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Mechanisms governing inflammasome activation, assembly and pyroptosis induction

Sannula Kesavardhana and **Thirumala-Devi Kanneganti**

Department of Immunology, St. Jude Children's Research Hospital, MS #351, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA

Correspondence to: T.-D. Kanneganti; E-mail: thirumala-devi.kanneganti@stjude.org

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REVIEW

Abstract

Inflammasomes are multimeric protein complexes that regulate inflammatory responses and pyroptotic cell death to exert host defense against microbes. Intracellular pattern-recognition receptors such as nucleotide-binding domain and leucine-rich repeat receptors (NLRs) and absent in melanoma 2 like receptors (ALRs) assemble the inflammasome complexes in response to pathogens and danger or altered-self signals in the cell. Inflammasome sensors, in association with an adaptor protein—apoptosis-associated speck-like protein containing a caspase-activation and -recruitment domain (ASC)—activate inflammatory caspase-1 to enable the release of inflammatory cytokines and induce cell death, conferring host defense against pathogens. Beyond infectious diseases, the importance of inflammasomes is implicated in a variety of clinical conditions such as auto-inflammatory diseases, neuro-degeneration and metabolic disorders and the development of cancers. Understanding inflammasome activation and its molecular regulation can unveil therapeutic targets for controlling inflammasome-mediated disorders. In this review, we describe recent advances in inflammasome biology and discuss its activation, structural insights into inflammasome assembly and mechanisms for the execution of pyroptosis.

Keywords: ASC, caspase-1, cell death, NLRs, pathogens

Introduction

Innate immunity is the first line of defense to recognize pathogen-associated molecular patterns (PAMPs) and discriminate between self and non-self patterns. The innate immune system utilizes a fixed set of germline-encoded pattern-recognition receptors (PRRs) to engage PAMPs and eliminate the pathogens from the host system (1). The components of the bacterial cell wall, proteins of secretory systems and microbial nucleic acids are some of the conventional PAMPs recognized by the PRRs. Emerging evidence indicates that PRRs can also sense host-associated physiological aberrations called danger-associated molecular patterns (DAMPs) (2). The DAMPs can vary from host cell damage to the release of cellular contents such as ATP, perturbations in potassium and uric acid crystals.

The PRRs are evolutionarily conserved in most living organisms including plants. These PRRs are localized to various cellular compartments to engage microbial PAMPs arriving through various routes of entry. On the basis of their subcellular localization, PRRs are classified as membrane-anchoring PRRs and intracellular PRRs. Membrane-anchored PRRs include Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) that are localized to the plasma membrane and endosomes to sense the PAMPs and DAMPs that are present in the extracellular compartment (3). Nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR) receptors (NLRs), retinoic acid inducible gene I (RIG-I)-like receptors (RLRs) and absent in melanoma 2 (AIM2)-like receptors (ALRs) are intracellular PRRs localized within the cytoplasm (4).

NLRs and ALRs are distinct from the other PRRs in their domain architecture and function (5, 6). After the discovery of the first member of the NLR family—nucleotide-binding oligomerization domain-containing protein 1 (NOD1), also called NLR-family caspase-activation and -recruitment domain (CARD)-containing 1 (NLRC1)—multiple NLRs have been identified in humans and mice (2, 6). Upon activation, some of the NLRs assemble macro-molecular protein complexes called inflammasomes in the cytosol (7). This inflammasome assembly consists of NLR or ALR and a bipartite protein called apoptosis-associated speck-like protein containing a caspase-activation and -recruitment domain (ASC) to initiate caspase-1 activation. The NLR-nucleated assembly of the ASC specks in the cytosol is considered as a hallmark for inflammasome assembly (4). The activation of caspase-1

results in the proteolytic processing of pro-inflammatory cytokines IL-1β and IL-18 and execution of a type of inflammatory cell death called pyroptosis (8, 9).

Inflammasomes are widely recognized for their role in pathogen recognition and host defense. However, constitutive or deregulated activation of inflammasomes is associated with multiple autoimmune and auto-inflammatory disorders and with the development of cancers, neuro-degeneration and metabolic diseases. Therefore, the activation of inflammasome and its functions need to be tightly regulated in order to avoid unintended host tissue damage while inducing the pathogen-killing inflammatory responses. Thus, a better understanding of inflammasome biology enables therapeutic modulation of its activity in multiple disorders. In this review, we report recent advances pertaining to inflammasome activation, its structural assembly and functional effector mechanisms.

Inflammasomes

The ability of NLRs to assemble into inflammasomes was first ascribed to NLR-family pyrin domain (PYD)-containing 1 (NLRP1) (10, 11). On the basis of the current literature, five PRRs are confirmed to assemble the inflammasome complex after sensing their respective stimuli: NLRP1, NLRP3, NLRC4, AIM2 and pyrin (9, 12). These inflammasomes are considered as canonical inflammasomes as they convert pro-caspase-1 into the catalytically active capsase-1. The canonical inflammasome activation is complemented by a non-canonical pathway, which promotes activation of capsase-11 (in mice) and caspase-4 and caspase-5 (in humans) (13). These caspases in turn activate NLRP3 inflammasomes or caspase-1 (14). Caspase-1 converts substrates such as pro-IL-1β, pro-IL-18 and gasdermin-D (a pyroptosis inducer) into their active forms upon inflammasome activation (9). In addition to these inflammasome-forming receptors, other innate receptors of NLR and ALR families such as NLRP2, NLRP6, NLRP7, NLRP12, IFI16 (interferon-γ-inducible protein 16) and RIG-I are also reported to activate caspase-1 (15–20). The activation mechanism of each inflammasome is described below.

The NLRP1 inflammasome

NLRP1 was the first NLR reported to form an inflammasome complex (7). Whereas humans have only one NLRP1 protein, the mouse NLRP1 gene is highly polymorphic and encodes multiple paralogs of the NLRP1: NLRP1a, 1b and 1c. Human NLRP1 contains an N-terminal PYD, a NOD, LRRs, the function to find domain (FIIND) and a CARD at the C-terminus, but mouse NLRP1 molecules lack a PYD (Fig. 1A).

The NLRP1b allele of mice is polymorphic and exists as five different variants. Two of these variants are susceptible for Anthrax lethal toxin from *Bacillus anthracis*. The Anthrax toxin consists of a pore-forming protective antigen and a lethal factor that enters through the pore created by protective antigen (Fig. 1A) (21). The lethal factor activates NLRP1b by cleaving at its N-terminal domain. New studies suggest that the human NLRP1 and mouse NLRP1a and NLRP1b are activated by proteolysis within an N-terminal linker region (22, 23). This proteolysis processing is reported as a general mechanism of NLRP1 activation (22).

Evolutionary analysis of NLRP1 in primates indicates that the N-terminal linker region has evolved under positive selection, suggesting pathogen-elicited selective pressure at this region. However, lethal factor cleaves both lethal toxin-sensitive and lethal toxin-resistant forms of NLRP1, indicating the requirement for an additional activation step after the cleavage (23, 24). In addition, further studies reported that FIIND in the human NLRP1 and the lethal toxin-resistant mouse NLRP1b undergoes auto-proteolytic processing that leads to the assembly of the inflammasome (25).

A very recent study suggests that gain-of-function mutations in the human NLRP1 gene enhance its self-oligomerization and lead to spontaneous inflammasome activation in primary keratinocytes (26). These gain-of-function mutations interfere with the auto-inhibitory function of the PYD and disrupt interactions of PYD and LRRs of NLRP1 to relieve it from its inactive conformation (26). All these studies indicate that the spatial separation of PYD and LRRs and cleavage of NLRP1 at its N-terminus might relieve its auto-inhibition and perhaps cleavage at FIIND may separate the N-terminus of the NLRP1 from ASC complexes once the ASC speck formation is initiated.

The NLRP3 inflammasome

NLRP3 (also known as cryopyrin and NALP3) is the best-studied inflammasome. Initial observations suggested that NLRP3 triggers activation of caspase-1 under *in vitro* conditions (27). Further, three independent studies demonstrated NLRP3-dependent caspase-1 activation under physiological conditions and its role in host defense and immune responses (28–30). Studies from our laboratory and others demonstrated a critical role for the NLRP3 inflammasome in host responses against influenza and fungal infections (31–35). The NLRP3 is activated in response to a diverse array of PAMPs derived from various microbes and DAMPs such as nucleic acids, uric acid crystals, asbestos, aluminium hydroxide and silica (4, 8, 36).

The activation of NLRP3 inflammasomes in macrophages requires two stimuli. The first signal, called priming, is provided by an inflammatory stimulus such as TLRs and TNF-α receptor (TNFR) that leads to NF-κB-mediated NLRP3 expression and post-translational modifications of NLRP3 (37). In addition, this NF-κB signaling induces the expression of pro-IL-1β and pro-IL18 cytokines, which are the important effectors of inflammasome activation. The second signal, called the activation signal, provided by PAMPs or DAMPs initiates inflammasome assembly to promote caspase-1-mediated IL-1β and IL-18 release and pyroptosis. However, human monocytes require only a priming signal for IL-1β secretion and perhaps this priming signal is sufficient to mediate the activation of caspase-1 (38). The sensing of a variety of stimuli by NLRP3 with no evidence of direct interaction of these stimuli with NLRP3 strongly indicates that NLRP3 may be activated by a common cellular mechanism, which converges the signals from different stimuli (Fig. 1B).

Different mechanisms have been proposed for NLRP3 activation, including potassium efflux, calcium influx, reactive oxygen species (ROS), oxidized mitochondrial DNA, translocation of cardiolipin from the inner mitochondrial

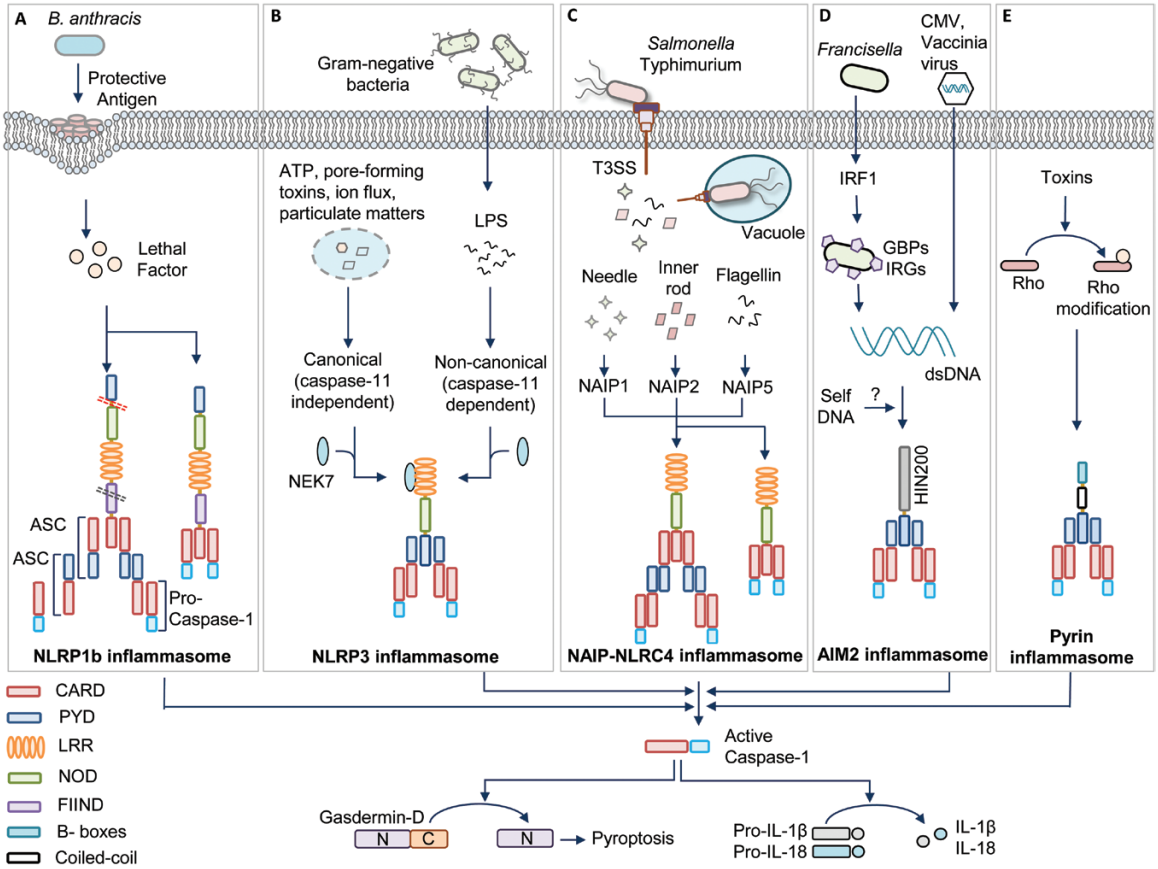

Fig. 1. Inflammasome activation mechanisms. (A) *Bacillus anthracis* toxin containing protective antigen and lethal factor activate the human (contains pyrin domain) NLRP1 inflammasome by inducing cleavage at the N-terminal linker region (red dotted lane). Auto-proteolysis at the FIIND domain (black dotted lane) is also required for NLRP1 activation. The NLRP1 inflammasome activates caspase-1 via ASC-dependent recruitment to the inflammasome complex or by direct association with caspase-1 through CARD–CARD interactions. (B) Various pathogen-derived ligands (PAMPs) and physiological aberrations (DAMPs) activate NLRP3. Assembly of the NLRP3 inflammasome is categorized into canonical (caspase-11 independent) and non-canonical (caspase-11 dependent) inflammasome activation. NEK7 is an upstream activator of NLRP3 inflammasome assembly. (C) Pathogenic bacteria such as *Salmonella enterica* subspecies Typhimurium operate a T3SS to release effector proteins into the cytosol. These pathogen-associated proteins are recognized by a family of NAIPs and they further recruit NLRC4 to assemble the inflammasome complex. NLRC4 enables ASC-dependent or direct CARD–CARD interaction-dependent casapse-1 activation. (D) The DNA viruses such as cytomegalovirus (CMV) and vaccinia virus and intracellular bacteria such as *Francisella* release DNA during infection for activating the AIM2 inflammasome. IRF1 induces the expression of GBPs and IRGs to liberate DNA for AIM2 recognition. (E) Pyrin detects the modifications of Rho induced by Rho-inactivating toxins. All these inflammasomes recruit an adaptor protein called ASC, which contains PYD and CARD. ASC further brings caspase-1 to the inflammasome complex by CARD–CARD interactions for its activation. Activated caspase-1 drives the cleavage of pro-inflammatory cytokines pro-IL-1β and pro-IL-18 and also the processing of gasdermin-D protein for executing pyroptosis.

membrane, phagosome destabilization, perturbation in cell volume and pore-formation mechanisms driven by the host or bacteria (4, 39). Several studies suggest that potassium efflux is associated with many of these stimuli and low intracellular potassium is sufficient to activate the NLRP3 inflammasome assembly. This indicates that potassium efflux can be a downstream intracellular convergence point to activate NLRP3 (40). However, it is yet to be determined whether NLRP3 can directly sense a decrease in the levels of potassium or NLRP3 requires additional cellular proteins to sense the low levels of potassium. Several potassium-independent mechanisms of NLRP3 activation were also reported recently (41, 42). Imiquimod and CL097, small molecular ligands of TLR7, inhibit quinone oxidoreductase NQO2 and mitochondrial complex I. This results in the induction of ROS production and thiol oxidation, which triggers NIMA-related kinase 7 (NEK7)-dependent NLRP3 activation (41). In addition, a drop in NADH levels and mitochondrial ROS production leads to disruption of glycolytic flux in the cell that activates NLRP3 inflammasome and pyroptosis (42). Recent studies from our laboratory suggest that Z-DNA-binding protein 1/DNA-dependent activator of interferon regulatory factors (ZBP1/DAI), the apoptosis adaptor protein Fas-associated death domain (FADD), caspase-8 and Toll or interleukin-1 receptor containing adaptor inducing interferon-β (TRIF) act as apical mediators of canonical and non-canonical NLRP3 activation (43–46). In addition, recent reports identified an unexpected role for NEK7, an important protein in the formation of mitotic spindles and separation of centrosomes, for the activation of NLRP3 in response to both canonical and non-canonical stimuli (Fig. 1B) (47–49). NEK7 is required downstream of potassium efflux, physically interacts with NLRP3 and is required

for NLRP3 oligomerization. Surprisingly, the kinase activity of NEK7 is not important for NLRP3 activation, but its catalytic domain is vital for NLRP3 interaction. In addition, mitotic cells show reduced inflammasome activation compared with the cells in interphase (49). This suggests that NEK7 enables a regulatory switch to balance cell division and inflammasome activity.

Current studies suggest that post-translational modifications of NLRP3, such as ubiquitination and phosphorylation, play an important role in NLRP3 activation. NLRP3 is reportedly ubiquitinated at its NOD and LRRs before its activation (50). Removal of ubiquitin chains by a deubiquitinase, BRCA1/BRCA2-containing complex subunit 3 (BRCC3), is required for the activation of the NLRP3 inflammasome (50). The phosphorylation of NLRP3 is also reported to regulate its function. Protein tyrosine phosphatase, non-receptor type 22 (PTPN22) dephosphorylates NLRP3 leading to efficient activation of NLRP3 inflammasome and IL-1β release (51). These studies suggest that the priming of NLRP3 expression is immediately followed by its ubiquitination and/or phosphorylation to regulate aberrant activation of NLRP3. More studies are needed to further understand the intricate mechanisms of NLRP3 activation.

The NLRC4 inflammasome

Initial reports on NLRC4 indicated its similarity to apoptotic protease-activating factor (APAF1). Mouse NLRC4 is activated by bacterial flagellin to assemble the inflammasome complex (52–54). Further studies showed that NLRC4 is also activated by the inner rod and needle proteins of the type III secretion system (T3SS) of bacteria (Fig. 1C) (55, 56). Similar to NLRP3, the bacterial ligands do not directly interact with NLRC4.

It was later identified that proteins belonging to the NLR-family apoptosis inhibitory proteins (NAIPs) are the sensors acting upstream of NLRC4 inflammasome assembly. NAIPs directly bind to the pathogen ligands such as flagellin (NAIP5 and NAIP6) or rod and needle proteins of the T3SS (NAIP1 and NAIP2) and recruit NLRC4 to assemble the inflammasome complex (57). Interestingly, the ligand sensing by NAIPs is conferred by the NOD, but not the LRR domain, which was considered to be essential for ligand binding (58). Humans have only one NAIP protein, and it senses the needle protein of the bacterial T3SS but does not respond to the rod proteins or flagellin (56, 59). In contrast, a recent report showed that human NAIP has two isoforms and one of the isoforms expressed in human monocyte-derived macrophages responds to *Salmonella* flagellin (60).

The CARD domain of NLRC4 can directly interact with the CARD domain of caspase-1 in the absence of ASC (Fig. 1C). This direct interaction of NLRC4 with caspase-1 partly explains its ability to activate pyroptosis independently of ASC (61). NLRC4 also recruits caspase-8 into its inflammasome complex, which is speculated to induce cell death functions of NLRC4 (62). The presence of ASC in the NLRC4 complex enhances NLRC4-mediated IL1-β and IL-18 release by aggregating large ASC specks. It is reported that the activating mutation in the nucleotide-binding domain (NBD), a subdomain of NOD, of the NLRC4 leads to spontaneous inflammasome activation that causes recurrent macrophage-activation syndrome (63).

The phosphorylation of the highly conserved S533, mediated by the kinase PCKδ, is reported to be required for NLRC4 inflammasome assembly during *Salmonella typhimurium* infection (64). However, another report argues that PKCδ is dispensable for NLRC4 activation (65). A recent study found that the phosphorylation of NLRC4 at S533 occurs upstream of flagellin detection by NAIP5 (66). This suggests that the NLRC4 activation requires a two-step mechanism where phosphorylation of S533 primes the NLRC4 for its subsequent activation by NAIP5.

The AIM2 inflammasome

AIM2 is a cytosolic receptor for double-stranded DNA (dsDNA) and assembles an inflammasome complex to activate caspase-1 (67–70). The discovery of AIM2 has been made based on the fact that the DNA from host and microbes can trigger ASC-dependent caspase-1 activation, which is independent of NLR signaling (71). AIM2 consists of a PYD and a hematopoietic interferon-inducible nuclear protein with a 200-amino acid repeat (HIN200) domain. The positively charged surface of the HIN domain binds to the DNA and the PYD recruits ASC to assemble the inflammasome complex (67, 68, 70).

Various DNA viruses including vaccinia virus and cytomegalovirus and intracellular bacterial infections such as *Listeria monocytogenes*, *Francisella tularensis*, *Streptococcus pneumonia* and *Mycobacterium tuberculosis* activate the AIM2 inflammasome to orchestrate host defense (Fig. 1D) (72–75). In the case of intracellular bacterial infections, type I interferon signaling contributes to the activation of the AIM2 inflammasome (72, 76). Molecular mechanistic studies from our laboratory and others show that *Francisella* infection induces type I interferon-dependent interferon regulatory factor-1 (IRF1) expression that further induces the expression of guanylate-binding proteins (GBPs) and immunity-related GTPases (IRGs) for promoting the lysis of the bacteria to liberate DNA ligands (Fig. 1D) (77–79).

Apart from responding to infectious agents, AIM2 is also associated with several other human diseases. Increased expression of AIM2 is associated with auto-inflammatory diseases such as systemic lupus erythematosus, psoriasis and abdominal aortic aneurysm (80–82). Recognition of self-DNA in the cytosol of keratinocytes by AIM2 drives IL-1β release and auto-inflammation in psoriasis (81). However, a reduction in AIM2 expression is associated with prostate and colorectal cancer (83, 84). Further studies from our laboratory and others indicated that AIM2-deficient mice are highly susceptible to colorectal cancer and this is because of the role of AIM2 in restraining stem cell proliferation (83). Further, a new study showed that PKM2, a pyruvate kinase that catalyses a final rate limiting step of glycolysis, promotes AIM2 and NLRP3 inflammasome activation in sepsis (85). In addition, a recent study shows that AIM2 mediates caspase-1-dependent cell death in response to radiation-induced DNA damage and the AIM2-deficient mice are protected from radiation-induced gastrointestinal syndrome and hematopoietic failure (86). Another study showed that an inhibitor of HIV aspartyl

protease, Nelfinavir, disrupts nuclear envelope integrity. This leads to the release of DNA into the cytosol, which activates AIM2 inflammasomes (87). These additional roles of AIM2 suggest the possibility that AIM2 could sense self-DNA to regulate cellular functions.

The pyrin inflammasome

Pyrin is encoded by the gene *MEFV*, mutations in which are associated with an auto-inflammatory disease called familial Mediterranean fever (FMF) (88, 89). The mouse pyrin molecule consists of a PYD, two B-boxes and a coiled-coil domain, and the human pyrin molecule consists of an extra C-terminal domain called the B30.2 domain. Some of the mutations in the B30.2 domain are associated with FMF.

In initial studies, pyrin was recognized as a negative regulator of inflammasomes and secretion of IL-1β (90, 91). However, Chae *et al*. showed that FMF is caused by gain-of-function mutations in the pyrin-encoding gene *Mefv* (88, 89). Supporting this evidence, Gavrilin *et al*. (92) reported that type VI secretion system (T6SS) effectors of *Burkholderia cenocepacia* activates pyrin inflammasome. In addition, recent studies demonstrate that the pyrin inflammasome is triggered by Rho-modifying toxins that are produced by bacteria, such as *Clostridium difficile* (TcdB), *Histophilus somni* (IbpA), *Vibrio parahemolyticus* (VopS), *Clostridium botulinum* (C3) and *Burkholderia cenocepacia* (93, 94). These toxins induce covalent modifications in the Rho switch I region such as glycosylation, adenylation, ADP ribosylation and deamination (Fig. 1E) (93, 94). Pyrin senses these modifications instead of directly interacting with Rho. Rho modifications are essential for other cellular functions and perhaps pyrin is repressed under these homeostatic conditions.

Multiple pathways that inhibit the pyrin inflammasome have been identified including pyrin phosphorylation, cytoskeletal alterations and the mevalonate pathway. Two recent studies indicate that Rho activates the serine threonine kinases protein kinase N1 (PKN1) and PKN2 and they in turn phosphorylate pyrin (95, 96). Further, 14-3-3 proteins bind to the phosphorylated form of pyrin to regulate its activation. A mutation at phosphorylation site S242 of pyrin, which attenuates 14-3-3 binding, is reported to cause pyrin-associated auto-inflammation with neutrophilic dermatosis (95). A direct interaction of pyrin with actin has also been reported, suggesting a possibility for the sensing of Rho-mediated downstream events by pyrin (97). A recent study demonstrated that a mutation of WD-repeat-containing protein 1 (Wdr1), which encodes a factor that regulates actin depolymerization, causes pyrin-mediated auto-inflammation and thrombocytopenia (98). Another study identified negative regulation of pyrin inflammasome through protein geranylgeranylation, which is a protein post-translational modification catalyzed by geranylgeranyl transferase I (GGTase I). Perturbations in the mevalonate pathway that generate substrates for protein geranylgeranylation or absence of GGtase I lead to constitutive activation of pyrin inflammasome (99).

Pyrin is also categorized as a member of the tripartite motif (TRIM) family and these proteins actively participate in autophagy. Recent evidence indicates that the B30.2 domain is involved in recognizing autophagy-targeted cargo and participates in the autophagic degradation of NLRP1, NLRP3 and caspase-1 (100). However, the understanding of precise regulatory mechanisms of the pyrin inflammasome needs further studies.

Structural insights into inflammasome activation and assembly

Research done over the last decade in the inflammasome field described the signals that trigger inflammasome assembly. However, the structural mechanism of inflammasome assembly is poorly understood, and studies are emerging to describe the structure of the inflammasome.

It was proposed that inflammasomes attain a wheel-shaped structure similar to the apoptosome complex because of their functional similarity (39). Recent studies used negative-stain cryo-electron microscopy (cryo-EM) of purified NLRC4 in association with NAIP2–flagellin or NAIP5–PrgJ (a rod protein) to describe inflammasome assembly (101–104). These studies show that the NLRC4 inflammasome attains a wheel-like or disc-like structure with 10–12 protomers unlike the apoptosome, which is a heptameric wheel structure.

Typically, the binding of NAIPs to their respective ligand initiates recruitment of the NLRC4 protomers to NAIPs and NAIP nucleates NLRC4 oligomerization (Fig. 2). Assembly of wheel-shaped inflammasomes requires NAIP–NLRC4 and NLRC4–NLRC4 interactions, and these interactions are predominantly mediated by the NOD of these proteins (Fig. 2). On the basis of these studies, activated NAIP binds to an NLRC4 protomer and induces conformational changes in NLRC4 that make its oligomerization surface accessible for the subsequent NLRC4 protomer interaction to propagate the self-oligomerization process. These structures of the NLRC4 inflammasome lack CARDs, which precludes the understanding of their spatial orientation in the inflammasome complex.

These proposed structures provide the basis for understanding the process of inflammasome assembly. The importance of the NOD in this assembly is highlighted by the fact that the NOD is conserved amongst NLRs and mutations within the NOD are highly associated with auto-inflammatory disorders. Future studies should investigate (i) why NAIPs do not undergo self-oligomerization, (ii) how the CARD is arranged in the inflammasome wheel-like structure and (iii) whether all inflammasome-forming NLRs attain a similar structure.

Interestingly, previous literature suggests that NLRC4 physically interacts with NLRP3 and both are present in the same inflammasome complex during *Salmonella* infection (105, 106). Our recent study also indicates that the AIM2 and NLRP3 activation during *Aspergillus* infection leads to the assembly of a single inflammasome platform (107). The interaction of different NLRs indicates the possibility that multiple NLRs might be recruited to a single inflammasome complex during infections. AIM2 does not contain a NOD that induces self-oligomerization but it still assembles into an inflammasome complex. Therefore, it was proposed that DNA bound to the HIN domain of AIM2 acts as a scaffold to assemble the AIM2 inflammasome (108). A recent study suggests that the

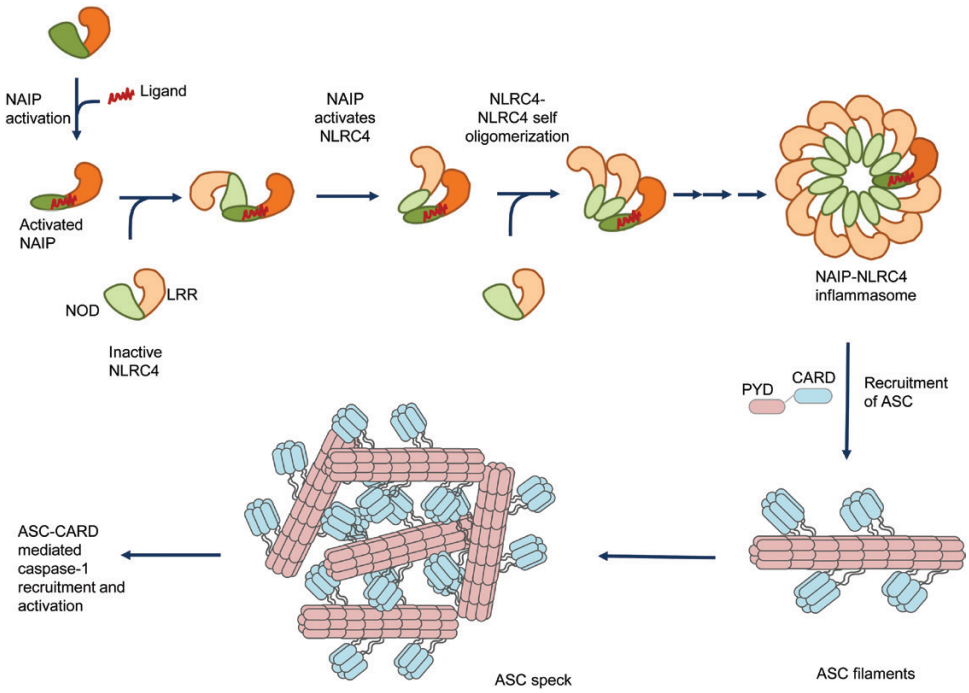

Fig. 2. Assembly of the NLRC4 inflammasome complex and ASC specks. Bacterial ligands such as flagellin, rod and needle proteins of the T3SS bind to NAIPs, which relieves the auto-inhibitory conformation and enables the accessibility of the oligomerization/interacting surface. The interacting surface of NAIP acts as a scaffold and recruits inactive NLRC4. The binding of NLRC4 monomer to the NAIP leads to the fully activated conformation of the NLRC4. The fully activated NLRC4 in turn exposes its interaction surface to further recruit another NLRC4 protomer to progress the self-oligomerization process. This chain of co-operative recruitment assembles a wheel- or disc-shaped inflammasome complex, which has inner ring of NODs and an outer ring of LRR domains. The available structures for the NLRC4 inflammasome lack CARD domains, which preclude the prediction of its orientation in the inflammasome complex. The activated inflammasome recruits ASC, an adaptor protein. ASC further undergoes self-polymerization to form filamentous structures. These ASC filaments aggregate to form macro-molecular complexes known as ASC specks. The exposed CARD domains of ASC in turn recruit caspase-1 that also forms filamentous structures (not shown). The recruitment of caspase-1 to the ASC filaments leads to caspase-1 activation.

HIN domain of AIM2 forms filament-like structure with dsDNA (109).

Inflammasome assembly recruits ASC to the complex through PYD–PYD interaction, and it further recruits caspase-1 through CARD–CARD interactions (Fig. 2). The recruitment of ASC to the inflammasome complex induces macro-molecular aggregate structures of 1–2 μM in size named ASC puncta or specks (4, 110, 111). ASC specks are formed after receptor activation and are released into the extracellular space to further enhance inflammatory responses.

Structural studies by cryo-EM and solid-state nuclear magnetic resonance spectroscopy indicate that ASC forms filament-like structures and through the PYD it can further form long helically shaped filaments (111). ASC specks promote recruitment of caspase-1 to the filaments through CARD-mediated interactions (112). A recent study suggests that mutations in the CARD of ASC led to the formation of ASC filaments but not ASC specks (113). Further, ASC speck formation is required for efficient processing of IL-1β but dispensable for the activation of gasdermin-D and pyroptosis induction (113).

In summary, ligands bound to the NAIP trigger assembly of the NLRC4 inflammasome that recruits the ASC and caspase-1 to generate the macro-molecular ASC specks, which mediate robust cellular responses.

Recent insights into the mechanisms of pyroptotic cell death

Canonical inflammasome-induced caspase-1 activation or the non-canonical inflammasome-induced caspase-4, caspase-5 (humans) and caspase-11 (mice) triggers an inflammatory form of cell death called pyroptosis (114). Pyroptosis is a lytic form of programmed cell death characterized by cell swelling and release of cellular contents through lysis. Pyroptosis is initiated in response to sensing of pathogens or host-derived perturbations.

The molecular mechanisms that mediate pyroptosis were poorly understood for a long time. Recent studies show that the proteins from the gasdermin family execute inflammatory caspase-induced pyroptosis (13, 115). The gasdermin gene family is highly conserved in vertebrates and consists of four paralogous genes in humans (*GSDMA*, *GSDMB*, *GSDMC* and *GSDMD*), whereas mice lack *Gsdmb* (116). All gasdermin family members share a similar N-terminal domain. Gasdermin-D is a substrate for inflammatory caspases and is cleaved between the N-terminal and C-terminal domains (Fig. 3). The N-terminal domain of gasdermin-D is sufficient to induce pyroptotic cell death, and the C-terminal domain blocks the cell death function through auto-inhibition (Fig. 3) (115, 117).

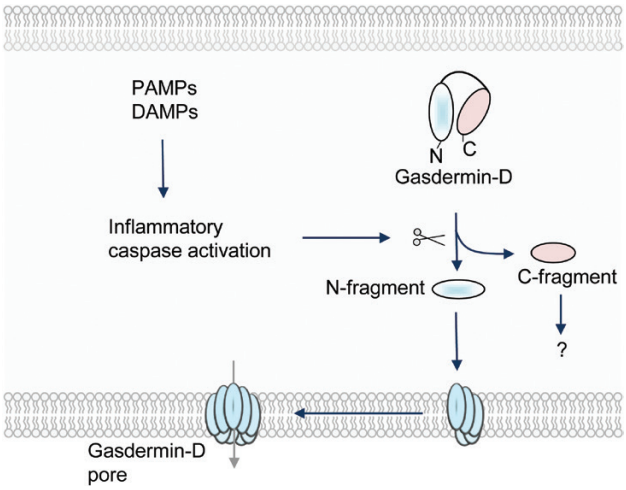

Fig. 3. Induction of pyroptosis mediated by gasdermin-D. Pathogen-derived PAMPs or physiological aberrations (DAMPs) activate inflammatory caspases. These caspases cleave gasdermin-D to separate its N- and C-terminal domains. The N-terminal domain of gasdermin-D is targeted to the membrane and assembles large, permeable pore complexes in the plasma membrane of the cell to induce pyroptosis.

Biochemical and structural studies have further unraveled the mechanism of gasdermin-mediated cell death. These studies show that gasdermin family members (GSDMD, GADMA3 and GSDMA) bind membrane lipids and exhibit membrane-disrupting cytotoxicity (118–120). Dye-loaded liposome-based assays indicated that the N-terminal domain of gasdermin-D targets liposome membranes to form pores sized ~10–20 nm, whereas the C-terminal domain or full-length gasdermin-D remains soluble (Fig. 3). These studies confirmed that the pyroptotic death is executed through the recruitment of the N-terminal domain of gasdermin-D to the plasma membrane to assemble large pores that impair cell membrane integrity. Ding *et al.* (119) further show that the pores formed by most gasdermin proteins contain 16 symmetric protomers.

In addition, the crystal structure of GSDMA3 showed the auto-inhibitory conformation of gasdermin domains and explains their inhibition in the absence of caspase-dependent cleavage. The understanding of gasdermin function and its mechanism of action will open up new therapeutic targets for controlling inflammatory diseases.

Conclusions

Inflammasome biology is a rapidly expanding field and the past 2–3 years have provided a tremendous advancement in understanding of inflammasome activation and function. Most importantly, studies detailing the structural components of inflammasome assembly, the mechanism of pyroptosis induction and genetic mutations in the NLRs associated with disease progression have expanded the existing knowledge about inflammasomes. These exciting findings have also raised many new questions in the field. However, the functions and specific ligands for many NLRs still remain unknown. The interplay between NLR activation and cellular homeostasis and its impact on physiological functions is still under investigation. Furthermore, ambiguities about receptors that activate inflammasome-complex formation still exist in the field. Therefore, in the coming years, it will be crucial to understand receptor activation mechanisms and the receptor structural requirements for ligand recognition to design novel immunotherapies.

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