands and sensitizing factors. Activation of the proteins that initiate signaling for necroptosis is controlled at two mechanistic levels. The first is induction, mainly by extracellular host-derived ligands such as TNF. This induction does not affect healthy cells because it is normally blocked by cellular inhibitory activities, such as that mediated by the proteolytic function of the caspase-8/cFLIP$_L$ complex (upper panel). The second level is sensitization, by pathogens and other agents that counteract the cellular inhibitory activities, allowing phosphorylation and oligomerization of MLKL and hence triggering of necrotic cell death (lower panel). The figure also depicts the various reported molecular consequences of MLKL activation. Those that might contribute to the induction of cell death are marked in orange.

cells occurs whenever the pathogen acts to block signaling for caspase-mediated apoptotic death. Some pathogens have further evolved to express proteins that block the induction of necroptosis. Conversely, some have usurped mechanisms of necroptosis induction for their own benefit [reviewed in (*46*)].

Pyroptosis—a pathogen-induced lytic form of death, associated with generation of inflammatory cytokines

Pyroptosis was discovered as a form of death that is inflicted on cells upon their infection by certain pathogens. It is usually associated with secretion of IL-1β and IL-18, proinflammatory cytokines generated by proteolyic processing of precursors located within the cytoplasm. Similarly to apoptosis, pyroptosis, as well as its accompanying generation of processed forms of IL-1β and IL-18, is mediated by specific members of the caspase family of cysteine proteases (the "inflammatory caspases"). These caspases—caspase-1 and -11 in mice and caspase-1 and two orthologs of caspase-11 (caspase-4 and -5) in humans—are structurally similar to the initiator caspases that signal for apoptosis. As alluded to in the term "pyroptosis" ("pyro" relating to fire or fever), the effects of DAMPs released from the dying cells, in combination with effects of the mature forms of IL-1β and IL-18, are believed to trigger inflammation. Such induced inflammation following rupture of the cellular membrane can effectively facilitate destruction of intracellular pathogens that have triggered the death program upon their exposure to the extracellular milieu (*47*).

Whereas necroptosis occurs in a wide variety of cell types, pyroptosis was observed mostly in professional phagocytes—macrophages, monocytes, and dendritic cells, where it contributes to defense against pathogens (*47*)—although it has also been reported in other cells. Similarly to necroptosis, pyroptosis can be triggered by intracellular pathogens—bacteria, viruses, and nucleated parasites—and, like necroptosis, it is facilitated by exposure of the cell to extracytoplasmic stimuli such as IFNs, TLR ligands, or TNF. However, the way in which these two groups of stimuli contribute to pyroptosis differs diametrically from how they contribute to necroptosis. In necroptosis, death signaling is usually initiated by extracytoplasmic ligands, and is facilitated by pathogens via the effects of pathogen-derived proteins that release the inhibition of this signaling by host-derived proteins. In contrast, extracytoplasmic ligands like TNF or those of the TLRs do not trigger signaling for pyroptosis, but only "prime" the cell for death induction by facilitating synthesis of the proteins that signal it. The actual triggering event is the recognition of intracellular pathogen components by "pattern recognition receptors" (PRRs), cellular proteins that have evolved to specifically bind components of pathogens, and consequently to initiate signaling for defense (Fig. 2).

Caspase-4, -5, and -11 are themselves PRRs; they are directly activated by Gram-negative bacteria in the cytoplasm within macromolecular signaling complexes called "noncanonical inflammasomes." This activation is mediated by binding of the lipid-A portion of lipopolysaccharide (LPS) to the caspases (*48*).

In the case of caspase-1, however, activation by pathogen components (as well as by DAMPs) occurs through the function of distinct proteins with PRR activity. These proteins bind caspase-1 by death-fold associations within "canonical inflammasomes." The currently known caspase-1–activating PRRs belong to three protein families: the NOD-like receptor (NLR) and the tripartite motif–containing (TRIM) families that mediate activation of caspase-1 by bacterial proteins such as the flagellin of *Legionella pneumophila* and by DAMPs, and the Pyrin and HIN domain (PYHIN; also known as AIM2-like receptors, ALRs) family of PRRs that mediate activation of caspase-1 by cytoplasmic or nuclear DNA of pathogens or by misplaced host nucleic acids.

Some of the caspase-1–activating PRRs bind caspase-1 directly. Others employ ASC (apoptosis-associated speck-like protein containing a CARD), an intermediate adapter protein that serves as a means of signaling amplification. Upon activation, ASC self-associates, forming in each cell a single huge aggregate ("Spek") that massively activates caspase-1 and also induces its self-processing [reviewed in (*49*)].

The functional roles of caspase-1 and of caspase-4, -5, and -11, although interrelated, are apparently somewhat different. Whereas all of them cause pyroptosis, the accompanying processing of IL-1β and IL-18 appears to require caspase-1. The processing of IL-1β and IL-18 by caspase-4, -5, and -11 is indirectly mediated via activation of caspase-1 by the inflammasome containing the PRR NLRP3 (*49*).

Besides cleaving the precursors for IL-1β and IL-18, the inflammatory caspases also cleave several other proteins. This cleavage serves various functions (*50*, *51*). Recent studies revealed that cleavage of gasdermin-D (GSDMD) by these caspases contributes to death induction (*52*–*54*). The N-terminal fragment of GSDMD generated by this cleavage is by itself cytotoxic. Moreover, cells deficient in GSDMD are fully resistant to the induction of pyroptosis by caspase-11. They are also less sensitive to the pyroptotic effect of caspase-1, but are not fully resistant to it (*52*–*54*).

Identifying programmed necrosis in inflamed tissues: A chicken-and-egg conundrum

As mentioned above, inflammation is frequently associated with cell death. This phenomenon has attracted particular attention in situations where cell death is a major pathological determinant—for example, in the massive destruction of liver tissue in acute hepatitis, the loss of lymphocytes in septic shock, or damage to the heart muscle as a result of infarct or to the brain as an outcome of stroke. The identification of distinct sets of mechanisms that dictate programmed death raises hopes that drugs designed to target these mechanisms will be therapeutic in such pathological situations (*42*, *55*). Moreover, the finding that specific molecules control certain forms of necrotic death that can initiate inflammation suggests that drugs targeting these molecules might allow arrest of some inflammatory processes.

To achieve such goals, however, we must first find ways to identify those pathological situations in which specific forms of programmed cell death occur. We must then identify those situations in which these death programs serve an initiating role. Acquiring such knowledge is hampered, however, by the lack of means to distinguish between necrotic death that was inflicted in a programmed and in a nonprogrammed manner. Nonprogrammed necrotic death is likely to occur in inflammation, which characteristically involves isolation of the injured site. Restriction of the access of nutrients and oxygen to the isolated cells may suffice to cause their death. Mere dissociation of cells through effects of extracellular digestive enzymes generated by leukocytes may also result in death, because cells must constantly receive survival signals from their neighbors and from the extracellular matrix. Cells can be killed by toxic compounds released by leukocytes, by ROS produced by leukocytes, by complement proteins, and by other agents generated at the site of inflammation [reviewed in (*2*)].

In the absence of a reliable analytical tool to distinguish necrotic death inflicted by any of the above-mentioned causes from necrotic death induced in a programmed manner, we will continue to encounter a chicken-and-egg conundrum. We will be unable to tell whether the co-occurrence of necrotic death and inflammation in a particular situation reflects an initiating role of the death in the observed inflammation or elicitation of this death by the inflammatory process. Because the morphological analysis of cells that die in the course of inflammation does not suffice to permit this distinction, we must base it on our mechanistic knowledge of programmed necrotic processes. For this, however, we must identify molecular parameters that are specific enough to allow definitive identification of such processes.

Rigorous definition of parameters is always required for specifying causal relationships between disease and co-occurring, potentially pathogenic, events. An example of such rigorous definition is the set of "Koch postulates" formulated over a century ago in an attempt to define the causal roles of specific infectious agents in diseases. Similar rigorous criteria must be applied to the molecular probes used to identify distinct forms of programmed cell death and establishing the causal role of these death forms in inflammation (Table 1).

Are we approaching identification of molecular probes specific to distinct forms of programmed cell death?

Attempts over the years to define molecular probes specific to programmed cell death have been hampered by the fact that those same molecules with important roles in death also contribute to various functions of living cells (*56*).

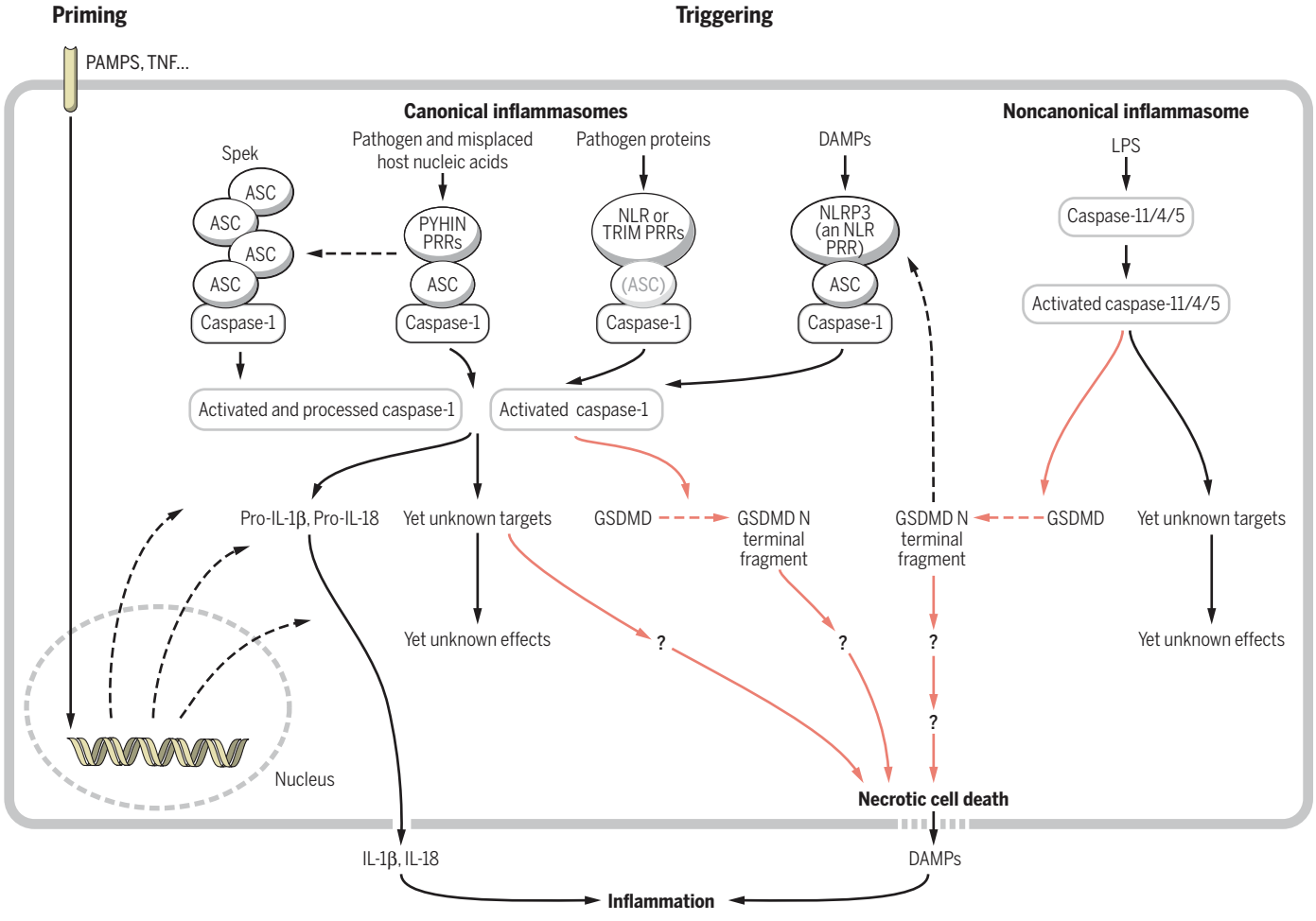


Fig. 2. Induction of pyroptosis: distinct roles of priming and triggering stimuli and of "canonical" and "noncanonical" inflammasomes. Signaling for pyroptosis is regulated on two mechanistic levels, as in the case of necroptosis, but in a sort of inverse manner. The first level has to do with effects of extracytoplasmic ligands like TNF and PAMPs that "prime" the cell by activating genes encoding the signaling proteins. The second level is the actual triggering of signaling by components of intracellular pathogens and by DAMPs. This induction involves two distinct groups of signaling mechanisms. Most of the agents that induce pyroptosis do so by binding specific PRRs of the NLR, TRIM, or PYHIN families, thereby triggering the activation of caspase-1 within macromolecular complexes called the "canonical inflammasomes." Some of these inflammasomes also contain the protein ASC, which, once activated, self-associates in a huge complex ("Spek") that both activates caspase-1 and facilitates its self-processing. In addition, intracellular LPS triggers pyroptosis by directly binding caspase-11 (in the mouse) or caspase-4 or -5 (in humans), activating these inflammatory caspases within the so-called "noncanonical inflammasome." The caspases cleave a variety of target proteins that serve different functions. Their cleavage of GSDMD triggers pyroptosis through a yet-unknown effect of the GSDMD N-terminal domain. Other yet-unknown targets of caspase-1 also trigger pyroptotic death. Activation of IL-1β and IL-18 in the course of pyroptosis is mediated by processing of the precursors of these inflammatory cytokines by caspase-1. Caspase-11, -4, and -5 are incapable of processing these precursors. However, they impose this processing indirectly via activation of caspase-1.

The function of the protein kinase RIPK1 provides a typical example of the pleiotropic effects of proteins serving proximal roles in signaling pathways activated by TNF. A C-terminal death-domain motif in RIPK1 mediates activation of the extrinsic apoptotic pathway through association with the adapter protein FADD/MORT1. Another region within it (the "intermediate domain") is targeted to ubiquitination, and then initiates signaling for nuclear factor κB (NF-κB) activation and hence induction of inflammatory and death-inhibitory genes. It also blocks activation of the extrinsic apoptotic death pathway through mechanisms independent of gene activation. Association of the RHIM domain in RIPK1 with a homologous region in RIPK3 has a dual effect on necroptosis induction: It restricts the spontaneous activation of RIPK3 and hence its spontaneous initiation of necroptosis, but is also required for necroptosis initiation by TNF and by some other inducing agents (*57*).

This complexity of RIPK1 function is underscored in recent studies of immunogenic cell death that occurs when cells undergo necroptosis. As discussed above, upon engulfment of a dying cell by a dendritic cell, antigens associated with the dying cell can be transferred to the class I MHC of the dendritic cell by "cross-priming," a process important for activation of antigen-specific T cells. Cells that have undergone necroptosis are particularly good at inducing such cross-priming. One study (*58*) suggested, however, that this process is dependent upon the activation of NF-κB by RIPK1 and that necroptotic cells that do not engage in such activation do not cross-prime, even though they induce inflammatory effects in dendritic cells.

Pleiotropy has also been observed in the functions of all other molecules found to participate in the proximal events of programmed cell death induction. The ubiquitin ligases cIAP1 and cIAP2 and the protease caspase-8, prominent negative players in necroptosis initiation, each has a variety of other functional roles. Such is also the case with RIPK3; this protein kinase, apparently crucial for the induction of necroptosis, also contributes, independently of cell death, to a growing number of other cellular activities (*59*–*62*).

Table 1. Defining "Koch postulates"–like rigorous tests for identification of death-effector molecules and their causative roles in inflammation.

Criteria that are indicative of causative role	Criteria indicative of a causative role of microbes in disease: the Koch postulates	Criteria indicative of the identity of death-effector molecules and their causative role in inflammation		
			The case of MLKL	The case of GSDMD
Documentation of co-occurrence	The microorganism is found in abundance in all organisms suffering from the disease, but not in healthy organisms.	The molecule is generated or activated in the dying cell prior to death.	Phosphorylation and membrane translocation of MLKL co-occur with necroptosis.	Caspase-mediated generation of an N-terminal fragment of GSDMD co-occurs with pyroptosis.
Characterization of the potentially causative element/effector molecule	The microorganism can be isolated from a diseased organism and grown in pure culture.	(a) Functional studies of the molecule demonstrate cell-killing potential. (b) Studies of the particular form of death induced by the molecule confirm the potential induction of inflammation by it.	*(a) MLKL might be able to form pores in membranes or activate ion channels.* (b) The expected release of DAMPs by necroptotic cells can potentially initiate inflammation.	*(a) No "deadly feature" of the GSDMD N-terminal fragment as yet identified.* (b) The expected release of DAMPs by pyroptotic cells can potentially initiate inflammation. Generation of IL-1β and IL-18
Documentation of exclusive cause-and-effect relationship	(a) The cultured microorganism causes disease when introduced into a healthy organism. (b) The microorganism can be re-isolated from the inoculated diseased experimental host and identified as identical to the original specific causative agent.	(a) Expression/activation of the molecule alone suffices to inflict death and consequent inflammation. (b) Ablation of the expression of the molecule prevents cell death and consequent inflammation. (c) The molecule can be shown to be incapable of triggering inflammation independently of death.	(a) Mere expression or mutational exposure of the MLKL coiled-coil domain triggers necroptosis *but has not yet been shown to trigger inflammation.* (b) Knockout and knockdown of MLKL block induction of necroptosis in vitro and of some inflammatory processes in vivo. *(c) MLKL seems also to have nondeadly functions. Those functions might yield inflammation in vivo.*	(a) Mere expression of the GSDMD N-terminal fragment triggers death *but has not yet been shown to trigger inflammation.* (b) GSDMD knockout abolishes caspase-11–mediated pyroptosis and decreases caspase-1–mediated pyroptosis. *(c) Generation of the GSDMD N-terminal fragment by caspase-11 triggers NLRP3-inflammasome–mediated generation of inflammatory cytokines, apparently independently of death.*

The caspases that serve crucial roles in the induction and execution of apoptosis, as well as those that induce pyroptosis, are also not death specific. Restrained activation of the "apoptotic caspases" contributes to induction of cell growth and differentiation (*63*). Activation of the inflammatory caspase-1, besides inducing pyroptosis, activates IL-1β and IL-18. It also has other functions; for example, it triggers activation of the sterol regulatory element–binding proteins (SREBPs), transcription factors that up-regulate lipogenic genes and thus promote cell survival, probably by facilitating membrane repair (*64*). Caspase-4, -5, and -11 not only induce pyroptosis but also trigger activation of IL-1β and IL-18 through the NLRP3 inflammasome. Their reported ability to cleave various other target proteins suggests that they serve other functions as well (*50*, *65*).

Among the nondeadly functions of the proteins that signal for programmed necrotic death are several that affect inflammation. Through such effects, these proteins might therefore also inflict necrotic cell death indirectly [e.g., (*55*, *66*)].

Of the early components of the pathways leading to programmed cell death, none has been found that is sufficiently specific to serve as a probe for its identification. It might nevertheless be possible to design such probes, based on our knowledge of those later events that are the most directly related to death. Recent developments in elucidation of the mechanisms underlying necroptosis and pyroptosis raise hopes that we are nearing the identification of such "death-specific" late events. MLKL, the last known component of the necroptotic signaling pathway, might be an effector molecule of death. The N-terminal coiled-coil region in MLKL, whose oligomerization is necessary and sufficient for induction of necroptosis, can bind certain lipids. This may suggest that it causes death by forming holes in the plasma membrane (*34*–*36*, *67*). Other reports suggest, however, that MLKL affects cell function and viability by association with intracellular membranes and specific protein targets (*32*, *40*, *68*). Activated MLKL molecules also translocate to the nucleus, and contain a nuclear localization signal in the C-terminal part. The nuclear translocation occurs independently of cell death and might contribute to some nondeadly functions (*69*). MLKL has also been reported to mediate activation of the inflammasome in dendritic cells, without any apparent association with cell death (*60*) (Fig. 1).

The recent discovery of the central role of GSDMD in pyroptosis has probably made a similar contribution to our state of knowledge of the mechanisms of programmed necrosis. There are still major gaps in our knowledge of this role. The biochemical activity of the caspase-generated fragment of GSDMD that induces death is unknown. It also seems that signaling for pyroptosis is not the only functional consequence of GSDMD cleavage, because cells deficient in GSDMD also fail to display caspase-11–mediated activation of IL-1β and IL-18 through the NLRP3 inflammasome (*52*). Besides, although GSDMD deficiency prevents induction of pyroptosis via caspase-11, it only partly decreases such induction by caspase-1, implying that its role in pyroptotic death is not absolute (*52*).

Box 2. Are MLKL and GSDMD the effector molecules in necroptosis and pyroptosis?

Table 1 summarizes the evidence pointing to MLKL and GSDMD as effectors of programmed necrosis, as well as the evidence indicating that, as a consequence, they are potential inducers of inflammation. Italic type indicates that the evidence is uncertain or lacking, or might argue against the above notions.

With regard to their effector abilities: Activated MLKL can associate with membranes in cells and form pores in artificial membranes, but it is not yet known whether the latter also occurs in living cells. Some studies provide evidence that MLKL might also mediate cell death in other ways.

For GSDMD, as yet, no evidence of a potential effector function of its death-inducing N-terminal fragment has been reported.

Also lacking is evidence that release of DAMPs in response to MLKL or GSDMD indeed yields inflammation. Nor is there any in vivo evidence that activation of MLKL alone or expression of its N-terminal fragment alone can initiate inflammation.

Concerning whether the functions of these two proteins are restricted to induction of necrotic death or inflammation, there is some evidence that they can serve other, nondeadly functions as well.

It has yet to be determined whether MLKL and GSDMD are indeed the actual effector molecules in the induction of necroptosis and pyroptosis (Table 1 and Box 2). However, the knowledge acquired about these molecules has undoubtedly brought us closer to identifying such effectors, and hence to establishing reliable tools for defining the in vivo relevance of programmed necrotic cell death.

REFERENCES AND NOTES

1. R. A. Lockshin, Z. Zakeri, Programmed cell death and apoptosis: Origins of the theory. *Nat. Rev. Mol. Cell Biol.* **2**, 545–550 (2001). doi: 10.1038/35080097; pmid: 11433369
2. D. Wallach, T. B. Kang, A. Kovalenko, Concepts of tissue injury and cell death in inflammation: A historical perspective. *Nat. Rev. Immunol.* **14**, 51–59 (2014). doi: 10.1038/nri3561; pmid: 24336099
3. A. Hochreiter-Hufford, K. S. Ravichandran, Clearing the dead: Apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harb. Perspect. Biol.* **5**, a008748 (2013). doi: 10.1101/cshperspect.a008748; pmid: 23284042
4. K. Segawa, S. Nagata, An apoptotic 'eat me' signal: Phosphatidylserine exposure. *Trends Cell Biol.* **25**, 639–650 (2015). doi: 10.1016/j.tcb.2015.08.003; pmid: 26437594
5. G. Kroemer, L. Galluzzi, O. Kepp, L. Zitvogel, Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72 (2013). doi: 10.1146/annurev-immunol-032712-100008; pmid: 23157435
6. A. Giodini, M. L. Albert, A whodunit: An appointment with death. *Curr. Opin. Immunol.* **22**, 94–108 (2010). doi: 10.1016/j.coi.2010.01.023; pmid: 20171862
7. E. D. Crawford *et al.*, Conservation of caspase substrates across metazoans suggests hierarchical importance of signaling pathways over specific targets and cleavage site motifs in apoptosis. *Cell Death Differ.* **19**, 2040–2048 (2012). doi: 10.1038/cdd.2012.99; pmid: 22918439
8. V. A. Fadok *et al.*, Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.* **101**, 890–898 (1998). doi: 10.1172/JCI1112; pmid: 9466984
9. S. W. Tait, D. R. Green, Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Biol.* **11**, 621–632 (2010). doi: 10.1038/nrm2952; pmid: 20683470
10. T. Moldoveanu, A. V. Follis, R. W. Kriwacki, D. R. Green, Many players in BCL-2 family affairs. *Trends Biochem. Sci.* **39**, 101–111 (2014). doi: 10.1016/j.tibs.2013.12.006; pmid: 24503222
11. I. Budihardjo, H. Oliver, M. Lutter, X. Luo, X. Wang, Biochemical pathways of caspase activation during apoptosis. *Annu. Rev. Cell Dev. Biol.* **15**, 269–290 (1999). doi: 10.1146/annurev.cellbio.15.1.269; pmid: 10611963
12. H. H. Park *et al.*, The death domain superfamily in intracellular signaling of apoptosis and inflammation. *Annu. Rev. Immunol.* **25**, 561–586 (2007). doi: 10.1146/annurev.immunol.25.022106.141656; pmid: 17201679
13. G. A. Granger, W. P. Kolb, Lymphocyte in vitro cytotoxicity: Mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. *J. Immunol.* **101**, 111–120 (1968). pmid: 5690881
14. N. H. Ruddle, B. H. Waksman, Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. *J. Exp. Med.* **128**, 1267–1279 (1968). doi: 10.1084/jem.128.6.1267; pmid: 5693925
15. S. M. Laster, J. G. Wood, L. R. Gooding, Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. *J. Immunol.* **141**, 2629–2634 (1988). pmid: 3171180
16. N. Holler *et al.*, Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat. Immunol.* **1**, 489–495 (2000). doi: 10.1038/82732; pmid: 11101870
17. A. Degterev *et al.*, Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat. Chem. Biol.* **4**, 313–321 (2008). doi: 10.1038/nchembio.83; pmid: 18408713
18. R. J. Thapa *et al.*, Interferon-induced RIP1/RIP3-mediated necrosis requires PKR and is licensed by FADD and caspases. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E3109–E3118 (2013). doi: 10.1073/pnas.1301218110; pmid: 23898178
19. C. P. Dillon *et al.*, RIPK1 blocks early postnatal lethality mediated by caspase-8 and RIPK3. *Cell* **157**, 1189–1202 (2014). doi: 10.1016/j.cell.2014.04.018; pmid: 24813850
20. W. J. Kaiser *et al.*, Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J. Biol. Chem.* **288**, 31268–31279 (2013). doi: 10.1074/jbc.M113.462341; pmid: 24019532
21. J. W. Upton, W. J. Kaiser, E. S. Mocarski, DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. *Cell Host Microbe* **11**, 290–297 (2012). doi: 10.1016/j.chom.2012.01.016; pmid: 22423968
22. Z. Huang *et al.*, RIP1/RIP3 binding to HSV-1 ICP6 initiates necroptosis to restrict virus propagation in mice. *Cell Host Microbe* **17**, 229–242 (2015). doi: 10.1016/j.chom.2015.01.002; pmid: 25674982
23. X. Wang *et al.*, Direct activation of RIP3/MLKL-dependent necrosis by herpes simplex virus 1 (HSV-1) protein ICP6 triggers host antiviral defense. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 15438–15443 (2014). doi: 10.1073/pnas.1412767111; pmid: 25316792
24. D. W. Zhang *et al.*, RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* **325**, 332–336 (2009). doi: 10.1126/science.1172308; pmid: 19498109
25. Y. S. Cho *et al.*, Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* **137**, 1112–1123 (2009). doi: 10.1016/j.cell.2009.05.037; pmid: 19524513
26. S. He *et al.*, Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* **137**, 1100–1111 (2009). doi: 10.1016/j.cell.2009.05.021; pmid: 19524512
27. D. Vercammen *et al.*, Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J. Exp. Med.* **187**, 1477–1485 (1998). doi: 10.1084/jem.187.9.1477; pmid: 9565639
28. A. Oberst *et al.*, Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* **471**, 363–367 (2011). doi: 10.1038/nature09852; pmid: 21368763
29. T. Tenev *et al.*, The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. *Mol. Cell* **43**, 432–448 (2011). doi: 10.1016/j.molcel.2011.06.006; pmid: 21737329
30. L. Sun *et al.*, Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **148**, 213–227 (2012). doi: 10.1016/j.cell.2011.11.031; pmid: 22265413
31. J. Zhao *et al.*, Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 5322–5327 (2012). doi: 10.1073/pnas.1200012109; pmid: 22421439
32. Z. Cai *et al.*, Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nat. Cell Biol.* **16**, 55–65 (2014). doi: 10.1038/ncb2883; pmid: 24316671
33. J. M. Murphy *et al.*, The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* **39**, 443–453 (2013). doi: 10.1016/j.immuni.2013.06.018; pmid: 24012422
34. J. M. Hildebrand *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. U.S.A.* **111**, 15072–15077 (2014). doi: 10.1073/pnas.1408987111; pmid: 25288762
35. X. Chen *et al.*, Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. *Cell Res.* **24**, 105–121 (2014). doi: 10.1038/cr.2013.171; pmid: 24366341
36. H. Wang *et al.*, Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. *Mol. Cell* **54**, 133–146 (2014). doi: 10.1016/j.molcel.2014.03.003; pmid: 24703947
37. Y. Dondelinger *et al.*, RIPK3 contributes to TNFR1-mediated RIPK1 kinase-dependent apoptosis in conditions of cIAP1/2 depletion or TAK1 kinase inhibition. *Cell Death Differ.* **20**, 1381–1392 (2013). doi: 10.1038/cdd.2013.94; pmid: 23892367
38. K. Newton *et al.*, Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. *Science* **343**, 1357–1360 (2014). doi: 10.1126/science.1249361; pmid: 24557836
39. P. Mandal *et al.*, RIP3 induces apoptosis independent of pronecrotic kinase activity. *Mol. Cell* **56**, 481–495 (2014). doi: 10.1016/j.molcel.2014.10.021; pmid: 25459880
40. Z. Wang, H. Jiang, S. Chen, F. Du, X. Wang, The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell* **148**, 228–243 (2012). doi: 10.1016/j.cell.2011.11.030; pmid: 22265414
41. T. Zhang *et al.*, CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. *Nat. Med.* **22**, 175–182 (2016). doi: 10.1038/nm.4017; pmid: 26726877
42. M. Pasparakis, P. Vandenabeele, Necroptosis and its role in inflammation. *Nature* **517**, 311–320 (2015). doi: 10.1038/nature14191; pmid: 25592536
43. K. D. Marshall, C. P. Baines, Necroptosis: Is there a role for mitochondria? *Front. Physiol.* **5**, 323 (2014). doi: 10.3389/fphys.2014.00323; pmid: 25206339
44. W. K. Joklik, T. C. Merigan, Concerning the mechanism of action of interferon. *Proc. Natl. Acad. Sci. U.S.A.* **56**, 558–565 (1966). doi: 10.1073/pnas.56.2.558; pmid: 5229977
45. D. Aderka, D. Novick, T. Hahn, D. G. Fischer, D. Wallach, Increase of vulnerability to lymphotoxin in cells infected by vesicular stomatitis virus and its further augmentation by interferon. *Cell. Immunol.* **92**, 218–225 (1985). doi: 10.1016/0008-8749(85)90003-6; pmid: 2986852
46. E. S. Mocarski, H. Guo, W. J. Kaiser, Necroptosis: The Trojan horse in cell autonomous antiviral host defense. *Virology* **479-480**, 160–166 (2015). doi: 10.1016/j.virol.2015.03.016; pmid: 25819165
47. E. A. Miao *et al.*, Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.* **11**, 1136–1142 (2010). doi: 10.1038/ni.1960; pmid: 21057511
48. J. Shi *et al.*, Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* **514**, 187–192 (2014). pmid: 25119034
49. I. Jorgensen, E. A. Miao, Pyroptotic cell death defends against intracellular pathogens. *Immunol. Rev.* **265**, 130–142 (2015). doi: 10.1111/imr.12287; pmid: 25879289

50. N. J. Agard, D. Maltby, J. A. Wells, Inflammatory stimuli regulate caspase substrate profiles. *Mol. Cell. Proteomics* **9**, 880–893 (2010). doi: 10.1074/mcp.M900528-MCP200, pmid: 20173201
51. K. Labbé, M. Saleh, in *Progress in Inflammation Research: The Inflammasomes*, I. Couillin, V. Pétrilli, F. Martinon, Eds. (Springer Basel AG, Basel, 2011), pp. 17–36.
52. N. Kayagaki *et al*., Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* **526**, 666–671 (2015). doi: 10.1038/nature15541; pmid: 26375259
53. J. Shi *et al*., Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* **526**, 660–665 (2015). doi: 10.1038/nature15514; pmid: 26375003
54. W. T. He *et al*., Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res.* **25**, 1285–1298 (2015). doi: 10.1038/cr.2015.139; pmid: 26611636
55. D. E. Christofferson, Y. Li, J. Yuan, Control of life-or-death decisions by RIP1 kinase. *Annu. Rev. Physiol.* **76**, 129–150 (2014). doi: 10.1146/annurev-physiol-021113-170259; pmid: 24079414
56. D. Wallach, T.-B. Kang, A. Kovalenko, The extrinsic cell death pathway and the élan mortel. *Cell Death Differ.* **15**, 1533–1541 (2008). doi: 10.1038/cdd.2008.41; pmid: 18794887
57. R. Weinlich, D. R. Green, The two faces of receptor interacting protein kinase-1. *Mol. Cell* **56**, 469–480 (2014). doi: 10.1016/j.molcel.2014.11.001; pmid: 25459879
58. N. Yatim *et al*., RIPK1 and NF-κB signaling in dying cells determines cross-priming of $CD8^+$ T cells. *Science* **350**, 328–334 (2015). doi: 10.1126/science.aad0395; pmid: 26405229
59. J. E. Vince *et al*., Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* **36**, 215–227 (2012). doi: 10.1016/j.immuni.2012.01.012; pmid: 22365665
60. T. B. Kang, S. H. Yang, B. Toth, A. Kovalenko, D. Wallach, Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasome. *Immunity* **38**, 27–40 (2013). doi: 10.1016/j.immuni.2012.09.015; pmid: 23260196
61. X. Wang *et al*., RNA viruses promote activation of the NLRP3 inflammasome through a RIP1-RIP3-DRP1 signaling pathway. *Nat. Immunol.* **15**, 1126–1133 (2014). doi: 10.1038/ni.3015; pmid: 25326752
62. K. Moriwaki *et al*., The necroptosis adaptor RIPK3 promotes injury-induced cytokine expression and tissue repair. *Immunity* **41**, 567–578 (2014). doi: 10.1016/j.immuni.2014.09.016; pmid: 25367573
63. E. Kuranaga, M. Miura, Nonapoptotic functions of caspases: Caspases as regulatory molecules for immunity and cell-fate determination. *Trends Cell Biol.* **17**, 135–144 (2007). doi: 10.1016/j.tcb.2007.01.001; pmid: 17275304
64. L. Gurcel, L. Abrami, S. Girardin, J. Tschopp, F. G. van der Goot, Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* **126**, 1135–1145 (2006). doi: 10.1016/j.cell.2006.07.033; pmid: 16990137
65. B. F. Py *et al*., Caspase-11 controls interleukin-1β release through degradation of TRPC1. *Cell Rep.* **6**, 1122–1128 (2014). doi: 10.1016/j.celrep.2014.02.015; pmid: 24630989
66. D. Wallach, T. B. Kang, S. H. Yang, A. Kovalenko, The in vivo significance of necroptosis: Lessons from exploration of caspase-8 function. *Cytokine Growth Factor Rev.* **25**, 157–165 (2014). doi: 10.1016/j.cytogfr.2013.12.001; pmid: 24411566
67. Y. Dondelinger *et al*., MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep.* **7**, 971–981 (2014). doi: 10.1016/j.celrep.2014.04.026; pmid: 24813885
68. W. Chen *et al*., Diverse sequence determinants control human and mouse receptor interacting protein 3 (RIP3) and mixed lineage kinase domain-like (MLKL) interaction in necroptotic signaling. *J. Biol. Chem.* **288**, 16247–16261 (2013). doi: 10.1074/jbc.M112.435545; pmid: 23612963
69. S. Yoon, K. Bogdanov, A. Kovalenko, D. Wallach, Necroptosis is preceded by nuclear translocation of the signaling proteins that induce it. *Cell Death Differ.* **23**, 253–260 (2016). doi: 10.1038/cdd.2015.92; pmid: 26184911

10.1126/science.aaf2154

Programmed necrosis in inflammation: Toward identification of the effector molecules

David Wallach, Tae-Bong Kang, Christopher P. Dillon, and Douglas R. Green

Science, 352 (6281), aaf2154.

Apoptosis, necrosis, and pyroptosis

The routes to cell death are many, and distinguishing which path a particular cell may have taken remains a challenge. Wallach *et al.* review current understanding of how programmed necrotic cell death contributes to inflammation.

Science, this issue p. 10.1126/science.aaf2154

Science (ISSN 1095-9203) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.