Pyroptosis: A Caspase-1-Dependent Programmed Cell Death and a Barrier to Infection

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Abstract Infection elicits a number of innate protective responses in the host that cooperate to promote effective pathogen clearance. Increasingly, the inflammatory response to infection appears to be coupled to cell death as an important mediator of host defence. In this chapter we review the modalities of "pyroptosis", a highly inflammatory form of cell death mediated by the inflammasome and caspase-1 activation. Occurring in the context of infection, pyroptosis is morphologically, mechanistically and physiologically distinct from other forms of cell death. The pathogenic factors that initiate pyroptosis and the cellular mechanisms and signalling pathways responsible for its execution are examined, with a focus on the role of the inflammasome in these processes. Finally, we discuss the possible physiological significance of this unique form of cell death during infection, that is, how pyroptosis can favour pathogen elimination on one hand, while contributing to the pathophysiology of disease on the other.

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1 Introduction

One of the most primitive antimicrobial responses consists of the elimination of the infected cell by programmed cell death, a response found in all metazoan phyla, including plants [1]. Although mammals have developed several additional layers of immune defence, cell death remains a key component of the host response against infection. Eliminating an infected cell results in the death of the infectious agent, promoting effective pathogen clearance and the elimination of a pathogenic niche. Intracellular pathogens require a viable host cell within which to replicate and bacteria, such as *Mycobacterium tuberculosis* [2], *Rickettsia rickettsii* [3] and *Chlamydia* spp. [4], have evolved mechanisms to prevent host cell death to assure their own survival. Conversely, certain pathogens have devised strategies to use cell death to their advantage, to subvert normal host defence mechanisms or as a way to penetrate the epithelial barrier and reach deeper layers of tissue or the blood stream. *Bacillus anthracis* [5], *Actinobacillus actinomycetemcomitans* [6, 7] and *Pseudomonas aeruginosa* [8, 9] secrete cytotoxic exotoxins to kill macrophages before they themselves are phagocytosed and destroyed. *Bordetella pertussis* adenylate cyclase haemolysin promotes successful colonization of alveolar tissue by eliminating the local monocyte population [10]. Cell death therefore plays a major role in determining the outcome of host–pathogen interactions.

During infection, recognition by the innate immune system is achieved through a number of pattern-recognition receptors including Toll-like, Nod-like and RIG-I-like receptors. Activation of these receptors initiates an array of signalling networks that culminate in the mounting of a proinflammatory immune response [11]. These innate mechanisms are essential for primary pathogen clearance as well as the development of an adequate adaptive response to the infection. Increasingly, the inflammatory response to infection appears to be coupled to the induction of cell death as an important mediator of host defence. Understanding the modalities of cell death is therefore critical to the elucidation of pathogenic mechanisms. Here, we review the current knowledge on the mechanisms and functions of "pyroptosis", an inflammatory form of cell death initiated by infection and mediated by the activities of the inflammasome and caspase-1.

2 Cell Death Pathways

Cell death is generally described dichotomously as either programmed or passive. The former requires metabolic energy and is mediated by specific cellular pathways and effector molecules, while the latter occurs uncontrollably due to extracellular stresses. Programmed cell death can be further classified as either apoptosis (type I) or autophagic cell death (type II), each with a unique set of cellular mechanisms and morphologies (Fig. 1). Apoptosis is the best described form of cell death and is mediated by the apoptotic caspase enzymes, a family of cysteinyl aspartate-specific

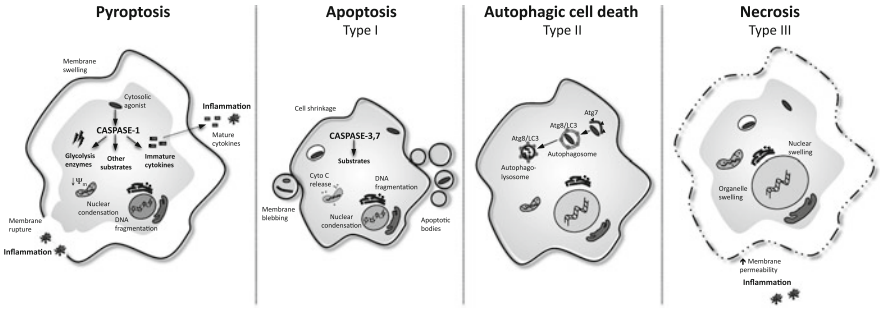

Fig. 1 Cell death pathways. Cell death is generally described as either programmed (apoptosis, or type I and autophagic cell death, or type II) or passive (necrosis or type III). Pyroptosis is categorized as programmed cell death, as it requires metabolic energy and is mediated by specific cellular pathways, namely, the inflammasome and caspase-1. Pyroptotic cells display a distinct set of morphological and biochemical characteristics, some of which are shared with apoptosis and necrosis. Unlike apoptosis and autophagic cell death, which do not induce inflammation, cytokine release and escape of cytoplasmic content during pyroptosis are highly inflammatory events

proteases. Eleven human caspases have been identified and are grouped into two major subfamilies according to their function in either apoptosis or cytokine maturation [12]. There are two major apoptosis pathways, extrinsic or intrinsic, and apoptotic caspases are classified as either upstream initiators (caspase-2, -8, -9 and -10) or executioners (caspase-3, -6 and -7) of these pathways. Caspases are synthesized as inactive precursors but in response to apoptotic signals become enzymatically active through processing or by a conformational change induced by oligomerization [13]. The intrinsic pathway is initiated when intracellular stresses induce the activation of Bcl-2 homology 3 (BH3)-only proteins, which leads to the oligomerization of the pro-apoptotic Bcl-2 family proteins BAX and BAK that form pores in the outer mitochondrial membrane [14]. This mitochondrial outer membrane permeabilization (MOMP) causes the release of apoptogenic factors, including Smac/DIABLO and cytochrome *c*, into the cytosol [15]. Cytochrome *c* associates with the apoptosis protease activating factor-1 (Apaf-1) to recruit and activate caspase-9 in a protein complex termed the "apoptosome" [16]. Active caspase-9 cleaves and activates the executioner caspases that in turn process cellular substrates to ultimately kill the cell. The extrinsic apoptosis pathway is induced by stimulation of death receptors of the TNF receptor family. At the active receptor, the adaptor proteins Fas-associated via death domain (FADD) and TNFR1-associated death domain (TRADD) recruit caspases-8 and -10 to form the death inducing signalling complex (DISC) [17]. Active caspase-8 cleaves and activates the executioner caspases, and, in certain cells, amplifies the cell death signal by cleaving the BH3-only protein Bid to induce MOMP and caspase-9 activation. Apoptotic cells are characterized by DNA fragmentation and chromatin condensation, nuclear fragmentation, cell shrinkage, loss of membrane asymmetry and the formation of cytoplasmic blebs and apoptotic bodies (Table 1).

Table 1 Pyroptosis is distinct from apoptosis. Despite sharing characteristics with apoptosis, the morphological, mechanistic and physiological features of pyroptosis make it a distinct form of programmed cell death

	Characteristics	Apoptosis	Pyroptosis	References
Morphology	Cell lysis	✖	✔	35, 38, 51
	Cell swelling	✖	✔	44, 51, 53
	Pore formation	✖	✔	35, 42, 44, 53
	Membrane blebbing	✔	✖	51, 52
	Nuclear condensation	✔	✔	40, 51
	DNA fragmentation (TUNEL positive)	✔	✔	42, 45, 47, 53
Mechanisms	Caspase-1 activation	✖	✔	26, 27
	Caspase-3 activation	✔	✖	34
	Caspase-7 activation	✔	✔	58
	MOMP/Cytochrome C release	✔	✖	38, 39, 53
	ICAD cleavage	✔	✖	53
	PARP cleavage	✔	✖	53
	Glycolysis enzyme inactivation	✔	✔	57
Out-come	Inflammation	✖	✔	112

Autophagy is also a tightly regulated process. Orchestrated by the ATG/Beclin proteins, autophagy results in lysosomal enzyme degradation of intracellular components captured within a double-membraned vacuole termed the autophagosome [18]. This catabolic process is essential during starvation conditions to maintain energy homeostasis and cell survival. Excessive autophagy has, however, been associated with a form of "autophagic cell death" characterized by massive accumulation of autophagic vacuoles in the cytoplasm in the absence of chromatin condensation [19]. Cytoplasmic content is not spilled into the extracellular space and thus, like apoptosis, autophagic cell death is non-inflammatory.

Passive cell death, or necrosis (type III), is often identified in negative terms for cells that do not have the markers of type I or type II programmed cell death pathways. Necrosis is thought to occur accidentally and uncontrollably as a result of

environmental perturbations. Cells undergo cytoplasmic and organellar swelling, resulting in plasma membrane lysis and release of intracellular content, which is a highly inflammatory event [19]. The causative agents of necrosis are still unclear, as are the cellular events they initiate. Mitochondrial dysfunction such as production of reactive oxygen species and membrane permeabilization has been implicated, as have ATP depletion, loss of Ca^{2+} homeostasis, protease activation and lysosomal rupture [20]. Increasingly, there is evidence that necrotic cell death may be regulated by signal transduction and catabolic mechanisms and is not a completely passive event [21].

Recently, a novel form of programmed cell death has been described that occurs specifically in the context of infection. Like apoptosis, it is mediated by caspases, but rather than depending on the action of classical apoptotic caspases, it is an inflammatory caspase, caspase-1, that is critical. Caspase-1 was the first caspase to be described as the enzyme responsible for the cleavage of pro-IL-1β and was thus initially named Interleukin-1β converting enzyme, or ICE [22]. Caspase-1 is the prototypical member of the inflammatory caspase family, which includes caspases-4, -5 and -12 (and -11 in rodents). Caspase-1 activation is triggered by the formation of a cytosolic complex termed the "inflammasome" (see previous chapters for a detailed description). The 44-kDa pro-caspase-1 consists of a 10-kDa CARD domain (caspase-activation and recruitment domain), a large subunit (p20) and a small subunit (p10). Proximity-induced oligomerization of caspase-1 [13] results in auto-processing, release of the CARD domain and tetramerization of two small and two large subunits to form the active enzyme [22, 23]. Residues from both the p10 and the p20 subunits form the active site of the enzyme. The catalytic cysteine, Cys^{285}, and histidine, His^{237}, are found in the p20, while substrate specificity is determined by residues of the p10 [24]. Like all caspases, caspase-1 has an absolute requirement for Asp in the P1 position of its substrates, immediately N-terminal of the scissile bond. The optimal caspase-1 amino acid recognition sequence is $Tyr_{(P4)}$–$Val_{(P3)}$–$Ala_{(P2)}$–$Asp_{(P1)}$, though it can tolerate conservative substitutions at P2 and P3, and has a preference for hydrophobic amino acids at P4 [25]. Substrate cleavage by caspase-1 does not contribute to classical apoptosis pathways [26, 27]. Rather, activation of caspase-1 results in the cleavage of a unique array of proteins, including the preferred substrates pro-IL-1β and pro-Il-18, that are converted into their secreted, biologically active forms. Both cytokines are highly inflammatory and play important roles in the immune response by recruiting and activating immune cells [28]. Indeed, caspase-1 activation is essential for the mounting of an efficient immune response to a number of infectious pathogens [29].

There are striking parallels between the pathways controlling intrinsic apoptosis and those that activate caspase-1. Caspase-1-dependent cell death is initiated by infection, while apoptosis is can be induced by the mitochondria, an organelle reminiscent of bacteria. Both release stimulatory products (PAMPs and cytochrome *c*) into the cytosol to activate sensors (NLR/PYHIN and Apaf-1) that undergo oligomerization to form an activation platform (inflammasome and apoptosome). CARD-containing caspases are subsequently recruited (caspase-1 and caspase-9) where they are activated by proximity-induced catalysis, resulting in substrate

recognition and cleavage. In the case of caspase-9, this leads to apoptosis, a tolerogenic form of cell death. For caspase-1, the outcome is a form of cell death tightly coupled to inflammation and the anti-microbial response. Such similarities may be the result of the co-evolution of these pathways under the pressures imposed by infection. This homology also provides important insight into the mechanisms of cell death and inflammation, furthering our understanding of the roles played by both in the host response to infection.

3 Caspase-1-Dependent Cell Death: Pyroptosis

The first report of a caspase-1-dependent cell death was in mouse macrophages infected with the gram-negative bacteria *Shigella flexneri* [30], the etiological agent of bacillary dysentery. In its human host, *Shigella* invades the colonic mucosa, where it encounters and infects the phagocytes of the lamina propria, resulting in extensive macrophage death and abscess formation [31]. *Shigella* was the first invasive bacteria reported to induce host cell death, which was initially described as apoptosis [32]. Further mechanistic studies uncovered a cell death pathway occurring independently of the apoptotic effector caspase-3, but contingent on the activity of the inflammatory caspase-1 [30]. Pharmacological inhibition of caspase-1 by Ac-YVAD-CHO [30, 33] or genetic ablation in *casp1*$^{-/-}$ mice [34] rendered macrophages fully resistant to *Shigella*-induced cytotoxicity while caspase-3, caspase-11 or p53 deficiency did not [34].

The findings in *Shigella*-infected macrophages were further corroborated by reports of caspase-1-dependent cell death induction by *Salmonella typhimurium* [35–37]. Macrophages are killed within minutes of *Salmonella* infection but are rescued by YVAD treatment [35] or if derived from *casp1*$^{-/-}$ mice [37]. Furthermore, caspases-3, -6 and -7 remain inactive in these cells [35, 38] and cytochrome *c* release does not occur [38]. Together, these findings firmly established the existence of a caspase-1-mediated cell death pathway distinct from apoptosis. Caspase-1-dependent cell death has since been reported in macrophages infected with a number of pathogens including *Listeria monocytogenes* [39], *Legionella pneumophila* [40, 41], *Yersinia pseudotuberculosis* [42], *P. aeruginosa* [43], *Burkholderia pseudomallei* [44] and *Francisella tularensis* [45], though the possible contribution of other inflammatory caspases to the cell death induced by these bacteria has not been fully investigated.

Concomitant with cell death are the inflammatory consequences of caspase-1 activation. Secretion of IL-1β promotes inflammatory cell recruitment and further production of proinflammatory mediators, resulting in important and sometimes severe physiological consequences such as fever, hypotension and metabolic derangements [46]. The release of mature IL-18 further amplifies the inflammatory response by stimulating immune cell activation and cytokine secretion [28]. The cleavage and release of IL-1β and IL-18 are not, however, required for caspase-1-mediated cell death [47]. Indeed, the action of caspase-1 is not limited to the

processing of these cytokines, as demonstrated by the resistance of *casp1*$^{-/-}$ mice [27] but not *IL-1β*$^{-/-}$ [48] or IL-1β/IL-18 double knockout animals [49] to endotoxemia and septic shock. Although neither IL-1β nor IL-18 is required for its execution [47], caspase-1-dependent cell death is a highly inflammatory event, a key feature distinguishing it from apoptosis. The term "pyroptosis" has been proposed to describe this unique form of programmed cell death [50] as "pyro" or fire, denotes the release of proinflammatory mediators, while "ptosis" denotes falling, a term commonly used to describe cell death.

4 Execution of Pyroptosis

Pyroptosis is a distinct form of cell death [19] with a unique combination of morphological and mechanistic features (Table 1).

4.1 Morphology

Morphologically, pyroptosis is most notably characterized by loss of plasma membrane integrity and release of cytoplasmic content into the extracellular milieu [35, 38, 51]. This feature is shared with necrosis but not with apoptosis, in which cytosolic content is contained within cytoplasmic blebs and apoptotic bodies [52]. Microscopically, the pyroptotic plasma membrane appears to rupture, then rapidly reseal and swell, forming a "balloon-shaped" vesicle around the nucleus [51] (Fig. 2). Indeed, during pyroptosis, cells undergo a measurable size increase [44, 53]. As the membrane swells, the nucleus also undergoes rounding and condensation [40, 51], but, unlike apoptosis, nuclear integrity is maintained. Pyroptotic cells undergo DNA fragmentation and, like apoptotic cells, show positive TUNEL staining [42, 45, 47, 53] (Table 1). The TUNEL positivity of *S. flexneri* and *S. typhimurium* infected cells initially led to the assumption that cell death induced by these pathogens was apoptotic.

4.2 Mechanisms

The cellular mechanisms that mediate caspase-1-dependent cell death are still largely unknown, but are distinct from the classical apoptotic pathways. The apoptotic caspases, including caspases-3, -6 and -7, are not involved in pyroptosis [34, 35, 38]. During apoptosis, the poly-ADP ribose polymerase (PARP) is cleaved by executioner caspases in an attempt to preserve cellular ATP energy stores [54]. PARP is also cleaved by caspase-1 [55] but studies using PARP inhibitors [53] and *PARP1*$^{-/-}$ macrophages [56] suggest that PARP activity does not significantly

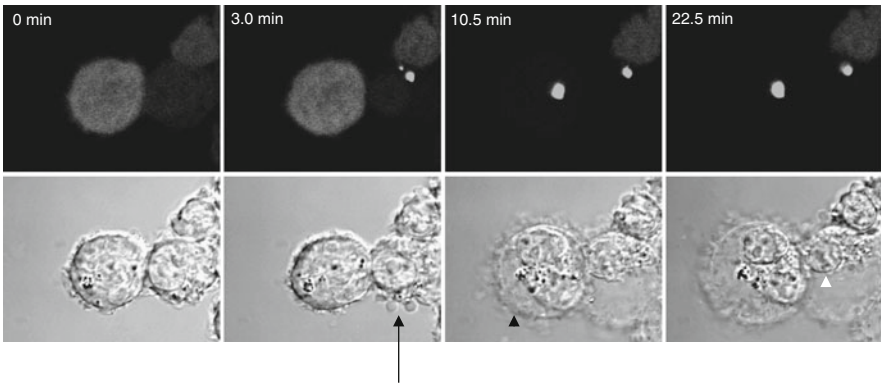

Fig. 2 Imaging of pyroptosis by time-lapse confocal microscopy. Pyroptosis was induced in differentiated THP-1-ASC-GFP cells with crude LPS. *Top row*; fluorescence images depicting the formation of the cytoplasmic ASC-GFP pyroptosome. *Bottom row*; plasma membrane rupture was observed rapidly (*black arrow*), followed by membrane re-sealing and swelling (*black arrowhead*) and nuclear condensation (*white arrowhead*) (these images are courtesy of Dr. E. Alnemri; reprinted with the permission of Nature Publishing Group)

impact pyroptosis. During apoptosis, DNA is degraded by the caspase activated DNase (CAD). The activity of CAD is controlled by its inhibitor, ICAD, a caspase-3 substrate [57]. During pyroptosis, DNA fragmentation depends on nuclease activity, but the lack of detectable ICAD processing suggests that this is a CAD-independent process [53]. Finally, a crucial step in apoptosis is the induction of MOMP, which causes the release of cytochrome c and Smac/DIABLO into the cytosol [14]. In pyroptosis, mitochondrial integrity is maintained and cytochrome c is not released [38, 39, 53].

Although distinct from apoptotic pathways, the question remains, what are the pro-death mechanisms occurring downstream of caspase-1 (Fig. 3)? The finding that mice genetically deficient in both IL-1β and IL-18 do not phenocopy caspase-1-deficient animals in septic shock models prompted investigation into the discovery of novel caspase-1 substrates and the description of novel caspase-1 mediated signalling pathways. Some insight into these signals has been gained with the report of the caspase-1 digestome using the diagonal gel approach on the human monocytic THP-1 cell line [55]. Caspase-1 was shown to target substrates involved in cellular functions as diverse as maintenance of the cytoskeleton, ATP metabolism, detoxification, trafficking, RNA/protein synthesis and degradation, signal transduction and cytokine production. Caspase-1 was further shown to cleave and inactivate a number of glycolysis enzymes, linking inactivation of bioenergetic pathways to cell death. An interesting conclusion of this study is the seemingly dual function of caspase-1 as both an initiator and executioner caspase.

Another report of caspase-1 substrates used the gel-free COFRADIC peptide sorting methodology on the mouse Mf4/4 macrophage cell line [58]. This study identified caspase-7 as a specific caspase-1 substrate. Caspase-7 cleavage was

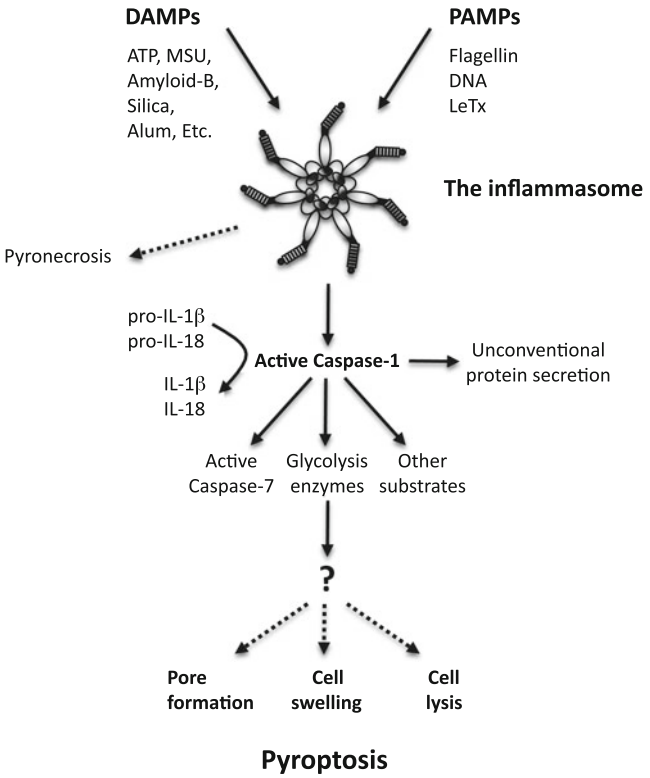

Fig. 3 Cellular mechanisms of pyroptosis. Caspase-1 is activated within the "inflammasome" a cytosolic multiprotein complex assembled in response to cytosolic PAMPs or host-derived DAMPs. Caspase-1 activation results in proinflammatory cytokine processing, as well as the cleavage of a number of additional substrates such as pro-caspase-7 and glycolysis enzymes. The nature of the pathways downstream of caspase-1 that ultimately result in cell lysis and death are still unknown

identified downstream of a number of caspase-1 activators, but *casp7*$^{-/-}$ cells were not deficient in IL-1β or IL-18 production and were not protected from death during *S. typhimurium* infection. A follow-up study using *L. pneumophila*-infected macrophages did demonstrate that caspase-7 was required for efficient bacterial clearance by promoting phagosome fusion with the lysosome [58]. Both studies reported caspase-3 processing in pyroptotic macrophages, but this occurred independently of caspase-1 activity and caspase-3-deficiency did not impair cytokine processing or cell death, leaving the function of this processing undetermined.

A downstream mechanism proposed to mediate pyroptotic cell lysis during *S. typhimurium* infection is the formation of small-ion permeable pores in the plasma membrane [35, 42, 44, 53]. Through size-exclusion studies and flow cytometry, the pore size has been estimated to be 1.1–2.4 nm in diameter [53]. Addition of extracellular glycine or osmoprotectants of 2.4 nm or greater to pyroptotic

macrophages prevented cell swelling and lysis [35, 53]. Thus, it is suggested that plasma-membrane pores dissipate the cellular ionic gradient, producing an increase in osmotic pressure that results in water influx, cell swelling and membrane lysis [53]. Whether these events are downstream of substrate processing by caspase-1 remains to be examined.

5 The Role of the Inflammasome

The inflammasome is activated by bacterial [60], viral [61] and parasitic infection [62], as well as by host-derived danger-associated molecular patterns (DAMPs) [63]. The generation of knock-out mice for the various components of the inflammasome has allowed the investigation of which pathways are required for caspase-1 activation and pyroptosis.

5.1 NLRC4 and NAIP5

The NLRC4 inflammasome is most closely associated with the induction of pyroptosis. It is activated by the flagellin [64] and the rod proteins of the type-three secretion system [65] of a number of bacterial pathogens. Expression of NLRC4 is essential for pyroptosis induced by *S. typhimurium* [66], *P. aeruginosa* [43, 67, 68] and *S. flexneri* [69]. The role of ASC in mediating NLRC4 signalling differs between cytokine production and cell death. Indeed, macrophages infected with *S. typhimurium* require both NLRC4 and ASC for IL-1β production, but only *Nlrc4*$^{-/-}$ cells are resistant to pyroptosis [64, 66, 70, 71]. The same conditions are also true for *S. flexneri* and *P. aeruginosa*-infected macrophages [43, 67–69].

The facultative intracellular bacteria *L. pneumophila* is distinct in that it has an absolute requirement for a functional NAIP5 to stimulate caspase-1 activity. The action of NAIP5 is coupled to that of NLRC4 as both mediate cytotoxicity but require the additional presence of ASC to permit IL-1β secretion [40, 41, 72, 74]. NAIP5 was identified as the effector gene of the *Lgn* locus that controls macrophage permissiveness to *Legionella* replication [75]. Cells derived from A/J mice express a mutant NAIP5 and are resistant to pyroptosis, whereas those derived from C57BL/6 mice, which carry the functional *Lgn* allele, are susceptible. To better understand the function of NAIP5, complete *Naip5*$^{-/-}$ mice were recently generated on a C57BL/6 background [73]. Infectious studies in macrophages derived from these animals have now demonstrated a partial dependence on NAIP5 expression for *S. typhimurium* and *P. aeruginosa* induced pyroptosis [73].

5.2 NLRP1

A critical factor in the virulence of *B. anthracis* is the production of anthrax lethal toxin (LeTx). LeTx is produced during infection and is secreted as two proteinaceous subunits, protective antigen (PA) and lethal factor (LF). When PA-LF complexes are endocytosed and trafficked to acidic vesicles, conformational changes in PA allow LF to translocate to the cytosol [76]. LF, a zinc-dependent metalloprotease, specifically cleaves MAPKKs, disrupting MAPK signalling pathways [77]. Although MAPKK cleavage occurs in all infected mammalian cells, LeTx is not universally lethal. In mouse macrophages, cell death is strain dependent, where C57BL/6-derived cells are resistant and 129S1 or BALB/c cells are susceptible. Genetic studies have mapped susceptibility to the *Ltxs1* locus and subsequently shown that *Nlrp1b* mediates strain susceptibility. NLRP1b is essential for caspase-1 activation by lethal toxin and is required for both IL-1β production and cytotoxicity, suggesting that LeTx kills cells by pyroptosis [78]. Macrophages derived from 129S1 or BALBc mice express a functional NLRP1, and are susceptible to LeTx pyroptosis, whereas the C57Bl/6 strain *Nlrp1b* gene is mutated and non-functional, conferring LeTx-resistance to macrophages derived from these mice. *B. anthracis* spores activate caspase-1 and promote IL-1β release by macrophages, but do not induce cell death [79]. It is not yet known whether live *B. anthracis* infection activates caspase-1.

5.3 NLRP3

The list of activators of the NLRP3 inflammasome is increasingly long and includes pathogen-derived signals (viral [80], fungal [81, 82] and bacterial infection [83], pore-forming toxins [71]), environment-derived factors (silica [84], asbestos [85], alum [84, 86]) and host-derived danger signals (ATP [71], uric acid [87], hyaluronan [88], amyloid-β [89]). Yet despite this list of agonists, NLRP3 is not known to mediate caspase-1-dependent cell death. Mutant, overactive forms of NLRP3 are capable of driving an inflammatory form of cell death during infection, distinct from pyroptosis, termed "pyronecrosis". It is entirely caspase independent but requires the activity of cathepsin B [90]. Pyronecrosis promotes and the release of HMGB1, a cellular DAMP [91], and is induced by high multiplicity of infection with *S. flexneri* [90], *K. pneumoniae* [92] and *N. gonorrhoeae* [83].

5.4 AIM2

During infection by intracellular pathogens, the presence of foreign double-stranded DNA (dsDNA) leads to robust caspase-1 activation. The inflammasome component involved in this case is AIM2, a cytosolic member of the HIN-200

protein family that specifically binds dsDNA and associates with ASC to activate caspase-1 [93–96]. Transfection of the synthetic dsDNA poly(dA:dT) results in AIM2-dependent IL-1β processing and macrophage death, both of which require the presence of ASC [95]. Pyroptosis of macrophages infected with *F. tularensis* or *L. monocytogenes* was long known to depend on ASC but the PRR involved was unknown until AIM2 was identified as the activated inflammasome [97, 98]. Cytosolic release of dsDNA by these intracellular Gram-negative bacteria is directly sensed by AIM2, resulting in caspase-1 activation, IL-1β secretion and pyroptosis.

5.5 ASC

The role of the adaptor protein ASC in mediating pyroptosis is still unclear. As described above, NLRC4/NAIP5 inflammasome-dependent pyroptosis does not require ASC whereas AIM2-mediated cell death does. A recent study [99] determined that it is the presence of a CARD domain that determines whether an inflammasome requires ASC to induce pyroptosis. It was further reported that ASC-independent pyroptosis does not induce caspase-1 auto-proteolysis as does ASC-dependent pro-IL-1β processing, suggesting the existence of two different caspase-1 activation pathways.

ASC has been implicated in other pyroptotic conditions. For instance, ASC has been shown to induce pyroptosis in a human THP-1 monocytic cell line engineered to stably express an ASC-GFP fusion protein. These cells were used to demonstrate the formation of a large supramolecular ASC complex termed the "pyroptosome" in response to a number of inflammasome agonists [51, 94]. Upon stimulation, a single ASC pyroptosome is formed in the cell and is required for caspase-1 activation and the induction of pyroptosis. ASC oligomers were also shown to form in primary macrophages from both wild-type and *casp1*$^{-/-}$ mice, though only the former succumbed to cell death [51].

The observation that caspase-1 activation does not always result in cell death is indicative of the existence of a unique, pro-death inflammasome signalling pathway. A certain level of signalling specificity is achieved by the type of inflammasome activated by an agonist; while NLRC4 activation is closely associated with pyroptosis, NLRP3 responses are predominantly cytokine based. The types of inflammasome agonists that induce pyroptosis also reflect this specificity. NLRC4 is primarily activated by bacteria in the context of a live infection. In these conditions, pyroptosis would result in the elimination of the infected cell and promote a strong inflammatory response at the site of infection. NLRP3, on the other hand, is mainly activated by danger signals derived from the environment or from other cells. In this case, a cytokine-only response would promote tissue repair without causing cell death.

6 Physiological Significance

During infection, pyroptosis can be a beneficial event for the host. The compromised cell is eliminated, effectively destroying a protective milieu in which infectious agents can thrive. For instance, macrophages deficient in caspase-1, NLRC4 or NAIP5 are protected from pyroptosis during *L. pneumophila* infection and support higher intracellular bacterial loads [41, 73]. Pyroptosis also promotes pathogen clearance by acting as an alarm signal that recruits immune cells to the site of infection. The production of IL-1β and IL-18 promotes leukocyte infiltration and activation [46] and cell lysis releases immuno-stimulatory factors into the extracellular milieu. Several cytosolic products are potent DAMPs, such as HMGB1 [100], ATP [71], uric acid [87], heat-shock proteins [101] and DNA–chromatin complexes [102], that promote proinflammatory cytokine production through the activation of pattern-recognition receptors.

The hypothesis that pyroptosis is detrimental to the pathogen is also supported by the description of several microbial inflammasome-evasion mechanisms. One such strategy is to reduce the presence of pyroptosis-inducing agonists, as does *S. typhimurium*. By downregulating flagellin expression during *in vivo* infection, *Salmonella* evades detection by the inflammasome, thus rendering $Nlrc4^{-/-}$ mice as susceptible to infection as wild-type animals [37, 64]. Other pathogens have evolved effector proteins capable of directly inhibiting inflammasome activation, such as the *Y. pseudotuberculosis* T3SS Rho-GTPase activating protein YopE [103], the *M. tuberculosis* zinc metalloprotease Zmp1 [104], the poxvirus M13L [105] or the influenza virus NS1 protein [106]. Recently, a screen for *F. tularensis* virulence genes identified two targets involved in the delay of IL-1β release and cell death in infected cells [107]. Similarly, virulent *Pseudomonas* strains express the effector protein ExoU, which blocks caspase-1-dependent cell death and promotes necrosis [43]. The evolution of such mechanisms would suggest that preventing inflammasome activation contributes to pathogen fitness, and that the activity of caspase-1 leading to pyroptosis represents an important selective pressure that prevents replication and spread of the pathogen to neighbouring cells.

Yet, as with any physiological process, excessive pyroptosis is detrimental to the host and may contribute to histopathology and disease. Macrophage cell death prevents appropriate elimination of the infectious agent and pyroptosis of dendritic cells [108, 109] leads to immunosuppression by impairing cytokine production and antigen presentation. Excessive pyroptosis also leads to the development of severe sepsis and septic shock, through the release of the alarmin HMGB1. When released into the circulation, HMGB1 acts as a critical mediator of severe sepsis [110] and administration of HMGB1 neutralizing antibodies confers resistance from lethality [111]. During apoptosis, oxidation of HMGB1 neutralizes its stimulatory activity [112].

Finally, it is difficult to determine the immunological function of pyroptosis, as caspase-1 activities are numerous and extend beyond cell death. Furthermore, there is enormous redundancy in cell death pathways, and cells in which pyroptosis

is blocked will often die by alternative pathways. Thus, although *casp1*$^{-/-}$ macrophages are protected from pyroptosis during *S. typhimurium* infection, they nonetheless succumb to a delayed form of cell death [38]. In *S. flexneri*-infected macrophages, deficiency in either caspase-1 or NLRC4 results in increased activation of autophagy [69]. The induction of alternate cell death pathways could provide a backup mechanism for the infected cell in cases where the inflammasome is not activated or actively is inhibited by the pathogen in an attempt to clear the infection. On the other hand, these alternate routes may provide time for the pathogen to replicate before the cell is destroyed. One study [113] sought to tease out the contribution of pyroptosis to the host response by using a strain of *S. typhimurium* engineered to persistently express flagellin. Enhanced clearance of these bacteria did not depend on cytokine production, but on the pyroptotic release of the pathogen from macrophages, where it was subsequently killed by neutrophil ROS production. A similar result was obtained for *L. pneumophilia* and *B. thailandensis*, illustrating that pyroptosis is an important innate effector mechanisms protecting from bacterial infection *in vivo*.

7 Conclusion

Cell death is an important factor in host–pathogen interactions. The elimination of an infected cell can be beneficial or detrimental to both parties, and each utilizes a number of strategies to regulate the outcome in its favour. Both host and pathogen responses to infection also rely on the modulation of the proinflammatory response to promote own survival. It should come as no surprise, then, that the pathways responsible for mounting the inflammatory response are also involved in the regulation of certain forms of cell death. Elucidation of cell death mechanisms will undoubtedly reveal information on inflammatory processes, and vice versa. Thus, a better understanding of pyroptosis will provide information on the function and regulation of the inflammasome, as well as important insights into the role of cell death during infection.

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