Trends in Immunology

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Opinion

Inflammasome diversity: exploring novel frontiers in the innate immune response

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Pathogens elicit complex mammalian immune responses by activating multiple sensors within inflammasomes, which recognize diverse pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). This simultaneous activation induces the formation of protein complexes referred to as multiple inflammasomes, that orchestrate a spectrum of programmed cell death pathways, including pyroptosis, apoptosis, and necroptosis. This concept is crucial for comprehending the complexity of the innate immune system's response to diverse pathogens and its implications for various diseases. Novel contributions here include emphasizing simultaneous sensor activation by pathogens, proposing the existence of multiple inflammasome complexes, and advocating for further exploration of their structural basis. Understanding these mechanisms may offer insights into disease pathogenesis, paving the way for potential therapeutic interventions targeting inflammasome-mediated immune responses.

Emergence and significance of multiple sensor inflammasomes

The mammalian immune system involves various defense mechanisms that protect organisms from pathogens, among which the innate immune response represents a nonspecific but rapid mechanism that swiftly eliminates pathogens. Innate immune responses are activated through the recognition of **PAMPs** (see Glossary) or **DAMPs** produced by viruses, bacteria, or other pathogens, aiding in the differentiation between self- and non-self-antigens. The recognition of PAMPs/DAMPs stimulates the innate immune response and initiates the release of proinflammatory cytokines, leading to **programmed cell death** in pathogen-infected cells. Hence, innate immunity responds to external pathogens through various sensing mechanisms (Box 1).

Conventionally, programmed cell death is thought to be triggered by sensors responding to a single PAMP/DAMP. Indeed, several studies have elucidated the mechanisms underlying the activation of a specific **inflammasome** sensor by a single PAMP/DAMP and activation of inflammasome-induced programmed cell death [1–6]. However, pathogens often carry multiple PAMPs/DAMPs that can regulate various programmed cell death pathways through multiple sensors (Figure 1) [7–13].

Live pathogens activate various PAMPs/DAMPs, leading to the formation of multiple inflammasome complexes, generally shown in mouse models and mouse or human cell lines. For instance, the absent in melanoma 2 (AIM2) and nucleotide oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasomes become activated during *Aspergillus* infection [9] and contribute to inflammasome-dependent cytokine release in response to *Plasmodium* infection [11]. Meanwhile, **NLR** family CARD domain-containing protein 4 (NLRC4) and NLRP3 mediate *Salmonella enterica* serovar Typhimurium clearance [7,8]. AIM2, Pyrin, and Z-DNA-binding protein 1 (ZBP1) form multiple sensor inflammasomes in single cells to drive inflammatory cell death and host

Highlights

Alone, pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) elicit the activation of single-sensor inflammasomes, activating caspases. Live pathogens harbor multiple PAMPs/DAMPs that may prompt recognition by multiple-sensor inflammasomes.

The synchronized detection and activation in response to diverse PAMPs and DAMPs are regulated by collaboration among multiple inflammasome sensors, instigating cell death pathways and bolstering the host's immune response.

Inflammasomes have an indispensable role in integrating various cell death pathways, showcasing their adaptability in modulating immune responses and influencing outcomes following pathogen infection.

Elucidating the nuanced mechanisms through which inflammasome sensors can orchestrate immune responses and programmed cell death pathways presents promising avenues for developing targeted therapeutics against infectious diseases and inflammatory disorders.

Significance box

Insights into the orchestration of multiple sensor inflammasomes highlight the intricate mechanisms of mammalian immunity. Understanding how diverse pathogens activate multiple sensors simultaneously can enhance our comprehension of the innate immune system's ability to mount tailored responses against various threats. This advanced understanding of immune system dynamics may provide avenues for developing targeted therapeutic interventions for infectious and autoimmune diseases, as well as cancers.

Box 1. Pathogen sensing by the innate immune system

The mammalian innate immune system involves a limited number of predetermined receptor-reporter pathways [1–6]. To overcome this limitation during the recognition process, sensors have evolved to detect multiple PAMPs, DAMPs, and missing self-antigens [1–15].

The Toll-like receptor family makes important contributions to innate immune responses in mammals [51]. However, other receptors, including retinoic acid-inducible gene-I (RIG-I)-like receptors and C-type lectin-like receptors, also participate in these responses. These receptors recognize microbial components, such as LPS, flagellin, modified self-antigens, and lipoteichoic acid, activating the innate immune response [52–56].

The nucleotide oligomerization domain (NOD)-like receptors (NLRs) represent another family of prominent sensors involved in innate immunity. NLRs include a common nucleotide-binding domain (NACHT) and sense PAMPs through variable N-terminal interaction domains containing a caspase recruitment domain (CARD), pyrin domain (PYD), acidic domain, and baculovirus inhibitor repeats. On the basis of the type of N-terminal interaction domains, the NLR family is classified into subfamilies NLRA, NLRB, NLRC, NLRP, and NLRX [57]. NLRP3 – one of the most representative members of the NLR family – is activated by stimuli, such as silica and K^+ efflux, and recruits apoptosis-associated speck-like proteins containing CARD (ASC) and activating caspase-1. It acts as a trigger, inducing inflammatory responses by activating and secreting proinflammatory cytokines (IL-1β and IL-18).

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defense in response to herpes simplex virus 1 (HSV1) or *Francisella novicida* infection [10] (see Clinician's corner). Moreover, NLRP3, AIM2, NLRC4, and Pyrin collectively induce inflammasome activation and assembly, simultaneously regulating multiple programmed cell death

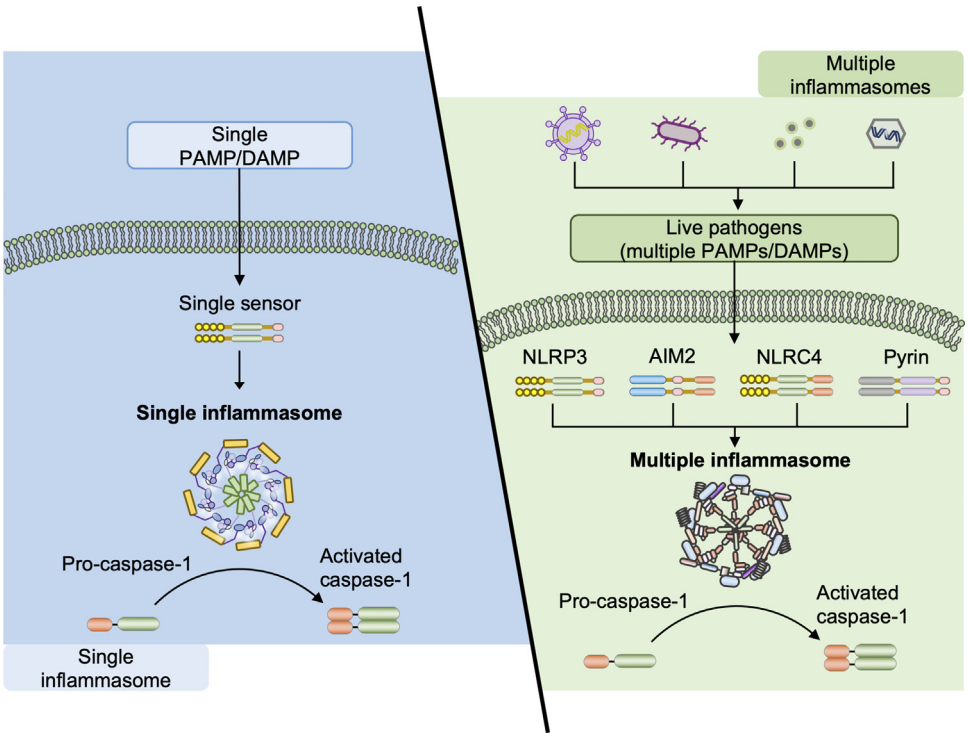

Figure 1. Model and comparative depiction of single-sensor inflammasomes versus multiple-sensor inflammasomes. Single pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) such as lipopolysaccharide, combined with ATP double-stranded DNA, flagellin, and lipoteichoic acid, can trigger a response from single-sensor inflammasomes such as nucleotide oligomerization domain (NOD)-like receptor protein 3 (NLRP3), absent in melanoma 2 (AIM2), NLR family CARD domain-containing protein 4 (NLRC4), or NLRP6, stimulating inflammasome assembly and subsequent caspase-1 activation [1–6]. However, live pathogens such as *Salmonella*, *Plasmodium*, herpes simplex virus 1 (HSV1), or *Francisella novicida*, may carry multiple PAMPs/DAMPs, which are sensed by purported multiple-sensor inflammasomes (e.g., NLRC4 + NLRP3, AIM2 + NLRP3, or Pyrin + AIM2 + ZBP1) [7–11]. These multiple-sensor inflammasomes presumably trigger the assembly of multiple inflammasome complexes, culminating in caspase-1 activation.

pathways [13]. A study on inflammatory programmed cell death hypothesized that understanding multiple inflammasomes is crucial when multiple PAMPs/DAMPs act simultaneously [13]. This stems from the observation that cell death becomes more robust when multiple inflammasomes are activated compared with single inflammasome activation [13]. Furthermore, its significance is underscored by the fact that pathogens often induce multiple PAMPs/DAMPs simultaneously. Accordingly, here we propose that attention should be directed toward the putative formation of multiple-sensor rather than single-sensor inflammasomes when evaluating innate immunity and programmed cell death.

Inflammasome sensing of single PAMPs/DAMPs

Various inflammasomes are associated with different cascades for sensing pathogens and triggering the inflammatory response. For instance, NLRP3 senses ion flux or reactive oxygen stress caused by K^+ efflux, reactive oxygen species (ROS) formation, or lysosomal rupture, leading to the activation of caspase-1 and secretion of IL-1β and IL-18 in primary murine bone marrow-derived macrophages (BMDMs) [1,2]. The NLRP3 complex comprises the PYD, NACHT, and C-terminal LRR domains and responds to a wide range of stimuli. In Gram-negative bacteria, the release of lipopolysaccharide (LPS) into the cytosolic space and subsequent activation of caspase-11 induces NLRP3 activation via the noncanonical pathway, leading to IL-1β secretion; thus, an inflammatory response is triggered, as shown in primary murine BMDMs [3].

Although **canonical** and **noncanonical inflammasome activation** induce **pyroptosis** and trigger IL-1β and IL-18 secretion, the processes leading to these outcomes are distinct. Canonical inflammasome activation involves inflammasomes containing NLRP1, NLRP3, NLRC4, NLRP6, NLRP7, and NLRP9b, ultimately leading to the activation of caspase-1. Meanwhile, the noncanonical pathway differs in that caspase-4 and caspase-5 act as sensors and effector molecules in humans, whereas caspase-11 serves this role in mice, resulting in pyroptosis [4,14]. Another representative NLR family inflammasome sensor is NLRC4, which collaborates with NLR family apoptosis inhibitory protein (NAIP)5 and NAIP6 to detect intracellular flagellin. For example, following *S. enterica* Typhimurium flagellin sensing, NAIP5 and NAIP6 transmit activating signals to NLRC4 in primary murine BMDMs [5]. NLRC4 is an adaptor that initiates inflammasome formation and induces cell death and immune responses. Such collaboration is a crucial mechanism promoting rapid and effective immune responses following the bacterial infection of cells [5].

NLRP6 is another prominent inflammasome sensor that senses lipoteichoic acid (LTA) and initiates an immune response following Gram-positive bacterial infection [6]. NLRP12 – an NLR family sensor – reportedly induces programmed cell death in response to the hemolytic effect, which causes red blood cell (RBC) lysis and heme release. However, unlike other NLR sensors activated by a single PAMP/DAMP, NLRP12 is not activated by heme alone in primary murine BMDMs or human peripheral blood mononuclear cells (PBMCs). Rather, it causes inflammatory cell death by simultaneously recognizing heme and PAMPs, such as Pam3CSK4, LPS, or other similar molecules [15]. The discovery of NLRP12's unique activation mechanism, requiring simultaneous recognition of heme and PAMPs, underscores the complexity of inflammasome sensor responses.

A prominent example of a non-NLR family inflammasome sensor is AIM2, which belongs to the AIM2-like receptor (ALR) family of inflammasome sensors and is induced by type 1 interferons. In contrast to the NLR sensors with common NATCH and LRR domains, AIM2 possesses CARD and PYD domains and is characterized by an HIN domain. AIM2 detects the double-stranded DNA of various pathogens, including *F. novicida* and HSV1, leading to inflammasome

Glossary

Apoptosis: routine and highly regulated mechanism of programmed cell death; it includes the breakdown of cell content that is packaged into small membrane vesicles.
Canonical inflammasome activation: associated with caspase-1 activation via NLRP1, NLRP3, NLRC4, NLRP6, NLRP7, and NLRP9b, resulting in pyroptosis.
DAMPs: endogenous molecules released by cells in response to various stressors, such as trauma, ischemia, and tissue damage, initiating and perpetuating immune responses.
Inflammasome: cytosolic molecular factory that orchestrates the processing of inflammatory responses within the cell.
Necroptosis: programmed inflammatory cell death pathway driven by phosphorylated MLKL and membrane pore formation.
NLR: large family of pattern recognition receptors that includes three main subtypes, NODs, NLRPs, and NLRCs.
Noncanonical inflammasome activation: associated with caspases 4, 5, and 11, resulting in pyroptosis.
PAMPs: pathogen-associated molecular patterns are molecular signatures of infection.
Programmed cell death: tightly regulated suicide process by which cells that are no longer needed or are a threat to the organism are destroyed.
Pyroptosis: inflammatory mode of regulated cell death that accompanies an increase in active caspase-1 and gasdermin D.

activation in primary murine BMDMs [10]. Similar to the functions of NLR sensors, AIM2 triggers cell death and secretion of IL-1β and IL-18.

ZBP1, also known as the DNA-dependent activator of interferon-regulatory factor (DAI), is another prominent non-NLR family inflammasome sensor. It contains a Z-domain and a receptor-interacting protein homotypic interaction motif that binds to Z-DNA and Z-RNA [12,16–18]. This cytoplasmic sensor detects viral nucleic acids and is crucial for recognition and response to viral pathogens, such as the influenza A virus (IAV). ZBP1 binds to viral RNA and triggers a signaling cascade that activates inflammatory cytokines, including type I interferons, by forming inflammasomes in primary murine BMDMs [12,19,20]. Hence, ZBP1 plays a key role as a non-NLR family inflammasome sensor, detecting viral nucleic acids and initiating a signaling cascade that leads to the activation of inflammatory cytokines.

Hence, inflammasomes have single sensors that specifically respond to a single ligand. For example, NLRC4, NLRP1b, NLRP12, and NLRP6 respond to bacterial flagellin [21], metalloproteinase anthrax lethal factor [22], HIF-1 alpha [23], and LTA [6], respectively. In contrast, the un-ligand sensor NLRP3 is activated by K^+ efflux and ROS, triggering immune responses through the activation of a single inflammasome after sensing a single stimulant in primary murine BMDMs (Figure 2) [1,24]. Collectively these data demonstrate the intricate specificity between single inflammasomes and single PAMPs/DAMPs, helping elucidate the mechanisms by which host cells mount tailored immune responses to distinct pathogenic signals.

Diverse functions of multiple sensor inflammasomes in the innate immune response

A single ligand triggers a specific sensing mechanism leading to inflammasome activation, which induces downstream programmed cell death and IL-1β and IL-18 secretion [1–6]. However, recent studies have revealed that during infection with a pathogen, multiple PAMPs/DAMPs are detected by multiple host inflammasome sensors that respond simultaneously, forming a multiple inflammasome complex (Figure 3) [7–11]. These complexes regulate various programmed cell death mechanisms, including pyroptosis, **apoptosis**, and **necroptosis**, employed by primary murine BMDMs to defend against infection [10,13,25,26]. This simultaneous activation of multiple inflammasome sensors by live pathogens underscores a complex orchestration of immune responses involving diverse cell death mechanisms and representing a crucial aspect of the innate immune response.

Previously, these programmed cell death mechanisms were considered to occur independently from a single PAMP/DAMP-stimulated inflammasome, exhibiting no interconnection. Investigation of these relationships provided important insights regarding the specific pathways triggered by individual ligands, advancing the understanding of host–pathogen interactions and immune responses. However, this single-sensor–single-inflammasome perspective does not align with physiological pathogen invasion scenarios, which typically involve multiple PAMPs/DAMPs. Hence, the significance of multiple inflammasomes has rapidly garnered considerable research attention. Recently, ZBP1 was identified as a master regulator of apoptosis, pyroptosis, and necroptosis, becoming activated during viral infection in primary murine BMDMs [10,26,27]. In our view, this further validates the hypothesis of mutual interactions among these three cell death mechanisms [28].

Each cell death mechanism possesses independent sensing mechanisms and is activated through distinct cascades; hence, crosstalk between each programmed cell death pathway is not intuitive. For example, within the peritoneal exudate macrophages of C3H/HeNHsd mice, research on inflammasome sensors is centered primarily on the induction of cell death through

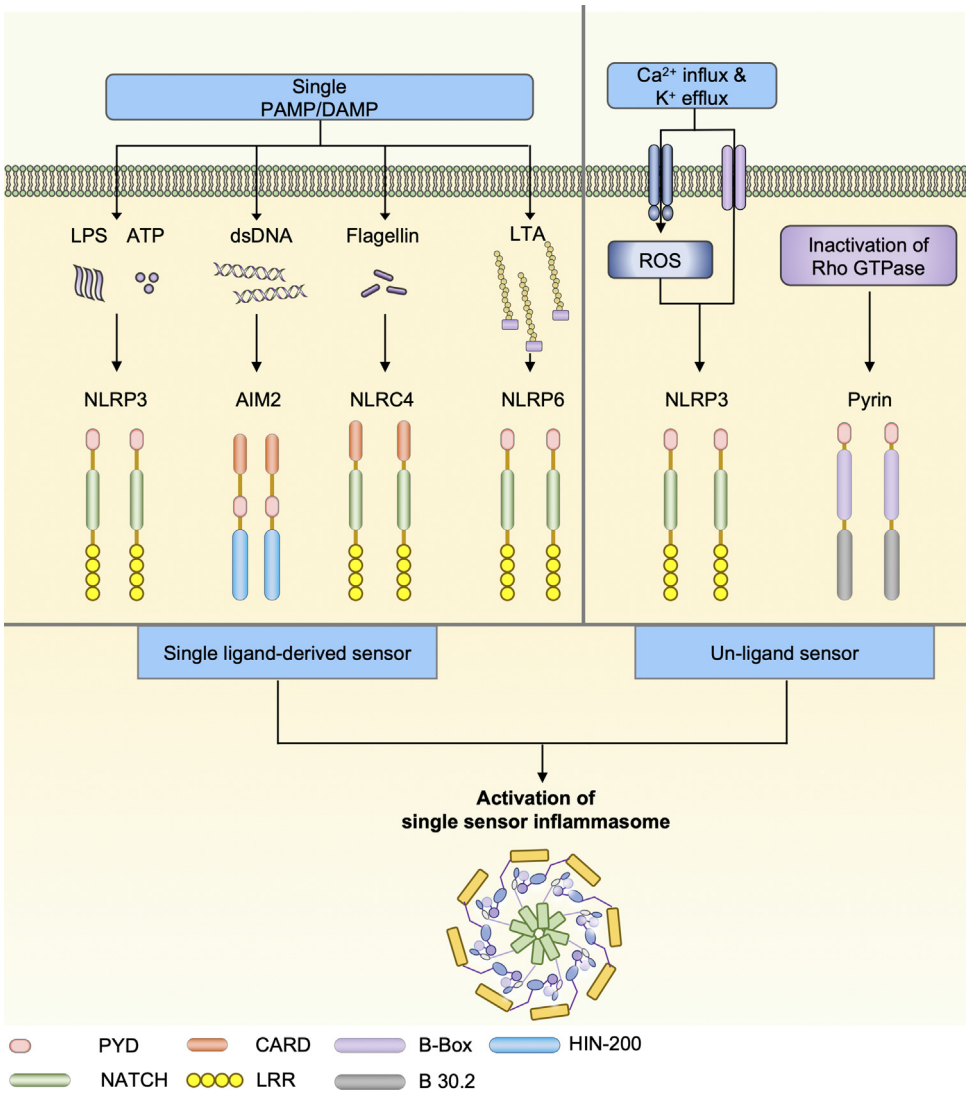

Figure 2. Pathogen-associated molecular pattern (PAMP)/damage-associated molecular pattern (DAMP)-derived single-inflammasome sensors. The activation of single-sensor inflammasomes is categorized into ligand-derived sensors and un-ligand sensors. Nucleotide oligomerization domain (NOD)-like receptor protein 3 (NLRP3) detects lipopolysaccharide and ATP [1,2,12], whereas absent in melanoma 2 (AIM2) senses double-stranded DNA (dsDNA) in the cytosolic space [32,58]. Bacterial flagellin and lipoteichoic acid (LTA) are recognized by NLR family CARD domain-containing protein 4 (NLRC4) [5,21] and NLRP6 [6,59], respectively (i.e., ligand-derived sensors). NLRP3 and pyrin respond to un-ligand stimuli such as Ca^{2+} influx/K^{+} efflux or inactivation of Rho-GTPase activity [1,24,60]. Subsequently, the single-inflammasome sensors recruit apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1, forming a single-sensor inflammasome. This complex triggers the activation of caspase-1 and an inflammatory response, culminating in the release of activated IL-1β and IL-18. NLRP3 and NLRP6 contain LRR, NACHT, and PYD domains; AIM2 comprises hematopoietic interferon-inducible nuclear proteins with a 200-amino acid repeat (HIN-200), PYD, and caspase recruitment domain (CARD) domains; NLRC4 contains LRR, NACHT, and CARD domains; pyrin is characterized by B 30.2, B-Box, and PYD domains.

pyroptosis, further supporting the nonintuitive nature of these outcomes [29]. However, recent evidence increasingly supports the notion that inflammasomes may be responsible for orchestrating multiple cell death mechanisms in a compounded manner [30,31]. For instance, using *in vivo* (male Sprague-Dawley rats) and *in vitro* retinal neuronal models of ischemia/reperfusion

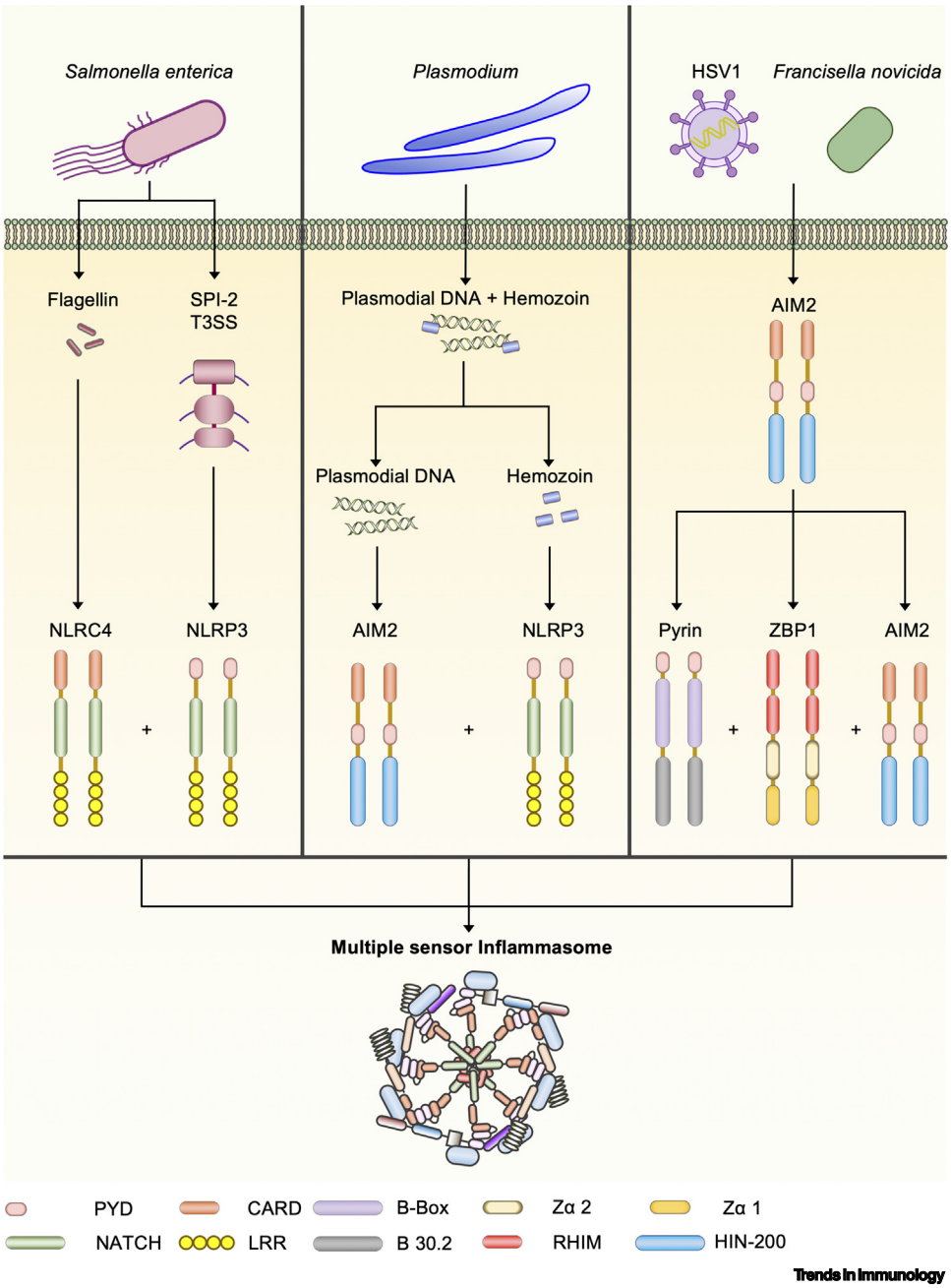

Figure 3. Activation of multiple-sensor inflammasomes in response to pathogens. The activation of multiple inflammasomes can occur in response to pathogens carrying various pathogen-associated molecular patterns (PAMPs)/damage-associated molecular patterns (DAMPs), sensed by distinct inflammasome sensors, resulting in the purported formation of multiple sensor inflammasomes. For instance, in *Salmonella enterica* infection, NLR family CARD domain-containing protein 4 (NLRC4) senses flagellin, whereas nucleotide oligomerization domain (NOD)-like receptor protein 3 (NLRP3) detects *Salmonella* pathogenicity island-2 (SPI-2) [7,8]. Plasmodial DNA and hemozoin of *Plasmodium* are sensed by absent in melanoma 2 (AIM2) and NLRP3, respectively [11]. Although the specific PAMPs/DAMPs of herpes simplex virus 1 (HSV1) and *Francisella novicida* that are sensed remain unclear, complex inflammasome sensors involving AIM2, ZBP1, and pyrin are regulated by AIM2 [10]. These multiple-sensor inflammasomes may initiate the assembly of putative multiple inflammasome complexes. Notably, in LRR, NACHT, and Pyrin (PYD) domains, AIM2 comprises

(Figure legend continued at the bottom of the next page.)

Table 1. Comparison of single-sensor inflammasome and multiple-sensor inflammasome[a,b]

	Sensor	Stimuli	Ligand	Un-ligand	Cytokine	Species/cell line	Refs
Single-sensor inflammasomes	NLRP3	IAV, SARS-CoV2	LPS, ATP	K^+ efflux, ROS	IL-1β, IL-18	Murine BMDMs	[1,2,12]
	AIM2	HSV, VACV	dsDNA	N/A		African green monkey: Vero cells Rabbit: skin cells Human: 293FT cells Murine: BMDMs	[32,58]
	NLRC4	*Salmonella*	Flagellin			Murine: BMDMs	[5,21]
	NLRP6	*Listeria monocytogenes*, *Staphylococcus aureus*	LTA				[6,59]
	Pyrin	Bacteria	N/A	Rho-A GTPase		Murine: Immortalized BMDMs Human: THP-1 monocytes	[60,63]
	NLRP1b	*Bacillus anthracis*	Lethal factor	N/A		Human: T cells, B cells, NK cells, monocytes	[64,65]
	NLRP11	Gram-negative bacteria	LPS			Murine: BMDMs	[66]
	NLRP12	*Yersinia pestis*	Heme			Murine: BMDMs	[15,34]
	MxA	IAV	Viral NP			Human: PL16T cells, NHBEs	[67,68]
Multiple-sensor inflammasomes	NLRC4 + NLRP3	*Salmonella*	PAMPs/DAMPs	N/A	IL-1β, IL-18, TNF- α, IL-12, IL-6, IL-10, etc.	Human: THP-1, monocytes, MDMs	[7,8]
	NLRP3 + AIM2	*Aspergillus*				Murine: BMDCs	[9]
	NLRP3 + NLRP12	*Y. pestis*				Murine: BMDMs	[34]
	ZBP1 + NLRP3	IAV				Murine: BMDMs	[12,36]
	AIM2 + Pyrin + ZBP1	HSV, *Francisella novicida*				Murine: BMDMs	[10,13]
	NLRP3 + AIM2 + NLRC4 + Pyrin	LPS + ATP, Poly(dA:dT), flagellin, and Tcd				Murine: BMDMs	[13]

[a]Each inflammasome and its sensor, stimuli, (un-)ligands, the cell line used in experiments, and the resulting elicited responses and cytokines are shown. Both ultimately induce pyroptosis and other programmed cell death pathways. Single-sensor inflammasomes refer to inflammasomes where one sensor interacts with one stimulus, particularly ligands or un-ligands, to trigger inflammasome activation. Conversely, multiple-sensor inflammasomes may involve multiple sensors acting in concert to sense various PAMPs/DAMPs present in stimuli, thereby triggering inflammasome activation. As a result, they release a variety of cytokines, including IL-1β, IL-18, tumor necrosis factor (TNF)-α, IL-6, and IL-12.
[b]Abbreviations: MDM, monocyte-derived macrophage; N/A, not applicable.

(I/R) injury, researchers reported that I/R induced morphological changes as well as gene and protein expression changes of molecules important in all three forms of cell death [30]. The notion suggests that multiple inflammasome sensors targeting the same pathogen simultaneously act to induce these complex forms of cell death, although this remains conjectural.

Regarding AIM2, this molecule acts as a principal regulating sensor of inflammatory cell death during *in vitro* HSV1 or *F. novicida* infection in dendritic, Vero, 293FT, or rabbit skin cells, as well as in primary murine BMDMs [32,33]. Specifically, AIM2 orchestrates the regulation of pyrin and ZBP1, forming an inflammasome complex that triggers a host protective mechanism leading

hematopoietic interferon-inducible nuclear proteins with a 200-amino acid repeat (HIN-200), PYD, and caspase recruitment domain (CARD) domains; NLRC4 contains LRR, NACHT, and CARD domains; ZBP1 contains Zα1, Zα2, and the RIP homotypic interaction motif (RHIM) domains; whereas pyrin is characterized by B 30.2, B-Box, and PYD domains.

to inflammatory cell death [10]. During this process, AIM2, pyrin, and ZBP1, accompanied by apoptosis-associated speck-like protein containing a CARD (ASC), caspase-1, caspase-8, receptor-interacting protein kinase (RIPK)3, RIPK1, and FAS-associated death domain (FADD), may collaboratively form an extensive multiple-inflammasome complex to concurrently govern various cell death mechanisms in primary murine BMDMs [10].

Additionally, NLRP3 may form multiple inflammasomes by interacting with other inflammasome sensors. For instance, during *Aspergillus fumigatus* infection of murine bone marrow-derived dendritic cells (BMDCs), AIM2 and NLRP3 concomitantly create a single cytoplasmic inflammasome; in this case, a multiple inflammasome is formed comprising ASC, caspase-1, and caspase-8 [9]. Furthermore, the role of NLRP3 and AIM2 in *Plasmodium* infection of primary murine BMDMs and human PBMCs has been reported; in this case, hemozoin is formed and encapsulates plasmodial DNA. Hemozoin and DNA simultaneously induce inflammation, leading to NLRP3 and AIM2 inflammasome activation, highlighting the simultaneous involvement of NLRP3 and AIM2 in the *Plasmodium* infection-specific immune response [11]. This further suggests an interplay between different inflammasome sensors in orchestrating host defense mechanisms against specific pathogens.

Moreover, the coactivation of NLRP3 with NLRC4 or NLRP12 represents another example of multiple inflammasome sensor-derived inflammatory cell death. During *Salmonella* infection, NLRP3 and NLRC4 cooperate in host protection mechanisms [7,8]. Comparably, the coactivation of NLRP12 and NLRP3 also encompasses a multiple sensing mechanism that releases cytokines IL-18 and IL-1β during *Yersinia pestis* infection of primary murine BMDMs [34].

As multiple inflammasome sensors simultaneously sense diverse PAMPs/DAMPs of pathogens and become coactivated [10,35], multiple caspases and RIPK proteins are recruited, concurrently modulating different inflammatory cell death mechanisms in primary murine BMDMs [10,36–39]. Hence, inflammasome-derived caspases and RIPK proteins engage in complex interplay, challenging conventional views of single-triggered pyroptosis. In fact, multiple inflammasome ligands reportedly lead to a higher rate of cell death than a single inflammasome sensing a single PAMP/DAMP [13]. This further supports the concept of simultaneous activation of multiple inflammasomes leading to enhanced inflammatory cell death compared with death associated with a single activated inflammasome. One of the most prominent examples of this concept is the purported NLRP3, AIM2, NLRC4, Pyrin, and ASC multiple inflammasome complex formed in primary murine BMDMs [13]. The formation of such complexes seems to be crucial for the comprehensive regulation of these cell death pathways and can also influence immune responses related to IAV and HSV1 infections in an RIPK3- and caspase-8-dependent manner in embryonic murine cells [10,40]. Hence, because live pathogens carry multiple PAMPs/DAMPs simultaneously, further research on multiple inflammasomes is essential to better dissect the physiological characteristics of these processes (Table 1).

Physiological relevance of inflammasomes

Inflammasomes can regulate pyroptosis and other programmed cell death pathways, making them crucial players in myriad diseases, including infectious and autoimmune diseases, metabolic disorders, and cancer [41–43]. On the basis of analysis of synovial fibroblasts, macrophages, and chondrocytes from patients in Chengdu city, *NLRP1* gene mutations causing upregulation or activation of the NLRP3 inflammasome have been associated with an increased risk of rheumatoid arthritis [42,44]. Furthermore, in patients with systemic lupus erythematosus (SLE), SNPs in the *NLRP1* and *ZBP1* genes, encoding inflammasome-associated proteins, are closely related to SLE pathogenesis [45,46]. Despite the absence of a direct correlation between

Clinician's corner

In the case of COVID-19, a potentially lethal cytokine storm is triggered by the host's immune response [61,62]. During the immune response necessary to initiate the cytokine storm, multiple-sensor inflammasomes are activated to recognize the virus [26]. With infections from *F. novicida*, herpes simplex virus 1, or *Y. pestis*, the immune response activates multiple-sensor inflammasomes [10,34]. When treated with a cocktail of PAMPs/DAMPs, including bacterial LPS with ATP, poly (dA:dT), and flagellin, a significant increase in multiple programmed cell death pathways occurs, along with elevated protein expression associated with cell death [13]. Thus, a detailed understanding of coactivated inflammasome sensors and the regulation of these responses might help reduce the occurrence of cytokine storms, potentially enhancing survival.

metabolic disorders and immune responses, recent research has focused on NLRP3 inflammasome activation in type 2 diabetes, identifying the upregulation of IL-1β and IL-18 secretion [41,47].

On the basis of analysis of tumor tissues excised from an ethnic Chinese individual's upper gastrointestinal endoscopy, tumor growth was associated with inflammatory responses and the interactions of various inflammasome sensors [43,48,49]. Additionally, analysis of mRNA sequencing metadata for skin cutaneous melanoma has suggested that *ZBP1* might contribute to modulating the tumor microenvironment [50]. The role of ZBP1 in other forms of programmed cell death suggests that physiological crosstalk may occur within programmed cell death pathways [50]. Given the close association between the pathogenesis of various diseases, the inflammatory response, and programmed cell death, a better understanding of multiple inflammasomes might serve as a foundation for developing approaches to prevent or treat various diseases.

Concluding remarks

Pathogens harbor multiple PAMPs/DAMPs, which trigger programmed cell death by activating multiple sensors within inflammasomes. The simultaneous activation of multiple sensors may lead to the formation of protein complexes. These complexes, referred to here as 'multiple inflammasomes', may have a comprehensive regulatory role in multiple programmed cell death pathways.

Although several studies have confirmed the existence of physiological multiple inflammasomes, there is a lack of structure-based evidence for multiple inflammasomes, rendering this model hypothetical. Moving forward, exploration of the domains and structure-based interactions facilitating the assembly of individual inflammasome sensors remains a central research focus (see Outstanding questions). Furthermore, the elucidation and characterization of multiple inflammasome complex mechanisms can advance our current understanding of the complex innate immune response to various diseases.

Outstanding questions

How do multiple inflammasome sensors collectively respond to the presence of diverse PAMPs and DAMPs, and what are the implications of their simultaneous activation in regulating programmed cell death pathways?

What are the key differences in the signaling cascades triggered by single-sensor inflammasomes compared with the presumed multiple-sensor inflammasomes, particularly in terms of the downstream effects on inflammatory responses and cell death mechanisms?

How does the coactivation of the presumed multiple-sensor inflammasomes, such as NLRP3, NLRC4, AIM2, and ZBP1, influence the regulation of various programmed cell death pathways, including pyroptosis, apoptosis, and necroptosis, during infections by different pathogens?

In what ways do multiple-sensor inflammasomes contribute to the pathogenesis of autoimmune diseases, metabolic disorders, and cancer, and how can a deeper understanding of multiple-inflammasome-mediated inflammatory responses inform the development of targeted therapeutic interventions for these conditions?

Author contributions

G.Y., Y.K.C., and S.L. conceived this study, prepared the manuscript, and critically revised and approved the final submitted version of the manuscript. All authors contributed to the manuscript and approved the submitted version.

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Declaration of interests

The authors declare that they have no competing interests.

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