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REVIEW

Caspase-8: not so silently deadly

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Apoptosis is a caspase-dependent programmed form of cell death, which is commonly believed to be an immunologically silent process, required for mammalian development and maintenance of cellular homoeostasis. In contrast, lytic forms of cell death, such as RIPK3- and MLKL-driven necroptosis, and caspase-1/11-dependent pyroptosis, are postulated to be inflammatory via the release of damage associated molecular patterns (DAMPs). Recently, the function of apoptotic caspase-8 has been extended to the negative regulation of necroptosis, the cleavage of inflammatory interleukin-1β (IL-1β) to its mature bioactive form, either directly or via the NLRP3 inflammasome, and the regulation of cytokine transcriptional responses. In view of these recent advances, human autoinflammatory diseases that are caused by mutations in cell death regulatory machinery are now associated with inappropriate inflammasome activation. In this review, we discuss the emerging crosstalk between cell death and innate immune cell inflammatory signalling, particularly focusing on novel non-apoptotic functions of caspase-8. We also highlight the growing number of autoinflammatory diseases that are associated with enhanced inflammasome function.
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INTRODUCTION

Apoptosis is a genetically encoded process essential for the removal of superfluous or damaged cells. Apoptotic cell death can promote phagocytic clearance of infected cells to limit pathogenic infections,[1,2] and is required to delete lymphocytes to prevent autoimmune disease.[3] Intrinsic 'mitochondria-dependent' apoptosis is triggered by cellular stressors (for example, growth factor withdrawal) and is tightly regulated by the pro- and anti-apoptotic members of the BCL-2 protein family (reviewed in Delbridge *et al.*[4]). In contrast, the extrinsic 'death receptor-mediated' apoptotic pathway is induced by ligand binding, which via a series of events can activate the death receptor complex or ripoptosome that activates caspase-8 (Figure 1). Both pathways merge following initiator caspase activation to trigger the activation of effector caspases, caspase-3 and caspase-7, resulting in ordered cellular breakdown.

Death receptor ligation not only induces caspase-8-mediated apoptosis but also results in transcription factor activation (Figure 1). Pro-inflammatory cytokines, such as tumour necrosis factor (TNF), are potent activators of NF-κB, regulating the transcription of a variety of inflammatory genes, including TNF itself. In fact, enhanced TNF production and TNF receptor 1 (TNFR1) signalling is associated with macrophage accumulation and inflammatory cytokine production in common autoimmune pathologies, such as Crohn's disease, rheumatoid arthritis and psoriasis. Although biologics targeting TNF have proven relatively efficacious in the treatment of these common diseases, understanding the pathways that regulate TNFR1 signalling is key to identifying next-generation therapies. This is particularly true in view of the crosstalk, and shared signalling components, of cell death and pattern recognition receptor (PRR)-mediated innate immune signalling pathways. Similar to the death receptors, Toll-like receptors (TLRs) detect pathogen molecules and host-derived damage-associated molecular patterns (DAMPs) to induce the transcription of death ligands, including TNF and directly trigger caspase-8-dependent apoptosis and caspase-independent necroptotic cell death. TLR signalling may therefore contribute to the pathogenic effects of TNF in autoimmune diseases, such as arthritis.

In this review, we give an overview of innate immune and cell death signalling pathways, particularly focusing on pathways that signal to caspase-8. We outline non-apoptotic functions for caspase-8, including its ability to repress necroptosis, regulate cytokine transcription, and interact with inflammasomes to direct their signalling. Importantly we describe emerging evidence suggesting that in specific circumstances caspase-8 activity determines how pro-inflammatory interleukin-1β (IL-1β) processing and activation occurs. Finally, we highlight the increasing number of autoinflammatory diseases linked to mutations in cell death machinery, which can lead to excessive inflammasome activation.

DEATH RECEPTOR SIGNALLING

The TNF superfamily comprises a number of type I plasma membrane proteins that feature a common cysteine rich extracellular-ligand binding domain, a membrane-spanning region and a C-terminal intracellular tail. Notably, only a subset of these receptors also harbour a death domain (DD) that can directly induce apoptosis. Of these, TNFR1 (p55), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1/DR4) and 2 (TRAIL-R2/DR5) and Fas (CD95/APO-1) are the most widely studied in response to their respective ligands TNF/lymphotoxin-α, TRAIL and Fas ligand (FasL; Figure 1).

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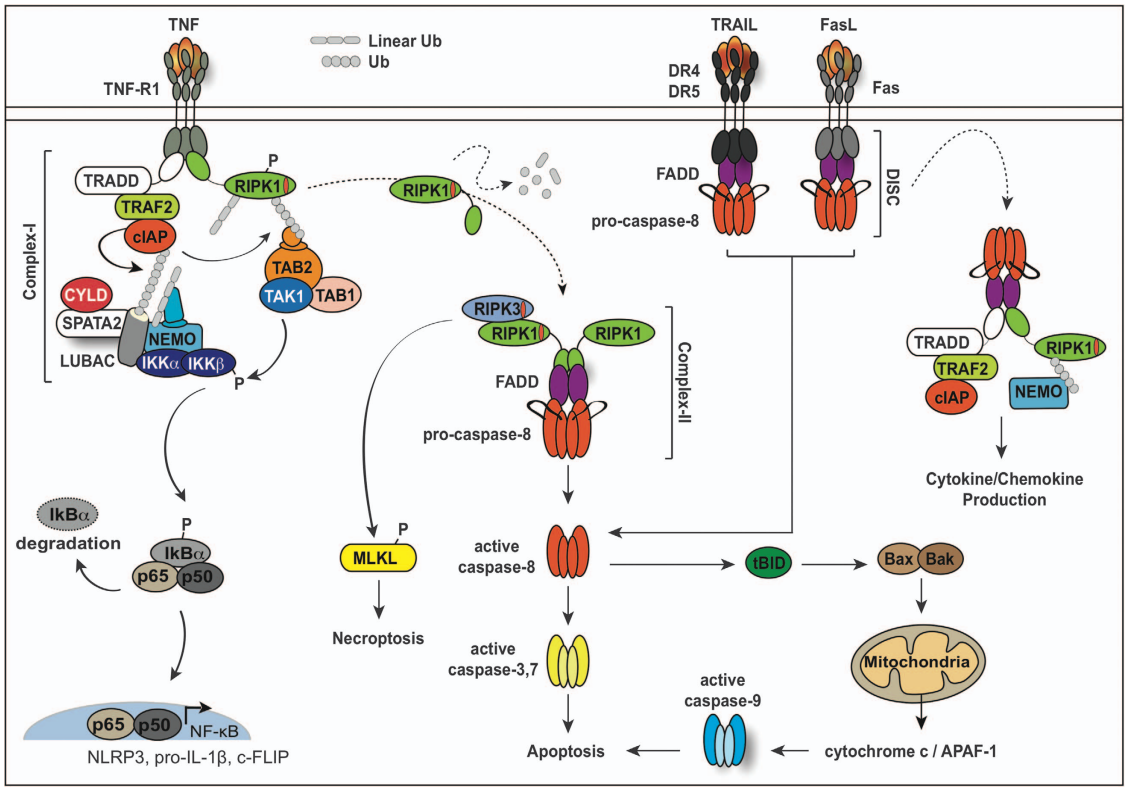

Figure 1 Death receptor signalling pathways. Schematic depicting the activation of the death receptor signalling pathways upon ligation of TNF-TNFR1, FasL-Fas and TRAIL-DR4/DR5. TNF interacts with TNF-R1 and induces timerisation of the receptor. This stimulates recruitment of the adaptor proteins TRADD and RIPK1. TRADD interacts with TRAF2 allowing the recruitment of the cIAP proteins and subsequently LUBAC to the receptor complex. The cIAPs and LUBAC ubiquitylate RIPK1, which stimulates the recruitment and activation of the downstream signalling effectors, culminating in the activation of NF-κB. These ubiquitylation events are important to limit the association of RIPK1 with caspase-8. Similarly, the IKK complex (comprising the kinases IKKα, IKKβ and regulatory subunit NEMO) stimulates the phosphorylation of RIPK1 preventing the association of RIPK1 with caspase-8.[121] Dysregulation of either the ubiquitylation or phosphorylation of RIPK1 causes the dismantling of complex-I and stimulates the formation and activation of complex-II, which has the ability to drive apoptosis or necroptosis (when caspase activity is inhibited). TRAIL or FasL bind their cognate receptors which induces receptor trimerisation and formation of the death inducing signalling complex (DISC), comprising FADD and caspase-8. Caspase-8 activation can induce death via direct cleavage of downstream caspases, caspase-3/7 in type I cells, or in type II cells caspase-8 can cleave Bcl-2 family member BID to its truncated form that can activate intrinsic apoptosis. Through a less defined mechanism a secondary complex, the ripoptosome, can also form comprising signalling proteins, such as RIPK1, RIPK3 and the cIAPs, which stimulates the upregulation of cytokines and chemokines.

TNFR1 signalling, ripoptosome formation and activation of caspase-8

TNF is expressed predominantly by activated macrophages and T lymphocytes, as a 26 kDa protein on the plasma membrane. Cleavage of transmembrane TNF by the metalloproteinase, TACE (TNF-α-converting enzyme), results in the release of a 17 kDa soluble form.[5] Despite many studies focusing specifically on the soluble form of TNF, both membrane bound (mTNF) and soluble TNF (sTNF) are biologically active and accumulating evidence suggests that each form of TNF have distinct roles in inflammatory responses. For example, anti-human TNF monoclonal antibodies (infliximab and adalimumab) and soluble human TNFRII (etanercept) are highly efficacious in neutralising sTNF to treat rheumatoid arthritis. In contrast, anti-TNF biologicals, particularly etanercept fail in Crohn's disease possibly due to ineffective targeting of mTNF that drives localised pathology.[5,6]

Binding of TNF to TNFR1 results in the formation of a pro-survival TNF receptor signalling complex (TNFRSC; also known as complex-I), comprising the core proteins, TNFR1-associated death domain protein (TRADD), TNF receptor-associated factor 2 (TRAF2), Receptor-Interacting Protein Kinase 1 (RIPK1), cellular inhibitor of apoptosis proteins (cIAP1/cIAP2) and the linear ubiquitin chain assembly complex (LUBAC, a hetero-trimeric complex comprising SHARPIN, HOIL-1 and HOIP) (Figure 1). Conformational changes in TNFR1 facilitate the intracellular recruitment of TRADD via common death domains (DD), which allows interactions between TRADD, TNFR1 and RIPK1.[7–10] TRAF2 associates directly with TRADD via a TRAF binding domain and acts as an adaptor for the recruitment of the E3 ubiquitin ligases, cIAP1 and cIAP2, via a cIAP1/2-interacting motif (CIM).[11] The cIAPs are arguably the most important E3 ubiquitin ligases recruited to the TNFRSC, where they function to recruit LUBAC (Figure 1). Subsequently, the cIAPs and LUBAC ubiquitylate NEMO and RIPK1 to promote cell survival through TAK1- and IKK-dependent transcriptional activation of canonical NF-κB, resulting in cytokine (for example, TNF and IL-6) and pro-survival protein (for example, c-FLIP and cIAPs) induction.[12]

The activation of NF-κB only constitutes one arm of the TNFRSC. When TNFR1 induced pro-survival responses are compromised (loss of the IAPs or inhibition of TAK1), complex-I dissociates from the receptor, and RIPK1 together with TRADD associate with the adaptor protein FADD (Fas-associated protein with death domain) and pro-caspase-8 to form complex-II (Figure 1).[7] This complex has the ability to drive apoptosis through the dimerisation and auto-activation of caspase-8, resulting in cleavage and activation of downstream effector caspases, caspase-3 and caspase-7. Formation of this secondary

complex is tightly regulated by the inducible caspase-8 inhibitor c-FLIP (cellular FLICE-inhibitor protein), which dictates the activity of caspase-8 and determines if apoptosis ensues.[13] If caspase-8 activity is compromised, TNF stimulation under appropriate circumstances, such as IAP depletion, triggers necroptosis (Figure 1). TNF-induced necroptosis requires the kinase activity of RIPK1 and RIPK3, and the RIPK3 substrate, the pseudokinase Mixed Lineage Kinase domain-Like protein (MLKL). The phosphorylation of MLKL by RIPK3 activates MLKL, and is thought to result in a conformational change that leads to the exposure of the N-terminal four-helix bundle (4HB) killing domain.[14–16] In its oligomerised form, MLKL migrates to plasma membranes, where it is purported to cause death via pore formation,[17] thus leading to inflammatory DAMP release (for example, IL-1α and HMGB1).

In contrast to the cIAPs, X-linked IAP (XIAP) has not yet been detected in the TNFRSC, but is widely reported to bind and directly inhibit apoptotic caspases, caspase-3, -7 and -9. Recently, however, XIAP has also been shown to critically regulate both cell death and innate immune responses following TNF and TLR ligation (discussed later in this review). Despite this fact, no major perturbation of TNF-induced canonical NF-κB, p38 or JNK signalling pathways has been reported in XIAP-deficient dendritic cells.[18] Nevertheless, inhibition of both the cIAPs and XIAP with compounds that mimic the natural inhibitor of IAPs, Smac/DIABLO, termed 'Smac mimetics', or the genetic loss of all three IAPs, leads to the formation of a complex akin to complex-II, termed the 'ripoptosome', comprising RIPK1, RIPK3, FADD, caspase-8 and cFLIP.[19,20] This complex forms without the need for receptor ligation and functions to drive caspase-8-mediated apoptosis or caspase-independent necroptosis.

TRAIL- and FAS-mediated activation of caspase-8

Distinct from TNFR1, FAS and TRAIL-RI and -RII signalling culminates from the formation of a death inducing signalling complex (DISC) at the receptor cytoplasmic tail (Figure 1). This DISC complex is remarkably similar to the TNFR complex-II, and the ripoptosome, in that it forms from a DD interaction between the receptor and FADD, which is followed by death effector domain (DED)-mediated recruitment, oligomerisation and activation of caspase-8 or -10. FasL and TRAIL receptors are considered classic examples of 'death receptors', as their primary function is to drive caspase activation and apoptosis, unlike TNFR1 whose primary role is to activate NF-κB to drive inflammatory gene transcription. However, recently it has been appreciated that FasL and TRAIL receptor engagement can lead to pro-inflammatory cytokine and chemokine expression.[21–23] Not surprisingly, this inflammatory programme is somewhat dependent on RIPK1, but more critically dependent on caspase-8.[21] A scaffolding function for caspase-8 seems likely, as inhibiting caspase activity does not suppress Fas-mediated cytokine/chemokine expression or function.[21] Similar to complex-II and the ripoptosome, cFLIP is also recruited to the DISC to determine the cellular fate. Although $cFLIP_S$ acts as a direct inhibitor of caspase-8, $cFLIP_L$ is incorporated into the DISC thereby preventing pro-caspase-8 interdomain processing but facilitating non-apoptotic caspase-8 cleavage of a limited number of substrates around the DISC.[24,25]

Like TNFR1, FAS and TRAIL-R signalling events are important for regulating pathogen clearance and immune responses, as well as for driving inflammatory disease via effects on cell viability and/or pro-inflammatory cytokine and chemokine induction.[26] The critical role of Fas/FasL signalling in disease is highlighted by the autoimmune lymphoproliferative syndrome (ALPS) that occurs in patients harbouring Fas/FasL mutations, and C57BL/6.Fas^{lpr} and C57BL/6.$FasL^{gld}$ mice, respectively.[3]

CROSSTALK BETWEEN TLR AND TNFR1 SIGNALLING PATHWAYS: NON-APOPTOTIC ACTIVITIES FOR CASPASE-8

PRRs, including TLRs and inflammasome-forming NOD-like receptors (NLRs), act as the major sensors for invading pathogens and, like TNFR1, have an essential role in coordinating the innate immune response to clear microbial infections. Recent work has revealed significant crosstalk between TNFR1 and TLR signalling pathways, where RIPK1 and RIPK3 interact with TIR-domain-containing adaptor-inducing interferon-β (TRIF) via common RIP Homotypic Interacting Motif domains (RHIM) to induce cell death signalling.[27,28] Work has also highlighted that caspase-8 is not simply the initiator caspase for cell death but is a key player in regulating inflammatory responses.

Caspase-8-mediated repression of necroptosis

The best recognised non-apoptotic functions for FADD and caspase-8 is the repression of necroptotic signalling, where inhibition of caspase-8 by pathogen/mammalian inhibitors (for example, CrmA, vICA and cFLIPs),[29] chemical inhibition (for example, ZVAD-fmk),[28,30] or genetic loss,[31] promotes necroptosis upon death receptor or TLR signalling. The seminal studies performed by the laboratories of Mocarski and Green, and more recently Strasser, highlighted this fact when they rescued the embryonic lethality of caspase-8 knockout mice (embryonic day 10.5) by co-deletion of the necroptotic regulator *Ripk3* or *Mlkl* itself (Table 1).[32–34] $Ripk3^{-/-}Caspase\text{-}8^{-/-}$ and $Mlkl^{-/-}Caspase\text{-}8^{-/-}$ mice are born viable and healthy, although similar to mice lacking Fas/FasL they succumb to SLE-like lymphoproliferative disease.[32–35] Not surprisingly, mice deficient in the caspase-8 adaptor FADD,[36,37] or c-FLIP (*Cflar* gene)[38,39] are also embryonic lethal. However, only $Fadd^{-/-}$ mice are rescued by blockade of necroptotic activity via deletion of *Ripk3* or *Mlkl*,[34,39,40] suggesting that in the absence of c-FLIP, FADD-caspase-8 homodimers induce lethal apoptotic signalling. Consistent with this, loss of *Fadd* in the $Cflar^{-/-}Ripk3^{-/-}$ mouse restores viability.[39] Interestingly, early embryonic lethality of $Fadd^{-/-}$ and/or $caspase\text{-}8^{-/-}$ mice is driven by TNFR1 signalling, as RIPK1 or TNFR1 deficiency but not TRIF loss prolongs survival, although mice still exhibit perinatal lethality akin to $Ripk1^{-/-}$ mice (Table 1).[28,41–43] The lethality in *Ripk1*-deficient mice is partially rescued by loss of MyD88, TRIF and/or TNFR1.[41,43] More importantly, loss of RIPK3 or MLKL also delays death, demonstrating a repressive role of RIPK1 on inflammatory necroptotic signalling.[41,43] Intriguingly, complete rescue of $Ripk1^{-/-}$ mice was only achieved by elimination of both necroptotic (that is, RIPK3) and apoptotic (that is, caspase-8 or FADD) machinery.[41–43]

Caspase-8 has also been shown to repress necroptosis in a cell and tissue-specific manner. In gut epithelial cells, caspase-8 maintains the gut integrity to microbial challenge by preventing lethal necroptotic cell death and epithelial cell shedding.[44] Likewise, caspase-8 expression in the skin prevents inflammatory necroptotic cell death.[45] In the haematopoietic system caspase-8 apoptotic activity is widely thought to restrict lymphocyte accumulation, where ALPS develops in caspase-8-deficient or Fas-deficient patients, as well as in T cell-specific caspase-8 knockout mice, Fas-, FasL- or membrane FasL-deficient mice.[3,46] However, unexpectedly, NF-κB1 signalling has recently been shown to drive the lymphoproliferation in mice deficient in Fas.[47] Necroptosis has been suggested to contribute to the contraction of T cell responses, as cell death in caspase-8 deficient T cells upon T cell

Table 1 Rescue of inflammatory disease phenotypes in mice lacking ripoptosome machinery

Mouse Model	*Ripk3*$^{-/-}$	*Ripk1*$^{-/-}$	*Ripk1*$^{D138N/D138N}$	*Mlkl*$^{-/-}$	*Casp8*$^{-/-}$	*FADD*$^{-/-}$	*Tnfr1*$^{-/-}$	*MyD88*$^{-/-}$	*Trif* $^{-/-}$	*Reference*
Caspase-8 deficiency (*Casp8*$^{-/-}$) Embryonic lethality E10.5	Viable	E19-P0	—	Viable	NA	—	E17	—	No	28,32, 33,43,119
Caspase-8 deficiency Intestinal epithelium (*Casp8*$^{Villin/ER.cre}$) Ileitis Adult onset	Viable[e]	—	Viable	—	NA	—	—	—	—	122–124
Caspase-8 deficiency epidermis (*Casp8*$^{K5/ER.Cre}$) Atopic dermatitis P0–P7	—[e]	—	—	—	NA	—	P9+[f]	No	No	45,123
Ripk1 deficiency (*Ripk1*$^{-/-}$) Perinatal lethality E19–P0; P2–P5[a]	P3-P4[b+] P2-P21[a,b+,c+]	NA NA	NA NA	P3–P4 —	E19–P0[b] P2–P16[a,b]	— —[a,b]	— P2–P18[a,c,d+]	P3–P4 —	— P2-P7[a,d]	41–43
Ripk1 deficiency intestinal epithelium (*Ripk1*$^{Villin.Cre}$) Lethal intestinal disease P1–P30	No[b+]	NA	NA	—	P60+[f,g]	P50+[b,f,g]	P30–P80+[f]	P30-P60+[f]	No[g]	124,125
Ripk1 deficiency epidermis (*Ripk1*$^{K14.Cre}$) Atopic dermatitis P8–P21+	Viable[b]	NA	NA	Viable	—	P28+[b+]	P28+	—	P28+	125
RIPK3 kinase deficiency (*Ripk3*$^{D161N/D161N}$) Embryonic lethality E11.5	NA	E19-P0	No	No	Viable	—	No	—	No	126
c-FLIP-deficient (*cflar*$^{-/-}$) Embryonic lethality E10.5	E10.5-E11.5[b+]	—	—	—	—	—[b]	E14–E17[b]	—	—	39,41
c-FLIP deficiency Intestinal epithelium (*cflar*$^{Villin.Cre}$) Fatal intestinal disease P1	No	—	—	—	—	—	P1–P140+ days[f]	—	—	127
FADD-deficient (*FADD*$^{-/-}$) Embryonic lethality E10.5	Viable	E19-P0	—	Viable	—	NA	E14–E17	—	—	39,40,128
FADD deficiency Intestinal Epithelium (*FADD*$^{Villin.Cre}$) Intestinal disease P1–P21(50%)	Viable[b]	No[b+,g]	No	—	—	NA	—	—	—	129
FADD deficiency epidermis (*FADD*$^{K14.Cre}$) Lethal skin lesions P4–P8	Viable[b]	P21+[b+,g]	P21+	—	—	NA	P14–P35	P8–P21	—	111,125
A20 deficiency (*A20*$^{-/-}$) Fatal multi-organ disease P1–P250	P1-P400+	—	P1-300+	No	—	—	—		—	119
cIAP double deficiency (*cIAP1*$^{-/-}$*cIAP2*$^{-/-}$) Embryonic lethality E10	E14.5	E12.5	—	—	—	—	P0–P2	—	—	130

Rescue of lethal/inflammation mouse model to E, ~embryonic day; No, no effect, —, not done; NA, not applicable; P, ~post-natal day; viable, no lethality or health restored in viable animals.
[a]*Ripk1*$^{-/-}$129 derived.
[b]Co-deletion of genes viable.
[c,d]Enhanced survival with co-deletion.
[e]Protection shown in adult tissues.
[f]Delayed or altered disease progression.
[g]Tissue-specific deletion.

receptor ligation is prevented by RIPK1 kinase inhibition or RIPK3 co-deletion.[48] However, recent reports in MLKL and caspase-8 deficient mice show subtle differences in a discrete subset of inflammatory genes, suggesting closer examination of non-necroptotic activities of RIPK3 and RIPK1 is warranted.[34] Caspase-8 loss in mature myeloid cells (that is, dendritic cells, macrophages and neutrophils) also does not lead to spontaneous or TLR-induced necroptotic cell death,[49] however, reduced levels of IAPs renders cells permissive to necroptosis.[50]

How caspase-8 prevents necroptotic signalling is not fully understood, although the catalytic activity of caspase-8, rather than its autocatalytic processing, appears to be essential to block necroptosis.[32] Caspase-8-mediated cleavage of RIPK1 and RIPK3 is widely believed to limit necroptotic signalling, where necroptosis activation requires oligomerisation of full length RIPK1 and RIPK3 via RHIM-RHIM interactions to form an amyloid-like fibril structure.[51] Indeed, over-expression of mutant RIPK1 and RIPK3 RHIMs blocked necroptosis, however, these RHIM mutations may also inhibit upstream RIPK1/3 kinase activity. Nevertheless, increased phosphorylated MLKL activity is associated with reduced caspase-8 levels and amyloid-like RIPK1 and RIPK3 deposits in the cortical lesions found in the brains of multiple sclerosis patients.[52]

The levels of the different cFLIP isoforms, and the interaction of these isoforms with caspase-8 at the DISC, have also been suggested to dictate ripoptosome stability, and thus influence apoptotic and necroptotic death signalling. For instance, low $cFLIP_L$ levels trigger caspase-8 oligomerisation and activation to induce apoptosis and inhibit necroptosis, whilst high levels block apoptotic activity. In the case of $cFLIP_s$, caspase-8-c-$FLIP_s$ complexes inhibit apoptosis and induce necroptosis.[53] Finally, caspase-8 also cleaves the deubiquitylase CYLD (cylindromatosis), which is required to deubiquitylate and activate RIPK1-mediated necroptosis in response to TNF,[54] thus potentially providing an alternative or additional mechanism for silencing necroptosis. Recently, TLR4-TRIF-caspase-8 signalling was also shown to cleave CYLD to limit autocrine TNF-driven, type 1 IFN-dependent necroptosis in macrophages.[55] Intriguingly, this work contrasts a similar study that suggested type 1 IFN induces necroptosis in the absence of TNFR1/2.[56]

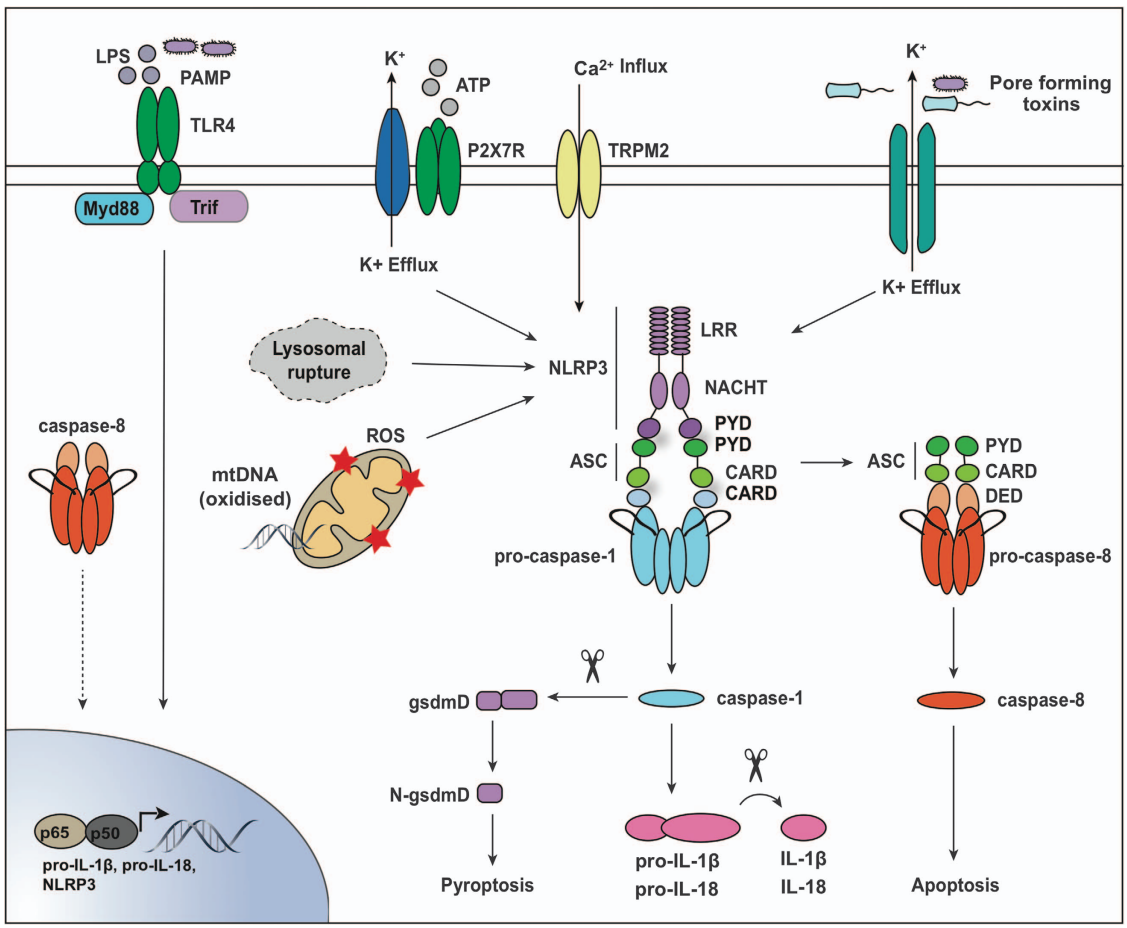

Figure 2 Models for canonical inflammasome activation. Activation of the canonical NLRP3 inflammasome requires a priming step to induce transcription of inflammasome machinery, namely pro-IL-1β and NLRP3. This priming step can be initiated via caspase-8, although this is not well characterised. Upon sensing a range of DAMPS, PAMPS or environmental irritants, NLRP3 oligomerises via its NACHT domain, subsequently the N-terminal pyrin domain (PYD) facilitates homotypic interactions with the PYD domain of ASC. ASC oligomerisation into prion-like fibril structures, visualised as discrete specks, can interact with caspase-1 via common CARD domains to facilitate caspase-1 proximity-associated activation. Active caspase-1 cleaves pro-IL-1β and pro-IL-18 to their mature bioactive forms, and induces gasdermin D-mediated pyroptosis. The exact mechanism for NLRP3 activation remains contentious, but potassium (K^+) efflux is widely believed to be the common trigger. Other potential triggers include calcium (Ca^{2+}) influx, lysosomal rupture, ROS production and mitochondrial cell death (e.g., oxidised DNA, ROS, cardiolipin). Of note, under certain circumstances ASC can interact via its PYD domain with the DED of caspase-8 leading to its oligomerisation and activation to induce apoptosis and cleave IL-1β.

Transcriptional role for caspase-8 in cytokine production

In response to TNFR and TLR ligation cytokine transcription can be activated in a RIPK1 and RIPK3 dependent manner.[57–59] Subsequent studies have sought to discern if caspase-8 also regulates transcription of inflammatory genes. Cuda *et al.* recently documented that deletion of caspase-8 in dendritic cells (*Caspase-8*^{CD11cCre}) is associated with heightened inflammatory cytokine and chemokine levels and autoimmune SLE-like disease, which is driven by RIPK1 and MyD88, but not RIPK3.[60] Myeloid-specific loss of caspase-8 (*Caspase-8*^{LysMCre}) also led to mild autoimmunity, albeit with a less prominent inflammatory cytokine/chemokine profile that could be rescued by RIPK3 co-deletion.[61] In line with *in vivo* findings, caspase-8 deficient macrophages exhibited altered transcriptional responses to TLR ligation *in vitro*, where enhanced TLR4-induced TNF and IL-6 production was reduced by either necrostatin-1 or RIPK3 loss.[61] Unexpectedly, *in vivo* cytokine responses to LPS were blunted in *Caspase-8*^{LysMCre} mice,[61] akin to defective cytokine production reported in *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$, *Mlkl*$^{-/-}$*Fadd*$^{-/-}$ and *Ripk3*$^{-/-}$*Fadd*$^{-/-}$ mice, and macrophages exposed to TLR3/4 stimuli or Gram-negative bacteria (for example, *Citrobacter rodentium*).[40,58,62] Results therefore suggest that caspase-8 is required for optimal TLR-induced cytokine transcriptional responses. However, the fact there are conflicting reports as to whether defective canonical NF-κB activation is responsible for reduced cytokine production in *Ripk3*$^{-/-}$*Fadd*$^{-/-}$, *Mlkl*$^{-/-}$*Fadd*$^{-/-}$ and *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$ macrophages to TLR ligation,[40,58,62,63] suggests that further studies are warranted. Globally, results point to an important role for caspase-8 levels in dictating TLR-induced transcriptional responses in a cell-by-cell manner.

CASPASE-8 REGULATION OF THE INFLAMMASOME AND IL-1B ACTIVATION

Inflammasome activation

Inflammasomes are large multimeric protein complexes that typically comprise a NOD-like receptor (NLR), the adaptor ASC (apoptosis-associated speck-like proteins containing a CARD, encoded by the *PYCARD* gene) and caspase-1 (Figure 2). The primary function of inflammasomes is to activate caspase-1 to cleave precursor IL-1β and IL-18 into their mature bioactive forms. However, a physiological role for the lytic form of cell death, pyroptosis, which ensues following inflammasome activation has also been described. To date there are at least 6 NLR proteins suggested to form inflammasomes, NLRP1, NLRP3, NAIP/NLRC4 (IPAF), NLRP6, NLRP7, NLRP12, as well as non-NLR inflammasomes, the HIN 200 family members AIM (Absent in melanoma)-like receptors, AIM2 and IFI-16 (Interferon-gamma inducible protein-16), and the tripartite motif-containing family member Pyrin.[64] In addition to these canonical inflammasomes, caspase-11 (human orthologues caspase-4 and caspase-5) has recently been revealed to be the cytosolic receptor for intracellular LPS derived from Gram-negative bacteria (for example, *Burkholderia spp.* and *Eschericia coli.*).[65–67] Termed the noncanonical inflammasome, LPS derived from Gram-negative bacteria induces caspase-11 activation. Caspase-11, like caspase-1, cleaves gasdermin D to cause pore formation and pyroptotic cell death. However, caspase-11 also activates the NLRP3 inflammasome indirectly, reportedly as a result of gasdermin D-mediated potassium (K^+) efflux.[67,68]

Canonical inflammasome activation is generally thought of as a two-step model. TLR/TNFR ligation provides the first signal, termed inflammasome priming, which is required for the transcriptional upregulation of inflammasome machinery (for example, NLRP3) and pro-IL-1β. The second signal, the trigger, such as a PAMP (Pathogen associated molecular pattern) or DAMP, is then required to induce inflammasome assembly and activation in the cytosol (Figure 2). A number of inflammasomes have well-defined pathogen triggers that implicate them in innate immune responses to infection. NLRP1 senses lethal toxin from *Bacillus anthracis*, AIM2 senses cytosolic DNA, and pyrin is triggered by Rho GTPase inactivating bacterial toxins/effectors, such as *Clostridium difficile* toxin A/B.[69,70] In contrast, the most widely studied inflammasome, NLRP3, is triggered by a diverse range of PAMPs (for example, *Streptococcus pneumoniae* and *Staphylococcus aureus*), host-derived DAMPs (for example, monosodium urate crystals, islet amyloid polypeptide and ATP), and environmental irritants (for example, silica and alum).[70] These diverse triggers implicate the NLRP3 inflammasome in the pathogenesis of a variety of inflammatory and autoimmune diseases, including type 1 diabetes, gout, and silicosis.[70] Yet the importance of tight regulation of the NLRP3 inflammasome and IL-1β is best reflected by the potentially lethal autoinflammatory diseases, termed cryopyrin associated periodic syndromes (CAPS), which occur in humans and mice harbouring various activating mutations in *NLRP3*.[71]

Based on the diverse stimuli that trigger the NLRP3 inflammasome one might predict a unifying activation mechanism exists. Gabriel Nunez's laboratory proposed K^+ efflux as the universal trigger for NLRP3.[72] However, recent reports suggest that K^+ efflux is not required for NLRP3 inflammasome activation triggered by peptidoglycan-N-acetylglucosamine-induced hexokinase release from the mitochondrial outer membrane,[73] or for caspase-8-mediated activation of the NLRP3 inflammasome in human monocytes.[74] Other common models for NLRP3 activation include phagolysosomal rupture and cathepsin release, ion channel flux and calcium influx, and reduced cyclic AMP. Activation of the mitochondrial cell death pathway is also postulated to trigger canonical NLRP3, via mitochondrial reactive oxygen species (ROS), oxidised mitochondrial DNA release, or direct NLRP3 binding by the mitochondrial membrane lipid cardiolipin.[75,76] However, recent studies have genetically disputed the role of the mitochondria and associated proteins in canonical NLRP3 activation.[58,72,75] Hence, the elusive common NLRP3 activating mechanism remains of outstanding interest.

Caspase-8-mediated IL-1β activation

Interest in caspase-8 as an inflammatory caspase stemmed from a study showing that TLR3 or TLR4 ligation and protein synthesis inhibition resulted in caspase-8 dependent activation and cleavage of precursor IL-1β at the same site as caspase-1.[77,78] Subsequently, a range of stimuli have been shown to activate caspase-8 to cleave IL-1β (and IL-18) in TLR or TNF primed cells (Figure 3a), where TLR4 ligation alone can trigger spontaneous caspase-8-induced IL-1β activation in bone marrow derived dendritic cells (BMDCs).[79] Other stimuli include natural death receptors Fas[80] and DR3,[81] bacterial and fungal c-type lectin receptor, dectin-1,[82] as well as chemicals that induce ER stress (for example, tunicamycin)[83] or chemotherapeutic compounds, such as doxorubicin, staurosporine[84] and histone deacetylase inhibitors (Figure 3a).[85] Fas-mediated cleavage of IL-1β, draws parallels to the TLR-mediated pathway in dendritic cells, as it too is dependent on FADD and caspase-8 and occurs independent of the NLRP3 inflammasome, however, it does not require RIPK3.[80] Interestingly, while Dectin-1 signalling can activate caspase-8 to cleave IL-1β (Figure 3a),[82] other studies suggest that dectin receptors also utilise the canonical NLRP3 inflammasome.[86,87] How chemical stressors trigger apoptosis, caspase-8 and thus IL-1β activation is unclear, but is likely to involve changes in expression of cell death inhibitory components.[84] In this regard, recent work shows that in LPS- or TNF- primed murine macrophages and dendritic cells, genetic

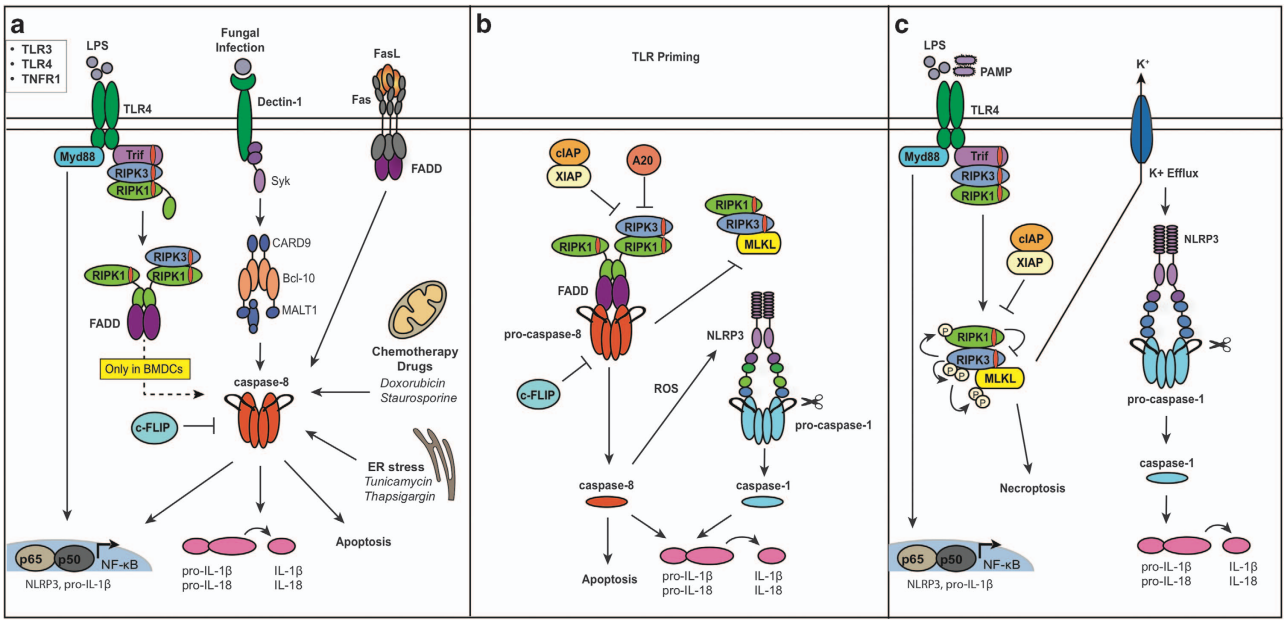

Figure 3 Novel roles for caspase-8 in IL-1β regulation. Following exposure to TLR/TNFR stimuli to induce transcription of pro-IL-1β (and NLRP3) caspase-8 levels can regulate IL-1β activity. (**a**) Caspase-8 can be activated by a wide variety of stimuli to directly cleave IL-1β, including TLR4 or Fas death receptor ligation, fungal protein binding of dectin-1 that induces a CARD9-Bcl-10-MALT1 complex to trigger activation, as well as apoptosis-inducing stimuli. Of note, reports illustrating that TLR4 stimulation can directly stimulate caspase-8-mediated cleavage of IL-1β are restricted to bone marrow derived dendritic cells (BMDCs). (**b**) When IAPs are inhibited/genetically removed (or A20 is absent), the ripoptosome forms and caspase-8 can either directly cleave IL-1 and/or trigger the NLRP3 inflammasome, whereby caspase-1 cleaves IL-1β. In this scenario caspase-8 causes apoptosis and limits necroptosis, possibly by cleavage of RIPK1 and RIPK3. (**c**) When caspase-8 levels are low/absent and IAPs are inhibited/genetically removed, the necrosome is formed and MLKL is activated through RIPK3 kinase activity to trigger necroptosis and NLRP3 inflammasome, possibly via K^+ efflux.

or chemical loss of all three IAP proteins, and pivotally XIAP, can promote caspase-8-mediated IL-1β activation (Figure 3b).[18,50,78] Furthermore, loss of c-FLIP enhances caspase-8 activation of IL-1β secretion upon treatment with Smac mimetic, FasL or heat-killed *Candida albicans* (Figures 3a and b).[88]

Caspase-8 and the activation of the NLRP3 inflammasome

A number of recent studies have also suggested that caspase-8 can activate NLRP3 (Figure 3b). For example, upon chemical or genetic loss of IAP activity in LPS-primed murine macrophages and dendritic cells, TLR-TRIF-RIPK1-RIPK3-caspase-8 signalling has been shown to not only directly cleave IL-1β, but to also induce NLRP3 inflammasome activation,[18,50,78] independent of the kinase activity of RIPK1 and RIPK3.[50] In contrast, Gurung *et al.* suggested that FADD-RIPK3-caspase-8 associates with the NLRP3 inflammasome to promote canonical (for example, ATP and nigericin) and non-canonical caspase-11 (for example, *C. rodentium*) inflammasome activation.[62] However, as other groups have reported relatively normal canonical NLRP3 inflammasome activation (for example, ASC oligomerisation and Caspase-1 cleavage) in *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$ (and *Caspase-8*$^{-/-}$) myeloid cells upon sufficient priming signals, this finding warrants re-examination.[49,50,89] A further model proposed in response to *Yersinia* infection is that a RIPK1-FADD-caspase-8 complex can directly cleave caspase-1 in the absence of NLRP3 and NLRC4.[63,90] Despite these variations, we recently established an upstream position for caspase-8 in ripoptosome-mediated, but not canonical, NLRP3 inflammasome activation.[50] In this study, to avoid issues in defective priming in *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$ macrophages, NLRP3 was triggered in unprimed macrophages (which express low levels of NLRP3) and caspase-1 cleavage assessed as an activation measurement. Upon Smac mimetic-induced inhibition of IAPs, caspase-1 activity was blocked in *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$ and *Nlrp3*$^{-/-}$ macrophages. In contrast, in response to canonical NLRP3 stimulus, nigericin, caspase-1 cleavage was only blocked in the absence of NLRP3 demonstrating that caspase-8 is not required for canonical inflammasome activation.[50]

In bone marrow derived dendritic cells a TLR-TRIF-RIPK3 platform not only activates caspase-8 to directly cleave IL-1β, but also triggers NLRP3 inflammasome activation.[79] Intriguingly this IL-1β activation occurs largely in the absence of cell death, and independent of the kinase activities of RIPK1 and RIPK3. However, unexpectedly, a RIPK3 kinase inhibitor, which blocks necroptotic activity and amplifies caspase-8 activity,[91] actually heightened IL-1β activation.[79] An alternative TLR-TRIF-RIPK1-FADD-caspase-8-mediated, K^+ independent, route to NLRP3 inflammasome activation was also recently described in human BlaER1 monocytes.[74] Of note, ROS activity was suggested to be involved in ripoptosome-triggered NLRP3 activation upon IAP loss in TLR-primed macrophages.[78] Further studies are, therefore, needed to address how RIPK1-RIPK3-FADD-caspase-8 complexes can trigger the NLRP3 inflammasome.

Recent work has also documented that when caspase-8 levels are reduced TLR ligation can also trigger NLRP3 inflammasome activation via necroptotic activity (Figure 3c). Caspase-8 deficiency in dendritic cells sensitised mice to LPS-induced lethality through RIPK3-driven NLRP3 inflammasome activation.[49] Subsequently, TLR2/4 ligation in caspase-8 deficient murine bone marrow derived dendritic cells and macrophages, as well as human BalER1 monocytes, was shown to trigger necroptotic activation of the NLRP3 inflammasome, in a RIPK1 and RIPK3 kinase-dependent manner.[49,50,74] Likewise, in TLR-primed macrophages IAP loss and inhibition/genetic loss of caspase-8 leads to RIPK3 kinase-dependent MLKL-driven activation of NLRP3.[50] It has been proposed that following TLR3 pathway activation, catalytically inactive caspase-8 (that is, by chemical

Table 2 Autoinflammatory-like syndromes associated with mutations in death receptor signalling machinery

Death Receptor machinery (*aliases*)	*Human disease* *Gene (chromosome position) mutation*	*Mouse model(s)* *Gene mutation*	*Clinical features*	*Reference*
TNFR1 (p55, CD120a)	TNF Receptor-Associated Periodic Fever Syndrome (TRAPS) *TNFRS1A* (12p13.2) High penetrance: C29F/Y, C30Y/R/S/F, C33G/Y, C43R/Y/S, C52W/F/Y/R, C55R/S/Y, C70G/R/S/Y, C88R/Y. Low penetrance: P46L, T50M/K, L57P, S86P and R92Q	$p55^{\Delta NS}$ mice (non-sheddable TNFR1 knock-in) T50M & C33Y TNFR1-mutant mice	Periodic fever Rash Myalgia Arthralgia Abdominal pain Periorbital oedema Lymphadenopathy Serum A protein amyloidosis (kidney)	71,100,131
XIAP (BIRC4, MIHA, IAP-3)	X-linked Lymphoproliferative disease Type 2 (XLP2) *XIAP* (Xq25) P482R, I494N, G188E, Y290fsX294, Q33X, Q104X, G188E, DelExon6 and DelExon1-5	$Xiap^{-/-}$ mice (MHV68-infected) $cIAP1^{LysM.Cre}$ $Xiap^{-/-}$ $cIAP2^{-/-}$	Periodic fever Rash Heptosplenomegaly Cytopenia Chronic haemorrhagic colitis Arthralgia	18,50,101,132
LUBAC -HOIL-1/HOIP/ SHARPIN complex	LUBAC deficiency autoinflammatory disease *HOIL* gene *RBCK1* (20p.13) Biallelic: L41fsX7 Compound heterozygote Q185X/TRIB3:g-1272_HOIL: g9780del, *HOIP* gene *RNF31* (14q12) Biallelic: L72P	$HOIL\text{-}1^{-/-}$ (MHV68-infected) $Sharpin^{cpdm/cpdm}$	Periodic fever Rash Hepatosplenomegaly Lymphadenopathy Abdominal pain (IBD) Muscular amylopectinosis (e.g., myocardium, oesophagus, bowel) Lymphangiectasia	105,106,109,110
A20 (TNFAIP3)	Haploinsufficiency of A20 (HA20) *A20* gene *TNFAIP3* (6q23.3) High penetrance: F224SfsX4, L227X, P268LfsX19, R271X, Y306X and T604RfsX93	$A20^{-/-}$ $A20^{LysM.Cre}$	Fever Oral ulcers Genital ulcers Rash (erythaema nodosum-like lesions)	113,118

Abbreviations: MHV68, murine gamma herpes virus 68; TNF, tumour necrosis factor.

inhibition) acts as a scaffold to recruit a FADD-RIPK1-RIPK3 complex that subsequently, via the kinase activity of RIPK3, triggers MLKL-dependent NLRP3 inflammasome activation.[92] Supporting this idea is the fact that TLR3-induced necroptotic activation of the NLRP3 inflammasome was not restored by complementing RIPK3 into $Ripk3^{-/-}Caspase\text{-}8^{-/-}$ macrophages.[92] However, defective TLR3/4 induced inflammasome priming in $Ripk3^{-/-}Caspase\text{-}8^{-/-}$ macrophages may complicate these findings.[58] Furthermore, the fact TLR-induced RIPK3-MLKL can trigger NLRP3 inflammasome activation upon genetic loss of caspase-8, or RIPK1, also suggests that a scaffolding function for caspase-8 and RIPK1 may only be relevant to dsRNA.[43,49,50,92]

Inflammasome-induced caspase-8 activation and apoptosis

Biochemically and structurally, caspase-8 has been shown to interact via its DED domain with the PYD domain of inflammasome adaptor ASC, thereby making it plausible that multiple ASC-containing inflammasomes utilise caspase-8 signalling.[93,94] Studies have shown that in the absence of caspase-1, canonical NLRP3 (for example, nigericin) and AIM2 (for example, *Francisella tularensis* and DNA) inflammasome activation can cause ASC-induced caspase-8 oligomerisation into filamentous structures capable of inducing apoptosis.[93–95] Furthermore, in the absence of caspase-1/11, canonical NLRP3-ASC activation of caspase-8 induces not only apoptosis but also IL-1β activation in dendritic cells, albeit with delayed kinetics compared with caspase-1.[89] Notably, ASC-caspase-8 triggered apoptosis may occur preferentially in wild type dendritic cells exposed to low concentrations of inflammasome stimuli.[94] Perhaps in this scenario, apoptotic cell death would be a preferable immunologically silent route for the cells demise. The strongest physiological evidence for an ASC-caspase-8 containing inflammasome is the fact that *Francisella tularensis* activates a caspase-1 independent AIM2-ASC inflammasome to drive IL-18-dependent IFN-γ production.[96] In the case of *Salmonella* infection, an NLRC4-ASC-caspase-8-caspase-1 inflammasome has also been shown to induce pro-IL-1β via caspase-8 and pyroptosis via caspase-1 activity.[97] Overall it appears possible that ASC may recruit caspase-8 to trigger appropriate responses to a microbial insult.

DEATH RECEPTORS AND CASPASE-8 IN AUTOINFLAMMATORY DISEASE

Evidence now suggests that death receptor signalling affects NLRP3 inflammasome activity at three levels; inflammasome priming, activation and assembly, and post-translational modification. First, the RIP kinases and caspase-8 have a critical step in inflammasome priming, as demonstrated by reduced cytokine production and pro-IL-1β in $Ripk3^{-/-}Caspase\text{-}8^{-/-}$ mice.[58,63] Second, not only can the NLRP3 inflammasome be triggered by RIPK3-caspase-8 and RIPK3-MLKL signalling, but caspase-8 can bind ASC-containing inflammasomes to signal apoptosis and activate IL-1β.[50,95] Finally, TNFR1 death receptor signalling molecules have been implicated in NLRP3 inflammasome assembly (that is, c-$FLIP_L$),[88] or post-translational modifications of inflammasome components. For example, LUBAC has been suggested to ubiquitylate the adaptor ASC.[98] Importantly, mutation of a number of key death receptor components that have previously been linked to autoimmunity or immunodeficiency, also mirror features of autoinflammatory syndromes that are associated with heightened NLRP3 inflammasome activity, IL-1β and/or TNF levels (Table 2).

TNFR1 and TRAPS

TNF receptor-associated periodic syndrome (TRAPS; Table 2) was one of the first genetically defined autoinflammatory diseases. TRAPS occurs due to autosomal dominant mutations of the TNFR1 *(TNFRS1A)* gene and leads to a hereditary recurrent fever syndrome. Mechanistically, missense mutations in the first two cysteine rich domains of the TNFR1 extracellular domain, critical for receptor association and ligand binding, have been linked to aberrant folding and defective receptor shedding. Defective receptor expression reportedly leads to overexpression, aggregate formation in the endoplasmic reticulum and stress responses, as well as mitochondrial ROS production.[71,99,100] These events have been linked to constitutive TNFR1 signalling and pro-inflammatory cytokine release. Even in a heterozygous state, TRAPS TNFR1-mutant cells exhibit both spontaneous and sustained LPS-induced MAPK activation, resulting in heightened cytokine production, including TNF, IL-6 and IL-1β.[100] How IL-1β is activated in TRAPS patients remains unclear, yet like TNF inhibition, IL-1 blockade is also used therapeutically.

XIAP and XLP2

Until recently the E3 ligase XIAP was cast as a direct inhibitor of caspase-3, -7 and -9. However, it has now become abundantly clear in monocyte, macrophages and dendritic cells that XIAP and its E3 ligase activity, has an obligatory role in repressing ripoptosome activation and consequently preventing cell death and IL-1β activation.[18,50,78] Despite functional redundancy of individual IAPs, macrophages lacking XIAP, or XIAP and cIAP2, are sensitised to LPS/TNF-induced RIPK3-caspase-8-mediated apoptosis, RIPK3-MLKL-mediated necroptosis and NLRP3 inflammasome activation.[50] Of note, myeloid-specific cIAP1 and cIAP2 loss did not sensitise macrophages to death *in vitro* or activate IL-1β, yet mice developed a severe TNF-driven inflammatory arthritis.[50] In contrast, co-deletion of XIAP with cIAP1 and cIAP2, or inhibition of all three IAPs and TLR/TNFR ligation, resulted in maximal ripoptosome-mediated apoptotic and necroptotic signalling, and IL-1β activation in myeloid cells (Figure 3).[50] Remarkably, mice lacking all IAPs in myeloid cells exhibited multi-organ inflammation and arthritis associated with heightened inflammatory cytokines, including IL-1β and TNF.[50,101]

The importance of XIAP in repressing inflammatory signalling in mice is of significant interest, as mutations in *XIAP* are commonly associated with inflammatory bowel disease,[102] and loss of function mutations in *XIAP* are found in X-linked lymphoproliferative disease 2 (XLP2) patients.[103] XLP2, which is often associated with Epstein Barr Virus (EBV) infection, results in hemophagocytic lymphohistiocytosis (HLH) in 60–90% of patients.[103] The characteristic macrophage hyperactivation is associated with elevated levels of the inflammasome-caspase-1 (or caspase-8) substrate IL-18 and clinical features reminiscent of CAPS patients with NLRP3 activating mutations, and mice with myeloid-specific IAP loss (Table 2).[104] Hence XLP2 with HLH could be reclassified as an inflammasome-driven autoinflammatory syndrome.

LUBAC and LUBAC autoinflammation

LUBAC, comprised of HOIL-1, HOIP and SHARPIN, is responsible for linear ubiquitylation of RIPK1 and NEMO to allow efficient NF-κB activation. The importance of LUBAC for activating NF-κB signalling in both humans and mice has been demonstrated by blunted cytokine transcription in fibroblasts lacking HOIP, HOIL-1 or SHARPIN in response to TLR ligands, TNF and IL-1β.[9,105,106] In line with this, defects in CD40 ligand signalling have also been observed upon loss of HOIL-1, HOIP and SHARPIN in B cells.[9,105,106] In the myeloid compartment the role of LUBAC in inflammatory signalling is less clear. Similar to the responses seen in fibroblasts, TLR-induced NF-κB activation and cytokine production is defective in dendritic cells and macrophages derived from *Sharpin*$^{cpdm/cpdm}$ mice.[105,107,108] However, in contrast, LPS or TNF-induced NF-κB activation and cytokine transcription is normal in HOIL-1-deficient macrophages,[98] although defective cytokine production has been described upon *Listeria* infection.[109] Surprisingly, *HOIP* or *HOIL-1* mutations in humans triggers systemic autoinflammation, immunodeficiency and amylopectinosis (Table 2).[105,106] Likewise, loss of SHARPIN in mice (*Sharpin*$^{cpdm/cpdm}$) causes a TNF/TNFR1 and partly IL-1 driven spontaneous autoinflammatory disease featuring chronic proliferative dermatitis, splenomegaly, and liver inflammation.[9,110] In contrast, HOIL-1-deficient mice do not exhibit spontaneous autoinflammation, although animals do develop amylopectin-like deposits in the myocardium with age and exhibit immunodeficiency.[109] Of note, spontaneous inflammatory responses are triggered in HOIL-1-deficient mice infected with chronic MHV68 and *M. Tuberculosis*.[109] Consistent with this, *HOIL-1*- and *HOIP*-deficient human monocytes display cytokine hyper-production in response to IL-1β or TNF,[105,106] and anti-TNF therapy reduced clinical inflammation in a *HOIL-1* deficient patient.[106] This contradictory role for LUBAC in autoinflammation highlights the fact that we do not fully appreciate the roles of these proteins on a cell-by-cell basis and in different disease settings.

Epidermal-specific caspase-8 or FADD loss causes necroptotic skin lesions.[45,111] Hence, it was somewhat surprising that the dermatitis featured in *Sharpin*$^{cpdm/cpdm}$ mice is driven by TNF-induced keratinocyte apoptosis, as shown by the absence of lesions upon epidermal-specific deletion of FADD (on a *Ripk3*$^{-/-}$ background) or heterozygous deletion of caspase-8, but not MLKL loss.[110,112] In contrast, other disease manifestations were driven by both apoptotic and necroptotic signalling. For example, MLKL deficiency prevented the leukocytosis, and partly rescued the liver pathology and splenomegaly, in *Sharpin*$^{cpdm/cpdm}$ mice.[110]

Considering IL-1 is a pathogenic factor in skin lesion development in SHARPIN-deficient mice, the mechanism behind IL-1β activation was recently evaluated. Deficiency in NLRP3 or caspase-1/11 in *Sharpin*$^{cpdm/cpdm}$ mice delayed dermatological symptoms, however, whether NLRP3 inflammasome activity was restricted to keratinocytes versus macrophages remains unclear.[107] This is particularly puzzlingly, as LUBAC activity appears to be essential for inducing, rather than inhibiting, inflammasome activation in macrophages. HOIL-1, for example, has been implicated in the linear ubiquitiylation of ASC and assembly of ASC-containing AIM2 and NLRP3 inflammasomes.[98] Although a further study suggested LUBAC component SHARPIN regulated canonical and noncanonical NLRP3 inflammasome priming (that is, pro-IL-1β and caspase-11), and not AIM2 activation.[107] These discrepancies suggest that inflammasome activation regulation by LUBAC requires further study.

A20 and HA20

A20 is widely recognised for its ability to dampen TNF/TLR-induced NF-κB signalling via the enzymatic removal of ubiquitin chains from RIPK1, TRAF6 and NEMO. In humans, A20 polymorphisms (reduced A20 protein) are associated with a range of inflammatory diseases, such as rheumatoid arthritis. Recently, inactivating germline mutations in A20 were also discovered to cause a Bechet's like autoinflammatory syndrome, now termed Haploinsufficiency of A20 (HA20). HA20 patients exhibit exaggerated NF-κB responses, constitutive NLRP3 activation and elevated serological inflammatory cytokines, including

Table 3 Contribution of necroptotic and apoptotic signalling to disease

	Ripk3$^{-/-}$	*Ripk1*$^{kinase\ dead}$/*Inhibitor*	*Mlkl*$^{-/-}$/*inhibitor*	*Casp8*$^{-/-}$/*inhibitor*	*Ripk3*$^{-/-}$ *Casp8*$^{-/-}$	*Reference*
Injury						
Renal ischaemic reperfusion injury	↓[a]	↓	↓[b]	No effect	↓	d,119
Oxalate crystal-induced renal injury[c]	↓	↓	↓	—	—	133
Myocardial ischaemic reperfusion injury	↓	↓	—	—	—	d,119,133
Brain Hypoxic Injury	No effect	↓	—	↓	—	d,119
Retinal Degeneration[c]	↓	↓	—	No effect	—	d,134
Pancreatitis	↓/No effect	↑/No effect	↓	↑	—	d,119
Inflammation						
Imiquimod-induced psoriasis	—	No effect	—	—	—	119
K/BxN arthritis[c]	↓[a]	—	No effect	—	↓	50
DSS colitis[c]	↑/No effect	↓/No effect	—	—	—	59,119
EAE	—	↓	—	—	—	52
OVA-induced asthma[c]	No effect	—	—	—	↓	135
Cecal ligation and puncture	No effect	↓	No effect	↓	—	d
TNF-induced	↓	↓/↑	↓	↓/↑	↓	d,119
LPS-induced	↓/No effect	No effect	No effect	↑	↓	d,58,119
Infectious disease						
Murine cytomegalovirus	↑	—	—	↑	—	d,29
Vaccinia virus	↑	—	—	—	—	d,29
HSV-1	↑	—	—	—	—	136
Influenza A virus	↑	—	No effect	—	—	137
Serratia marcescens[c]	↓	↓	↓	—	—	120
Staphylococcus aureus[c]	↓	↓	—	—	—	138
Yersinia pestis[c]	No effect	—	—	—	↑	63,90

Abbreviations: DSS dextran sulphate sodium; EAE experimental autoimmune encephalomyelitis; HSV-1 herpes simplex virus-1; OVA ovalbumin.
Key, Not done,↓, reduced disease,↑, increased disease.
[a]*Ripk3*$^{-/-}$ *Caspase-8*$^{-/-}$ show equivalent protection to Ripk3$^{-/-}$.
[b]Partial contribution.
[c]NLRP3 inflammasome or caspase-8 activity implicated in IL-1β activation in study.
[d]Original references in Khan *et al.*[27] due to space constraints.

IL-1β.[113] Interestingly, deletion of A20 in mice causes early onset lethality due to spontaneous multi-organ inflammation and severe cachexia caused by excessive TLR-MyD88-TRIF-RIPK3 signalling, independent of TNF/TNFR1.[114–116] Impressively, cell/tissue-specific A20 deletion also often recapitulates aspects of inflammatory diseases associated with A20 dysfunction. For example, myeloid-specific deletion of A20 causes spontaneous inflammatory arthritis that is dependent on the NLRP3 inflammasome and the IL-1 receptor but not TNF.[117] These results largely parallel findings in myeloid-specific IAP knockout mice, although IL-1β-associated pathology was TNF-dependent in these animals.[50] Similar to IAP deficient macrophages, TLR stimulation of A20-deficient macrophages resulted in spontaneous IL-1β activation that was dependent on RIPK3 and partially on NLRP3 inflammasome activation, whereby residual IL-1β was presumably driven by direct cleavage by caspase-8.[50,117]

How A20 restricts spontaneous TLR-induced RIPK3 mediated NLRP3 activation is unclear. A20 is obviously important in restricting NF-κB transcription and therefore NLRP3 inflammasome priming. Furthermore, A20 reportedly acts downstream to impede NLRP3 signalling by cleaving K63-linked ubiquitin chains from K133 of IL-1β to limit IL-1β processing and secretion.[118] Additionally, A20 has recently been shown to deubiquitylate RIPK3 on K5 to limit necrosome formation,[116] however, surprisingly in contrast to RIPK3 loss, MLKL deletion does not protect A20 knockout mice from lethality.[119] Perhaps RIPK3-caspase-8 inflammatory signalling, rather than necroptotic signalling, drives disease in the absence of A20.

DEATH RECEPTOR INDUCED CASPASE-8 SIGNALLING AND DISEASE

The role of the ripoptosome and caspase-8 signalling in common inflammatory disease and IL-1 activation has been difficult to study due to the early onset embryonic lethality in caspase-8 deficient mice due to excessive necroptotic signalling. Tissue- and cell-restricted deletion of caspase-8 has also been complicated by necroptotic inflammatory phenotypes (Table 1) and the fact caspase-8 regulates cytokine transcription. In fact, loss of key cell death receptor signalling components commonly precipitates in early onset lethality, or increased morbidity due to severe inflammatory disease and tissue destruction (Tables 1 and 2). However, utilising a range of knockout mice and inhibitors of core death machinery the role of the ripoptosome in inflammatory disease is becoming more clear (see Table 3 for recent examples and Khan *et al.*[27]). The discovery that co-deletion of caspase-8 and RIPK3 leads to the birth of viable and healthy animals has led to a greater understanding of the role of caspase-8 signalling has in disease. However, as *Ripk3*$^{-/-}$*Caspase-8*$^{-/-}$ mice eventually develop lymphoproliferative disease (>12 weeks of age), and exhibit defects in TLR-induced cytokine production (ie. pro-IL-1β), results are still difficult to interpret. Nevertheless, caspase-8 cleavage of IL-1β and IL-18, or activation of NLRP3 is likely to be of physiological relevance. For example, the IL-1β dependent murine K/BxN serum transfer arthritis model has been reported to be caspase-1-and MLKL independent, and is instead dependent on RIPK3 and caspase-8 for local and systemic IL-1β secretion during the resolution phase of disease.[50] Results suggest that caspase-8 activity

is required for optimal priming and may directly cleave IL-1β in this model.

The contribution of RIPK3 and necroptotic signalling to a range of diseases reportedly driven by necroptotic signalling was recently re-examined by Newton *et al.*[119] Contradicting previous findings, no role for RIPK3 and necroptosis was observed in acute pancreatitis, brain injury (major cerebral artery occlusion and hypoxia-induced cerebral oedema) or DSS colitis (Table 3).[119] Differences in experimental procedures or the microbiome were suggested as plausible explanations for these discrepancies. In contrast, in kidney ischaemia-reperfusion injury and TNF-induced systemic inflammatory syndrome, both MLKL necroptotic and apoptotic caspase-8 signalling contributed to disease pathogenesis.[119] Of note, the strongest role for necroptosis in disease resides around work exploring the mechanism behind death, rather than NLRP3 activation, induced by viral pathogens (for example, CMV and vaccinia virus) or pore-forming toxin producing bacteria (for example, *Serratia marcescens*).[27,29,120] Collectively these results highlight that previous findings in RIPK3-deficient mice suggesting pathological necroptotic signalling in disease need to be revisited utilising MLKL-deficient mice. Moreover, they suggest that caspase-8 activity is more pivotal in driving inflammatory disease pathologies and IL-1β than previously thought.

CONCLUSIONS

This review summarises an extensive body of research, which suggests there is substantial crosstalk between innate PRR and cell death signalling pathways, and that caspase-8 levels dictate the net outcome. Caspase-8 activity is now recognised to critically suppress necroptosis, regulate cytokine transcription, and to act downstream of RIPK3 or inflammasome-related ASC to induce apoptosis. More remarkably, caspase-8 levels have been shown to act as a rheostat for activation of IL-1β. Ripoptosome-associated caspase-8 can either directly cleave IL-1β or indirectly trigger the NLRP3 inflammasome, and when caspase-8 levels are low RIPK3-MLKL necroptotic signalling can also trigger NLRP3. These findings have major ramifications for our understanding of innate immune cell responses during disease, where apoptotic or necroptotic cell death combined with IL-1β and IL-18 activation could be beneficial for pathogen clearance. Moreover, identification of whether apoptotic versus necroptotic cell death pathways contribute to NLRP3 activation and/or IL-1β activation in common inflammatory disease, and in rare autoinflammatory diseases caused by mutations in cell death regulators, is of major interest and will inform rationale therapeutic drug design.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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