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PANoptosome signaling and therapeutic implications in infection: Central role for ZBP1 to activate inflammasome and PANoptosis

Rajendra Karki[1,2,*], **Thirumala-Devi Kanneganti**[1,*]

[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, 38105, USA

[2]Current affiliation: Department of Biological Sciences, Seoul National University, Seoul, 08826, Republic of Korea

Abstract

The innate immune response provides the first line of defense against infection and disease. Regulated cell death (RCD) is a key component of innate immune activation, and RCD must be tightly regulated to clear pathogens while preventing excess inflammation. Recent studies have highlighted a central role for the innate immune PANoptosome sensor ZBP1 as an activator of a form of inflammatory RCD called PANoptosis, which is regulated by multifaceted cell death complexes called PANoptosomes. In response to influenza A virus infection, ZBP1 activates the NLRP3 inflammasome, which then acts as an integral component of the ZBP1-PANoptosome to drive inflammatory cell death, PANoptosis. In this context, the NLRP3 inflammasome is critical for proinflammatory cytokine IL-1β and IL-18 maturation, but dispensable in the larger PANoptosome for cell death due to functional redundancies between PANoptosome molecules. Similarly, ZBP1 is also central to the AIM2-PANoptosome, which forms in response to *Francisella novicida* and herpes simplex virus 1 infections and incorporates the AIM2 inflammasome as an integral component. In this review, we will discuss the critical roles of ZBP1 in mediating innate immune responses through inflammasomes, PANoptosomes, and PANoptosis during infection. Improved understanding of the molecular mechanisms of innate immunity will be essential for the development of targeted modalities that can improve patient outcomes by mitigating severe disease.

[*]**Correspondence should be addressed to:** Thirumala-Devi Kanneganti, Department of Immunology, St Jude Children's Research Hospital, MS #351, 262 Danny Thomas Pl., Memphis, TN 38105-2794, Tel: +1 (901) 595-3634; Fax: +1 (901) 595-5766, Thirumala-Devi.Kanneganti@StJude.org Or Rajendra Karki, Department of Biological Sciences, Seoul National University, Gwanak-ro 1, Gwanak-gu, Seoul 08826, Republic of Korea, Tel: +82-02-880-2656, rkarki@snu.ac.kr.

Declaration of Competing Interest

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Keywords

ZBP1; innate immunity; inflammation; cell death; pyroptosis; apoptosis; necroptosis; PANoptosis; PANoptosome; inflammasome; caspase; caspase-1; caspase-8; caspase-3; caspase-7; caspase-6; infection; bacteria; virus; fungi; influenza virus; AIM2; NLRP3; RIPK1; RIPK3; MLKL

Introduction

Innate immunity is a multifaceted response that provides the first line of host defense against invading pathogens. The innate immune system uses pattern recognition receptors (PRRs) to detect pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) and activate signaling cascades for the production of proinflammatory cytokines and chemokines and to induce regulated cell death (RCD) [1,2]. There are multiple RCD pathways, including non-lytic pathways such as apoptosis and lytic pathways that drive inflammatory responses such as pyroptosis, necroptosis, and PANoptosis. In the context of infection, inflammatory innate immune-mediated RCD can be beneficial to clear infected cells, but excess activation can lead to systemic inflammation and pathology.

PANoptosis has recently been implicated as a key RCD pathway at the intersection of host defense and pathology. PANoptosis is a unique, lytic innate immune inflammatory RCD pathway that is regulated by PANoptosome complexes, which integrate components from other RCD pathways [3–13]. PANoptosomes are formed by cytosolic PRRs in response to pathogens, PAMPs, DAMPs, or the cytokines produced downstream. These complexes often contain inflammasomes (cytosolic PRR sensor, ASC, and caspase-1) as integral components to drive the maturation of the proinflammatory cytokines IL-1β and IL-18, and they also generally contain caspase-8, RIPKs, and other molecules to induce cell death [6,10–12]. One of the key PANoptosome sensors is ZBP1 (Fig. 1A), which was initially identified as an innate immune sensor of influenza A virus (IAV) infection to induce NLRP3 inflammasome activation and PANoptosis [4] (Fig. 1B). Subsequently, ZBP1 has also been identified as a key component of the AIM2-PANoptosome, which forms in response to *Francisella novicida* and herpes simplex virus 1 (HSV1) infections [12] (Fig. 1C), and recent studies have demonstrated a regulatory connection between ADAR1 and ZBP1 via their Zα domains that is central to development, survival, and tumorigenesis [5,14–17]. These recent studies highlight the importance of ZBP1 and PANoptosis across development and disease. In this review, we will discuss the critical roles of ZBP1 in innate immune responses and host defense through PANoptosis during infection. Understanding innate immune sensing and RCD mechanisms in host defense is critical for the identification of therapeutic strategies to promote the clearance of infections while preventing inflammatory pathology.

Molecular characteristics of ZBP1

ZBP1, initially named DLM-1 and then DAI (DNA-dependent activator of interferon (IFN)-regulatory factors), is a prototypic IFN-inducible gene; its expression is strongly upregulated in response to type I IFN signaling. ZBP1 contains two N-terminal Zα domains, Zα1 and Zα2, followed by two RHIM domains and an undefined C-terminal domain (Fig. 1A). Its central conserved regions have sequence similarity to the RHIM domains of RIPK1, RIPK3,

and TRIF [18,19], which is important for its intermolecular interactions. ZBP1 is rather conserved between the human and murine homologs, with 47% protein sequence similarity between them. ZBP1 is predominantly expressed in normal lung, spleen, and liver tissues in mice, whereas it is highly expressed in small intestine and lymphatic tissues in humans [20,21]. Additionally, ZBP1 is primarily cytoplasmic, although it can form nuclear foci in activated cells [22,23].

Discovery of ZBP1-mediated NLRP3 inflammasome activation and PANoptosis

NLRP3 was initially characterized as an inflammasome sensor that induces proinflammatory cytokine maturation and caspase-1 activation in response to physiological ligand stimuli [24–26]. Soon after, IAV was the first pathogen identified to activate the NLRP3 inflammasome in vitro [27] and in vivo [28,29]. Despite many years of study focused on NLRP3 following these discoveries, the upstream regulatory mechanisms remained unknown. A quest to identify regulators of the NLRP3 inflammasome then identified ZBP1 as an innate immune sensor that induces NLRP3 inflammasome activation and cell death in response to IAV [4]. However, the biochemical nature of the cell death was distinct from previously identified RCD pathways, including pyroptosis, apoptosis, and necroptosis, which are executed by gasdermin family proteins (pyroptosis) [30–32], caspase-3 and -7 (apoptosis) [33,34], and MLKL (necroptosis) [35,36]. Moreover, IAV infection activates biochemical markers from multiple RCD pathways at the same timepoint, and genetic deletion of a single pathway is not sufficient to protect against cell death [4]; a similar phenomenon has been observed in response to other infections and under many other physiological conditions [5–8,37–39]. These findings suggest that defining this process by the previously identified RCD pathways or considering this as a sequential activation of RCD pathways does not accurately characterize the mechanism. This gap in our understanding of innate immune sensing and RCD led to the conceptualization of PANoptosis as a unique, lytic innate immune inflammatory RCD pathway that is regulated by PANoptosome complexes, which integrate components from other RCD pathways [3–13].

ZBP1 induces PANoptosome formation and PANoptosis in response to IAV [4,38], while other sensors and regulators, including FADD/caspase-8, RIPK1, and AIM2 have also been implicated in response to other infections, including RIPK1 in *Yersinia* infection [7] and AIM2 in *F. novicida* and HSV1 infections [12]. Mechanistically, IAV induces type I IFN signaling that triggers upregulation of ZBP1 expression to induce PANoptosome formation and cell death [4,40]. TLR and RIG-I signaling to produce IFNs are both involved in ZBP1 induction in immune cells, while RIG-I signaling alone is important for ZBP1 upregulation in nonimmune cells [40]. Furthermore, host cells lacking IFNAR1 are resistant to IAV-induced cell death, as ZBP1 upregulation does not occur in these cells [4].

Sensing of IAV by the ZBP1 Zα domain allows ZBP1 to interact with RIPK3 and other associated molecules, including caspase-8, caspase-6, and RIPK1, to form the ZBP1-PANoptosome and execute PANoptosis [9,11,41] (Fig. 1B). The ZBP1-PANoptosome also

recruits the NLRP3 inflammasome as an integral component, leading to inflammasome activation to drive IL-1β and IL-18 production. However, NLRP3 and caspase-1 are both dispensable for IAV-induced cell death at both early and late timepoints, as ZBP1-dependent activation of caspase-8, caspase-3, caspase-7, RIPKs, and MLKL drive the cell death in the absence of inflammasome components [4] (Fig. 2). This suggests that inflammasomes can act as integral components of PANoptosomes to drive inflammatory cytokine production, but that the inflammasomes are not essential for the IAV-induced cell death due to functional redundancies.

Most studies have shown that ZBP1 and its Zα domain are crucial for providing host defense against IAV infection [10,13,42,43], while a few studies found that *Zbp1*$^{-/-}$ and wild type (WT) mice are similarly susceptible to IAV infection [10,43]; the discrepancies in these outcomes are likely due to differences in the dose or route of administration used to challenge the mice. While ZBP1-dependent necroptosis was initially suggested to drive IAV-induced cell death to provide host protection, MLKL-deficient cells undergo cell death with similar sensitivity compared with wild type (WT) cells [4], and *Mlkl*$^{-/-}$ mice and WT mice are similarly susceptible to IAV infection [10,44–47], suggesting that ZBP1-mediated activation of other cell death mediators plays a compensatory role. In line with this concept, WT mice and mice expressing an inactive form of caspase-8, which are defective in the activation of apoptotic executioners, are similarly susceptible to IAV infection [45]. Furthermore, mice lacking NLRP3, which are defective in GSDMD-mediated cell death, are also susceptible to IAV infection [28,29,48]. Together, these studies suggest that ZBP1-mediated PANoptosis, rather than necroptosis, apoptosis, or pyroptosis individually, is critical for host protection against IAV infection.

ZBP1-mediated inflammasome activation and PANoptosis in infection—beyond IAV

In addition to IAV infection, ZBP1 has been implicated in inflammasome activation and PANoptosis during other infectious diseases. As a cytosolic nucleic acid sensor, the function of ZBP1 has largely been studied during infection with viruses such as coronaviruses, HSV1, cytomegalovirus (CMV), vaccinia (VACV), and IAV, as discussed above. There are limited studies on the role of ZBP1 during infection with bacteria and fungi.

Coronaviruses:

In the context of SARS-CoV-2 infection, increased TNF and IFN-γ levels in the pathogenic cytokine storm lead to PANoptosis through the STAT1-IRF1 signaling axis [3,8]. Loss of ZBP1 improves survival during lethal TNF and IFN-γ challenge in mice; however, the mortality rate is similar in WT and *Zbp1*$^{-/-}$ mice in response to TNF alone, suggesting that ZBP1 contributes to IFN-γ–induced inflammatory responses [49]. Furthermore, patients with progressive COVID-19, who succumb to infection, have increased *ZBP1* expression in their immune cells compared with patients with stable COVID-19, who do not require hospitalization [13]. These findings suggest that there may be a pathological role for ZBP1 in driving COVID-19 severity. SARS-CoV-2 infection is known to induce high levels of IFN in patients with severe disease [50], which could cause the increase in *ZBP1* expression.

Indeed, deletion of ZBP1 substantially reduces PANoptosis, cytokine storm, and lethality in mice infected with a murine coronavirus, MHV, following type I IFN treatment [13], providing an additional mechanistic link between ZBP1 and the pathogenesis of coronavirus infections.

HSV1:

The role of ZBP1 in cell death was first identified in HSV1 infection due to the presence of a RHIM-containing protein ICP6 in this virus [51]. ICP6 promotes cell death in murine cells but inhibits it in human cells [52–55], indicating species-specific functions. However, HSV1 expressing ICP6 carrying a mutated RHIM triggers virus-induced ZBP1-dependent cell death in both mouse and human cells [56]. In macrophages, HSV1 induces formation of the AIM2-PANoptosome, which is coordinated by the Zα domain of ZBP1 and Pyrin [12]. The AIM2 inflammasome is also an integral component of this PANoptosome [12]. Mice lacking both ZBP1 and Pyrin mimic *Aim2*$^{-/-}$ mice in terms of their increase in viral titer compared with WT mice in response to HSV1 infection [12], suggesting that ZBP1 and Pyrin co-operatively mediate protective AIM2 immune responses during HSV1 infection.

CMV:

CMV inhibits cell death in both human and mouse cells [57,58]. Murine CMV (MCMV) expresses M45, a RHIM-containing protein that competes with RIPK3 for binding to ZBP1 to prevent the formation of a ZBP1-RIPK3 complex and suppress cell death. During infection with MCMV that carries M45 with a mutated RHIM domain (MCMV^{mM45}), ZBP1 and RIPK3 do form a complex [59], providing further evidence that this complex is the target of the M45 protein. Footpad inoculation of WT MCMV, but not MCMV^{mM45}, induces footpad swelling in mice. However, MCMV^{mM45} infection can cause footpad swelling in *Zbp1*$^{-/-}$ mice, demonstrating the RHIM-dependent pathogenesis of MCMV infection [59]. When the M45-RHIM is replaced by ICP6-RHIM, the mutated M45 gains the ability to induce cell death in mouse cells [52], suggesting that these different RHIM domains perform distinct functions and can either promote or inhibit cell death. The role for the full ZBP1-PANoptosome requires further study in MCMV infection.

VACV:

VACV is thought to produce Z-nucleic acids in infected cells [60]. Unlike HSV1 and MCMV, which express RHIM-containing proteins, VACV and variola express a Zα domain-containing protein, E3L. Absence of E3L leads to the accumulation of Z-nucleic acids in infected cells, as E3L sequesters Z-nucleic acids that accumulate during infection and blocks ZBP1-mediated cell death [61,62]. Indeed, VACV expressing a Zα-deficient E3L protein ($Zα_{E3L}$) induces robust cell death in IFN-β–primed cells [62]. $Zα_{E3L}$ has structural similarities to the Zα domains of ZBP1. In fact, $Zα_{ZBP1}$ can functionally replace $Zα_{E3L}$ without affecting the viral pathogenicity [62,63]. Therefore, it is possible that $Zα_{E3L}$ antagonizes the function of ZBP1 via competition for binding to Z-nucleic acids during infection. Whether this affects the formation of the ZBP1-PANoptosome remains unclear.

Other viruses:

Similar to MCMV and HSV1, varicella-zoster virus encodes a protein containing a RHIM that forms a complex with ZBP1 to inhibit cell death, the open reading frame 20 (ORF20) protein [64]. In the context of Zika virus (ZIKV), *Zbp1*$^{-/-}$ mice experience increased paralysis upon peripheral infection and enhanced mortality upon intracranial infection [65]. Moreover, *Zbp1*$^{-/-}$ neuronal cultures show enhanced replication of ZIKV, suggesting that ZBP1 activation contributes to cell-intrinsic restriction of ZIKV replication. The anti-ZIKV activity of ZBP1 is cell death-independent and instead depends on altered cellular metabolism via upregulation of IRG1 and production of the metabolite itaconate to suppress viral genome replication [65]. In addition, *Zbp1*$^{-/-}$ mice show higher morbidity and mortality compared with WT mice upon infection with lethal and non-lethal West Nile virus (WNV), which could be due to an inability of *Zbp1*$^{-/-}$ mice to clear the virus. Indeed, *Zbp1*$^{-/-}$ neuronal cultures produce higher virus titers following infection with WNV [66].

Bacterial and fungal infection—While little is known about ZBP1 in bacterial and fungal infections, recent studies have identified mechanistic connections. ZBP1, along with Pyrin, has been implicated in AIM2-PANoptosome formation during *F. novicida* infection. Indeed, mice lacking both ZBP1 and Pyrin have increased bacterial titers similar to those observed in *Aim2*$^{-/-}$ mice [12], suggesting a role for ZBP1 in PANoptosis and immune responses against *F. novicida* infection for host defense [12]. Similarly, *Zbp1*$^{-/-}$ mouse macrophages show reduced PANoptosis during *Candida albicans* and *Aspergillus fumigatus* infections [39], suggesting that ZBP1 plays a role in mediating immune responses during fungal infection as well. However, the ligands that ZBP1 senses during bacterial and fungal infection are unknown. Since the ZBP1 Zα domain contributes to the cell death induced by fungal infections [39], it is possible that ZBP1 could sense extracellular nucleic acids released by bacteria or fungi during biofilm formation [67,68].

Summary and future directions

ZBP1-mediated inflammasome activation and PANoptosis is increasingly implicated in host defense, as well as development, inflammatory diseases, and cancers. Triggers that mimic the cellular conditions found during infection, such as treatment with the combination of IFN with a nuclear export inhibitor (NEI), also activate ZBP1-mediated PANoptosis [5]. Mechanistically, this combination upregulates the expression of ZBP1 and sequesters the key ZBP1 suppressor protein ADAR1 in the nucleus, unleashing ZBP1 in the cytosol to induce PANoptosis [5]. Therapeutically, this regulatory mechanism can be targeted for the treatment of cancer; in a murine model, treatment with IFN and the NEI KPT-330 regresses tumors in a ZBP1-dependent manner [5]. Following the initial discovery of a regulatory connection between ADAR1 and ZBP1, other studies subsequently confirmed ADAR1's suppression of ZBP1-mediated cell death in several cell types and disease models [14–17], further highlighting the importance of ZBP1 in cell death and PANoptosis in infection and beyond.

Our current understanding of PANoptosomes suggests that while inflammasomes can be integral components of these complexes that are critical for the production of the proinflammatory cytokines IL-1β and IL-18, in many physiological contexts, the

inflammasome is dispensable for cell death due to functional redundancies among PANoptosome molecules. Additionally, while PANoptosomes contain molecules that can be found in other cell death complexes, they are distinct in their multifaceted nature and in their ability to integrate these diverse molecules. As a prototypical example of the distinction between PANoptosis and other cell death pathways in host defense, IAV-induced lethality in murine models is not affected by deletion of NLRP3 or MLKL or in mice expressing an inactive form of caspase-8 [10,28,29,44–46,48], while many groups have reported that loss of ZBP1 or its Zα domain increase lethality [10,13,42,43]. These findings suggest a central role for the ZBP1-PANoptosome in host defense. As PANoptosis and its molecular components have been widely implicated across the disease spectrum, future research to understand the molecular mechanisms of PANoptosis will continue to identify therapeutic targets and strategies that can be applied across infections, as well as inflammatory diseases, cancers, and beyond.

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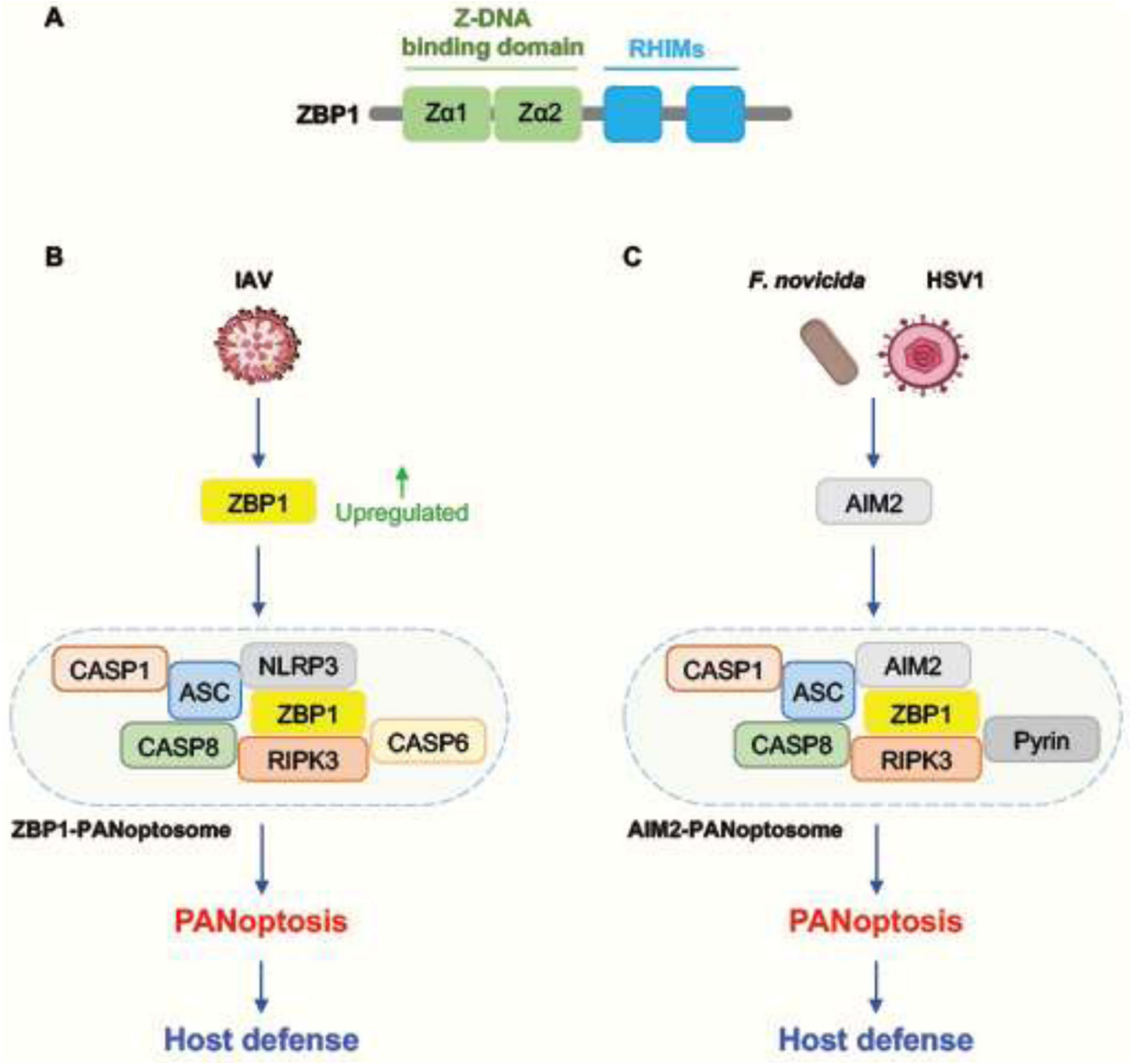

Figure 1: Assembly of ZBP1- and AIM2-PANoptosomses.

A) ZBP1 contains two Zα domains, two receptor-interacting protein homotypic interaction motif (RHIM) domains, and an undefined C-terminal domain. B) Type I interferon (IFN) induced during influenza A virus (IAV) infection upregulates the expression of ZBP1. Sensing of IAV by the Zα domain of ZBP1 results in its interaction with RIPK3 and the recruitment of several other molecules, including components of the NLRP3 inflammasome (NLRP3, ASC, and caspase-1 [CASP1]) and caspase-8 (CASP8) into the complex to form the ZBP1-PANoptosome. Caspase-6 (CASP6) is also part of the ZBP1-PANoptosome and facilitates the interaction of ZBP1 with RIPK3. Upon formation, the ZBP1-PANoptosome executes PANoptosis to drive host defense against IAV infection. C) AIM2 senses dsDNA from *Francisella novicida* or herpes simplex virus 1 (HSV1) to assemble the AIM2-PANoptosome complex consisting of the AIM2 inflammasome (AIM2, ASC, and CASP1),

as well as ZBP1, Pyrin, CASP8, and RIPK3. The AIM2-PANoptosome drives PANoptosis to promote host defense during these infections.

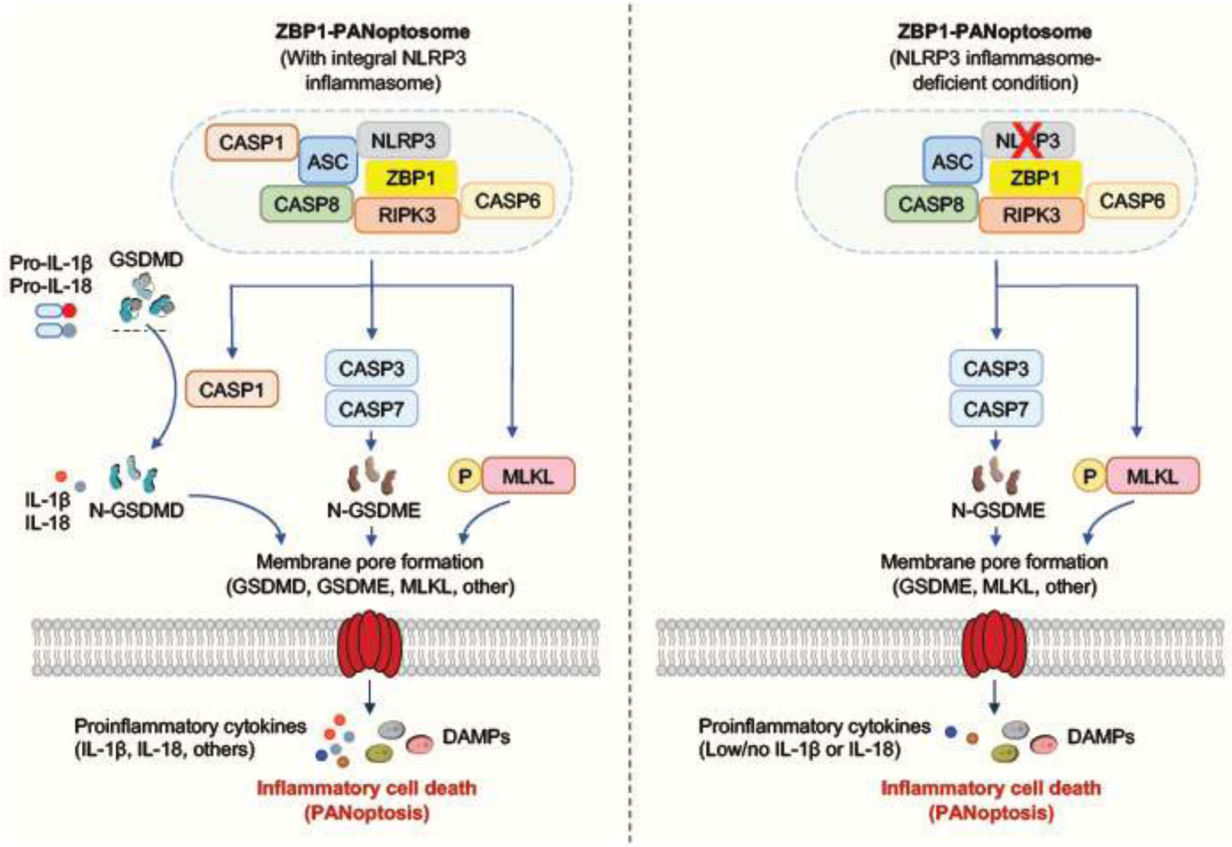

Figure 2: NLRP3 inflammasome is an integral component of the ZBP1-PANoptosome to drive IL-1β and IL-18 production but not cell death.

Formation of the ZBP1-PANoptosome involves the recruitment of the NLRP3 inflammasome (NLRP3, ASC, and caspase-1 [CASP1]), along with RIPK3, caspase-8 (CASP8), and caspase-6 (CASP6). Downstream of complex formation, CASP1 cleaves pro–IL-1β and pro–IL-18 to generate their mature forms. CASP1 also cleaves gasdermin D (GSDMD) to release its pore-forming N-terminal domain (N-GSDMD). In addition, caspase-3 (CASP3) and caspase-7 (CASP7) are activated and can cleave gasdermin E (GSDME) to release its pore-forming N-terminal domain (N-GSDME), and MLKL becomes phosphorylated. These events lead to membrane pore formation and the release of IL-1β and IL-18, as well as other cytokines, and damage-associated molecular patterns (DAMPs). When the NLRP3 inflammasome is missing from the ZBP1-PANoptosome, as can occur when NLRP3 or caspase-1 are inhibited or deleted, IL-1β and IL-18 maturation does not occur, but cell death proceeds due to functional redundancies in the PANoptosome molecules.