Published in final edited form as:

Trends Immunol. 2020 August ; 41(8): 648–651. doi:10.1016/j.it.2020.06.007.

Flipping the Switch from Inflammation to Cell Death

Hayley I. Muendlein¹, Alexander Poltorak^{1,2,3,*}

¹Graduate Program in Genetics, Tufts Graduate School of Biomedical Sciences, Boston, MA 02111, USA

²Department of Immunology, Tufts University School of Medicine, Boston, MA 02111, USA

³Petrozavodsk State University, Petrozavodsk, Republic of Karelia 185910, Russia

Abstract

Multiple research groups have demonstrated that caspase-8 (CASP8)-mediated gasdermin D (GSDMD) cleavage drives pyroptotic cell death. Here, we discuss a novel role for the enzymatically inactive homolog of CASP8, the long isoform of cellular FLICE-like inhibitory protein (cFLIP_L), in the regulation of this process. Specifically, cFLIP-deficiency provides a model in which to study the mechanisms regulating CASP8-mediated activation of cell death and inflammatory signaling.

Programmed cell death and inflammatory signaling are crucial host responses to infection; because of this, pyroptotic cell death is particularly important for promoting host survival and limiting pathogen replication. Canonically, pyroptosis in eukaryotes is facilitated via caspase-1 (CASP1)-mediated cleavage and activation of the pore forming protein GSDMD [1]. Pyroptosis is aptly named after the Greek word for fire, ‘pyro’, due to the release of inflammatory interleukin-1 (IL-1) β and IL-18 cytokines concomitantly occurring with cell death. In mice, IL-1 β maturation and release occurs in response to bacterial lipopolysaccharide (LPS); however, in murine macrophages *in vitro*, IL-1 β maturation requires two signals: (i) a priming signal through **Toll-like receptors (TLRs)** (see Glossary) to upregulate proIL-1 β and (ii) a variety of danger signals necessary to induce oligomerization of **inflammasome** complexes, including Nod-like receptor protein 3 (NLRP3) [2]. The NLRP3 inflammasome includes apoptosis-associated speck-like protein (ASC), which recruits CASP1 to cleave proIL-1 β , subsequently released via pores formed by GSDMD [2].

Although GSDMD cleavage has been extensively studied downstream of CASP1, recent years have seen major progress in our understanding of the role of CASP8 in pyroptotic cell death. Two publications have highlighted the importance of CASP8-mediated cleavage of GSDMD in the subsequent pyroptosis of mouse macrophages infected by *Yersinia* sp. bacteria [5,6]. This death was shown to be dependent on the *Yersinia* effector protein YopJ, as infection of murine macrophages with YopJ-deficient *Yersinia* failed to induce cell death

*Correspondence: Alexander.poltorak@tufts.edu (A. Poltorak).

Supplemental Information

Supplemental information associated with this article can be found online at <https://doi.org/10.1016/j.it.2020.06.007>.

comparable with wildtype (WT) infection [5,6]. Overexpression studies in *Drosophila* and human cell lines have shown that YopJ can inhibit the activity of mitogen-activated protein kinase (MAPK) transforming growth factor β (TGF β)-activated kinase (TAK1) [5], while in *Yersinia*-infected murine macrophages and dendritic cells, YopJ inhibits downstream MAPK pathways [6]. In both settings, the actions of YopJ result in severely attenuated host inflammatory responses to infection. Moreover, in both studies, infection-driven activation of CASP8 could be recapitulated with treatment of macrophages with LPS and the small-molecule TAK1 inhibitor, 5Z-7-oxozeaenol (5z7), experimentally used to mimic the effect of YopJ-mediated inhibition of inflammatory responses (see Figure 1A) [3,4]. Similar CASP8-mediated cleavage of GSDMD has been independently demonstrated in the context of tumor necrosis factor (TNF)-activated, TAK1-inhibited mouse macrophages (see Figure 1A) [7]. In addition to CASP8-mediated cleavage of GSDMD, the three groups also detected activation of apoptotic caspase-3 and caspase-7 (CASP3 and 7), suggesting that LPS or TNF treatment – in the context of TAK1 inhibition – could lead to a **mixed cell death phenotype**, with characteristics of both pyroptosis and apoptosis. CASP8-mediated cell death has been shown to be essential for host survival in response to *Yersinia* infection, as CASP8-deficient mice display uncontrolled bacterial proliferation, decreased IL-1 β production, and rapidly succumb to infection. This suggests a role for CASP8-mediated pyroptosis as an important host response to bacterial infection, the mechanism of which warrants thorough investigation [8].

Despite agreement between these reports regarding CASP8-mediated cleavage of GSDMD and the induction of pyroptotic cell death, different explanations have been provided concerning the mechanism of NLRP3 inflammasome activation. One study proposed that the NLRP3 inflammasome is activated via potassium (K⁺) efflux downstream of GSDMD pore formation [4]; another suggested a GSDMD-independent, pannexin-1-dependent mechanism of NLRP3 inflammasome activation [7]. In agreement with this, our group observed GSDMD-independent, K⁺ efflux-dependent activation of the NLRP3 inflammasome [3,9]. Although we did not confirm the role of pannexin-1 in our system, it is plausible that NLRP3 inflammasome activation occurred via K⁺ efflux downstream of pannexin-1 pores. These differences regarding the mode of NLRP3 inflammasome activation may likely be attributed to slight variations in the timing of the priming signal (LPS or TNF) and the second signal (5z7) between laboratories.

Since TAK1 inhibition attenuates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)- and MAPK-mediated proIL-1 β synthesis, we found that priming with LPS prior to the addition of 5z7 resulted in more robust IL-1 β release compared to concurrent treatment with LPS and 5z7 [3,9]. However, the timing of LPS addition had a significant impact on the kinetics of cell death, suggesting an effect of LPS-induced genes in the regulation of CASP8-mediated pyroptosis. Indeed, among the genes differentially regulated between macrophages stimulated with LPS alone and those stimulated with LPS in the context of TAK1 inhibition, was the enzymatically inactive homolog of CASP8, cFLIP. In primary mouse macrophages activated with LPS alone, knockdown (KD) of the long (cFLIP_L-KD), but not the short (cFLIP_R-KD), isoform resulted in CASP8-mediated pyroptosis (as defined by dependency on GSDMD) and NLRP3 inflammasome-mediated

IL-1 β production (see Figure 1B) [9]. This finding was particularly intriguing, since it suggested that cFLIP_L expression determined whether or not activation with LPS would elicit inflammatory cell death. Additionally, these data suggested that LPS activation alone was sufficient to drive NLRP3 inflammasome activation and IL-1 β production. Altogether, these data support a major protective role for cFLIP_L against LPS-induced pyroptosis *in vitro*. Further characterization of the mechanism of cFLIP_L-mediated regulation of pyroptosis revealed that treatment with LPS alone promoted formation of a complex containing FAS-associated death domain protein (FADD), receptor-interacting protein kinase (RIPK)1, and CASP8 in cFLIP_L-KD macrophages. This result confirmed that cFLIP_L protects macrophages from LPS-induced pyroptosis via inhibition of prodeath complex II formation [9]. While these findings support a role for cFLIP_L as a regulator of pyroptosis downstream of TAK1 inhibition, other studies have suggested additional mechanisms through which cell death progresses in the absence of TAK1. For instance, TAK1-deficient murine macrophages were reported to exhibit a mixed cell death phenotype, with features of apoptosis, pyroptosis, and necroptosis [10]. Similar to our findings in *Yersinia*-infected macrophages [3], TLR-mediated cell death and NLRP3 inflammasome activation were dependent on RIPK1, though the kinase activity appeared to be dispensable for cell death and inflammasome activation in TAK1-deficient macrophages [10]. Given the enumerable roles that TAK1 plays in the regulation of cell death and inflammation, including promoting NF- κ B and MAPK signaling and regulation of RIPK1 activity through direct [11] and indirect [12] phosphorylation of RIPK1, it is perhaps unsurprising that TAK1-deficiency results in multiple modes of cell death with diverse regulatory mechanisms (see the supplemental information online). Therefore, we propose that our findings using cFLIP_L-deficient macrophages provide us with a cleaner model of LPS-induced cytotoxicity and CASP8-mediated pyroptosis in the absence of the various effects of TAK1 inhibition.

A Single Signal from LPS Elicits IL-1 β Production

LPS activation led to the CASP8-dependent production and release of mature IL-1 β in cFLIP_L-KD mouse macrophages [9]. A number of studies have shown CASP8-mediated production of mature IL-1 β , including direct cleavage of proIL-1 β by CASP8 independent of NLRP3 inflammasome activation [13]. In contrast, we observed that IL-1 β release in LPS-activated cFLIP_L-deficient cells was completely dependent on NLRP3 and CASP1 [9]; this suggests that cFLIP_L can modulate CASP8-mediated activation of the NLRP3 inflammasome [8,9,14]. In further support of this concept, silencing of cFLIP_L in murine macrophages has promoted ASC speck formation and CASP1 cleavage upon LPS stimulation [9]. While IL-1 β release was abrogated in the absence of CASP8 and GSDMD, inflammasome activation occurred independently of GSDMD-mediated pore formation in cFLIP_L-deficient murine macrophages. However, inflammasome activation remained dependent on K⁺ efflux, as excess extracellular K⁺ eliminated ASC speck formation [9]. Similarly, although NLRP3-, ASC-, and CASP1-deficiency abrogated LPS-induced IL-1 β release, deficiency in these inflammasome components had no impact on GSDMD cleavage and pyroptosis in the absence of cFLIP_L, supporting a model in which CASP8 independently activates GSDMD to drive pyroptosis and the NLRP3 inflammasome to produce IL-1 β [9]. Altogether, these results challenge the ‘two signal’ dogma for IL-1 β production in

macrophages *in vitro* – as a single signal from LPS-induced inflammasome activation and IL-1 β release in the absence of cFLIP_L. Additionally, these findings suggest that differing amounts of cFLIP may reconcile differences seen in the requirements for IL-1 β maturation between *in vitro* and *in vivo* models.

cFLIP_L Regulates CASP8 Substrate Specificity

Although LPS or TNF activation of murine macrophages in the context of TAK1 inhibition resulted in CASP8-mediated cleavage of apoptotic CASP3 and 7 in addition to GSDMD, LPS activation in the context of cFLIP_L deficiency promoted GSDMD cleavage in the absence of CASP3/7 cleavage, providing a more strictly pyroptotic cell death phenotype [3,4,7,9]. Therefore, these two models of CASP8-mediated pyroptosis provide us with a setting in which to investigate the mechanisms determining the downstream targets of CASP8. Although 5z7 treatment inhibits de novo transcription of cFLIP_L, this approach does not eliminate cFLIP_L as efficiently as the KD. With sufficiently high cFLIP_L concentrations, CASP8:cFLIP_L heterodimers exhibit only partial enzymatic activity and, therefore, require downstream caspases, such as CASP3 and 7, for the execution of cell death [9]. However, in the absence of cFLIP_L, fully active CASP8 homodimers form readily and potentially cleave distant targets, such as GSDMD [9]. Therefore, we propose that the extent of CASP8 activation, regulated by cFLIP_L, determines the mechanism of cell death.

cFLIP_L – A Molecular Switch

In the context of bacterial infection, the first signal from macrophages is likely to be an inflammatory response to LPS, which upregulates cFLIP_L, sustaining the inflammatory nature of the response. Once the inflammatory response is inhibited, for example, via the *Yersinia* YopJ protein, cFLIP_L concentrations decrease, promoting homodimerization of CASP8 and the activation of cell death as an alternative to inflammatory signaling [9]. By controlling complex II formation, cFLIP_L can function as a molecular switch between inflammatory responses and cell death, supporting our current understanding of cell death and inflammation as two intertwined branches of the host response. Although, according to this model, complex formation occurs in the absence of cFLIP_L and requires the kinase activity of RIPK1 [9], the exact mechanism through which cFLIP_L regulates complex formation, and whether it does so across species, remains to be fully explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health grants [AI135369 and AI056234] and Russian Federation contract number 0752-2020-0007 to A.P.

Glossary

Inflammasomes

intracellular protein complexes that typically consist of a NOD-like family protein (NLR), such as NLRP3, the adaptor protein ASC, and procaspase-1; they play important roles in CASP1-mediated GSDMD and proIL-1 β cleavage, driving pyroptotic cell death and IL-1 β release.

Mixed cell death phenotype

refers to cell death in response to a specific stimulus, dependent on multiple effectors of established cell death pathways. For example, LPS or TNF activation in the context of TAK1 inhibition results in cell death that is partially dependent on GSDMD (a key effector of pyroptosis) as well as CASP3 and 7, which have well-established roles in the context of apoptosis.

Toll-like receptors (TLRs)

family of transmembrane proteins, which recognize specific pathogen-associated molecular components or endogenous damage-associated molecular components in the extracellular environment or within intracellular compartments. Upon ligand recognition, TLRs initiate signaling pathways, leading to the NF- κ B, MAPK, and interferon-regulatory factor (IRF)-mediated induction of cytokines, chemokines, and prosurvival factors.

References

1. He W et al. (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1 β secretion. *Cell Res* 25, 1285–1298 [PubMed: 26611636]
2. Liu X et al. (2016) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535, 153–158 [PubMed: 27383986]
3. Sarhan J et al. (2018) Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. *Proc. Natl. Acad. Sci. U. S. A* 115, E10888–E10897 [PubMed: 30381458]
4. Orning P et al. (2018) Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* 362, 1064–1069 [PubMed: 30361383]
5. Paquette N et al. (2012) Serine/threonine acetylation of TGF β -activated kinase (TAK1) by *Yersinia pestis* YopJ inhibits innate immune signaling. *Proc. Natl. Acad. Sci. U. S. A* 109, 12710–12715 [PubMed: 22802624]
6. Rosadini CV et al. (2015) A single bacterial immune evasion strategy dismantles both MyD88 and TRIF signalling pathways downstream of TLR4. *Cell Host Microbe* 18, 682–693 [PubMed: 26651944]
7. Chen KW et al. (2019) Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP3 inflammasome assembly. *EMBO* 38, e101638
8. Weng D et al. (2014) Caspase-8 and RIP kinases regulate bacteria-induced innate immune responses and cell death. *Proc. Natl. Acad. Sci. U. S. A* 111, 7391–7396 [PubMed: 24799678]
9. Muendlein HI et al. (2020) cFLIPL protects macrophages from LPS-induced pyroptosis via inhibition of complex II formation. *Science* 367, 1379–1384 [PubMed: 32193329]
10. Malireddi RKS et al. (2019) Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity-independent pyroptosis, apoptosis, necroptosis, and inflammatory disease. *J. Exp. Med* 217, jem.20191644
11. Geng J et al. (2017) Regulation of RIPK1 activation by TAK1-mediated phosphorylation dictates apoptosis and necroptosis. *Nat. Commun* 8, 359 [PubMed: 28842570]
12. Dondelinger Y et al. (2015) NF- κ B-independent role of IKK α /IKK β in preventing RIPK1 kinase-dependent apoptotic and necroptotic cell death during TNF signaling. *Mol. Cell* 60, 63–76 [PubMed: 26344099]
13. Maelfait J et al. (2008) Stimulation of Toll-like receptor 3 and 4 induces interleukin-1 β maturation by caspase-8. *J. Exp. Med* 205, 1967–1973 [PubMed: 18725521]

14. Allam R et al. (2014) Mitochondrial apoptosis is dispensable for NLRP 3 inflammasome activation but non-apoptotic caspase-8 is required for inflammasome priming. *EMBO Rep* 15, 982–990 [PubMed: 24990442]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Trends in Immunology

Figure 1. Lipopolysaccharide (LPS) and Tumor Necrosis Factor (TNF) Induce Caspase (CASP)8-Mediated Pyroptosis and Interleukin-1β (IL-1β) Release in the Context of Transforming Growth Factor β-Activated Kinase (TAK1) Inhibition or the Long Isoform of Cellular FLICE-like Inhibitory Protein (cFLIP_L) Deficiency in Mouse Macrophages.

(A) TAK1 inhibition, in conjunction with activating signals from LPS or TNF, results in the transcriptional inhibition of Nod-like receptor protein 3 (NLRP3) inflammasome components, *Il1b*, and various pro-survival factors in addition to the formation of a prodeath complex, including receptor-interacting protein kinase (RIPK)1 and CASP8 [3,4,7,9]. CASP8 activation within this complex results in CASP8-mediated cleavage of multiple targets, including gasdermin D (GSDMD) and CASP3/7. Cleavage of GSDMD drives pore formation that contributes to a pyroptotic cell death phenotype. Meanwhile, activation of CASP3/7 promotes the cleavage of gasdermin E (GSDME) and pannexin-1 (PANX1) as well as apoptotic death effectors that contribute to the observed mixed cell death phenotype [3,4,7]. Independent reports suggest differing roles for the pore-forming proteins GSDMD and PANX1 in the regulation of NLRP3 inflammasome activation via potassium (K⁺) efflux [4,7]. (B) LPS activation in the absence of cFLIP_L allows for the transcription of NLRP3 inflammasome components and *Il1b*, while promoting the formation of a prodeath complex composed of FAS-associated death domain protein (FADD), RIPK1, and CASP8, inhibited by cFLIP_L under homeostatic conditions. Activated CASP8 independently drives the cleavage of GSDMD and activation of the NLRP3 inflammasome, resulting in pyroptotic cell death and IL-1β maturation, respectively. However, LPS activation in the absence of cFLIP_L does not result in CASP3/7 activation, resulting in a purely pyroptotic phenotype [9]. While IL-1β release is dependent on GSDMD-mediated pore formation, NLRP3 inflammasome activation occurs through a yet undetermined K⁺ efflux-dependent mechanism. It is likely that TNF activation in the absence of cFLIP_L drives a similar mechanism of pyroptosis and IL-1β release; however, this has yet to be proven. Abbreviations: 5z7, 5Z-7-oxozeaenol; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; YopJ, *Yersinia* outer protein J.