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Chopping GSDMD: caspase-8 has joined the team of pyroptosis-mediating caspases

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Inflammatory and apoptotic caspases mediate two distinct forms of cell death: pyroptosis and apoptosis, respectively. Three independent studies have now demonstrated that the "apoptotic" caspase-8 can cleave gasdermin D (GSDMD) leading to pyroptosis-like cell death and IL-1β release in murine macrophages (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019). Orning *et al* and Chen/Demarco *et al* also show that the NLRP3 inflammasome is activated downstream of active caspase-8, but they attribute this inflammasome activation to different pore-forming proteins, GSDMD and pannexin-1, respectively (Orning *et al*, 2018; Chen *et al*, 2019).

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See also: **KW Chen *et al*** (May 2019)

Different forms of programmed cell death are distinguished by distinct morphological changes and the proteins involved (Fig 1A–C; Galluzzi *et al*, 2018). Apoptosis is well characterised as non-inflammatory cell death triggered either by extrinsic or intrinsic stimuli, followed by consequential activation of initiator caspases (caspase-8 or caspase-9) and executioner caspases (caspase-3 and caspase-7). This leads to cell shrinking, membrane blebbing, DNA fragmentation and formation of apoptotic bodies containing cellular contents. Necroptosis, a necrotic form of cell death, can occur upon ligation of death receptors by proapoptotic stimuli, such as TNFα, in the absence of caspase-8 activation. The pore-forming protein MLKL mediates plasma membrane disruption, cell swelling and eventual cell lysis. Pyroptosis, another form of necrotic cell death, is typically triggered by activation of pro-inflammatory caspase-1/caspase-4/caspase-5 in humans (caspase-1/caspase-11 in mice), which leads to GSDMD cleavage, pore formation and eventual cell lysis. Caspase-1 is activated by the formation of the multi-protein complexes termed inflammasomes consisting of a cytosolic pathogen-recognition receptor (e.g. NLRP3) and the adaptor protein ASC, which recruits caspase-1. Pyroptosis promotes inflammation by the release of the cytokines IL-1β and IL-18 as well as proteins and nucleic acids acting as danger signals.

Diverse forms of cell death were traditionally thought to be independent of each other, but emerging evidence is challenging this view. In a variety of scenarios including infection with different *Yersinia* species such as *Y. pestis* and *Y. pseudotuberculosis*, activation of the apoptotic caspase-8 and inflammatory cell death have been observed to coincide (Philip *et al*, 2014; Lawlor *et al*, 2015, 2017). Now, three independent studies have revealed synchronicity between caspase-8 activity and GSDMD-mediated cell death in multiple scenarios (Fig 1D). Utilising a combination of the apoptosis trigger TNFα and pharmacological inhibitors blocking pro-survival signals (e.g. SMAC mimetic or the TAK1 inhibitor 5z-7-oxozeaenol), Chen/Demarco *et al* observe inflammatory caspase-8-mediated cell death in murine macrophages (Chen *et al*, 2019). Sarhan *et al* and Orning *et al* also observe caspase-8-driven inflammatory cell death studying TNFα/TAK1 inhibitor co-stimulation or infection with *Yersinia*, which inhibits TAK1 through the bacterial protein YopJ (Orning *et al*, 2018; Sarhan *et al*, 2018). Sarhan *et al* (2018) established that the necrotic cell death they observed resembled pyroptosis based on the timing of uptake of the nuclear dye PI and Annexin V staining of the plasma membrane. Cells from various genetic knock-out mice and pharmacological inhibitors were used to dissect this pyroptosis-like pathway, and Orning *et al* (2018), Sarhan *et al* (2018) uniformly concluded that RIPK1-mediated activation of caspase-8 drives GSDMD cleavage and consequential pyroptosis, as measured by lactate dehydrogenase (LDH) release. GSDMD$^{-/-}$ cells were not, however, protected from cell death and upon stimulation, but instead underwent apoptosis with release of some LDH which was absent in GSDMD$^{-/-}$/caspase-3$^{-/-}$/-7$^{-/-}$ cells, suggesting that either GSDMD-independent apoptosis or GSDMD-dependent pyroptosis could occur (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019).

To assess whether active caspase-8 triggered inflammasome formation and subsequent GSDMD cleavage, the three groups assessed different inflammasome-deficient knock-out cells. They found that the NLRP3 inflammasome was formed as ASC oligomerised, caspase-1 was active and cells released IL-1β. Inflammasome activation, however, unexpectedly appeared to be downstream of GSDMD cleavage (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019). Orning *et al* showed that recombinant mouse GSDMD is cleaved by purified active caspase-8 (Orning *et al*, 2018), but although it was less efficient at GSDMD processing in comparison with caspase-1, its activity was sufficient to trigger pyroptotic cell death in murine macrophages.

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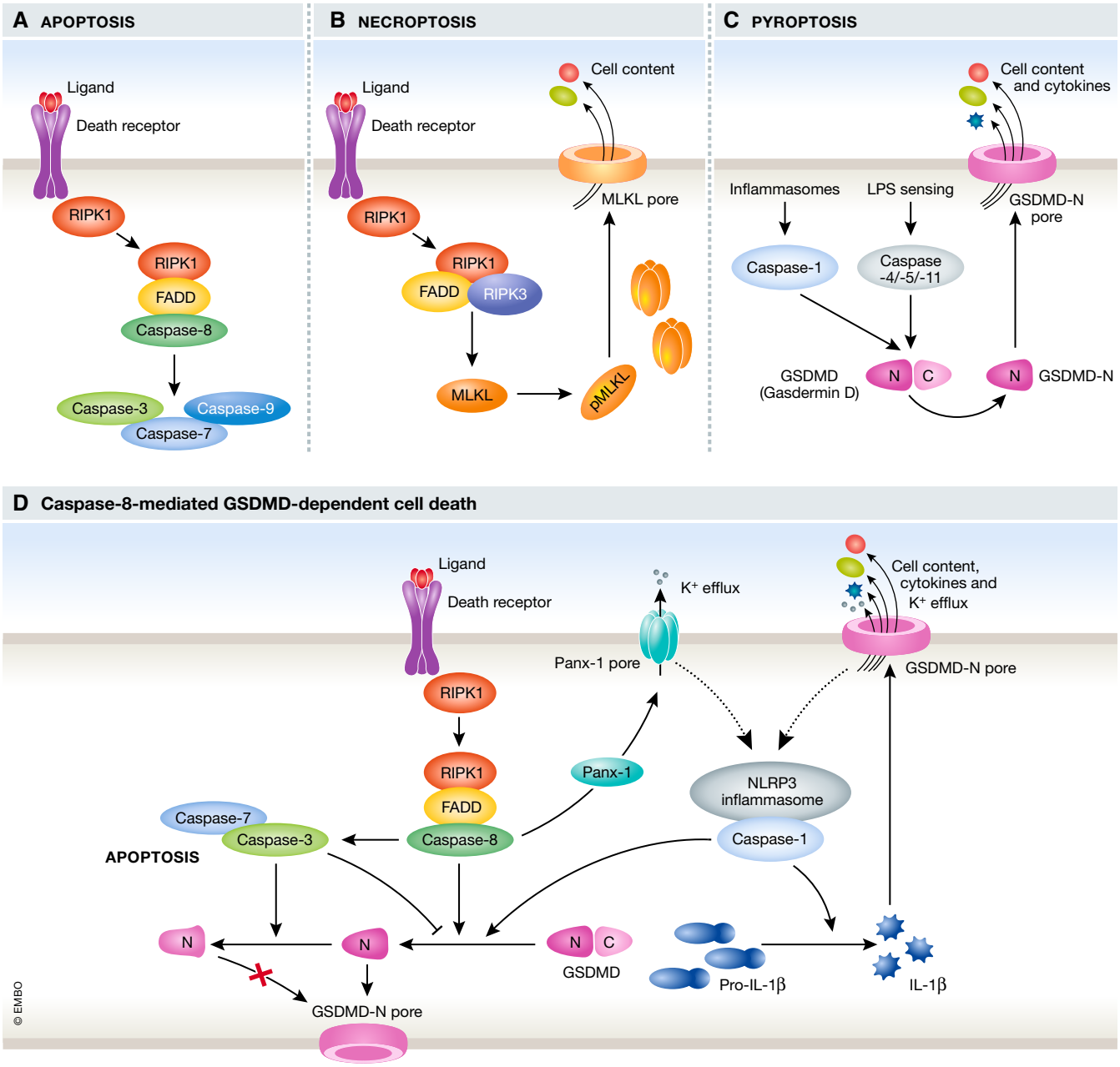

Figure 1. Mechanisms of cell death: the connections between caspases and gasdermin D.

Caspase-1-mediated GSDMD cleavage generates a pyroptotic p30 fragment, whereas upon *Yersinia* infection or TNFα/TAK inhibitor treatment, GSDMD was cleaved into p43, p30 and p20 fragments (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019). To assess whether caspase-8 generated the same pyroptosis-mediating p30 fragment as caspase-1, Chen/Demarco *et al* made use of a HEK reconstitution system in which they tested different non-cleavable GSDMD constructs. Caspase-8 activity led to cleavage at position D276, the cleavage site used by caspase-1 generating the p30 fragment (Chen *et al*, 2019). The p43 and p20 fragment were found to be generated by cleavage of full-length and the p30 fragment, respectively, at D88 in a caspase-3-dependent way (Chen *et al*, 2019). This suggests inactivation of GSDMD and thereby counteracting pyroptosis during caspase-3-mediated apoptosis. In line with this, Chen/Demarco *et al* reported that cells from a knock-in mouse bearing a GSDMD D88A mutation showed an increase in pyroptotic cell death (Chen *et al*, 2019).

Typical pyroptosis is characterised by the release of IL-1β and IL-18, and caspase-8-induced pyroptosis coincided with NLRP3 activation and IL-1β secretion (Orning *et al*, 2018; Chen *et al*, 2019). Conflicting conclusions emerge from these three studies, however, regarding the pathway leading to NLRP3 inflammasome activation and cytokine secretion. Orning *et al* suggest that

NLRP3 activation is GSDMD-dependent based on delays in ASC oligomerisation in GSDMD$^{-/-}$ cells. Conversely, Chen/Demarco *et al* observed normal caspase-1 processing in GSDMD$^{-/-}$ and/or GSDME$^{-/-}$ cells. Cells from the GSDMD D88A knock-in mouse also showed no increase of caspase-1 processing suggesting that GSDMD activation and caspase-1 processing were not linked (Chen *et al*, 2019). Chen/Demarco *et al* suggest that through RIPK3 involvement, the channel-forming pannexin-1 was activated and promotes downstream NLRP3 and caspase-1 activation. IL-1β release, however, was only partially decreased in pannexin-1$^{-/-}$ cells (Chen *et al*, 2019). They speculate that this is due to apoptosis-driven caspase-8-mediated IL-1β cleavage, which has also been reported by others (Chauhan *et al*, 2018; Vince *et al*, 2018). It should be noted, however, that all studies detected rather low levels of released IL-1β suggesting either inefficient transcription/translation or maturation of pro-IL-1β. Sarhan *et al* (2018) offer an interesting explanation as they report that IL-1β is not released from TAK1-inhibited cells, because TAK1 inhibition prevents transcription of pro-IL-β. Instead, IL-1β appeared to be secreted from TAK1-sufficient cells with the mechanism by which IL-1β is matured in those cells remaining to be determined. Here, the three studies disagree in how downstream inflammasome activation is mediated post-caspase-8 activation with GSDMD and pannexin-1 both suggested as relevant.

In summary, three studies show that *Yersinia* infection or extrinsic apoptosis triggers can lead to GSDMD cleavage and subsequent pyroptosis-like cell death in murine macrophages, which is mediated by the apoptotic caspase-8 (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019; Fig 1D). Downstream of GSDMD processing, the NLRP3 inflammasome becomes activated leading to cytokine maturation and release, thereby promoting inflammation. Another apoptotic caspase, caspase-3, appears to counteract the cell death observed by processing the pyroptotic-p30 fragment and full-length GSDMD further into the inactive p20 and p43 fragments, respectively. This caspase-3 activity together with the observation that, in the absence of GSDMD, cells underwent apoptosis demonstrates that several cell death pathways are triggered upon stimulation and that they do not operate exclusively. This raises the question of what shifts the balance between caspase-8-mediated GSDMD cleavage and caspase-3 activation and the subsequent GSDMD inactivation. Intriguingly, caspase-3 inhibition of pyroptosis has also been shown to cleave another Gasdermin family member, Gasdermin E (GSDME), leading to secondary necrosis (Rogers *et al*, 2017; Wang *et al*, 2017). Sarhan *et al* and Chen/Demarco *et al* also detected GSDME cleavage (Sarhan *et al*, 2018; Chen *et al*, 2019), which was mediated by caspase-3 (Sarhan *et al*, 2018). GSDME$^{-/-}$ cells, however, did not show a significant decrease in cell death. Understanding the role of caspase-3 in the crosstalk between cell death pathways as well as the consequential downstream effects of separate pathways should be a focus of future studies. Determining the physiological relevance of the described pathway is important, especially with the suggestion that this pathway does not occur in human macrophages under the same conditions (Sarhan *et al*, 2018). Further work should explore the balance of caspase-3 in inhibition and activation of pyroptosis and apoptosis, respectively, and how the crosstalk between the pathways mediates the response to infection or inflammation *in vivo*.

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