Review

THE EMBO JOURNAL

Beyond inflammasomes: emerging function of gasdermins during apoptosis and NETosis

Kaiwen W Chen, Benjamin Demarco & Petr Broz*

Abstract

Programmed cell death is a key mechanism involved in several biological processes ranging from development and homeostasis to immunity, where it promotes the removal of stressed, damaged, malignant or infected cells. Abnormalities in the pathways leading to initiation of cell death or removal of dead cells are consequently associated with a range of human diseases including infections, autoinflammatory disease, neurodegenerative disease and cancer. Apoptosis, pyroptosis and NETosis are three well-studied modes of cell death that were traditionally believed to be independent of one another, but emerging evidence indicates that there is extensive cross-talk between them, and that all three pathways can converge onto the activation of the same cell death effector—the pore-forming protein Gasdermin D (GSDMD). In this review, we highlight recent advances in gasdermin research, with a particular focus on the role of gasdermins in pyroptosis, NETosis and apoptosis, as well as cell type-specific consequences of gasdermin activation. In addition, we discuss controversies surrounding a related gasdermin family protein, Gasdermin E (GSDME), in mediating pyroptosis and secondary necrosis following apoptosis, chemotherapy and inflammasome activation.

Keywords apoptosis; gasdermin; inflammasome; NETosis; pyroptosis
DOI 10.15252/embj.2019103397 | Received 5 September 2019 | Revised 4 October 2019 | Accepted 6 November 2019 | Published online 3 December 2019
The EMBO Journal (2020) 39: e103397

Introduction

Pyroptosis is a form of necrotic cell death that has emerged as an important innate immune mechanism against intracellular pathogens. The existence of pyroptosis was first observed in the early 1990s when several laboratories documented that infection with *Shigella flexneri* or *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) triggered rapid cytotoxicity in murine macrophages (Zychlinsky *et al*, 1992; Monack *et al*, 1996). This peculiar form of pathogen-induced cell death features several characteristics of apoptosis such as DNA fragmentation and exposure of phosphatidylserine, in addition to hallmarks of necrosis such as rapid plasma membrane permeability (Brennan & Cookson, 2000). Subsequent studies revealed that these features of pathogen-infected cells were driven by inflammasomes, a large cytoplasmic, multiprotein complex that enables the activation of the proinflammatory protease, caspase-1 (Martinon *et al*, 2002). Thus in 2001, Cookson and Brennan coined the term "pyroptosis" to distinguish this form of cell death from apoptosis and accidental necrosis (Cookson & Brennan, 2001). While an increasing number of pathogens were documented to induce macrophage pyroptosis, the mechanisms by which pyroptosis drives host defence *in vivo* were unclear, although it was assumed that killing the infected cell was important. This mechanism was confirmed *in vivo* from elegant studies by Miao and colleagues, where they demonstrate that macrophage pyroptosis attenuates intracellular pathogens and present them for neutrophil-mediated killing (Miao *et al*, 2010; Jorgensen *et al*, 2016).

Early studies by Fink and colleagues indicated that pyroptosis was a form of regulated necrosis that was driven by membrane pores of a 1.1–2.4 nm (Fink & Cookson, 2006). However, the molecular mechanisms of plasma membrane pore formation were unclear until 2015, when two landmark studies from the laboratories of Vishva Dixit and Feng Shao, and subsequently by Jiahuai Han, identified Gasdermin D (GSDMD) as the essential pyroptosis mediator (He *et al*, 2015; Kayagaki *et al*, 2015; Shi *et al*, 2015). GSDMD consists of an N-terminal pyroptosis-inducing domain ($GSDMD^{NT}$ or p30) connected by a linker to a C-terminal regulatory domain ($GSDMD^{CT}$), which binds the N-terminus. Inflammasome-activated inflammatory caspase-1, caspase-4 and caspase-11 cleave GSDMD at a conserved site within the linker domain, thereby releasing the $GSDMD^{NT}$ from an intramolecular inhibition by $GSDMD^{CT}$. This cleavage event allows $GSDMD^{NT}$ to oligomerise in cellular membranes, assembling large pores with a diameter of around 18 nm, and to cause pyroptosis (Aglietti *et al*, 2016; Ding *et al*, 2016; Liu *et al*, 2016; Sborgi *et al*, 2016; Mulvihill *et al*, 2018; Ruan *et al*, 2018).

Emerging evidence suggests that GSDMD pores not only cause pyroptotic cell death, but that they are also essential for other consequences of inflammasome or caspase-1 activation, e.g. the release of mature IL-1 family cytokines, such as IL-1β and IL-18. Unlike other cytokines, IL-1β and IL-18 lack a signal sequence and are therefore secreted in an endoplasmic reticulum/Golgi-independent manner (Rubartelli *et al*, 1990). Since inflammasome activation usually

Department of Biochemistry, University of Lausanne, Lausanne, Switzerland
*Corresponding author. Tel: +41 21 692 5656; E-mail: petr.broz@unil.ch

elicits near-concurrent secretion of mature IL-1β and pyroptosis in macrophages, it is often proposed that IL-1β and IL-18 are passively released during cell lysis. In line with this model, *Gsdmd* deficiency severely abrogates IL-1β secretion upon canonical inflammasome activation (Kayagaki *et al*, 2015; Shi *et al*, 2015); and single-cell analysis of macrophages revealed that IL-1β release coincides with the uptake of membrane-impermeable nucleic acid dyes (e.g. SYTOX, propidium iodide), a widely used assay to measure the loss of plasma membrane integrity (Liu *et al*, 2014; Polykratis *et al*, 2019). By contrast, a number of studies reported that mature IL-1β can be secreted in the absence of intracellular lactate dehydrogenase release, a commonly used assay to quantify cell lysis in a bulk cell population (Kang *et al*, 2013; Chen *et al*, 2014; Gaidt *et al*, 2016; Wolf *et al*, 2016; Zanoni *et al*, 2016). Since the standard lactate dehydrogenase release assay may lack single-cell resolution, it remains plausible that mature IL-1β is indeed released by a small fraction of lysed cells upon inflammasome activation. However, several lines of evidence support the notion that that cell lysis is not an absolute requirement for IL-1β secretion. For example, ectopic expression of mature IL-1β in primary macrophages is sufficient to induce its secretion in the absence of inflammasome activation (Monteleone *et al*, 2018); and single-cell analysis of live, viable murine embryonic fibroblast revealed considerable IL-1β secretion after caspase-1 or caspase-8 activation (Conos *et al*, 2016). Consistent with these observations, a number of recent studies demonstrated that sublytic GSDMD pores (18 nm) are indeed large enough for the release of mature IL-1β (Evavold *et al*, 2018; Heilig *et al*, 2018) or entry of nucleic acid dyes (Russo *et al*, 2016; DiPeso *et al*, 2017), indicating that GSDMD can act as a conduit for IL-1β release in the absence of cell lysis. Studies carried out by us on ESCRT-III-dependent membrane repair have further strengthened the notion that cells can tolerate a certain number of GSDMD membrane pores (Ruhl *et al*, 2018). The model that emerges from these studies implies that caspase activation proceeds from a sublytic phase in which cells feature transient assembly of GSDMD pores to a lytic phase where GSDMD pores cause a complete breakdown of membrane integrity. Whether cells transit from the sublytic to the lytic phase depends on the strength of the activating signal, level of GSDMD expression and activation, cell type and the activity of membrane repair mechanism. Furthermore, recent findings indicate that while $GSDMD^{NT}$ is sufficient to assemble pores *in vitro* or when overexpressed, its activity might be regulated by additional mechanisms under physiological conditions. For example, it has been proposed that complete GSDMD-dependent cell lysis requires SARM1-dependent depolarisation of mitochondria in macrophages (Carty *et al*, 2019), indicating that mitochondrial damage is critical for the transition into the lytic phase of GSDMD activation in this cell type.

Altogether, these new findings highlight that more research is necessary to understand how GSDMD expression and activity is regulated on a translational and post-translational level, and which cellular membranes/organelles need to be targeted by $GSDMD^{NT}$ to induce pyroptotic cell death or to exert its lysis-independent functions. In the following, we however focus on an emerging host of studies that have begun to uncover cell type-specific and/or inflammasome-independent functions of GSDMD, and on the enigmatic role of GSDME, another member of the gasdermin family, in cell death.

GSDMD function in neutrophils

Neutrophils resist pyroptosis upon canonical inflammasome activation

Neutrophils express a repertoire of pattern recognition receptors (PRR) and are recruited in large quantity to a site of infection or inflammation, therefore are excellent candidates to drive inflammasome-dependent responses *in vivo* (Thomas & Schroder, 2013). However, earlier studies overlooked possible functions for neutrophil inflammasomes, after observing that neutrophils contributed to IL-1β processing through caspase-1-independent mechanisms in a mouse model of acute arthritis (K/BxN serum transfer) or upon FAS (CD95) ligation (Miwa *et al*, 1998; Guma *et al*, 2009; Joosten *et al*, 2009). In addition, two earlier studies proposed that neutrophils are unlikely to signal via inflammasomes during *Salmonella* Typhimurium or *Burkholderia pseudomallei* infection because these cells do not express NLRC4, an inflammasome-forming PRR that senses bacterial virulence factors (Miao *et al*, 2010; Ceballos-Olvera *et al*, 2011). Subsequent studies have now challenged these findings, as multiple groups readily detect expression of inflammasome-forming PRRs including NLRC4, NLRP3 and AIM2, and other components of the inflammasome signalling complex including the adaptor protein ASC, and the protease zymogen, caspase-1 in murine and human neutrophils (Mankan *et al*, 2012; Bakele *et al*, 2014; Chen *et al*, 2014, 2016; Karmakar *et al*, 2015, 2016). In agreement with this, exposure of neutrophils to the NLRC4 agonist *Salmonella* Typhimurium or the AIM2 agonist cytosolic double-stranded DNA triggered caspase-1 activation and caspase-1-dependent IL-1β processing (Chen *et al*, 2014). Although *Nlrp3* mRNA is basally expressed at much higher levels in neutrophils than macrophages (Chen *et al*, 2014), only soluble NLRP3 agonists such as ATP or the bacterial toxin nigericin, but not particulate or crystalline NLRP3 agonists (e.g. silica or monosodium urate crystals) are able to activate the neutrophil NLRP3 inflammasome (Chen *et al*, 2016). This highlights that inflammasome signalling is specialised even between the two closely related myeloid cell lineages. In agreement with this, while caspase-1 activation triggers rapid macrophage pyroptosis (Kayagaki *et al*, 2015; Shi *et al*, 2015), canonical inflammasome (e.g. NLRC4, NLRP3, AIM2) activation in neutrophils selectively triggers caspase-1-dependent IL-1β processing without concomitant pyroptotic cell death (Chen *et al*, 2014, 2018b; Karmakar *et al*, 2015, 2016). Although neutrophils are relatively short-lived cells and murine neutrophils have a half-life of 18 h in circulation (5.4 days in humans; Pillay *et al*, 2010), exposure of neutrophils to cytokines (e.g. GM-CSF, IL-1β, IFN-γ) and pathogen-derived products (e.g. LPS) can significantly increase their lifespan up to 96 h, indicating that neutrophils can significantly prolong their lifespan during infection (Colotta *et al*, 1992). This unique ability of neutrophils to resist pyroptosis enables the recruited neutrophils to maintain their lifespan to clear the microbial insult or cellular debris; and sustain IL-1β release to recruit, activate and prolong the lifespan of neutrophils at a site of infection (Chen *et al*, 2014; Karmakar *et al*, 2015, 2016). While inflammasomes are important for host defence, gain-of-function mutations in inflammasomes can also drive a variety of hereditary inflammatory disease (e.g. Muckle-Wells Syndrome, macrophage activating

syndrome; Agostini *et al*, 2004; Canna *et al*, 2014; Romberg *et al*, 2014). These diseases are currently attributed to inflammasome dysfunction in monocytes and macrophages, in which IL-1β/18 production is rapidly curtailed by pyroptotic cell death. Intriguingly, IL-1β production and inflammation are not self-limiting in these diseases, suggesting that the cellular source of IL-1β in these diseases may indeed be derived from other cell types. Since neutrophils express majority of the inflammasome signalling components, and that neutrophil IL-1β production proceeds in the absence of pyroptosis, it would be of interest to examine the contribution of neutrophil-derived IL-1β in human inflammatory disease in future studies.

The mechanisms by which neutrophils resist caspase-1-dependent pyroptosis is likely to be controlled by careful fine-tuning of the expression of specific pyroptotic machineries in these cells. Although GSDMD is expressed at comparable levels between neutrophils and macrophages (Chen *et al*, 2018b; Heilig *et al*, 2018), neutrophils express relatively low level of ASC and caspase-1, therefore, neutrophil inflammasomes assemble with a smaller ASC "speck" with reduced caspase-1 activity (Boucher *et al*, 2018; Chen *et al*, 2018a,b). Since caspase-1 cleaves pro-IL-1β better than it cleaves GSDMD (Chen *et al*, 2018b), this specific fine-tuning of caspase-1 activity in neutrophils ensures that caspase-1 only generates sublytic GSDMD pores to enable IL-1β secretion without concomitant cell lysis (Chen *et al*, 2018b; Fig 1). However, it is possible that additional mechanisms exist to restrict caspase-1-driven pyroptosis in neutrophils. For example, neutrophils may repair plasma membrane GSDMD pores via ESCRT-III repair mechanisms as reported for macrophages and HeLa cells (Ruhl *et al*, 2018). However, this hypothesis would be ambitious to demonstrate since it is technically challenging to manipulate primary neutrophils. SARM1 is a TIR-containing protein that is involved in TLR signalling. A recent study revealed a surprising role for SARM in driving optimal macrophage pyroptosis (Carty *et al*, 2019). Interestingly, *Sarm1*-deficient macrophages appear to be phenotypically similar to neutrophils, as both cell types release IL-1β in the absence of pyroptosis upon canonical inflammasome activation (Chen *et al*, 2014; Carty *et al*, 2019). Neutrophils were already documented to suppress TLR4-TRIF signalling to repress RIPK3-dependent cell death (Chen *et al*, 2018a); therefore, it is conceivable that neutrophils likewise suppress SARM1 expression to subvert caspase-1-dependent pyroptosis. Further studies should characterise the expression of SARM1 in neutrophils, and whether overexpression of SARM1 triggers neutrophil caspase-1-dependent pyroptosis.

Caspase-11 and neutrophil elastase cleave GSDMD to elicit NETosis

Although caspase-1 activation does not trigger pyroptosis in neutrophils, activation of the caspase-11 (non-canonical) inflammasome by cytosolic LPS or cytosolic Gram-negative bacteria triggered robust GSDMD cleavage and cell lysis in neutrophils, indicating that these cells are not intrinsically resistant to GSDMD pores (Chen *et al*, 2018b). Surprisingly, pyroptotic neutrophils appeared morphologically distinct from caspase-1 or caspase-11-activated macrophages. Instead, caspase-11 and GSDMD activation triggered classical hallmarks of NETosis, including nuclear delobulation, histone citrullination, DNA extrusion and rupture of nuclear, granule and plasma membrane (Fig 1). Strikingly, neutrophil elastase, myeloperoxidase and PAD4, three key enzymes involved in classical NETosis, are dispensable for caspase-11-dependent NET extrusion, indicating that caspase-11 and GSDMD may directly induce these hallmarks of NETosis (Chen *et al*, 2018b). In support of this, the combination of recombinant GSDMD and caspase-11 is sufficient to trigger neutrophil nuclear membrane rupture, chromatin relaxation and histone H3 degradation in a cell-free system. Further, application of exogenous DNase I to neutralise caspase-11 and GSDMD-driven NETs impairs *in vivo* host defence against a cytosolic mutant of *Salmonella* (*ΔsifA*), revealing a previously undescribed host protective function of NETs against cytoplasmic infection (Chen *et al*, 2018b). Given that cell type-specific signalling has such a profound impact on the phenotypical outcome of GSDMD-induced cell death, it will be very interesting to investigate the consequences of GSDMD activation in other granulocytes, as well as non-immune cells.

Neutrophil elastase cleaves GSDMD to trigger neutrophil cell death and NETs

Although GSDMD was initially identified as a substrate of inflammatory caspases, two recent studies documented that GSDMD is also processed by the serine protease, neutrophil elastase, in neutrophils (Fig 2; Kambara *et al*, 2018; Sollberger *et al*, 2018). Although neutrophil elastase cleaves GSDMD several amino acids upstream of the canonical caspase cleavage site, this did not affect the ability of the GSDMD N-terminal fragment to oligomerise and induce lytic cell death upon overexpression in HEK 293T cells, in line with the observation that the membrane insertion and lytic properties of GSDMD N-terminal fragment lie within the first 243 amino acid (Shi *et al*, 2015). However, despite these observations, the conclusion from the two studies was vastly different. In one study, neutrophil elastase-dependent GSDMD cleavage was proposed to trigger cell death in ageing neutrophils. Consequently, when challenged intraperitoneally with *E. coli* K12, *Gsdmd*-deficient mice accumulated more neutrophils at the site of infection and were more resistant to infection than wild-type animals (Kambara *et al*, 2018). However, whether GSDMD promotes spontaneous neutrophil cell death is controversial, as other studies documented similar rate of spontaneous neutrophil death in wild-type versus *Gsdmd*-deficient neutrophils (Chen *et al*, 2018b; Burgener *et al*, 2019). In agreement with macrophage studies showing that the $GSDMD^{NT}$ fragment triggers proinflammatory cell death, a second study reported that activation of GSDMD by neutrophil elastase drives neutrophil cell lysis and NET extrusion, a well-appreciated antimicrobial defence mechanism (Sollberger *et al*, 2018). Therefore, it appears that GSDMD activity in neutrophils can either promote or dampen host defence. The signalling mechanisms that dictate these differences have not been investigated in detail; however, it is tempting to speculate that the signal strength and cellular location of neutrophil elastase is a key regulator. It is well documented that a high concentration of neutrophil elastase translocates from azurophilic granules to the nucleus at the early stages of NETosis, and that nuclear membrane damage precedes cellular rupture (Papayannopoulos *et al*, 2010; Metzler *et al*, 2014; Sollberger *et al*, 2018). In this scenario, it is likely that the close proximity of cleaved GSDMD preferentially disrupts the nuclear membrane to initiate the hallmarks of NETosis. In contrast, it is conceivable that low levels of neutrophil elastase

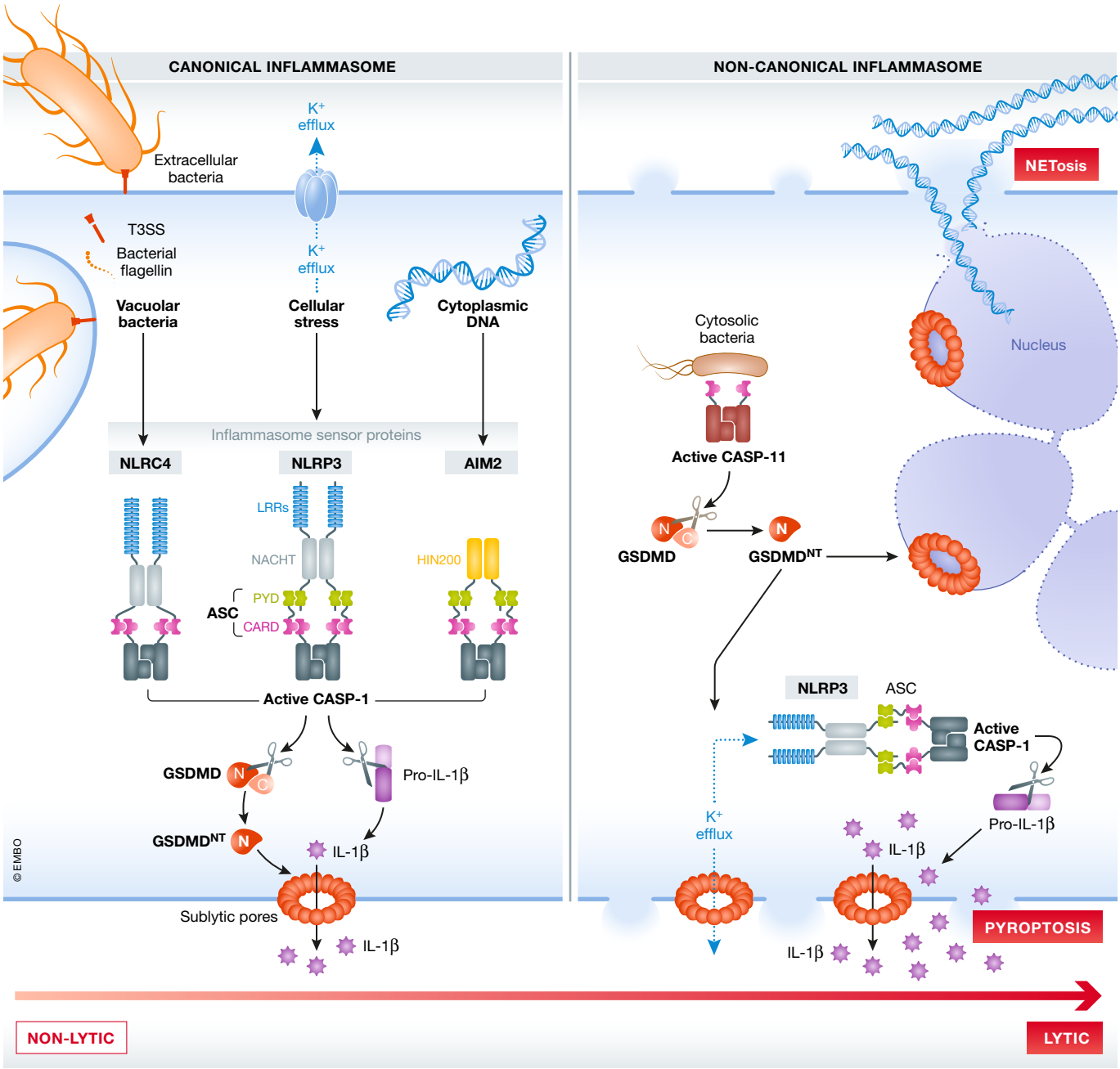

Figure 1. Canonical and non-canonical inflammasome activation in neutrophils.

Neutrophils express several inflammasome-forming PRR including NLRC4, NLRP3, AIM2 and caspase-11. Activation of canonical inflammasome selectively triggers IL-1β maturation without accompanying cell lysis. IL-1β secretion in living neutrophils requires the pore-forming protein GSDMD. Upon cytoplasmic Gram-negative bacterial infection, caspase-11 triggers robust GSDMD cleavage. $GSDMD^{NT}$ targets plasma membrane and nuclear membrane to elicit neutrophil extracellular traps (NETs). Caspase-11-driven GSDMD pores promotes potassium efflux and activation of the NLRP3 inflammasome.

"escapes" from azurophilic granules into the cytosol in aged neutrophils, which cleaves a low but steady amount of GSDMD to trigger neutrophil death without accompanying NETosis. Since GSDMD drives a variety of inflammatory disease and is thus an attractive pharmacological target, additional studies are clearly required to further characterise the function of GSDMD in neutrophils during inflammatory disease and infection.

GSDMD function during apoptosis

Emerging evidences of apoptosis-induced inflammation

Apoptosis is a form of programmed cell death that is important for embryonic development, removal of auto-reactive lymphocytes and clearance of damaged or superfluous cells. In contrast to pyroptosis, apoptotic cell death is generally regarded as an immunologically

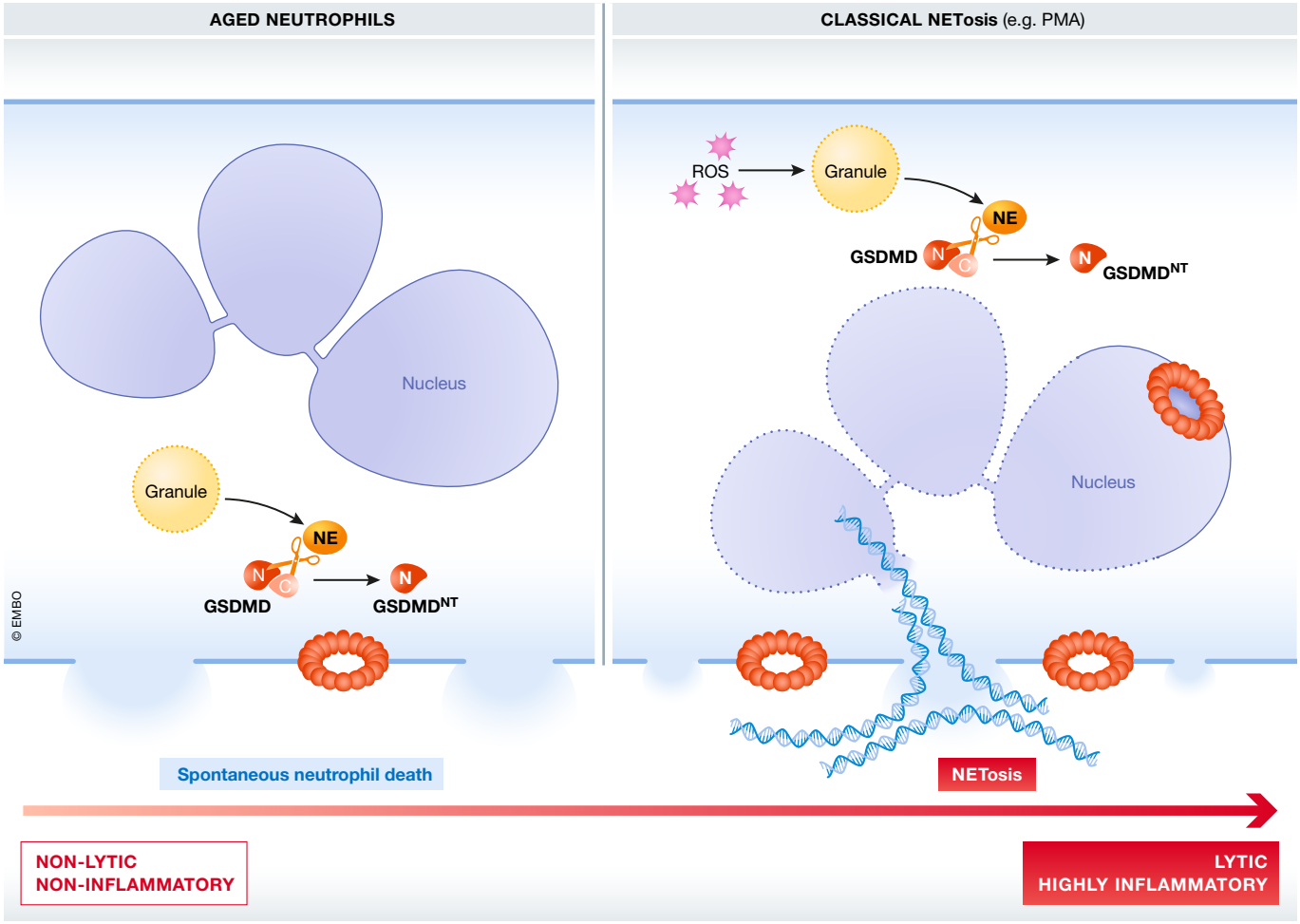

Figure 2. GSDMD promotes spontaneous neutrophil cell death and NET extrusion.

In ageing neutrophils, release of neutrophil elastase (NE) from specific neutrophil granules cleaves and activates GSDMD, resulting in neutrophil cell death. Upon treatment with classical NETosis activators (e.g. PMA), reactive oxygen species (ROS) promote the release of NE from the granules to cytosol in an ill-defined manner. NE cleaves and activates GSDMD, leading to nuclear and plasma membrane rupture and neutrophil cell lysis by NETosis.

silent process. This is achieved by several mechanisms, including sequential breakdown of the dying cell into small membrane-bound apoptotic bodies, the release of "find-me" and "eat-me" signals to promote efferocytosis of dying cells and caspase-mediated cleavage of innate immune sensors and proinflammatory cytokines (Luthi *et al*, 2009; Poon *et al*, 2014; Ning *et al*, 2019). However, despite these observations, *in vitro* studies revealed that genetic or pharmacological inhibition of endogenous apoptosis inhibitors such as the mammalian inhibitor of apoptosis proteins (IAPs) cIAP1, 2 and XIAP, or kinases such as transforming growth factor beta-activated kinase 1 (TAK1) and IκB kinase β (IKKβ), sensitise myeloid cells including macrophages, dendritic cells and neutrophils to caspase-8 activation, cell lysis and NLRP3 inflammasome activation (Vince *et al*, 2012; Yabal *et al*, 2014; Dondelinger *et al*, 2015; Lawlor *et al*, 2015, 2017; Wicki *et al*, 2016; Chen *et al*, 2018a). In agreement with these *in vitro* studies, global loss of *Map3k7* (TAK1), IKKβ or *Birc2* (cIAP1) in combination with *Birc3* (cIAP2) or *Birc4* (XIAP) similarly drives excessive inflammation that results in embryonic lethality (Tanaka *et al*, 1999; Sato *et al*, 2005; Shim *et al*, 2005; Moulin *et al*, 2012).

Direct cleavage of GSDMD by caspase-8 promotes cell lysis and inflammation

While the studies above clearly implicate an important function for caspase-8 in driving inflammation and even embryonic lethality, the molecular mechanisms by which caspase-8 promotes cell lysis and NLRP3 activation remain unsolved. By using pharmacological inhibitors of TAK1 or IAPs (e.g. SMAC mimetics), we and others recently demonstrate that the pyroptotic effector GSDMD plays a major role in this process (Orning *et al*, 2018; Sarhan *et al*, 2018; Chen *et al*, 2019b; Sanjo *et al*, 2019). Unexpectedly, under these conditions, GSDMD is processed into the lytic p30 fragment via two pathways. The first pathway involves direct cleavage of GSDMD by caspase-8 at position D276, similar to canonical caspase cleavage site described for caspase-1 and caspase-11. However, caspase-8 is 30-fold less efficient than caspase-1 in

processing GSDMD, and caspase-8-dependent GSDMD cleavage is only observed under conditions of strong caspase-8 activation (Chen *et al*, 2019b). This likely explains why early studies failed to observe GSDMD processing into the active p30 fragment by recombinant caspase-8 (Shi *et al*, 2015). The second pathway leading to GSDMD activation occurs via potassium efflux and activation of the NLRP3 inflammasome (Conos *et al*, 2017); however, the mechanisms by which caspase-8 drives NLRP3 activation are still a matter of debate and are discussed in greater details in the subsequent paragraphs.

The finding that caspase-8 triggers direct GSDMD activation is exciting and raises several important questions. For example, what is the physiological function of caspase-8-dependent GSDMD activation? Numerous pathogens are equipped with virulence factors that inhibit host NF-κB signalling pathways. This could in turn promote caspase-8 activation and induce GSDMD cleavage and pyroptosis, as recently reported for *Yersinia* infection (Orning *et al*, 2018; Sarhan *et al*, 2018). However, as pyroptosis is best known as an innate immune mechanism to restrict intracellular pathogen infection, how GSDMD activation can promote host defence against *Yersinia*, a predominantly extracellular pathogen is unclear, and has not been formally demonstrated. RIPK1/caspase-8-dependent apoptosis can promote the release of alarmins and activate neighbouring immune cells for cytokine production and anti-*Yersinia* defence (Peterson *et al*, 2017), yet whether GSDMD is also required in this scenario is unclear and warrants further investigation. Likewise, it would be of interest to investigate whether the caspase-8–GSDMD axis induces NET extrusion to combat extracellular pathogens, or whether this signalling axis is exploited by *Yersinia* to promote pathogen dissemination *in vivo*.

Another important question that arises from the discovery that caspase-8 cleaves GSDMD is the molecular mechanisms by which apoptosis remains immunologically silent during tissue homeostasis. Several lines of evidence suggest that executioner caspases play a key role in regulating the level of GSDMD activity in apoptotic cells, as previous studies documented that caspase-3, and a lesser extent caspase-7, cleave GSDMD at position D88 (D87 in humans) to disrupt its pore-forming ability (Rogers *et al*, 2017; Taabazuing *et al*, 2017). In keeping with this, $Gsdmd^{D88A/D88A}$ knock-in macrophages harbouring a caspase-3/7-uncleavable mutation accumulated $GSDMD^{NT}$ pores, resulting in enhanced pyroptosis compared to wild-type macrophages (Chen *et al*, 2019b). However, naïve $Gsdmd^{D88A/D88A}$ mice appear phenotypically similar to wild-type littermates (Chen *et al*, 2019b); thus, whether GSDMD inactivation is required to suppress pyroptosis during physiological conditions *in vivo* has not been formally demonstrated.

Aberrant caspase-8 activity has been implicated in a variety of inflammatory diseases and, in some cases, can even drive lethality. For example, caspase-8 drives lethal dermatitis in the absence of linear ubiquitin chain assembly complex (LUBAC; Taraborrelli *et al*, 2018), and caspase-8 activity triggers embryonic lethality observed in $Birc2^{-/-}$ $Birc3^{-/-}$ mice (Zhang *et al*, 2019). Furthermore, caspase-8-dependent intestinal damage is a key driver for septic shock in mice (Mandal *et al*, 2018). Since the caspase-8 is emerging a key mediator of cell death and inflammation, it would be of great interest to investigate whether caspase-8-dependent GSDMD activation is sufficient to drive pathogenesis of these diseases in the near future.

GSDMD and pannexin-1 control NLRP3 activation in apoptotic cells

Although apoptosis was traditionally considered an immunologically silent form of cell death, an increasing number of studies documented that apoptotic caspase-8 promotes assembly of the NLRP3 inflammasome (Vince *et al*, 2012; Lawlor *et al*, 2015, 2017; Wicki *et al*, 2016; Chen *et al*, 2019a,b). The existence of this signalling axis was first demonstrated by Vince and colleagues, who reported that loss of IAPs sensitised macrophages and dendritic cells to caspase-8-dependent cell death and NLRP3 activation upon TNF or TLR ligation (Vince *et al*, 2012). Although this signalling axis is implicated in a variety of physiological conditions, including the pathogenesis of X-linked lymphoproliferative syndrome type 2 in humans (Yabal *et al*, 2014; Lawlor *et al*, 2017), and during influenza or *Yersinia* infection (Kuriakose *et al*, 2016; Orning *et al*, 2018), the exact mechanism by which apoptotic caspases activate NLRP3 is still a matter of debate and might involve several pathways. Orning *et al* recently proposed that caspase-8-driven GSDMD pores triggers NLRP3 assembly (Orning *et al*, 2018), analogous to the non-canonical inflammasome pathway, where caspase-11-driven GSDMD pores promote membrane damage, potassium efflux and NLRP3 inflammasome activation (Fig 3; Kayagaki *et al*, 2015; Ruhl & Broz, 2015; Shi *et al*, 2015). In contrast, our study revealed that GSDMD is dispensable for caspase-1 activation during TNF-induced caspase-8 activation. Instead, we demonstrate that caspase-8-dependent NLRP3 activation requires the channel-forming transmembrane glycoprotein, pannexin-1. For this, caspase-8 promotes downstream executor caspase-3/7 activation, which cleave and activate pannexin-1 channel activity, membrane permeability and NLRP3 inflammasome activation (Fig 3; Chen *et al*, 2019a,b). Further support for the importance of pannexin-1 in driving NLRP3 activation during apoptosis comes from the fact that caspase-3/7 and pannexin-1 are also required also for NLRP3 activation upon caspase-9-dependent intrinsic apoptosis, which unlike caspase-8, does not have the ability to cleave GSDMD (Vince *et al*, 2018; Chen *et al*, 2019a,b).

The reasons for this discrepancy are unclear; however, it is tempting to speculate that the cellular activity of executor caspase-3/7 critically controls the amount of GSDMD pores and pannexin-1 activation in a given cell, and that dictates which pathway is preferentially activated. For example, a given cell with high caspase-3/7 activity would inactivate GSDMD pores and favour NLRP3 activation via pannexin-1 channels. On the other hand, cells with low caspase-3/7 activity would favour NLRP3 activation via GSDMD pores but not pannexin-1 channels. Given that executor caspase-3/7 activity is often suppressed in transformed cells and that many cancer chemotherapies induce tumour cell death through caspase-8, future studies should further characterise this pathway in the context of cancer chemotherapy, and whether modulating this signalling axis can promote tumour clearance.

GSDME activation by caspase-3 promotes pyroptosis in some but not all cells

The discovery that cleavage of GSDMD at the linker region by inflammatory caspases unleashes the pore-forming function of $GSDMD^{NT}$ has significantly enhanced the field's understanding of gasdermin family proteins. Indeed, recent studies found that GSDME features a caspase-3 cleavage motif in its linker region. Similar to GSDMD, cleavage of GSDME by caspases-3/-7 liberates the

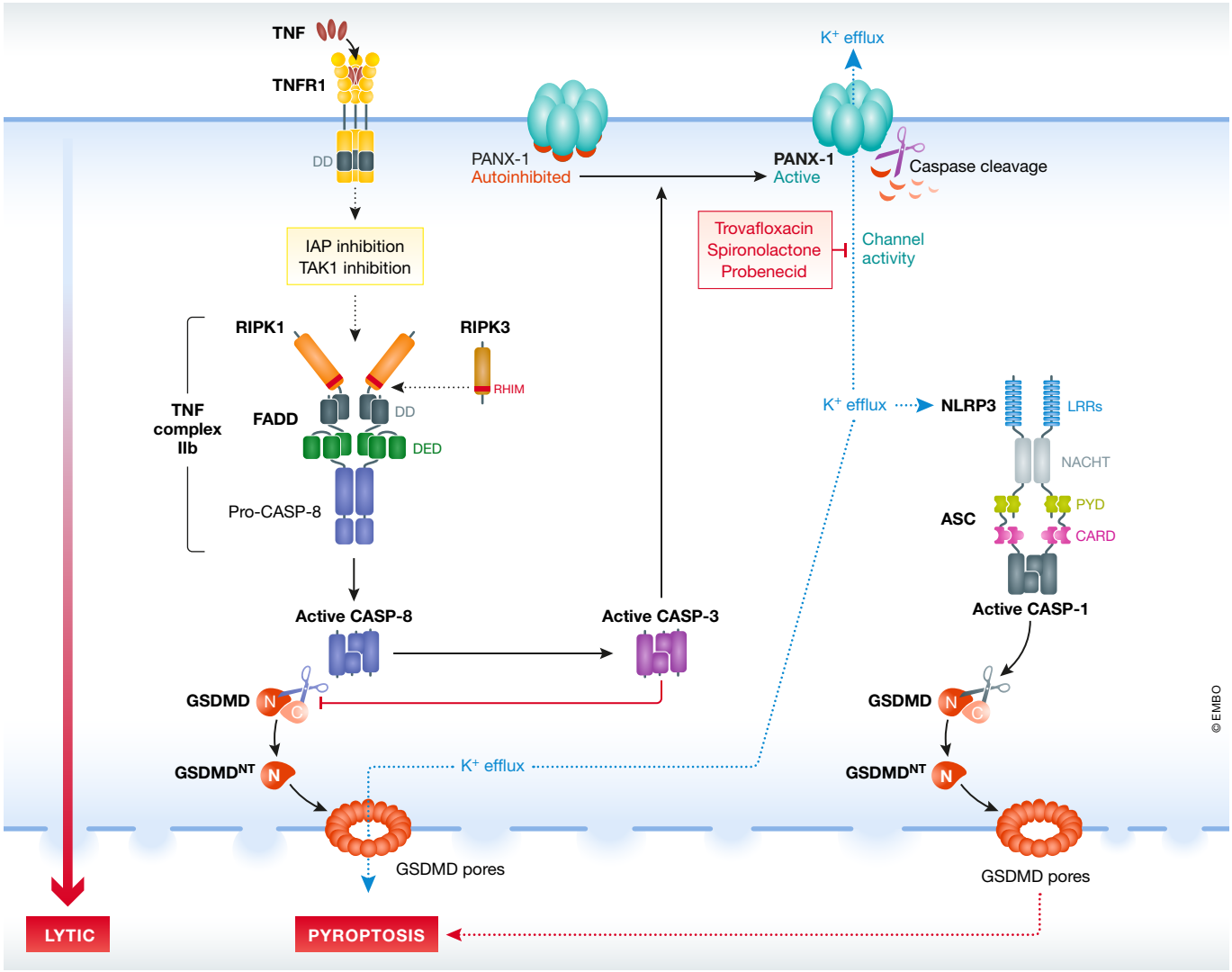

Figure 3. GSDMD is a novel effector protein in the extrinsic apoptosis pathway.

In TNF-stimulated cells, loss or inhibition of IAP and TAK1 function promotes assembly of a caspase-8-activating platform called TNF Complex IIb (also commonly referred as the ripoptosome). Active caspase-8 cleaves GSDMD at D276, leading to pyroptosis. Caspase-8-driven GSDMD activation, or caspase-3/7-dependent pannexin-1 activation promotes potassium efflux and NLRP3 assembly. NLRP3-dependent caspase-1 activation cleaves GSDMD to further drive pyroptosis. Probenecid, spironolactone and trovafloxacin are pannexin-1 channel inhibitors.

N-terminal pyroptosis-inducing domain (GSDME^{NT}) from its autoinhibitory C-terminal regulatory domain to trigger membrane pores and pyroptosis (Rogers *et al*, 2017; Wang *et al*, 2017). Interestingly, cleavage of GSDME by caspase-3 does not necessarily destine the cell to undergo pyroptosis. In this regard, immune cells appear to be the most resistant to GSDME pores. Indeed, despite evidence of GSDME processing into the active GSDME^{NT} fragment, a number of studies documented that GSDME is dispensable for pyroptosis or secondary necrosis upon extrinsic or intrinsic apoptosis in primary and immortalised murine macrophages, THP-1 monocytes and Jurkat T cells (Lee *et al*, 2018; Tixeira *et al*, 2018; Vince *et al*, 2018; Chen *et al*, 2019b). A simple explanation for this phenomenon is that GSDME pores need to surpass a critical threshold to initiate pyroptosis. In support of this, cancer cell lines that express high levels of GSDME are extremely susceptible to pyroptosis after exposure of apoptosis-inducing therapies such as cisplatin, doxorubicin and etoposide, while the same treatment triggers apoptosis in GSDME-deficient or low expressing cells (Wang *et al*, 2017). Although emerging studies demonstrate that MLKL-driven necrotic cell death promotes anti-tumour immunity (Brumatti *et al*, 2016; Snyder *et al*, 2019), whether GSDME-driven pyroptosis restricts tumour growth *in vivo* is still unclear and remains an open question. For example, a study reported that GSDME expression suppresses melanoma cell growth a murine xenograft model, whereas other studies documented that *Gsdme* deficiency had no impact on tumour formation during intestinal cancer (Zhou *et al*, 2018; Croes *et al*, 2019). Further studies are required to clarify the importance of GSDME during tumorigenesis.

Conclusion and outlook

Since the discovery of the GSDMD as executor of pyroptosis in 2015, it has taken centre stage in other cell death pathways as well, highlighting that inflammasomes are only one possible signalling pathway that can activate the protein. It is thus conceivable that other proteases, be it from the host or from pathogenic microorganisms, could also activate GSDMD or the other family members, as shown for caspase-3/7 and GSDME. However, proteolysis may not be the only mechanism of gasdermin activation, as point mutations in the $GSDM^{CT}$ result in activation without the removal of the C-terminal domain (Shi *et al*, 2015). It is thus clear that additional work will be necessary to better understand the activation and regulation mechanism that control this new family of cell death executors. Furthermore, given the importance of gasdermin-induced death in causing tissue damage and inflammation, additional efforts should be made to develop specific gasdermin inhibitors and to explore the possibility of therapeutical targeting of the gasdermin family.

Acknowledgements

This work was supported by a Swiss Government Excellence (ESKAS) postdoctoral fellowship and a Marie Skłodowska-Curie Actions Individual Fellowship (MSCA-IF-2018-838252) to K.W.C, and a European Research Council Grant (ERC2017-CoG-770988-InflamCellDeath) to P.B.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aglietti RA, Estevez A, Gupta A, Ramirez MG, Liu PS, Kayagaki N, Ciferri C, Dixit VM, Dueber EC (2016) GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc Natl Acad Sci USA* 113: 7858 – 7863

Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J (2004) NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20: 319 – 325

Bakele M, Joos M, Burdi S, Allgaier N, Poschel S, Fehrenbacher B, Schaller M, Marcos V, Kummerle-Deschner J, Rieber N *et al* (2014) Localization and functionality of the inflammasome in neutrophils. *J Biol Chem* 289: 5320 – 5329

Boucher D, Monteleone M, Coll RC, Chen KW, Ross CM, Teo JL, Gomez GA, Holley CL, Bierschenk D, Stacey KJ *et al* (2018) Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J Exp Med* 215: 827 – 840

Brennan MA, Cookson BT (2000) Salmonella induces macrophage death by caspase-1-dependent necrosis. *Mol Microbiol* 38: 31 – 40

Brumatti G, Ma C, Lalaoui N, Nguyen NY, Navarro M, Tanzer MC, Richmond J, Ghisi M, Salmon JM, Silke N *et al* (2016) The caspase-8 inhibitor emricasan combines with the SMAC mimetic birinapant to induce necroptosis and treat acute myeloid leukemia. *Sci Transl Med* 8: 339ra69

Burgener SS, Leborgne NGF, Snipas SJ, Salvesen GS, Bird PI, Benarafa C (2019) Cathepsin G inhibition by serpinb1 and serpinb6 prevents programmed necrosis in neutrophils and monocytes and reduces GSDMD-driven inflammation. *Cell Rep* 27: 3646 – 3656 e5

Canna SW, de Jesus AA, Gouni S, Brooks SR, Marrero B, Liu Y, DiMattia MA, Zaal KJ, Sanchez GA, Kim H *et al* (2014) An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet* 46: 1140 – 1146

Carty M, Kearney J, Shanahan KA, Hams E, Sugisawa R, Connolly D, Doran CG, Munoz-Wolf N, Gurtler C, Fitzgerald KA *et al* (2019) Cell survival and cytokine release after inflammasome activation is regulated by the toll-IL-1R protein SARM. *Immunity* 50: 1412 – 1424 e6

Ceballos-Olvera I, Sahoo M, Miller MA, Del Barrio L, Re F (2011) Inflammasome-dependent pyroptosis and IL-18 protect against Burkholderia pseudomallei lung infection while IL-1beta is deleterious. *PLoS Pathog* 7: e1002452

Chen KW, Gross CJ, Sotomayor FV, Stacey KJ, Tschopp J, Sweet MJ, Schroder K (2014) The neutrophil NLRC4 inflammasome selectively promotes IL-1beta maturation without pyroptosis during acute Salmonella challenge. *Cell Rep* 8: 570 – 582

Chen KW, Bezbradica JS, Gross CJ, Wall AA, Sweet MJ, Stow JL, Schroder K (2016) The murine neutrophil NLRP3 inflammasome is activated by soluble but not particulate or crystalline agonists. *Eur J Immunol* 46: 1004 – 1010

Chen KW, Lawlor KE, von Pein JB, Boucher D, Gerlic M, Croker BA, Bezbradica JS, Vince JE, Schroder K (2018a) Cutting edge: blockade of inhibitor of apoptosis proteins sensitizes neutrophils to TNF- but not lipopolysaccharide-mediated cell death and IL-1beta secretion. *J Immunol* 200: 3341 – 3346

Chen KW, Monteleone M, Boucher D, Sollberger G, Ramnath D, Condon ND, von Pein JB, Broz P, Sweet MJ, Schroder K (2018b) Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps. *Sci Immunol* 3: eaar6676

Chen KW, Demarco B, Broz P (2019a) Pannexin-1 promotes NLRP3 activation during apoptosis but is dispensable for canonical or Non-canonical inflammasome activation. *Eur J Immunol* https://doi.org/10.1002/eji.201948254

Chen KW, Demarco B, Heilig R, Shkarina K, Boettcher A, Farady CJ, Pelczar P, Broz P (2019b) Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP3 inflammasome assembly. *EMBO J* 38: e101638

Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80: 2012 – 2020

Conos SA, Lawlor KE, Vaux DL, Vince JE, Lindqvist LM (2016) Cell death is not essential for caspase-1-mediated interleukin-1beta activation and secretion. *Cell Death Differ* 23: 1827 – 1838

Conos SA, Chen KW, De Nardo D, Hara H, Whitehead L, Nunez G, Masters SL, Murphy JM, Schroder K, Vaux DL *et al* (2017) Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner. *Proc Natl Acad Sci USA* 114: E961 – E969

Cookson BT, Brennan MA (2001) Pro-inflammatory programmed cell death. *Trends Microbiol* 9: 113 – 114

Croes L, Fransen E, Hylebos M, Buys K, Hermans C, Broeckx G, Peeters M, Pauwels P, Op de Beeck K, Van Camp G (2019) Determination of the potential tumor-suppressive effects of Gsdme in a chemically induced and in a genetically modified intestinal cancer mouse model. *Cancers* 11: E1214

Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, Sun H, Wang DC, Shao F (2016) Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535: 111 – 116

DiPeso L, Ji DX, Vance RE, Price JV (2017) Cell death and cell lysis are separable events during pyroptosis. *Cell Death Discov* 3: 17070

Dondelinger Y, Jouan-Lanhouet S, Divert T, Theatre E, Bertin J, Gough PJ, Giansanti P, Heck AJ, Dejardin E, Vandenabeele P *et al* (2015) NF-kappaB-independent role of IKKalpha/IKKbeta in preventing RIPK1 Kinase-dependent apoptotic and necroptotic cell death during TNF signaling. *Mol Cell* 60: 63 – 76

Evavold CL, Ruan J, Tan Y, Xia S, Wu H, Kagan JC (2018) The pore-forming protein gasdermin d regulates interleukin-1 secretion from living macrophages. *Immunity* 48: 35 – 44 e6

Fink SL, Cookson BT (2006) Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell Microbiol* 8: 1812 – 1825

Gaidt MM, Ebert TS, Chauhan D, Schmidt T, Schmid-Burgk JL, Rapino F, Robertson AA, Cooper MA, Graf T, Hornung V (2016) Human monocytes engage an alternative inflammasome pathway. *Immunity* 44: 833 – 846

Guma M, Ronacher L, Liu-Bryan R, Takai S, Karin M, Corr M (2009) Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. *Arthritis Rheum* 60: 3642 – 3650

He WT, Wan H, Hu L, Chen P, Wang X, Huang Z, Yang ZH, Zhong CQ, Han J (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1beta secretion. *Cell Res* 25: 1285 – 1298

Heilig R, Dick MS, Sborgi L, Meunier E, Hiller S, Broz P (2018) The Gasdermin-D pore acts as a conduit for IL-1beta secretion in mice. *Eur J Immunol* 48: 584 – 592

Joosten LA, Netea MG, Fantuzzi G, Koenders MI, Helsen MM, Sparrer H, Pham CT, van der Meer JW, Dinarello CA, van den Berg WB (2009) Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. *Arthritis Rheum* 60: 3651 – 3662

Jorgensen I, Zhang Y, Krantz BA, Miao EA (2016) Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis. *J Exp Med* 213: 2113 – 2128

Kambara H, Liu F, Zhang X, Liu P, Bajrami B, Teng Y, Zhao L, Zhou S, Yu H, Zhou W *et al* (2018) Gasdermin D exerts anti-inflammatory effects by promoting neutrophil death. *Cell Rep* 22: 2924 – 2936

Kang TB, Yang SH, Toth B, Kovalenko A, Wallach D (2013) Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasome. *Immunity* 38: 27 – 40

Karmakar M, Katsnelson M, Malak HA, Greene NG, Howell SJ, Hise AG, Camilli A, Kadioglu A, Dubyak GR, Pearlman E (2015) Neutrophil IL-1beta processing induced by pneumolysin is mediated by the NLRP3/ASC inflammasome and caspase-1 activation and is dependent on K+ efflux. *J Immunol* 194: 1763 – 1775

Karmakar M, Katsnelson MA, Dubyak GR, Pearlman E (2016) Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1beta secretion in response to ATP. *Nat Commun* 7: 10555

Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, Cuellar T, Haley B, Roose-Girma M, Phung QT *et al* (2015) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526: 666 – 671

Kuriakose T, Man SM, Malireddi RK, Karki R, Kesavardhana S, Place DE, Neale G, Vogel P, Kanneganti TD (2016) ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Sci Immunol* 1: aag2045

Lawlor KE, Khan N, Mildenhall A, Gerlic M, Croker BA, D'Cruz AA, Hall C, Kaur Spall S, Anderton H, Masters SL *et al* (2015) RIPK3 promotes cell death and NLRP3 inflammasome activation in the absence of MLKL. *Nat Commun* 6: 6282

Lawlor KE, Feltham R, Yabal M, Conos SA, Chen KW, Ziehe S, Grass C, Zhan Y, Nguyen TA, Hall C *et al* (2017) XIAP loss triggers RIPK3- and Caspase-8-Driven IL-1beta activation and cell death as a consequence of TLR-MyD88-induced cIAP1-TRAF2 degradation. *Cell Rep* 20: 668 – 682

Lee BL, Mirrashidi KM, Stowe IB, Kummerfeld SK, Watanabe C, Haley B, Cuellar TL, Reichelt M, Kayagaki N (2018) ASC- and caspase-8-dependent apoptotic pathway diverges from the NLRC4 inflammasome in macrophages. *Sci Rep* 8: 3788

Liu T, Yamaguchi Y, Shirasaki Y, Shikada K, Yamagishi M, Hoshino K, Kaisho T, Takemoto K, Suzuki T, Kuranaga E *et al* (2014) Single-cell imaging of caspase-1 dynamics reveals an all-or-none inflammasome signaling response. *Cell Rep* 8: 974 – 982

Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, Lieberman J (2016) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535: 153 – 158

Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, Brumatti G, Taylor RC, Kersse K, Vandenabeele P *et al* (2009) Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 31: 84 – 98

Mandal P, Feng Y, Lyons JD, Berger SB, Otani S, DeLaney A, Tharp GK, Maner-Smith K, Burd EM, Schaeffer M *et al* (2018) Caspase-8 collaborates with caspase-11 to drive tissue damage and execution of endotoxic shock. *Immunity* 49: 42 – 55 e6

Mankan AK, Dau T, Jenne D, Hornung V (2012) The NLRP3/ASC/Caspase-1 axis regulates IL-1beta processing in neutrophils. *Eur J Immunol* 42: 710 – 715

Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 10: 417 – 426

Metzler KD, Goosmann C, Lubojemska A, Zychlinsky A, Papayannopoulos V (2014) A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. *Cell Rep* 8: 883 – 896

Miao EA, Leaf IA, Treuting PM, Mao DP, Dors M, Sarkar A, Warren SE, Wewers MD, Aderem A (2010) Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat Immunol* 11: 1136 – 1142

Miwa K, Asano M, Horai R, Iwakura Y, Nagata S, Suda T (1998) Caspase 1-independent IL-1beta release and inflammation induced by the apoptosis inducer Fas ligand. *Nat Med* 4: 1287 – 1292

Monack DM, Raupach B, Hromockyj AE, Falkow S (1996) Salmonella typhimurium invasion induces apoptosis in infected macrophages. *Proc Natl Acad Sci USA* 93: 9833 – 9838

Monteleone M, Stanley AC, Chen KW, Brown DL, Bezbradica JS, von Pein JB, Holley CL, Boucher D, Shakespear MR, Kapetanovic R *et al* (2018) Interleukin-1beta maturation triggers its relocation to the plasma membrane for gasdermin-D-dependent and -independent secretion. *Cell Rep* 24: 1425 – 1433

Moulin M, Anderton H, Voss AK, Thomas T, Wong WW, Bankovacki A, Feltham R, Chau D, Cook WD, Silke J *et al* (2012) IAPs limit activation of RIP kinases by TNF receptor 1 during development. *EMBO J* 31: 1679 – 1691

Mulvihill E, Sborgi L, Mari SA, Pfreundschuh M, Hiller S, Muller DJ (2018) Mechanism of membrane pore formation by human gasdermin-D. *EMBO J* 37: e98321

Ning X, Wang Y, Jing M, Sha M, Lv M, Gao P, Zhang R, Huang X, Feng JM, Jiang Z (2019) Apoptotic caspases suppress type I interferon production via the cleavage of cGAS, MAVS, and IRF3. *Mol Cell* 74: 19 – 31 e7

Orning P, Weng D, Starheim K, Ratner D, Best Z, Lee B, Brooks A, Xia S, Wu H, Kelliher MA *et al* (2018) Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* 362: 1064 – 1069

Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A (2010) Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol* 191: 677 – 691

Peterson LW, Philip NH, DeLaney A, Wynosky-Dolfi MA, Asklof K, Gray F, Choa R, Bjanes E, Buza EL, Hu B *et al* (2017) RIPK1-dependent apoptosis bypasses pathogen blockade of innate signaling to promote immune defense. *J Exp Med* 214: 3171 – 3182

Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L (2010) *In vivo* labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. *Blood* 116: 625 – 627

Polykratis A, Martens A, Eren RO, Shirasaki Y, Yamagishi M, Yamaguchi Y, Uemura S, Miura M, Holzmann B, Kollias G *et al* (2019) A20 prevents inflammasome-dependent arthritis by inhibiting macrophage necroptosis through its ZnF7 ubiquitin-binding domain. *Nat Cell Biol* 21: 731 – 742

Poon IK, Lucas CD, Rossi AG, Ravichandran KS (2014) Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol* 14: 166 – 180

Rogers C, Fernandes-Alnemri T, Mayes L, Alnemri D, Cingolani G, Alnemri ES (2017) Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat Commun* 8: 14128

Romberg N, Al Moussawi K, Nelson-Williams C, Stiegler AL, Loring E, Choi M, Overton J, Meffre E, Khokha MK, Huttner AJ *et al* (2014) Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet* 46: 1135 – 1139

Ruan J, Xia S, Liu X, Lieberman J, Wu H (2018) Cryo-EM structure of the gasdermin A3 membrane pore. *Nature* 557: 62 – 67

Rubartelli A, Cozzolino F, Talio M, Sitia R (1990) A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. *EMBO J* 9: 1503 – 1510

Ruhl S, Broz P (2015) Caspase-11 activates a canonical NLRP3 inflammasome by promoting K(+) efflux. *Eur J Immunol* 45: 2927 – 2936

Ruhl S, Shkarina K, Demarco B, Heilig R, Santos JC, Broz P (2018) ESCRT-dependent membrane repair negatively regulates pyroptosis downstream of GSDMD activation. *Science* 362: 956 – 960

Russo HM, Rathkey J, Boyd-Tressler A, Katsnelson MA, Abbott DW, Dubyak GR (2016) Active caspase-1 induces plasma membrane pores that precede pyroptotic lysis and are blocked by lanthanides. *J Immunol* 197: 1353 – 1367

Sanjo H, Nakayama J, Yoshizawa T, Fehling HJ, Akira S, Taki S (2019) Cutting edge: TAK1 safeguards macrophages against proinflammatory cell death. *J Immunol* 203: 783 – 788

Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, Tang AY, Rongvaux A, Bunnell SC, Shao F, Green DR *et al* (2018) Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during Yersinia infection. *Proc Natl Acad Sci USA* 115: E10888 – E10897

Sato S, Sanjo H, Takeda K, Ninomiya-Tsuji J, Yamamoto M, Kawai T, Matsumoto K, Takeuchi O, Akira S (2005) Essential function for the kinase TAK1 in innate and adaptive immune responses. *Nat Immunol* 6: 1087 – 1095

Sborgi L, Ruhl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H, Farady CJ, Muller DJ, Broz P, Hiller S (2016) GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J* 35: 1766 – 1778

Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526: 660 – 665

Shim JH, Xiao C, Paschal AE, Bailey ST, Rao P, Hayden MS, Lee KY, Bussey C, Steckel M, Tanaka N *et al* (2005) TAK1, but not TAB 1 or TAB 2, plays an essential role in multiple signaling pathways *in vivo*. *Genes Dev* 19: 2668 – 2681

Snyder AG, Hubbard NW, Messmer MN, Kofman SB, Hagan CE, Orozco SL, Chiang K, Daniels BP, Baker D, Oberst A (2019) Intratumoral activation of the necroptotic pathway components RIPK1 and RIPK3 potentiates antitumor immunity. *Sci Immunol* 4: eaaw2004

Sollberger G, Choidas A, Burn GL, Habenberger P, Di Lucrezia R, Kordes S, Menninger S, Eickhoff J, Nussbaumer P, Klebl B *et al* (2018) Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci Immunol* 3: eaar6689

Taabazuing CY, Okondo MC, Bachovchin DA (2017) Pyroptosis and apoptosis pathways engage in bidirectional crosstalk in monocytes and macrophages. *Cell Chem Biol* 24: 507 – 514 e4

Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, Goeddel DV (1999) Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. *Immunity* 10: 421 – 429

Taraborrelli L, Peltzer N, Montinaro A, Kupka S, Rieser E, Hartwig T, Sarr A, Darding M, Draber P, Haas TL *et al* (2018) LUBAC prevents lethal dermatitis by inhibiting cell death induced by TNF, TRAIL and CD95L. *Nat Commun* 9: 3910

Thomas CJ, Schroder K (2013) Pattern recognition receptor function in neutrophils. *Trends Immunol* 34: 317 – 328

Tixeira R, Shi B, Parkes MAF, Hodge AL, Caruso S, Hulett MD, Baxter AA, Phan TK, Poon IKH (2018) Gasdermin E does not limit apoptotic cell disassembly by promoting early onset of secondary necrosis in jurkat T cells and THP-1 monocytes. *Front Immunol* 9: 2842

Vince JE, Wong WW, Gentle I, Lawlor KE, Allam R, O'Reilly L, Mason K, Gross O, Ma S, Guarda G *et al* (2012) Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* 36: 215 – 227

Vince JE, De Nardo D, Gao W, Vince AJ, Hall C, McArthur K, Simpson D, Vijayaraj S, Lindqvist LM, Bouillet P *et al* (2018) The mitochondrial apoptotic effectors BAX/BAK activate caspase-3 and -7 to trigger NLRP3 inflammasome and caspase-8 driven IL-1beta activation. *Cell Rep* 25: 2339 – 2353 e4

Wang Y, Gao W, Shi X, Ding J, Liu W, He H, Wang K, Shao F (2017) Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature* 547: 99 – 103

Wicki S, Gurzeler U, Wei-Lynn Wong W, Jost PJ, Bachmann D, Kaufmann T (2016) Loss of XIAP facilitates switch to TNFalpha-induced necroptosis in mouse neutrophils. *Cell Death Dis* 7: e2422

Wolf AJ, Reyes CN, Liang W, Becker C, Shimada K, Wheeler ML, Cho HC, Popescu NI, Coggeshall KM, Arditi M *et al* (2016) Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell* 166: 624 – 636

Yabal M, Muller N, Adler H, Knies N, Gross CJ, Damgaard RB, Kanegane H, Ringelhan M, Kaufmann T, Heikenwalder M *et al* (2014) XIAP restricts TNF- and RIP3-dependent cell death and inflammasome activation. *Cell Rep* 7: 1796 – 1808

Zanoni I, Tan Y, Di Gioia M, Broggi A, Ruan J, Shi J, Donado CA, Shao F, Wu H, Springstead JR *et al* (2016) An endogenous caspase-11 ligand

elicits interleukin-1 release from living dendritic cells. *Science* 352: 1232 – 1236

Zhang J, Webster JD, Dugger DL, Goncharov T, Roose-Girma M, Hung J, Kwon YC, Vucic D, Newton K, Dixit VM (2019) Ubiquitin ligases cIAP1 and cIAP2 limit cell death to prevent inflammation. *Cell Rep* 27: 2679 – 2689 e3

Zhou B, Zhang JY, Liu XS, Chen HZ, Ai YL, Cheng K, Sun RY, Zhou D, Han J, Wu Q (2018) Tom20 senses iron-activated ROS signaling to promote melanoma cell pyroptosis. *Cell Res* 28: 1171 – 1185

Zychlinsky A, Prevost MC, Sansonetti PJ (1992) Shigella flexneri induces apoptosis in infected macrophages. *Nature* 358: 167 – 169