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MULTI-AUTHOR REVIEW

The intersection of cell death and inflammasome activation

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Abstract Inflammasomes sense cellular danger to activate the cysteine-aspartic protease caspase-1, which processes precursor interleukin-1β (IL-1β) and IL-18 into their mature bioactive fragments. In addition, activated caspase-1 or the related inflammatory caspase, caspase-11, can cleave gasdermin D to induce a lytic cell death, termed pyroptosis. The intertwining of IL-1β activation and cell death is further highlighted by research showing that the extrinsic apoptotic caspase, caspase-8, may, like caspase-1, directly process IL-1β, activate the NLRP3 inflammasome itself, or bind to inflammasome complexes to induce apoptotic cell death. Similarly, RIPK3- and MLKL-dependent necroptotic signaling can activate the NLRP3 inflammasome to drive IL-1β inflammatory responses in vivo. Here, we review the mechanisms by which cell death signaling activates inflammasomes to initiate IL-1β-driven inflammation, and highlight the clinical relevance of these findings to heritable autoinflammatory diseases. We also discuss whether the act of cell death can be separated from IL-1β secretion and evaluate studies suggesting that several cell death regulatory proteins can directly interact with, and modulate the function of, inflammasome and IL-1β containing protein complexes.

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Introduction

Pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and inflammasome-forming NOD-like receptors (NLRs), generate an efficient innate-immune response that is essential for host resistance to microbial infections [1]. In addition, research has documented the pathological stimulation of PRRs in common diseases such as rheumatoid arthritis, atherosclerosis, type II diabetes, psoriasis, liver disease and brain injury, or monogenic hereditary diseases such as Familial Mediterranean Fever and Cryopyrin-Associated Periodic Syndromes (CAPS) [2]. In several of these conditions, cell death and inflammatory cytokine production are inextricably intertwined. This is underlined by studies showing that the mutation or loss of the cell death regulatory machinery in mice and humans can result in pronounced autoinflammatory disease (see below). The ordering of which process comes first, death or inflammation, is often complicated by the fact that many PRRs, including the TLR and inflammasome complexes responsible for inflammatory cytokine production, respond to cellular constituents released as a consequence of cell death, but when activated may also directly cause cell death. Nevertheless, using well-defined genetic mouse models, recent research has begun to identify the mechanisms by which cell death influences cytokine production, and vice versa, to impact pathogenic infections and inflammation-driven disease in vivo [3]. In this review, we examine the emerging data implicating numerous regulated cell death signaling

pathways in activating pro-inflammatory IL-1β to drive immune responses. These include pathways such as intrinsic (mitochondrial) and extrinsic (death receptor) apoptosis, typically viewed as immunologically silent, as well as the recently characterized programmed RIPK3 (Receptor Interacting Protein Kinase-3) and MLKL (Mixed Lineage Kinase domain-Like) dependent necrotic cell death pathway termed necroptosis.

Inflammasomes

The term inflammasome was coined by Fabio Martinon and Jürg Tschopp to describe the large multimeric protein complex required for caspase-1 processing and activation of the inflammatory cytokines interleukin-1β (IL-1β) and IL-18 [4]. Inflammasome protein complexes are frequently comprised of a sensor protein, the adaptor protein ASC (Apoptosis-associated Speck-like protein containing a CARD) and caspase-1 (Fig. 1). Inflammasome sensor proteins include Nod-like receptor (NLR) family members, such as NLRP1, NAIP/NLRC4, NLRP3 and NLRP6, AIM2-like receptors and the tripartite motif containing family member Pyrin [5]. In general, the N-terminal domain of inflammasome sensor proteins consists of either a CARD or a PYD domain. PYD domain inflammasome sensors (i.e., NLPR3, AIM2, Pyrin) interact with ASC via a homotypic PYD–PYD domain interaction. ASC thereby promotes caspase-1 recruitment and dimerization through CARD–CARD homotypic interactions. The binding of ASC to an inflammasome sensor protein results in its oligomerization into prion-like filaments nucleated by ASC phosphorylation and are characteristically observed as a single ASC "speck" per cell [6–8]. Alternatively, inflammasomes containing a CARD, such NLRP1 and NLRC4, can directly recruit and activate caspase-1 in the absence of ASC. Activating mutations in the inflammasome machinery, including Pyrin [9], NLRP1 [10], NLRP3 [11] and NLRC4 [12, 13], cause heritable auto-inflammatory disorders in mice and/or humans that do not involve the adaptive immune system and are treated by neutralizing IL-1 activity. On the other hand, genetic deletion of

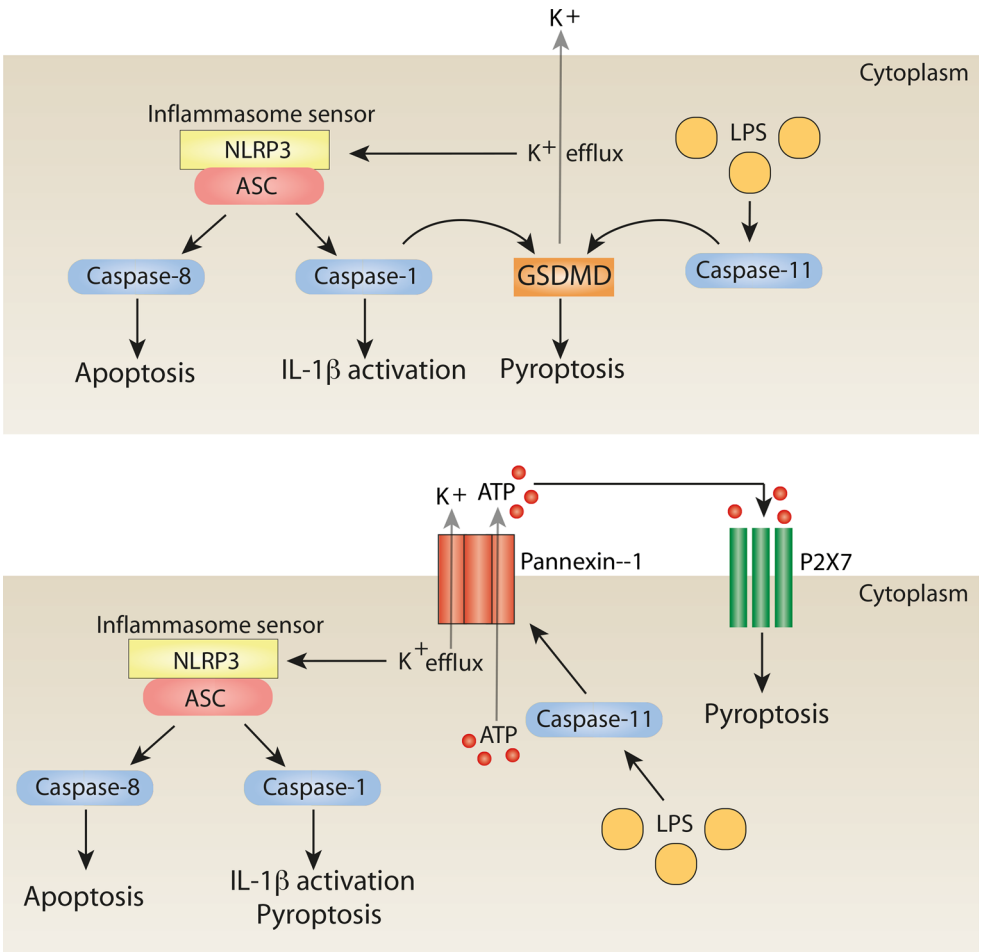

Fig. 1 Caspase-1 mediated pyroptosis. *Top panel*. Several reports indicate that pyroptosis is executed by Gasdermin D (GSDMD) which is processed and activated by inflammasome-associated caspase-1 (i.e. NLPR3, AIM2, NLRC4) or LPS-activated caspase-11. Caspase-11 activated GSDMD can also result in potassium efflux to indirectly activate NLRP3 and IL-1β processing and secretion. Inflammasome sensors (such as NLRP3 and AIM2) utilize the adaptor protein ASC that, other than recruiting and activating caspase-1, can also bind and activate caspase-8 to induce apoptotic cell death. *Bottom panel*. A recent study presented evidence suggesting that caspase-11-induced pyroptosis results from caspase-11 cleavage of Pannexin-1, triggering ATP release and P2X7-dependent cytolysis. Pannexin-1 cleavage also promotes potassium efflux to activate NLRP3 indirectly. See main text for references

inflammasome components and the IL-1 receptor (IL-1R) in mice has revealed the importance of inflammasome and IL-1 activity for immune responses and protection from a wide range of bacteria, viruses, protozoan parasites and fungi [2].

Inflammasome sensors survey the cellular cytosol for a wide range of pathogen molecules or metabolites (PAMPs; pathogen associated molecular patterns), environmental irritants (e.g. Asbestos, silica, UVB irradiation, nanoparticles) and host-derived danger molecules (e.g. cholesterol crystals, ATP, DNA) [5]. While several inflammasomes have relatively well-defined ligands or activators, such as DNA binding to AIM2, others, in particular NLRP3, appear to be activated in response to a wide variety of cellular stressors. In this regard, the mechanism of NLRP3 activation has attracted considerable attention and is not without controversy [14–16]. Models for NLRP3 activation include particulate-induced lysosomal rupture and cathepsin release into the cytosol [17], pore-forming toxin or ATP-mediated ionic flux [18], mitochondrial-derived reactive oxygen species [19], mitochondrial DNA [20, 21] or cardiolipin [20] release and binding to NLRP3, or finally, calcium influx and a decrease in cellular and NLRP3-associated cyclic AMP [23]. However, the most regularly reproduced and accepted trigger for NLRP3 activation is a requirement for potassium efflux [15], which may explain why diverse cellular and environmental molecules, and distinct cell death pathways, can all induce NLRP3 activation.

Despite the lack of mechanistic knowledge into signaling upstream of NLRP3, it is clear that NLRP3 responds to numerous cellular metabolites implicated in diseases where inflammation, and IL-1R signaling, have been reported to play a pathogenic role. These diseases include atherosclerosis (cholesterol crystals [24]), gout (uric acid crystals [25]), type 2 diabetes (islet amyloid polypeptide, ceramide and fatty acids [26–28]) and Alzheimer's (Amyloid-β [29, 30]). Several studies have successfully sought to identify NLRP3-specific inhibitors [31], although like NLRP3 activation itself, their mechanisms of action remain unknown.

The association of cell death and inflammasome activation

Inflammasomes can induce a form of cell death, called pyroptosis, and its mechanism and physiological function will be reviewed first. While other programmed cell death pathways are widely studied for their roles in development or cancer, it has become clear that they are also linked to inflammasome function, either to inhibit or promote. In the second part of the review we will highlight how distinct cell death pathways including extrinsic and intrinsic apoptosis, and necroptosis, may contribute to innate-immunity and IL-1β inflammatory responses (Fig. 2).

Pyroptosis

Initial studies documented how caspase-1 activation following bacterial sensing could trigger a lytic cell death [32–34] (Fig. 1). Cell swelling, plasma membrane pore formation (1–2.4 nm) and rapid cellular rupture are associated with caspase-1 killing, which can occur independently of apoptotic caspase activity [35]. This form of

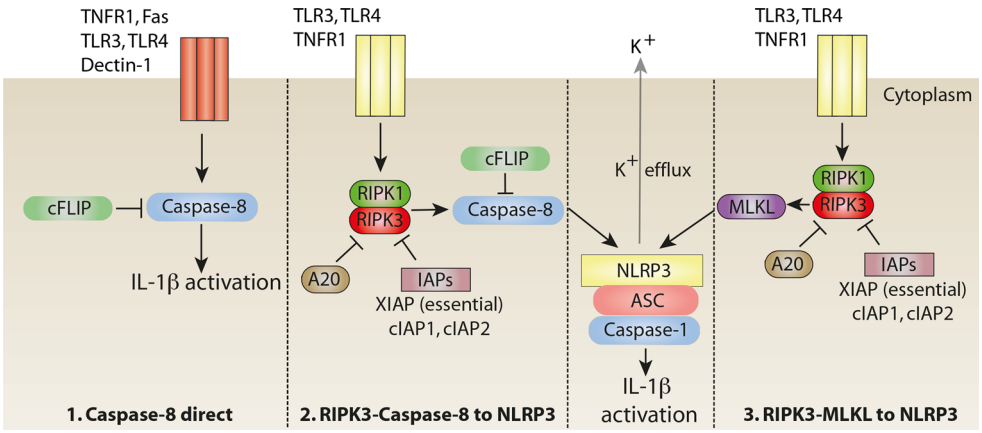

Fig. 2 Apoptotic and necroptotic signaling and their activation of IL-1β. **1**. Caspase-8 directly processes precursor IL-1β in response to death receptor, TLR or Dectin-1 signaling. **2**. Following IAP loss, and possibly A20, TLR- or TNF-induced RIPK3 promotes caspase-8 signaling that induces NLRP3 activation. Although experimental evidence has yet to be reported, this may occur via triggering potassium efflux at the onset of the cells demise. **3**. In the absence of caspase-8, TLR and TNF signaling triggers RIPK3 kinase activity and phosphorylation of its substrate MLKL. MLKL activates NLRP3 to induce IL-1β inflammatory responses. See main text for references

cell death, termed pyroptosis, is unlikely to exist only to facilitate IL-1β and IL-18 release. For example, pyroptosis can act to destroy a pathogen's replicative niche and expose it to immune attack independently of IL-1 and IL-18 signaling [36], is responsible for the death of CD4 T-cells in HIV infection [37], and can contribute to viral or chemotherapeutic-induced cytopenia and immune suppression [10]. Furthermore, although Cryopyrin Associated Periodic Syndromes (CAPS) caused by activating mutations in NLRP3 are treated successfully by neutralizing IL-1, murine models of CAPS have also documented how pyroptosis may contribute to pathology in the absence of IL-1R and IL-18 signaling [38]. This agrees with the idea that caspase-1 driven inflammation is not just a consequence of IL-1β or IL-18 maturation, as pyroptotic killing by caspase-1 also results in the release of pro-inflammatory Damage-Associated Molecular Patterns (DAMPs), such as HMGB1 and IL-1α [36]. Caspase-1 activity also promotes the non-conventional secretion of other leaderless proteins involved in inflammation and wound repair [39], and the generation of inflammatory signaling lipids, such as eicosanoids [40]. Whether caspase-1 killing versus cytokine maturation and secretion can be physiologically separated is an important and outstanding question in the field that is discussed in detail below.

Convergence of caspase-1 and caspase-11-induced pyroptosis

Unlike caspase-1 activation of IL-1β and IL-18, cell death appears to be the principal physiological function following activation of the related inflammatory caspase, murine caspase-11, or its human orthologues, caspase-4 and caspase-5. Cytosolic lipopolysaccharide (LPS) derived from Gram-negative bacteria reportedly binds directly to murine caspase-11 (and caspase-4 and -5) to trigger its oligomerization and activation [41] (Fig. 1). Consistent with this, caspase-11 is important for mounting innate immunity and host resistance against cytosolic gram-negative bacteria, such as *Burkholderia,* but not those that primarily reside in the vacuole, such as *Legionella* [42]. The N-terminal CARD domain of these inflammatory caspases is required for LPS binding [41] while, conversely, the hydrophobic hexa-acyl lipid A moiety of LPS is required for caspase-11 activation [43, 44]. Evidence suggests that caspase-11 killing promotes bacterial clearance through the destruction of a pathogen's immune-protected replicative niche and subsequent neutrophil-mediated clearance [42]. Although caspase-11 does not efficiently process IL-1β or IL-18, it can cause potassium efflux to indirectly activate NLRP3-caspase-1 and the processing of IL-1β [45, 46] (Fig. 1). However, it has been documented that this indirect canonical NLRP3 inflammasome activity is unimportant for caspase-11-mediated clearance of *Salmonella* genetically engineered to escape into the host cytosol [42]. Notably, caspase-11-deficient mice are resistant to LPS-induced endotoxic shock when compared to wild-type, NLRP3, ASC or caspase-1-deficient mice, implicating caspase-11-induced pyroptosis (rather than direct cytokine activation) in endotoxic shock pathogenesis [47].

Caspase-11-induced death resembles caspase-1 mediated pyroptosis, and both these caspases share the downstream substrate and pyroptotic effector molecule Gasdermin D [48–50]. Although caspase-11 mediated cell death is abrogated in Gasdermin D-deficient cells, or those harboring a caspase-11 cleavage site mutant, it remains unknown whether Gasdermin D is the terminal effector of pyroptosis (Fig. 1). Interestingly, however, the N-terminal domain of Gasdermin D released following caspase-11 cleavage is sufficient to cause pyroptosis, and other Gasdermins, such as Gasdermin A, may also induce cell death when C-terminal domain inhibition is relieved [49]. Unlike caspase-11 killing, canonical NLRP3-caspase-1-induced death in Gasdermin D-deficient cells, while retarded, is not completely blocked [48, 50]. This observation is likely to reflect the ability of NLRP3 to engage caspase-8 via ASC and induce apoptosis independently of caspase-1 (see below), making it likely that caspase-1 and caspase-11-induced Gasdermin D pyroptosis are, mechanistically, the same. In line with this idea, Gasdermin D loss also prevents caspase-1 killing resulting from other inflammasome sensor proteins, such as NLRC4 and AIM2.

Recently, a seemingly distinct caspase-11 killing pathway was proposed [51] (Fig. 1). In vitro and in vivo evidence using gene-deficient mice suggested that activated caspase-11 cleaves the cytosolic C-terminus of the cell surface pannexin-1 channel to cause ATP release and potassium efflux. Extracellular ATP activates the purinergic P2X7 receptor, which may form membrane pores to cause cytolysis and DAMP release. Meanwhile, pannexin-1 mediated potassium efflux nucleates NLRP3-caspase-1 activation independent of P2X7 activity (Fig. 1). Whether caspase-11 cleavage of Gasdermin D to induce cell death acts in this pathway, which is implied by the absence of pyroptosis in Gasdermin D-deficient macrophages, remains unclear. Studies to determine whether the active N-terminal Gasdermin D fragment can induce pyroptosis upon Pannexin-1 and P2X7 receptor deletion will be informative.

Is cell death required for IL-1β release?

IL-1β and IL-18 are classed as leaderless proteins, because they lack the sorting motif required for entry into the classical ER-Golgi secretory pathway, and are released

through a still poorly understood "unconventional" secretory pathway. Several mechanisms for active IL-1β release have been proposed, including via secretory lysosomes, multivesicular bodies (MVBs) and direct micro-vesicular shedding. Although significant caveats to all these models have been discussed [52], in some cell types genetic experiments have provided credence to the idea that IL-1β secretion can occur through an MVB-autophagic mediated process [53, 54]. However, other genetic data also exist demonstrating that IL-1β release is suppressed through basal autophagic pathways [20, 55, 56]. Therefore, MVB-autophagic-induced IL-1β release may not represent the mechanism of IL-1β secretion applicable to all cell types or conditions.

One attractively simple alternate hypothesis is that, because caspase-1 is required for both pyroptosis and IL-1β cleavage, IL-1β is released passively following plasma membrane rupture, alongside DAMPs such as HMGB1, and cellular cytosolic proteins such as Lactate Dehydrogenase (LDH). Recent studies using single-cell imaging technology on monocytic cell lines or macrophages have concluded that cell death is unavoidable following caspase-1 activation, and IL-1β is, therefore, only released from cells that also die [57–59]. Intravital imaging in vivo supports this idea, because inflammasome activation following viral infection was also observed to result in rapid macrophage death following ASC speck formation [60]. Similarly, NLRP3 activation in Gasdermin D-deficient macrophages does not appear to prevent caspase-1 processing of IL-1β into its mature form, but it does prevent IL-1β secretion [48, 49]. The simplest explanation for this observation is that caspase-1 cleaves IL-1β, but that Gasdermin D-induced cellular rupture is required for its release.

However, in contrast to the above evidence, other data support the hypothesis that IL-1β can be actively secreted independently of cellular lysis. Notably, two recent studies have reported that neutrophil NLRP3 and NLRC4 mediated IL-1β processing and release occur in the absence of detectable cell death [61, 62], which may act to sustain cytokine production and allow neutrophil-mediated clearance of pathogenic microbes. Similarly, the reconstitution of caspase-1 mediated IL-1β release in HEK293T cells can also occur with little or no cell death detected [54]. Whether Gasdermin D is robustly expressed and cleaved by caspase-1 in these cell types will be interesting to examine.

It has also been suggested that macrophage, dendritic cell (DC) and monocyte IL-1β release is an active process, even when cell death occurs. Several studies using populations of cells, rather than single cells, have reported that IL-1β is released into the supernatant prior to the detection of other intracellular proteins [63–65]. Similarly, while osmoprotectants such as glycine can prevent release of a number of intracellular proteins from cells undergoing pyroptosis, they do not block the secretion of mature IL-1β [45, 66, 67]. Perhaps more compellingly, it has been reported that caspase-11-induced macrophage pyroptosis is prevented upon deletion of P2X7, but this does not prevent NLRP3-dependent caspase-1 processing and IL-1β secretion into the supernatant [51]. Finally, significant levels of caspase-1-activated IL-1β are released from DCs or macrophages following activation of RIPK3 signaling, and this reportedly occurs in the absence of detectable cell death [68, 69]. Hence although caspase-1 killing and IL-1β often go hand-in-hand, there is a growing body of genetic and biochemical data that suggest these two events are separable, at least in some cell types.

Extrinsic apoptosis: capase-8-mediated activation of IL-1β

Extrinsic apoptosis of innate immune cells can occur following death receptor or TLR3 and TLR4 ligation and activation of the extrinsic apoptotic initiator caspase, caspase-8. While a principal function of receptors such as TNFR1 and TLRs is the transcriptional induction of inflammatory cytokines and chemokines, when pro-survival protein expression or activity is compromised cell death ensues. In general, extrinsic apoptosis is executed following the recruitment of death domain (DD) containing Receptor Interacting Protein Kinase 1 (RIPK1) and/or DD containing adaptor proteins, such TRADD and FADD, to activated TNFR1 and TLR3/4 complexes [70]. Subsequently, Death Effector Domain (DED) homotypic interactions between FADD and caspase-8 trigger proximity-induced caspase-8 autocatalytic processing and cleavage of the apoptotic effector caspases, caspase-3 and -7, to cause cell death. TNF or TLR-induced production of several anti-apoptotic proteins usually suppresses pro-apoptotic caspase-8 activity. These include cellular FLICE-inhibitory protein (cFLIP), which can dimerize with caspase-8 through its DED to prevent caspase-8 activation by FADD, and the E3 ubiquitin ligase inhibitor of apoptosis (IAP) proteins cellular IAP1 (cIAP1) and cIAP2, which ubiquitinate RIPK1 to promote NF-κB gene transcription and prevent RIPK1-mediated recruitment and activation of FADD and caspase-8 [71].

Direct proteolytic cleavage of IL-1β by caspase-8

Caspase-8 was first implicated in IL-1β activation in 2008 [72]. This work described how TLR3 or TLR4 stimulation under conditions of protein synthesis inhibition (which prevents pro-survival gene induction)

resulted in caspase-8 processing of inactive precursor IL-1β at the same site that is also cleaved by caspase-1. Although less efficient at cleaving recombinant IL-1β than caspase-1 [73], direct caspase-8 processing of IL-1β has now been reported under a variety of different conditions (Table 1). In all of these circumstances TLR or TNF stimulation provides a means for inducing inactive precursor IL-1β as well as formation of a caspase-8 activation platform. Other than protein synthesis inhibitors, cell stressors such as histone deacetylase inhibitors [74], ER stress-inducing agents [75] or chemotherapeutic compounds [73, 76] can all sensitize to TLR-induced caspase-8 cleavage of IL-1β. Although their mechanism of action has yet to be reported, it is possible that they all reduce the expression and/or activity of key anti-apoptotic proteins required to prevent TLR or TNF-induced death signaling. Consistent with this, it has been documented genetically how deletion of proteins required to suppress caspase-8 activity (IAP proteins or cFLIP) can promote caspase-8-mediated IL-1β processing and secretion following TLR or TNF stimulation [73, 77–79]. Similarly, TLR4 and death receptors such as Fas and DR3 can directly engage caspase-8 in myeloid cells to drive IL-1β or IL-18 maturation [80–82]. Dectin-1-mediated caspase-8 activity following bacterial or fungal infections may also contribute to IL-1β inflammatory responses [83–85]. It remains unclear if caspase-8 activation of the effector caspases, caspase-3 and -7, and subsequent apoptotic cell death, is required for IL-1β to be released, or whether its secretion can occur independently of effector caspase function.

Attempts to assess the importance of caspase-8 in cleaving IL-1β to drive inflammatory responses in vivo are complicated by the fact that caspase-8 deletion unleashes RIPK3 necroptotic signaling to cause embryonic lethality [86, 87], or upon tissue restricted deletion, induces transcriptional and/or necroptotic cell death-driven inflammation [3, 88, 89]. Further complicating matters, it has been shown that the loss of caspase-8 and RIPK3, although resulting in viable animals, can impair TLR-induced production of inactive precursor IL-1β [16, 90, 91]. Nevertheless, through the examination of IL-1β and IL-18 responses in caspase-1- (and caspase-11)-deficient mice, and comparing these to viable RIPK3 and caspase-8 double knockout mice, several studies suggest that caspase-8 cleavage of IL-1β and IL-18 is likely to be of physiological relevance. For example, early genetic studies documented FasL-dependent, caspase-1-independent, IL-18-induced liver injury in mice [81], and we now know that Fas-induced IL-1β and IL-18 processing is caspase-8 dependent [92]. Similarly, *Francisella* infection in vivo can drive AIM2-ASC-caspase-8 activation of IL-18 when caspase-1 is absent [93, 94]. Pathogenic serum-induced inflammatory arthritis in mice also occurs in a caspase-1-independent, but IL-1β-dependent manner, and disease resolution is significantly enhanced and correlates with reduced knee joint IL-1β levels in RIPK3 and caspase-8 double knockout (but not MLKL-deficient) animals [79]. Recent studies have also revealed that IL-1β driven osteomyelitis-like disease in proline-serine-threonine phosphatase interacting protein-2 (PSTPIP2) mutant mice requires both caspase-1 and caspase-8 deletion to prevent disease [95]. Notably, inflammatory disease in PSTPIP2 mutant mice is driven by the gut microbiota, adding to a weight of evidence now implicating the microflora in leukocyte pattern recognition receptor (PRR) and inflammasome signaling in prominent diseases such as metabolic syndrome, diabetes, colitis, respiratory tract infections and gout [96–102].

Caspase-8 activation of NLRP3-caspase-1

Several lines of evidence now support that, in addition to the direct cleavage of IL-1β, caspase-8 can act upstream to activate NLRP3 (Fig. 2; Table 1). For example, upon IAP loss, caspase-8 is essential for TLR-mediated, NLRP3-induced, caspase-1 processing [79]. Similarly, LPS stimulation combined with chemotherapeutic drugs (i.e. Doxorubicin) can also activate NLRP3, which is mediated by RIPK3 and caspase-8 signaling [73, 76, 103]. This is consistent with reports documenting how the RHIM (RIP homotypic interaction motif) of RIPK3 can recruit a RIPK1, FADD, caspase-8 activating complex to induce apoptosis when the kinase activity of RIPK3 is compromised [104, 105]. It has also been reported that catalytically inactivated caspase-8 (i.e. following pan-caspase inhibition) and poly(I:C) mediated TLR3 signaling allows caspase-8 to act as a scaffold to recruit and activate RIPK3 and subsequently trigger MLKL-dependent NLRP3 inflammasome activation [106]. However, it should be noted that the deletion of caspase-8 in other cell types, and even at the whole animal level, is sufficient to cause RIPK3-MLKL activity upon TLR or TNF stimulation, and therefore, a caspase-8 scaffolding role is not obligatory for RIPK3 activation under many conditions.

Inflammasome-induced caspase-8 activation and apoptosis

Oligomerization of the inflammasome adaptor protein ASC was linked to apoptotic cell death through the interaction of its PYD domain with caspase-8 several years ago [107, 108]. More recently, ASC-induced caspase-8 activation and apoptotic killing following AIM2 and NLRP3

Table 1 Cell death signaling-mediated IL-1β activation

Condition	Comments	References
Direct caspase-8 cleavage of IL-1β		
TLR3/4 stimulation with protein synthesis inhibition	Numerous human pathogens (such as *Legionella*) inhibit host cell protein synthesis	[72, 73]
TLR or TNF stimulation of IAP-deficient macrophages, or LPS stimulation of DCs	Triggers formation of a RIPK3-caspase-8-activating complex. XIAP mutation can trigger severe inflammatory disease, including markedly elevated IL-18, in humans	[73, 82]
Fas, Dectin-1 and IAP loss-induced IL-1 activation enhanced through hemizygotic deletion of the caspase-8 inhibitor cFLIP	cFLIP also proposed to directly modulate NLRP3 and AIM2 inflammasomes	[78]
TNFSF15 stimulation of DR3	TNFSF15 disease-risk polymorphism associated with increased cytokine activation	[80]
Fas activation	FasL-induced liver injury is dependent on IL-18	[81, 92]
Prolonged canonical NLRP3 stimulation (i.e. nigericin) or *Cryptococcus neoformans* internalization in caspase-1-deficient cells	NLRP3/ASC complexes activate caspase-8. May explain why some viral inhibitors such as CrmA target both caspase-1 and -8	[85, 111]
Mycobacteria-, Candida- and β-glucan-induced Dectin-1/CARD9 and complement receptor 3 signaling	Direct Dectin-1/caspase-8 cleavage of IL-1β dominates in human DCs, whereas both caspase-8 and NLRP3 participate in IL-1β processing in murine cells	[83, 84]
TLR4 stimulation combined with histone deacetylase inhibition	Possibly HDAC11 dependent. HDAC inhibitors enhanced intestinal IL-1β levels in a colitis model. Some caspase-1 activation also reported	[74]
TLR4 stimulation combined with chemotherapeutic drug treatment	Also results in caspase-1 activation. Correlates with reduced IAP levels	[73, 76]
TLR4 stimulation combined with ER stress inducing agents	Triggers caspase-8 and caspase-1 activation of IL-1β independent of the unfolded protein response	[75, 157]
Pstpip2 mutant mice	IL-1β-driven disease independent of NLRP3, but dependent on both caspase-1, caspase-8 and the microbiome	[95, 158, 159]
Pathogenic serum-induced arthritis	IL-1β-driven disease occurs independent of caspase-1, with disease resolution enhanced by caspase-8 and RIPK3 deletion	[79]
Francisella infection	AIM2-ASC-caspase-8-mediated IL-18 activation and subsequent IFNγ production in vivo	[93]
Caspase-8 activation of NLRP3 or caspase-1		
TLR or TNF stimulation of IAP-deleted or IAP-inhibited cells (XIAP loss essential), or following chemotherapeutic treatment	Caspase-8 activates NLRP3 signaling	[73, 76, 77, 79]
TLR priming and canonical NLRP3 activation (i.e. ATP or nigericin), or *Citrobacter* infection	Caspase-8 reported to process caspase-1 within canonical NLRP3 complexes. However, normal canonical NLRP3-caspasase-1 activation in caspase-8-deficient cells has been documented [69, 79, 106, 111, 160, 161]	[91]
Yersinia infection (mediated by *Yersinia* outer protein J)	RIP1-FADD-caspase-8 complex, and caspase-8 catalytic activity, required for caspase-1 activation independent of NLRP3, NLRC4 and, possibly, ASC	[160, 161]
Intraocular pressure-induced retinal ischemia	TLR4-caspase-8 activates NLRP3. Caspase-8 direct processing of IL-1β also likely to occur	[162]
Necroptosis (RIPK3-MLKL)-induced inflammasome activation		
TLR stimulation upon IAP loss (and caspase inhibition)	RIPK3-MLKL essential for NLRP3 activation	[79]
TLR stimulation of caspase-8-deficient DCs	RIPK3-MLKL-driven NLRP3 activation. Mice lacking caspase-8 in DCs are susceptible to endotoxin that is IL-1- and RIPK3-dependent	[69]
Poly(I:C) activation of TLR3 in the presence of the pancaspase inhibitor zVAD	Caspase-8 reported to act as a scaffold to recruit RIPK3 and MLKL resulting in NLRP3 activation	[106]

Table 1 continued

Condition	Comments	References
TLR stimulation of A20 (*TNFAIP3*)-deficient macrophages	RIPK3-dependent activation of NLRP3-caspase-1. Loss of RIPK3 prolongs survival of A20-deficient mice. MLKL KOs not tested. A20 prevents ubiquitination of RIPK3 and IL-1β-containing complexes [135]. Inflammatory markers in a *TNFAIP3* patient reduced following anakinra treatment [137]	[68]
Staphylococcus aureus toxins	MLKL inhibition prevented caspase-1 and IL-1β activation	[151]
LPS treatment of RIPK1-deficient macrophages	Caspase-1 and IL-1β activation is RIPK3-dependent and blocked by the NLRP3 inhibitor glyburide. MLKL dependency not tested	[163]

inflammasome activation was revealed through the use of caspase-1-deficient cells [94, 109]. Biochemical and structural studies have subsequently documented that the caspase-8 DED and ASC PYD domain interaction results in caspase-8 filament formation and promotes efficient caspase-8 activation [110].

Francisella tularensis infection of caspase-1-deficient cells drives caspase-8 binding to ASC following AIM2 activation, to trigger caspase-8 activation and apoptosis [94]. Similar results were obtained using DNA transfection to activate AIM2 or nigericin to activate the canonical NLRP3 inflammasome [109]. Notably, lower concentrations of inflammasome ligands (i.e. DNA for AIM2) revealed a shift from caspase-1-mediated pyroptosis to caspase-8-induced apoptosis in wildtype cells, which may better reflect physiological conditions, or the cells preferred mode of death (lytic vs. immunologically silent) in a manner determined by the concentration of the infectious agent. It is also likely that other inflammasomes that utilize ASC as an adaptor protein, such as NLRC4, are also capable of recruiting caspase-8 to induce apoptotic cell death in the absence of caspase-1 [107], although this has yet to be detected in wildtype cells [90].

The physiological implications of ASC-induced caspase-8 activation remain to be fully elucidated. Other than causing apoptotic cell death, it has been reported that NLRP3-ASC activation of caspase-8 may also result in caspase-1 processing [91], promote caspase-1 killing, or in the absence of caspase-1, activate IL-1β through caspase-8 cleavage [111]. However, several studies have also reported normal canonical NLRP3-induced caspase-1 activity in both *Casp8 Ripk3* double knockout, as well as *Casp8* single knockout, cells (see Table 1). Although caspase-8- (and RIPK3)-deficient mice are susceptible to bacterial infection with reduced serum IL-1β levels [91], this may reflect the defective transcriptional responses, including NLRP3 and IL-1β production, of these mice to TLR ligands [16, 90, 91], and/or a lack of direct processing of IL-1β by caspase-8. An alternate measure of the in vivo significance of ASC-driven caspase-8 activity is to compare caspase-1/11-deficient mice (still capable of ASC-caspase-8 activation) to ASC-deficient mice. In this regard, *Francisella tularensis* infection of these mice revealed that AIM2-ASC-activated caspase-8 can drive IL-18 activity to induce IFNγ independently of caspase-1 [93], highlighting that under some conditions, ASC-caspase-8 activation of IL-1β or IL-18 is likely to play a role in generating efficient anti-microbial responses.

Shared regulators of extrinsic apoptosis and inflammasome protein complexes

NLRP3 inflammasome activation typically requires two distinct stimuli. The first, termed inflammasome priming, induces the transcription of NLRP3 and precursor IL-1β and is normally provided by TLR stimulation. The second stimulus is then required to trigger NLRP3 activation, which results in caspase-1 and IL-1β processing. However, a transcription-independent event is also required for efficient TLR-mediated inflammasome priming [112]. TLR-induced reactive oxygen species [113], extracellular signal-regulated kinase 1 (ERK1) phosphorylation, proteasome activity [114] and NLRP3 de-ubiquitination [115] have all been suggested as post-translational inflammasome priming mechanisms. It is possible that TLR or TNF receptor signaling induces a protein complex that would allow for rapid post-translational inflammasome priming, which can take place within minutes. In support of this concept, the immunoprecipitation of endogenous LPS-induced IL-1β containing complexes in bone marrow-derived macrophages revealed interactions between pro-IL-1β, caspase-1, caspase-8, A20, RIPK1 and RIPK3, with A20-mediated deubiquitination of IL-1β required to limit its cleavage [68]. Although potential NLRP3 and ASC interactions and modifications were not examined in this study, a number of other publications have turned the spotlight onto the regulation of inflammasome complexes through modulators of TLR and extrinsic apoptosis signaling and are discussed below (Fig. 3).

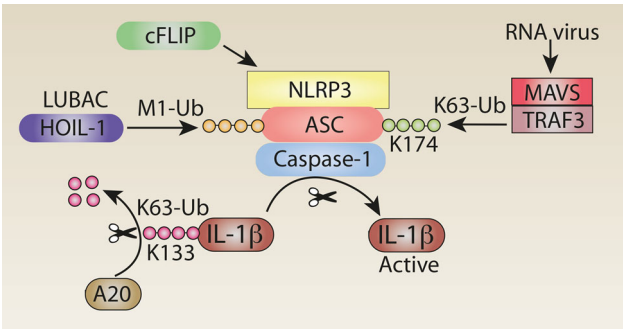

Fig. 3 Association of TLR and TNF receptor signaling components with inflammasome protein complexes. cFLIP reportedly interacts with NLRP3 (and AIM2) to promote inflammasome assembly, while HOIL-1, part of the LUBAC complex, decorates ASC with linear ubiquitin chains to induce ASC speck formation. Similarly, following RNA viral infection, MAVS-induced TRAF3 E3 ligase activity has been suggested to modify ASC with K63-linked ubiquitin chains to promote its aggregation. A20 removes K63-linked ubiquitin chains from K133 of IL-1β to limit its processing and activation in response to TLR signaling. See main text for references

IAPs

IAPs are a family of ubiquitin E3 ligases recruited into TNF receptor and TLR signaling complexes [116]. They target RIPK1 to promote NF-κB and prevent RIPK1 from associating with, and activating, caspase-8. In addition, they are required for the incorporation of the Linear Ubiquitin Chain Assembly Complex (LUBAC), comprised of HOIL-1, HOIP and SHARPIN, into TNF receptor signaling complexes [117, 118]. Intriguingly, while these functions have been ascribed to the cIAPs (cIAP1 and cIAP2) in various cell lines, in macrophages, XIAP plays a more important role [73, 77, 79]. For example, cIAP1/2 deletion, or inhibition, does not greatly sensitize macrophages to TNF or TLR-induced caspase-8 activation and apoptotic killing, nor RIPK3-MLKL mediated necroptosis, and these cell death pathways are only efficiently activated upon XIAP deletion [77, 79]. Similarly, the deletion or inhibition of all three of these IAPs dramatically sensitizes macrophages to IL-1β activation upon LPS stimulation alone, which in wildtype cells normally only suffices for inflammasome priming [73]. In this regard, XIAP deletion, but not cIAP1/2 loss, is essential for IL-1β activation, which can proceed via RIPK3-caspase-8 activation of NLRP3, direct caspase-8 processing of IL-β, or if caspase-8 is simultaneously inhibited, RIPK3-MLKL necroptotic-induced NLRP3 signaling (Fig. 2). Notably, however, the key XIAP substrate that inhibits TLR death signaling and inflammasome activation has yet to be identified. This is of significant interest because loss of function XIAP mutations in humans can cause colitis and X-linked lymphoproliferative disease (XLP2). XLP2 results in hemophagocytic lymphohistiocytosis (HLH) in 60–90 % of patients, and is characterized by the hyperactivation of macrophages and T-lymphocytes, and clinical features such as fever, hepatosplenomegaly and cytopenias [119]. Cytokine profiling of 10 XIAP-deficient patients revealed that the inflammasome-caspase-1 (or caspase-8) substrate, IL-18, was elevated in HLH episodes, and unlike other cytokines, did not return to normal levels following recovery. It was thus proposed that IL-18 is associated with HLH susceptibility [120], consistent with it being previously implicated in HLH disease pathogenesis [121]. Along similar lines, a XIAP mutant patient with auto-inflammatory features resembling those caused by activating mutations in the NLRP3 inflammasome was also recently identified [122].

cFLIP

cFLIP forms heterodimers with caspase-8 to inhibit TLR or death receptor caspase-8 apoptotic killing. Consistent with this, hemizygotic deletion of cFLIP in macrophages enhanced LPS-induced, caspase-8 dependent, IL-1β activation in response to IAP antagonism, or following Dectin-1 stimulation [78]. Surprisingly, however, it was also reported that the long isoform of cFLIP could interact with caspase-1, NLRP3 and AIM2 (but not ASC) and its expression was required for full inflammasome activity in both 293T inflammasome reconstitution assays, and following hemizygotic deletion in macrophages. The partial loss of cFLIP did not appear to influence cell TLR-mediated death responses, NF-κB signaling, or TNF production [78]. Because the majority of studies indicate that caspase-8 does not influence canonical NLRP3-caspase-1 activation directly (see Table 1), this implies cFLIP is a direct regulator of inflammasome functioning (Fig. 3). How it might do so remains an outstanding question.

LUBAC

As discussed above, LUBAC, comprised of HOIL-1, HOIP and SHARPIN, is required for efficient TNF and TLR-induced NF-κB activation, via linear ubiquitination of RIPK1 and NEMO. In humans, the destabilization of LUBAC resulting from HOIP or HOIL-1 mutation triggers systemic auto-inflammation and immunodeficiency [123, 124]. Consistent with LUBAC mutant mice, human fibroblasts lacking LUBAC activity also displayed defective NF-κB, and cytokine production, in response to TNF and IL-1β stimulation. However, human LUBAC-deficient monocyte IL-1β responses, in terms of cytokine induction, were exaggerated. Why this is the case remains unclear, although TNF-induced cell death has been implicated as a

driver of inflammatory disease in LUBAC-defective mice [125, 126]. Consistent with this, anti-TNF therapy also reduced clinical inflammation in a HOIL-1-deficient patient [123], although to our knowledge cell death *per se* has yet to be investigated in LUBAC mutant patient pathology.

SHARPIN deficiency in mice results in severe inflammatory disease, including chronic proliferative dermatitis (*cpdm*), with increased epidermal inflammatory cytokine levels, including IL-1 family members and TNF. Accordingly, skin lesion development is delayed, or abrogated, when *Sharpin*$^{cpdm/cpdm}$ mutant mice are crossed onto IL-1R or TNF signaling-deficient backgrounds, respectively [117, 125–127]. Dermatitis appears to result from DAMP-induced inflammatory responses following unrestricted TNF-induced apoptotic cell death of keratinocytes, because (1) whole animal or epidermal restricted removal of TNF, TNFR1 [117, 125, 126] or the death adaptor protein TRADD [126] prevented dermatitis development, (2) epidermal loss of FADD (on a *Ripk3*$^{-/-}$ background) or heterozygosity of caspase-8 prevented *cpdm* dermatitis [125, 126], (3) bone marrow reconstitution of wildtype mice with SHARPIN-deficient bone marrow failed to recapitulate the *Sharpin*$^{cpdm/cpdm}$ mutant phenotype, indicating a skin-intrinsic defect [125] and iv) keratinocytes derived from SHARPIN null mice are sensitive to TNF-induced apoptotic killing [117, 125, 126, 128]. One result that potentially speaks against this interpretation is that loss of RIPK1 kinase activity, which is normally associated with necroptosis, not apoptosis, also prevented the *Sharpin*$^{cpdm/cpdm}$ dermatitis [129]. However, it is fair to say that the roles of the kinase activity of RIPK1 are still being uncovered and given the other evidence; the simplest interpretation is that, in keratinocytes at least, RIPK1 kinase activity contributes to TNF-induced apoptosis.

It is also possible, although not tested, that TNF-drives intrinsic IL-1 responses through activation of NLRP3-caspase-1 (and caspase-8), in keratinocytes through enhanced cell death signaling. In support of this hypothesis, the loss of NLRP3 or caspase-1 and -11 also delayed dermatitis development in *Sharpin*$^{cpdm/cpdm}$ mice [130], although whether inflammasome signals primarily emanate from myeloid-derived cells such as macrophages, or keratinocytes, remains undetermined. Regardless, TNF-induced RIPK1 killing appears to be the apical driver of dermatitis in Sharpin mutant mice, because either TNF/TNFR1 ablation or the loss of RIPK1 kinase activity confers complete skin protection to at least 27-35 weeks post-birth, whereas caspase-1 and -11 deficiency only delays the onset of dermatitis.

As might be expected from its role in NF-κB activation [131], SHARPIN mutant macrophages also have a defect in TLR-mediated precursor IL-1β production [132, 133]. However, a recent study also proposed that HOIL-1 participates in the linear ubiquitination of ASC and is required for AIM2- and NLRP3-dependent ASC "speck" formation and caspase-1 activation [134] (Fig. 3). In contrast, AIM2 and NLRC4 activation was not affected by loss of SHARPIN, and defects in NLPR3 activity are likely to reflect a loss of inflammasome priming capacity [132]. The reason for these discrepancies in LUBAC-mediated modulation of inflammasome function remain unclear.

A20

The deubiquitinase A20 (encoded by *Tnfaip3*) is induced following TNF receptor or TLR ligation and acts to dampen NF-κB signaling via the enzymatic removal of ubiquitin chains from TLR and TNF receptor signaling components, such as RIPK1. Similar to IAP knock-out macrophages, TLR stimulation alone of A20-deficient cells was sufficient to activate IL-1β and induce its secretion in a RIPK3 and NLRP3-caspase-1 dependent manner [68]. Furthermore, like IAP null cells, LPS-induced IL-1β secretion in A20-deficient macrophages was not completely prevented following caspase-1 loss, and these cells were predisposed to caspase-8 processing, indicating a possible contribution of caspase-8 to IL-1β activation. Whether caspase-8 is also required for NLRP3 activation upon A20 deletion, akin to the mechanism of NLRP3 activation upon IAP loss, remains unknown.

Mechanistically it was proposed that A20 cleavage of K63-linked chains from K133 of IL-1β limits IL-1β processing and thus secretion (Fig. 3). However, A20 must also act upstream of IL-1β, because otherwise LPS stimulation following A20 deletion would not result in spontaneous RIPK3-driven NLRP3 activation. Consistent with this, the same laboratory recently reported that A20 deubiquitination of RIPK3 on K5 was essential for limiting RIPK1-RIPK3 complex formation, RIPK3 signaling and necroptotic cell death [135].

Several in vivo observations highlight the physiological significance of A20 in restricting RIPK3-driven inflammasome activity and necroptotic death. First, the premature death of A20-deficient mice is partly rescued through the co-deletion of RIPK3 [135]. Second, the cell-type restricted deletion of A20 in myeloid cells drives inflammatory arthritis, and this is dependent on NLRP3, caspase-1 and IL-1R signaling, but independent of TNFR1 [136]. Whether arthritis severity also depends on RIPK3, caspase-8 or MLKL signaling has not been reported. Third, inactivating A20 germline mutations in humans can trigger early onset auto-inflammatory disease with exaggerated NF-κB responses and elevated serum pro-inflammatory cytokines [137]. Like the loss of A20 in mice, patient cells also

responded to LPS with spontaneous NLRP3-caspase-1 and IL-1β activation, and in the one patient tested, the inhibition of IL-1 with anakinra normalized markers of systemic inflammation. Whether necroptotic cell death signaling may also impact inflammatory or autoimmune disease observed in A20-deficient humans has not been determined.

Intrinsic apoptosis: can it trigger IL-1β activation?

Apoptotic cell death has been divided into the extrinsic (death receptor) or intrinsic (mitochondrial) pathways, which both converge on activation of the apoptotic effector caspases, caspase-3 and -7. While apoptotic cell death has always been assumed to be non-inflammatory, recent reports have refined our understanding of this area. For example, in the absence of the effectors of the intrinsic apoptosis pathway: apaf1, caspase-9 and caspase-3, in the hematopoietic compartment, a systemic inflammation occurs [138, 139]. This inflammation is driven by type I interferons (IFNs) that are upregulated in a mitochondrial DNA (mtDNA)-cGAS/STING-dependent manner. Thus, one way that apoptosis inhibits inflammation is by preventing the detection of mitochondrial products, and particularly mtDNA, in the cytosol of signaling competent cells. In addition to dismantling the cell to prevent these intrinsic inflammatory responses, apoptosis also generates find-me and eat-me signals that promote efficient apoptotic body clear up and removal [140]. However, in situations where apoptosis is very high, it seems that this is insufficient to limit the onset of inflammation, as observed following keratinocyte apoptosis-triggered skin inflammation following Sharpin, TRAF2 or cFLIP loss [125, 126, 141, 142]. Thus, where there are high levels of apoptosis in a tissue it is possible that DAMPs are released which trigger inflammatory responses.

Notably, studies have linked intrinsic apoptosis, mitochondrial molecules, or mitochondrial reactive oxygen species (ROS) generation to NLRP3 inflammasome activation, although these connections are not without controversy [14]. For example, the viral nucleic acid sensing signaling adaptor MAVS (Mitochondrial Antiviral Signaling protein) may promote NLRP3 activation through potassium efflux and/or TRAF3-mediated ubiquitination of ASC [143, 144] (Fig. 3). However, whether MAVS is a direct regulator of canonical NLRP3 signaling is a matter of debate [16, 144, 145]. Similarly, the mediator of mitochondrial fission, mitofusin 2, has been reported to associate with NLRP3 and be required for optimal IL-1β secretion following viral infection [146]. The mitochondrial phosphatase PGAM5 was also recently suggested to be essential for efficient canonical and VSV (vesicular stomatitis virus)-induced NLRP3 activation in macrophages (but not in DCs) and was tentatively linked to maintaining mitochondrial homeostasis and sustained mitochondrial ROS production [147].

Cellular stresses, such as cycloheximide, staurosporine and doxorubicin, which have the potential to trigger intrinsic apoptosis, can also activate IL-1β. However, studies now suggest that when combined with a TLR inflammasome-priming signal, such as LPS, several of these agents may activate IL-1β via caspase-8 complexes, possibly through IAP or cFLIP degradation (see above and Table 1). More controversially, it has been proposed that mitochondrial apoptotic signaling is required for canonical NLRP3 activation [21]. It was reported that canonical NLRP3 activators such as ATP induce mitochondrial apoptosis, resulting in the release of (oxidized) mtDNA into the cytosol where it binds and activates NLPR3 [21]. However, this proposed model has several difficulties. First, as discussed above, apoptosis specifically prevents inflammatory mtDNA responses. Second, transfected mtDNA primarily activates the DNA-sensing AIM2 inflammasome, not NLRP3 [20, 21]. Third, canonical NLRP3 stimuli such as ATP and nigericin trigger NLRP3 to activate IL-1β within 5–10 min; however, most intrinsic apoptosis stimuli take significantly longer time to induce cytochrome c release from mitochondria, which is more consistent with mitochondrial damage occurring downsteam of NLRP3-caspase-1 activation [148]. Fourth, and most critically, the deletion of the essential mitochondrial apoptotic effector proteins Bax and Bak, or cyclophilin D (required for mitochondrial permeability transition pore opening) does not influence canonical NLRP3 activity [16]. Hence, while it remains possible that perturbations to mitochondrial homeostasis activate NLRP3 indirectly, mitochondrial apoptosis does not represent a unifying mechanism for how NLRP3 is triggered by diverse stimuli. It, therefore, remains of outstanding interest as to whether direct Bax/Bak activation can trigger IL-1β responses, or whether, akin to mtDNA responses, intrinsic apoptosis acts to shut down inflammasome-associated inflammatory signaling.

Necroptosis-driven IL-1β activation

Necroptotic cell death is associated with the release of DAMPs and inflammation. Proteins containing a RHIM, such as the TLR3 and TLR4 adaptor protein TRIF, the TNFR1/TLR signaling partner RIPK1 and the DNA receptor DAI (DNA-dependent activator of IFN-regulatory

factors), signal necroptosis by triggering homotypic interactions with the RHIM of RIPK3, resulting in RIPK3 oligomerization and activation of its kinase activity [149, 150]. Subsequently, RIPK3 phosphorylates MLKL, promoting the insertion of MLKL into the plasma membrane to induce cellular rupture. Caspase-8 activity prevents necroptotic signaling, possibly through cleavage-mediated inactivation of RIPK1, RIPK3 or CYLD (Cylindromatosis), and caspase-8 deletion suffices to activate RIPK3 and MLKL in most cell types following TNFR1 or TLR stimulation.

Several lines of evidence suggest that RIPK3-MLKL signaling also activates the NLRP3 inflammasome in a cell-intrinsic manner. TLR4-TRIF-induced RIPK3 and MLKL-dependent NLRP3 activation has been reported upon conditions of caspase-8 inhibition and IAP loss [79], or following TLR3-TRIF stimulation [106]. Notably, the in vivo deletion of *Casp8* in DCs sensitized mice to endotoxic shock, and this was prevented by blocking IL-1, but not TNF, signaling, and was also abrogated upon RIPK3 co-deletion [69]. Remarkably, despite the robust RIPK3-MLKL-driven NLRP3 response in caspase-8-deficient DCs, cell death following LPS stimulation was not detected. Regardless of whether these cells eventually die, the in vitro and in vivo findings strongly suggest that the primary pathological driver of disease in LPS-treated mice with DC-restricted caspase-8 loss is NLRP3-induced IL-1β activation, not DAMP release.

Recent findings also suggest that *Staphylococcus aureus* toxins such as Hla can trigger RIPK3-MLKL activation, resulting in caspase-1 and IL-1β activation, in addition to necroptosis [151]. The inhibition of necroptosis improved bacterial clearance by preventing pulmonary macrophage death, and also limited increases in pro-inflammatory cytokine levels, including IL-1β, in bronchoalveolar lavage fluid. Of note, pathological IL-1 activity and necroptotic signaling is often associated with the same disease models, such as multiple sclerosis and atherosclerosis [152–155]. It will, therefore, be informative to determine if MLKL-driven inflammasome responses might contribute to disease pathogenesis in conditions that necroptosis is implicated in.

It was recently proposed that poly(I:C) transfection or RNA viruses, such as vesicular stomatitis virus (VSV), can trigger RIPK3 to activate NLRP3 independently of MLKL. Unlike MLKL-independent RIPK3-caspase-8 activation of NLRP3 following IAP deletion, it was suggested that RIPK1-RIPK3 signaling activated the GTPase DRP1 to inflict mitochondrial damage, which resulted in NLRP3-caspase-1 inflammasome formation [156]. However, the results in this study have not been recapitulated by other laboratories using macrophages derived from gene targeted mice. For example, both caspase-1 and IL-1β activation following VSV infection of either RIPK1 and DRP1 knockout macrophages was not altered when compared to wildtype cells, and RIPK3 deficiency had either no impact, or only a minor impact, at best [106]. Similarly, poly(I:C) transfection also resulted in comparable inflammasome activation in RIPK1, RIPK3 and DRP1 gene-targeted cells. These findings were reproduced in a separate study, which documented that VSV-induced IL-1β secretion was not perturbed in either RIPK3 knockout macrophages or kinase inactive mutant RIPK1- and RIPK3-derived cells [147].

Conclusions

The programmed cell death pathways, pyroptosis, apoptosis and necroptosis can be genetically and biochemically separated. However, recent findings have demonstrated significant cross-talk between these pathways, such as caspase-8 suppression of necroptosis, and RIPK3 or ASC induction of apoptosis. In addition, there is mounting evidence that the apoptotic and necroptotic cell death machinery can both directly and indirectly modulate inflammasome and IL-1β activation in response to infection or cellular stresses, such as A20 modification of RIPK3- and IL-1β-containing protein complexes, or caspase-8 proteolysis of IL-1β itself. The act of cell death combined with a localized inflammatory IL-1β or IL-18 burst is likely to be a powerful stimulus to alert the host immune system to intracellular infections, or damaged tissues requiring repair. However, whether IL-1β activation and secretion mediated by RIPK3-MLKL, caspase-8, or caspase-1 signaling requires the death of the cell and membrane rupture, or can occur independently of death, remains unclear, but may vary depending on the cell type and stimulus. This idea is highlighted by research suggesting how the cell type and signal strength can dictate whether apoptosis is either pro-inflammatory or remains immunologically silent, or whether inflammasome activation results in a lytic pyroptotic cell death, causes apoptosis or induces no cell death. It will be of significant interest to investigate under what conditions necroptotic- and apoptotic-mediated IL-1β activity is either pathologic or required for optimal human health.

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