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Abbreviations: CARD: caspase recruitment domain · CIITA: MHC class II transactivator · NLR: NOD-like receptor · NOD: nucleotide-binding oligomerization domain · PGN: peptidoglycan · RLR: RIG-I-like receptor

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Pyroptosis – a cell death modality of its kind?

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The term “pyroptosis” was originally introduced to describe a particular form of cell death in macrophages, which is induced by bacterial infection, is accompanied by caspase-1 activation and hence leads to the release of pyrogenic interleukins; however, it is still controversial whether pyroptosis – which can also be triggered by non-bacterial pathological stimuli – truly represents a cell death modality on its own or whether it constitutes a special case of apoptosis or necrosis.

Morphological and biochemical features of programmed cell death

Depending on both cell-intrinsic and -extrinsic variables, programmed cell death (PCD) can be executed through several self-destructive cascades, which mostly manifest either apoptotic or necrotic morphological traits. Apoptosis is usually accompanied by cell shrinkage (pyknosis), nuclear condensation and fragmentation (karyorrhexis), as well as by the formation of apoptotic bodies

(which *in vivo* are engulfed by resident phagocytes). In contrast, necrotic cells are characterized by a swollen morphology (oncosis) and by the rather disorganized dismantling of intracellular contents [1, 2]. The most prominent biochemical features of apoptosis include a self-amplifying cascade leading to the activation of caspases (a specific class of proteases), internucleosomal DNA fragmentation and exposure of phosphatidylserine on the outer leaflet of the plasma membrane.

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It had been thought that PCD was exclusively mediated by apoptosis, while necrosis was considered a purely accidental cell death mode. Recently, however, it has become clear that necrosis can also be regulated, both in its occurrence and its mechanism [3]. Necroptosis is the term currently employed to define programmed necrosis, which depends on the activity of RIP1 and RIP3 kinases [4, 5].

Although autophagy has been proposed as a cell death mechanism, it exerts in most cases cytoprotective functions, and the term “autophagic cell death” might be considered a misnomer [6]. Still, cell death subroutines that do not fall in the categories of apoptosis and necroptosis have been described [1, 2]. These include (but are not limited to) mitotic catastrophe [7, 8] and pyroptosis, a cell death modality that was first observed in macrophages infected by *Salmonella typhimurium* [9]. Nevertheless, such cell death subroutines frequently constitute special instances of apoptosis or necroptosis [1, 2].

Morphological and biochemical features of pyroptosis

Pyroptosis was originally described as the caspase-1-dependent cell death of *S. typhimurium*-infected macrophages, and it can occur *in vitro* [9] and *in vivo* [10]. In contrast to apoptosis, which for years has been (mis)regarded as a completely non-

immunogenic cell death modality [11], the inherently pro-inflammatory nature of pyroptosis has immediately been recognized. The activation of caspase-1 (originally discovered as “IL-1 β -converting enzyme”) within the so-called inflammasome leads to the release of the pro-inflammatory cytokines IL-1 β and IL-18 and reportedly occurs before any morphological manifestation of cell death [12].

Notably, while apoptotic cells retain an intact plasma membrane until the final stages of the process (*i.e.* when secondary necrosis intervenes), pores of 1–2 nm rapidly form in the plasma membrane of pyroptotic macrophages, resulting in cytoplasmic swelling and osmotic lysis through rupture of the plasma membrane [13]. The formation of these pores reportedly requires caspase-1 activity as well as the rearrangement of the host cell actin cytoskeleton, yet is independent of poly(ADP-ribose) polymerase (PARP) and DNA fragmentation [13].

Such pores provide inflammatory molecules, including (but presumably not limited to) IL-1 β and IL-18 (both of which are generated within the cytosol) [12], with a direct route for release, and may therefore be instrumental for the pro-inflammatory character of pyroptosis. Of note, the early permeabilization of plasma membranes is shared by other immunogenic cell death subroutines like necroptosis and accidental necrosis [2, 3]. While pyroptotic DNA fragmentation has been shown to occur independently

of caspase-activated DNase [13], it remains to be determined whether caspase-independent nucleases like apoptosis-inducing factor or endonuclease G might be implicated in chromatinolysis [14]. Therefore, it appears that pyroptosis shares morphological features of both apoptosis and necroptosis (Table 1).

The biochemical cascades ignited by distinct pyroptotic triggers may involve different signal transducers, as each of these pathways depends on specific PAMP. PAMP like bacterial peptidoglycan or flagellin are detected by nucleotide-binding oligomerization domain-like receptors (NLR) such as NLRC4 and NLRP1, which consist of a variable N-terminal domain that includes a caspase recruitment domain (CARD), a central nucleotide-binding oligomerization domain, and a C-terminal leucine-rich domain (which acts as a PAMP-binding domain) [15]. Other NLR like NLRP3, which not only detects PAMP but also endogenous danger signals, lack a functional CARD in their N-terminus and strictly require the adaptor protein ASC for interacting with caspase-1 [11]. Caspase-1 activation may occur at the inflammasome (Fig. 1A), which involves both NLR and ASC [16], or it can be mediated by the so-called pyroptosome (Fig. 1A), a supramolecular ensemble of ASC dimers [17]. In both cases, caspase-1 activation is driven by induced proximity and results in the proteolytic maturation and release of IL-1 β and IL-18 [16, 17]. At present it is not clear why in some

Table 1. Main features of PCD subtypes

PCD type	Stimuli	Phenotype	Consequences
Apoptosis	Ligation of death receptors DNA damage Reactive oxygen species	Pyknosis DNA fragmentation Plasma membrane blebbing	Formation of apoptotic bodies Phagocytosis
Necroptosis	TNFR1 ligation+SMAC ^{a)} mimetics TNFR1 ligation+caspase inhibition TRAILR1 ligation+caspase inhibition	Cytoplasmic swelling Organelle swelling Disorganized dismantling	Release of intracellular content Inflammation
Pyroptosis	Bacterial infection Viral infection Stroke	Cytoplasmic swelling DNA fragmentation Pore formation	Release of cytokines Inflammation

^{a)} SMAC second mitochondria-derived activator of caspases.

instances caspase-1 activation leads to the mere production of cytokines (without cell death), while in others it triggers lethal caspase-3 activation. It remains to be determined to which extent intracellular inhibitors of caspase-3 activation and/or of mitochondrial outer membrane permeabilization determine the cell fate upon caspase-1 stimulation.

Pathophysiological relevance of pyroptosis

Macrophages undergo pyroptosis upon infection by bacterial pathogens including *S. typhimurium*, *Shigella flexneri*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* [18, 19] as well as in response to *Bacillus anthracis* toxin [20]. However, pyroptosis does not constitute a macrophage-specific cell death modality and can indeed be triggered in other cell types, either by bacterial agents or by non-bacterial stimuli including viruses, stroke, and cancer therapy [21]. Irrespective of the initiating event, the pyroptotic activation of caspase-1 has multiple pro-inflammatory consequences (Fig. 1B), which are mediated/facilitated by the secretion of: (i) IL-1 β , a potent endogenous pyrogen that favors leukocyte tissue migration as well as the expression of multiple cytokines and chemokines, thereby coordinating both local and systemic inflammation [22]; (ii) IL-18, which favors the activation of T cells and macrophages, and stimulates the production of IFN- γ [23]; (iii) IL-1 α , whose mechanism of caspase-1-dependent secretion has not been elucidated yet [24]; and (iv) IL-6 and TNF- α , which are optimally secreted only by caspase-1-proficient macrophages [25].

In summary, although it remains unclear whether pyroptosis truly constitutes a cell death modality *sui generis*, the activation of caspase-1 in pyroptotic cells has unquestionable pathophysiological implications. Thus, pharmacological interventions aimed at inhibiting caspase-1 may constitute a promising therapeutic approach to limit excessive/uncontrolled inflammatory reactions. Moreover, caspase-1-activat-

ing strategies may be combined with classical chemotherapeutic agents to favor inflammation and the elicitation of an anticancer immune response.

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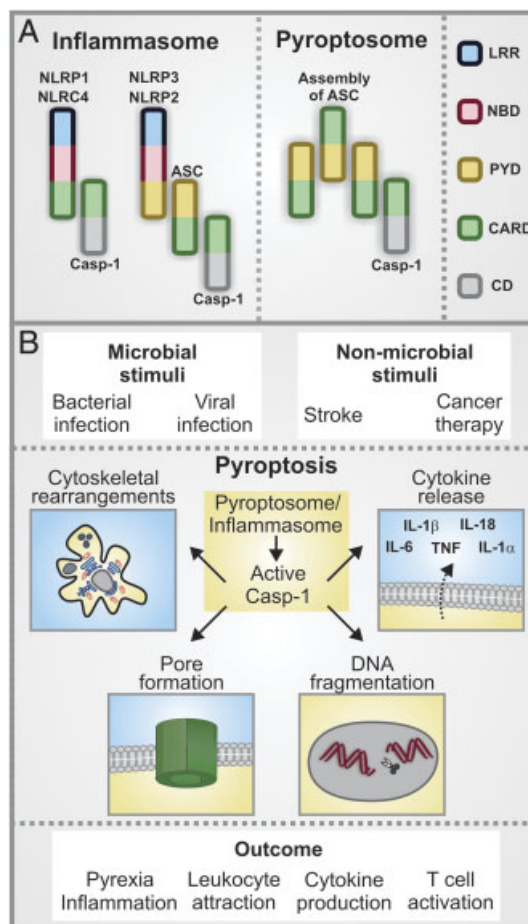


Figure 1. (A) Schematic representation of caspase-1-activating platforms. Caspase-1 (Casp-1) is activated within the inflammasome, a supramolecular complex whose assembly is driven by NLR, either alone (as for NLRP1 or NLRP4) or upon binding to the adaptor protein ASC (for instance when the inflammasome involves NLRP2 or NLRP3). Alternatively, Casp-1 activation can be mediated by the pyroptosome, a supramolecular ensemble of ASC dimers. CD, catalytic domain; LRR, leucine-rich repeat domain; NBD, nucleotide-binding and oligomerization domain; PYD, pyrin domain. (B) Illustration of the main features of pyroptosis. Macrophages (and other cell types) can undergo pyroptosis in response to both microbial and non-microbial stimuli. This results in the assembly of either the inflammasome or the pyroptosome, in turn leading to the activation of Casp-1. At a cellular level, pyroptosis is associated with cytoskeletal rearrangements, the formation of pores in the plasma membrane, variable extents of DNA fragmentation, and, most importantly, with the release of pro-inflammatory and pyrogenic cytokines such as IL-1 α , IL-1 β , IL-6, IL-18, and TNF- α . This results in a local inflammatory burst accompanied by leukocyte recruitment and T-cell activation, as well as in the activation of a systemic response to infection, fever.

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