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REVIEW

# Molecular mechanisms of inflammasome signaling

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## Abstract

The inflammasome is a macromolecular protein complex that mediates proteolytic cleavage of pro-IL-1$\beta$ and -IL-18 and induces cell death in the form of pyroptosis. Certain nucleotide-binding oligomerization domain-like receptors (NLRs), absent in melanoma 2 (AIM2)-like receptors (ALRs), or tripartite motif (TRIM) family receptors trigger the assembly of an inflammasome in response to pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). Recent studies have revealed a multitude of host components and signals that are essential for controlling canonical and noncanonical inflammasome activation and pyroptosis. These include pore-forming gasdermin proteins, the never in mitosis A-related kinase 7 (NEK7), IFN-inducible proteins (IFIs), reactive oxygen species (ROS), autophagy, potassium efflux, mitochondrial perturbations, and microbial metabolites. Here, we provide a comprehensive overview of the molecular and signaling mechanisms that provide stringent regulation over the activation and effector functions of the inflammasome.

KEYWORDS
AIM2, NLRP1/3, NLRC4, Pyrin, caspases

## 1 | INTRODUCTION

The inflammasome is a cytosolic multimeric signaling complex that coordinates host immune responses to invading pathogens and host-derived danger signals. Formation of the inflammasome is initiated by cytosolic PRRs, which are innate immune sensors capable of recognizing PAMPs and DAMPs. Whereas there are many families of PRRs, those known to form inflammasome complexes include members of the NLRs, ALRs, and the TRIM family member, Pyrin. The presence of multiple cytosolic PRRs, capable of forming an inflammasome, enables the host to respond to diverse stimuli.

NLRP1 was first described to form an inflammasome complex in 2002,[1] sparking intense interest to determine which other PRRs might also form inflammasome complexes. Since then, NLRP3, NLRC4, AIM2, and Pyrin have also been shown to form inflammasome complexes;[2] whereas less thoroughly characterized, there is also evidence that human NLRP2, NLRP7, and IFI16 and mouse NLRP6, NLRP9b, and NLRP12 can activate caspase-1.[3] In most cases, activated PRRs bind and engage the inflammasome adaptor protein ASC to recruit and activate the cysteine protease caspase-1.[4] ASC is a bipartite protein consisting of an N-terminal PYD and a CARD. Following PRR activation, ASC rapidly oligomerizes through homotypic PYD–PYD interactions into a large filamentous scaffold.[5,6] Inactive procaspase-1 monomers are recruited to ASC filaments through CARD–CARD interactions and thereby, brought into close proximity for optimal self-activation.[5,6] This sensor–ASC–caspase-1 inflammasome complex can be visualized as a distinct "speck" of 0.8–1 μm in diameter within the cytoplasm of macrophages and DCs;[7–10] indeed, formation of a speck is considered characteristic of inflammasome assembly.[11] Inflammasome specks released from pyroptotic cells have even been found to act as DAMPs and amplify the inflammatory response.[12,13]

Abbreviations: $\gamma$-H2AX, $\gamma$-H2A histone family member X; AIM2, absent in melanoma 2; ALR, absent in melanoma 2-like receptor; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; BMDM, bone marrow-derived macrophage; BRCC, BRCA1–BRCA2-containing complex; CARD, C-terminal caspase activation and recruitment domain; Cas, clustered regularly interspaced short palindromic repeat-associated system; cGAS, cyclic GMP-AMP synthase; DAMP, danger-associated molecular pattern; DC, dendritic cell; DPP, dipeptidyl peptidase; DSS, dextran sodium sulfate; DUB, deubiquitinase; FBXL2, F-box/leucine-rich repeat protein 2; FIIND, function-to-find domain; FMF, familial Mediterranean fever; GBP, guanylate-binding protein; HIN, hematopoietic expression; IAV, influenza A virus; IFI, IFN-$\gamma$-inducible protein; IFNAR, IFN-$\alpha/\beta$ receptor; IRAK, IL-1 receptor-associated kinase; IRF, IFN regulatory factor; IRG, immunity-related GTPase; LRR, leucine-rich repeat; MEFV, Mediterranean fever; MLKL, mixed lineage kinase domain-like protein; mtDNA, mitochondrial DNA; NAIP, nucleotide-binding oligomerization domain-like receptor family apoptosis inhibitory protein 1; NBD, nucleotide-binding domain; NEK7, never in mitosis A-related kinase 7; NLR, nucleotide-binding oligomerization domain-like receptor; P2X7R, P2X purinoceptor 7; PAAND, Pyrin-associated autoinflammation with neutrophilic dermatosis; PAMP, pathogen-associated molecular pattern; PKC, protein kinase C; PKN, serine/threonine protein kinase N; POP, Pyrin domain-only protein; PRR, pattern recognition receptor; PYD, Pyrin domain; RIPK, receptor-interacting serine/threonine protein kinase; ROS, reactive oxygen species; STING, stimulator of IFN genes; T3SS, type 3 secretion systems; TRIF, Toll/IL-1R domain-containing adaptor-inducing IFN-$\beta$; TRIM, tripartite motif

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After recruitment to the inflammasome complex and undergoing self-activation, caspase-1 executes several key cellular functions that are characteristic hallmarks of inflammasome activation. Caspase-1 proteolytically processes the proinflammatory cytokines pro-IL-1$\beta$ and pro-IL-18 into their biologically active forms. In addition, caspase-1 cleaves the propyroptotic factor gasdermin D.[14–16] The N-terminal fragment of gasdermin D oligomerizes and forms pores on the host cell membrane,[17–21] leading to cell swelling, lysis, and release of cytoplasmic contents in an inflammatory form of cell death, called pyroptosis. In the context of infection, the release of IL-1$\beta$ and IL-18 and induction of pyroptosis are largely beneficial in instigating an inflammatory immune response to fight the invading pathogen. However, inappropriate IL-1$\beta$ and IL-18 release can drive sterile inflammation and contribute to the development of autoimmune and inflammatory disease.

Whereas the downstream consequences of inflammasome activation are inflammation and cell death, the molecular mechanisms governing the regulation of ligand detection and activation of each inflammasome sensor are distinct, often in nuanced ways. This review aims to provide an extensive overview of the unique molecular mechanisms governing the different inflammasome complexes and highlight examples of dysregulated inflammasome activation in the context of disease.

## 2 | NLRP1 INFLAMMASOME

Human NLRP1 was the first protein identified to form an inflammasome complex.[1] This inflammasome complex contains NLRP1, ASC, and caspase-1 and -5.[1] The human NLRP1 protein is composed of a PYD, NBD, LRR, FIIND, and CARD.[1] Unlike human NLRP1, mice carry 3 NLRP1 paralogs (Nlrp1a, -b, and -c), all of which lack a PYD.[22] Mouse Nlrp1b and rat Nlrp1 are activated by the anthrax lethal toxin secreted by *Bacillus anthracis*.[23,24] The protective antigen component of the toxin transports the lethal factor component into the host cell, where the lethal factor induces N-terminal proteolytic cleavage of mouse Nlrp1b and rat Nlrp1 to catalyze the activation of the NLRP1 inflammasome[23–25] (Fig. 1). Mouse Nlrp1a is also activated by N-terminal proteolytic cleavage.[26] Likewise, human NLRP1 undergoes proteolysis within a specific N-terminal linker region between the PYD and NBD.[26] The observation that any protease capable of inducing site-specific N-terminal proteolytic cleavage is sufficient to activate the NLRP1 inflammasome suggests that proteolysis is a conserved mechanism of NLRP1 inflammasome activation in rodents and primates.

Owing to the presence of a CARD, mouse Nlrp1b licenses activation of caspase-1, secretion of IL-1$\beta$, and induction of pyroptosis in murine macrophages stimulated with lethal toxin in an ASC-independent manner.[27,28] However, ASC is required to amplify secretion of IL-1$\beta$ in response to low-dose lethal toxin[27] (Fig. 1). Mice bear a susceptible and resistant form of Nlrp1b,[22] and those carrying a susceptible variant are protected against *B. anthracis* infection as a result of the ability of the susceptible variant to respond to the lethal toxin compared with mice harboring a resistant form.[29,30] Whereas NLRP1 is best known for its role in sensing *B. anthracis* infection, it has also been implicated in the host response to the protozoan *Toxoplasma gondii*. For instance, mice deficient in Nlrp1b and Nlrp3 and infected with *T. gondii* produce less IL-1$\beta$ and IL-18, harbor increased parasitic loads, and succumb to the infection.[31] These findings suggest that Nlrp1b, in association with another NLR, can synergistically mount an effective host response.

Several studies have unraveled the biochemical mechanisms regulating activation of the NLRP1 inflammasome. The FIIND of NLRP1 undergoes autoproteolytic cleavage at the S1213 residue.[32,33] Furthermore, studies using point mutations and structural analysis revealed that the H1186 residue, located within a loop proximal to the cleavage site of the FIIND of NLRP1, is essential for autoproteolytic cleavage.[32,33] Mutagenesis studies targeting several residues within a fragment of Nlrp1b revealed that cleavage of the FIIND results in recruitment of procaspase-1.[34] Likewise, genetic disruption of the FIIND of Nlrp1b leads to impairment of self-oligomerization and activation of procaspase-1.[34] These studies demonstrate that cleavage of the FIIND contributes to activation of the Nlrp1b inflammasome.

Human NLRP1 is regulated by the autoinhibitory function of the PYD and LRR domains.[35] Mutations targeting the PYD (A54T, A66V, and M77T) or deletion of the PYD (aa 93–1474) or of residues in the LRR domain (F787–R843) promote increased production of IL-1$\beta$ in immortalized keratinocytes.[35] These results suggest that the PYD and LRR domains maintain NLRP1 in an inactive conformation rather than facilitating ASC oligomerization. In humans, germline gain-of-function mutations in the PYD and LRR domains of NLRP1 predispose individuals to the skin diseases, multiple self-healing palmoplantar carcinoma and familial keratosis lichenoides chronica.[35]

There is evidence to indicate that the NLRP1 paralog Nlrp1a has inflammasome functions. A single point mutation, causing a glutamine-to-proline substitution at aa 593 of Nlrp1a (*Nlrp1a*$^{Q593P}$), induces a systemic inflammatory disease in mice.[36] This inflammatory phenotype is prevented by genetic deletion of caspase-1, IL-1$\beta$, or IL-1R but not ASC,[36] implicating a role for Nlrp1a in assembling an Nlrp1a–caspase-1 complex, driving IL-1$\beta$-mediated immunopathology. Spontaneous caspase-1 activation induced by the *Nlrp1a*$^{Q593P}$ mutation results in pyroptosis of myeloid progenitor cells that affects their capacity to differentiate into mature myeloid cells.[36] The physiologic relevance of Nlrp1a has been examined in the context of infectious disease. The *Nlrp1a*$^{Q593P}$ *Il1r*$^{-/-}$ mouse strain, which does not develop systemic inflammation, suffers from prolonged cytopenia, bone marrow hypoplasia, and immunosuppression in response to lymphocytic choriomeningitis virus infection.[36] Further studies are required to decipher the differential roles of the 3 paralogs of Nlrp1 in mice. Whereas there has been progress in dissecting the molecular mechanisms contributing to the activation of the NLRP1 inflammasome, more comprehensive studies are required to understand fully its role in infection and autoinflammatory diseases.

## 3 | CANONICAL NLRP3 INFLAMMASOME

NLRP3 is known as a global sensor of PAMPs and DAMPs as a result of its ability to form an inflammasome in response to diverse

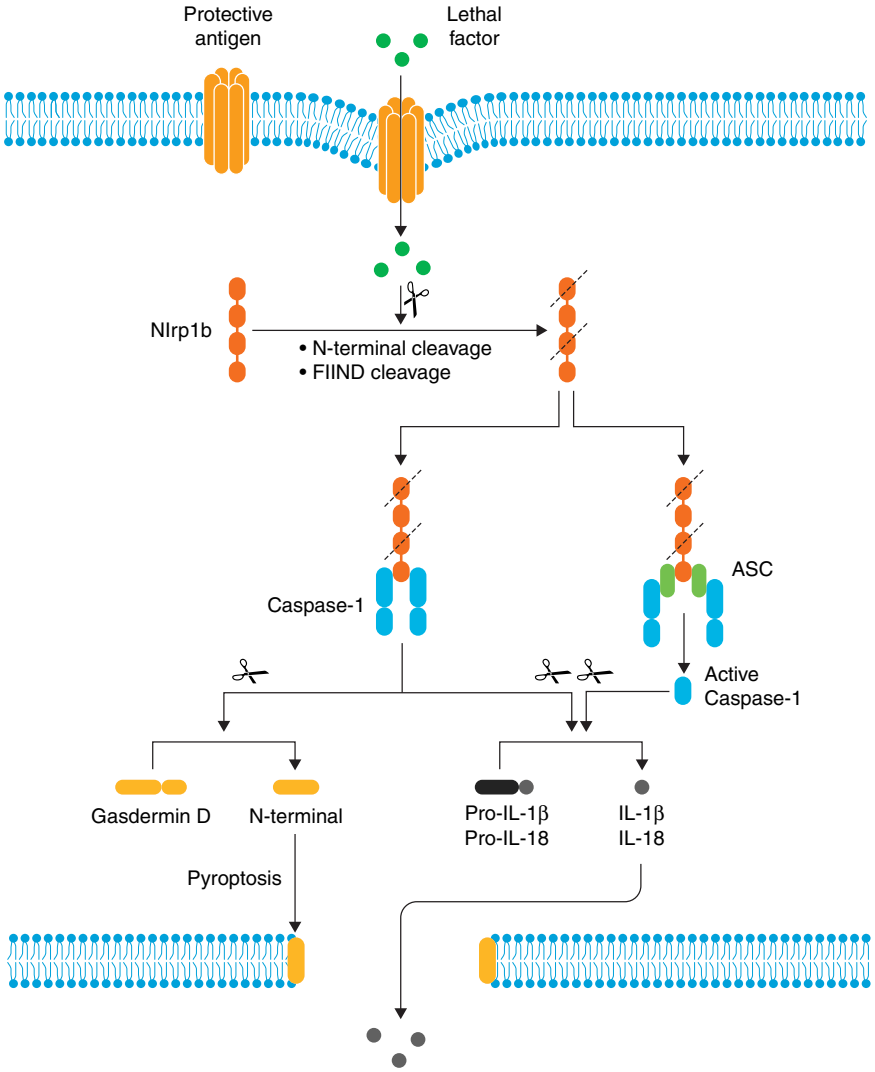


**FIGURE 1 NLRP1 inflammasome.** The anthrax lethal toxin released by the bacterium *B. anthracis* is composed of a protective antigen and a lethal factor. The protective antigen translocates the lethal factor across the cell membrane, where the lethal factor directly cleaves Nlrp1b at the N-terminal domain.[23–26] The FIIND of Nlrp1b may also undergo autoproteolytic cleavage.[32–34] Cleavage of Nlrp1b induces assembly of the inflammasome. In response to a high dose of lethal factor, Nlrp1b induces caspase-1-dependent cleavage of pro-IL-1$\beta$ and pro-IL-18 and pyroptosis independently of ASC or caspase-1 self-proteolysis.[27,28] In response to a low dose of lethal factor, Nlrp1b assembles an Nlrp1b–ASC–caspase-1 inflammasome complex that contributes to pro-IL-1$\beta$ and pro-IL-18 processing[27]

stimuli. NLRP3 is composed of an N-terminal PYD, a central NBD, and a C-terminal LRR domain. The PYD of NLRP3 interacts with the PYD of ASC, allowing the CARD of ASC to recruit and bind to caspase-1 through CARD–CARD interaction.[37] The LRR domain contributes to autoinhibition of NLRP3, whereas the NBD is responsible for its oligomerization.[38] Genome-wide-associated studies of human populations have revealed that mutations in the gene encoding NLRP3 are linked to the development of cryopyrin-associated periodic syndromes, a spectrum of clinical manifestations that include Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and neonatal-onset multisystem inflammatory disease.[39–41] NLRP3 has instrumental roles in the host defense against infection and contributes to the pathogenesis of rheumatoid arthritis, gout, type 1 diabetes, obesity, cancer, and neurodegenerative diseases.[11]

Activation of the canonical NLRP3 inflammasome normally requires 2 signals. The first signal, known as priming, up-regulates the expression of the NLRP3 protein (Fig. 2A). This priming process requires the engagement of TLRs, nucleotide-binding oligomerization domain 2, or TNFR; all of these pathways initiate NF-$\kappa$B-mediated expression of NLRP3.[42,43] However, there is evidence to suggest that activation of the canonical NLRP3 inflammasome can be achieved without extensive priming.[44–46] Simultaneous exposure of unprimed mouse BMDMs to TLR and NLRP3 activators (LPS plus ATP, LPS plus nigericin, or infection with *Listeria monocytogenes*) leads to activation of the NLRP3 inflammasome in 15 or 20 min in a manner dependent on the kinases IRAK1 and IRAK4.[45,46] This rapid mode of activation of the canonical NLRP3 inflammasome allows for instigation of a quick inflammatory response to PAMPs and DAMPs.

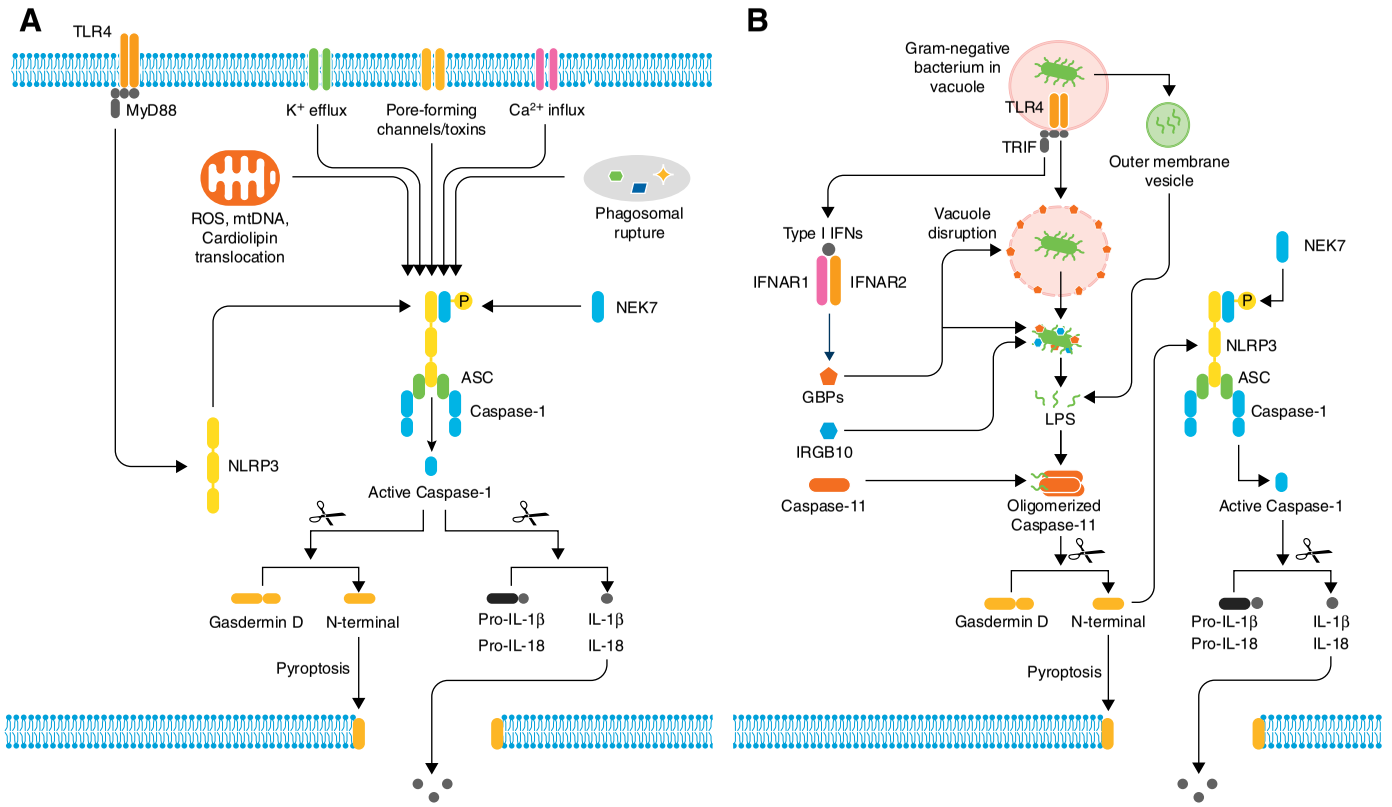


**FIGURE 2** **Canonical and noncanonical NLRP3 inflammasomes.** (A) Activation of the canonical NLRP3 inflammasome requires a priming signal (also known as Signal 1), often mediated by TLRs and activation of NF-$\kappa$B, inducing the expression of NLRP3.[42] An activating signal (also known as Signal 2) is provided by PAMPs or DAMPs.[48,49,55] PAMPs and DAMPs cause physiologic aberrations in the cell, which manifest in the forms of ion efflux and influx, mitochondrial dysfunction, or rupture of the phagosome.[63,64,66,67,69–72,74,75] These physiologic alterations are sensed by NLRP3, which induces the assembly of the NLRP3 inflammasome mediated by the kinase NEK7, ASC, and caspase-1.[79–81] Caspase-1 cleaves gasdermin D, releasing the N-terminal fragment of gasdermin D that assembles into pores on the membrane, resulting in pyroptosis.[14–16] Active caspase-1 also cleaves pro-IL-1$\beta$ and pro-IL-18, which are secreted through the pores formed by gasdermin D. (B) The non-canonical NLRP3 inflammasome is specifically activated by Gram-negative bacteria.[133] LPS from Gram-negative bacteria is recognized by TLR4 via the adaptor TRIF, resulting in the production of type I IFNs.[151–153] Type I IFNs induce the expression of IFIs GBPs and IRGB10 and caspase-11.[156,158] GBPs rupture the pathogen-containing vacuole, causing aberrant release of the bacteria into the cytoplasm,[156] and both GBPs and IRGB10 are recruited to the bacteria to disrupt and kill the pathogen.[158] LPS can also be released into the cytoplasm via bacterial outer-membrane vesicles.[148] Liberated LPS binds to caspase-11, promoting oligomerization and activation of caspase-11.[138] Active caspase-11 cleaves gasdermin D to induce pyroptosis.[14–16] The N-terminal fragment of gasdermin D also induces activation of the NLRP3 inflammasome and secretion of IL-1$\beta$ and IL-18[14,15]

The second signal, resulting in full activation of the NLRP3 inflammasome, is provided by an NLRP3 activator in the form of a PAMP or DAMP. The list of NLRP3 activators is extensive and includes Gram-positive and -negative bacteria, bacterial toxins, DNA and RNA viruses, fungi, and protozoa.[47–54] In addition, NLRP3 can sense a variety of DAMPs, including ATP, uric acid crystals, silica crystals, saturated fatty acids, asbestos, extracellular histones, lysophosphatidylcholine, aluminum hydroxide, and bee venom.[43,48,55–62] It is currently hypothesized that rather than direct binding of NLRP3 to the ligand, the repertoire of diverse ligands triggers a common set of cellular events that culminate in the activation of NLRP3. There are many proposed molecular mechanisms that lead to activation of the NLRP3 inflammasome (Fig. 2A). These molecular mechanisms include potassium efflux via the purinergic receptor P2X7R;[63–66] lysosomal disruption, resulting in the leakage of cathepsin B;[57] ROS;[67,68] oxidized mtDNA released from the mitochondria;[69] cardiolipin translocation from the inner to outer membrane of mitochondria;[70] calcium influx and reduction in cellular cAMP;[71–73] bacteria- or host-induced pores on the cell membrane;[74] and modulation in cell volume.[75] Although potassium efflux was proposed to be the converging signal,[66] a pathway leading to the activation of the NLRP3 inflammasome that does not require potassium efflux has been observed.[68]

Components of the NLRP3 inflammasome are localized to specific organelles; this subcellular localization has been proposed to govern activation of the inflammasome. A study demonstrated that NLRP3 activators induce translocation of ASC and NLRP3 from the mitochondria and endoplasmic reticulum, respectively, to the perinuclear region, where these components assemble an inflammasome complex.[67] A further study has shown that ASC migrates from the mitochondria to the endoplasmic reticulum, where NLRP3 resides—a process assisted by motor proteins kinesin and dynein.[76] In this case, NLRP3 activators decrease intracellular concentration of nicotinamide adenine dinucleotide$^+$, which leads to inactivation of tubulin deacetylase Sirtuin 2 and accumulation of acetylated $\alpha$-tubulin.[76] The accumulation of acetylated $\alpha$-tubulin drives ASC movement toward NLRP3, thereby facilitating inflammasome assembly.[76] A further study has suggested that localization of NLRP3 to the mitochondria requires interaction between the N-terminal residues of NLRP3 and the mitochondrial adaptor protein mitochondrial antiviral signaling.[77] The ability of NLRP3 to form oligomers and initiate prion-like nucleation allows rapid oligomerization of ASC to form larger polymers.[5,6,12,13] Given that multiple models of the inflammasome complex have been proposed,[78] additional comparative structural analysis of endogenous proteins from cells stimulated with a range of activators would reveal all possible architectures of the inflammasome.

Progress has been made to identify further novel components of the NLRP3 inflammasome. Recent studies have shown that the kinase NEK7 is specifically required for the activation of the NLRP3 inflammasome[79–81] (Fig. 2A). Phosphorylated NEK7 binds to the LRR domain of NLRP3,[79,80] suggesting that NEK7 may relieve autoinhibition of NLRP3. Overexpression studies revealed that NEK7 binds more strongly to NLRP3 carrying missense mutations associated with neonatal onset multisystem inflammatory disease compared with wild-type NLRP3.[79] Interaction between NEK7 and NLRP3 can be reduced by cytochrome C,[82] implying that the level of cytochrome C may drive the cell fate toward apoptosis rather than pyroptosis.

Further evidence demonstrates that the NLRP3 inflammasome and cell death components regulate one another, especially in relation to the apoptotic caspase, caspase-8. The activity of the NLRP3 inflammasome can be positively and negatively regulated by caspase-8. For example, caspase-8 and its adaptor Fas-associated protein with death domain are required for priming and possibly activation of NLRP3 inflammasome in macrophages and DCs.[83–94] Caspase-8 is recruited to the inflammasome speck, indicating that it is a component of the canonical NLRP3 inflammasome complex.[87,88] Direct activation of caspase-1 by caspase-8 or redundant activation between caspase-1 and caspase-8, leading to the proteolytic processing of pro-IL-1$\beta$, has also been reported.[10,95–99,110] By contrast, studies have also shown that caspase-8 suppresses the NLRP3 pathway in DCs.[111] Mouse DCs lacking caspase-8 and treated with LPS, in the absence of the activation signal (also known as signal 2), result in the activation of the canonical NLRP3 inflammasome.[111] This effect was mediated by the kinases RIPK1 and RIPK3, the necroptotic effector MLKL, and the phosphatase phosphoglycerate mutase 5.[111] A further study has suggested that the caspase-8 inhibitor c-FLIP might form a complex with the NLRP3 inflammasome to mediate caspase-1 activation.[112]

A link between the NLRP3 inflammasome and the necroptotic cell death pathway has emerged. In response to necroptotic activators, the kinase RIPK3 and the necroptotic effector MLKL are required to activate the NLRP3 inflammasome and induce IL-1$\beta$ release.[113,114] In this case, MLKL oligomerizes on the cell membrane to induce pore formation, resulting in necroptosis and a reduction in the level of intracellular potassium triggering activation of the NLRP3 inflammasome.[113,114] Of particular interest is that MLKL-dependent secretion of IL-1$\beta$ does not rely on the pyroptosis executor gasdermin D,[113,114] suggesting that IL-1$\beta$ might be released through MLKL-induced pores instead. These studies further accentuate the complex interplay between the NLRP3 inflammasome and nonpyroptotic cell death pathways.

Owing to the large number of stimuli that can activate NLRP3, post-translational modifications exist at multiple levels to modulate and fine tune NLRP3 inflammasome activation. NLRP3 is maintained in its inactive form via binding of its LRR domain to the ubiquitin ligase-associated protein suppressor of the G2 allele of skp1 (SGT1).[115] Likewise, NLRP3 is ubiquitinated and undergoes proteasomal degradation mediated by ubiquitin ligases S-phase kinase-associated protein 1–cullin–F-box protein and FBXL2.[116] FBXL2 binds to the W73 residue within the PYD and the K689 residue within the LRR domain of NLRP3 and further ubiquitinates at the K689 residue within the LRR domain of NLRP3.[116] In addition to NLRP3 expression being induced by the priming step, NLRP3 is post-translationally activated by deubiquitination, which is mediated by a DUB enzyme in both mice and humans.[117,118] A later study clarified that activation of NLRP3 requires deubiquitination of its LRR domain by the DUB enzyme BRCC3 in mice or BRCC36 in human.[119] Furthermore, phosphorylation of residue Y144 and linear ubiquitination of ASC are required for the activation of the NLRP3 inflammasome.[120,121]

Further studies have shown that NLRP3 inflammasome is activated when the kinase Syk is phosphorylated in response to fungal, malarial,

and mycobacterial pathogens.[122–125] Mass spectrometry analysis revealed 3 corresponding conserved and phosphorylated serine residues in mouse (S3, S157, and S725) and human (S5, S161, and S728) NLRP3.[126] Mutation of the S5 residue located in the PYD of NLRP3 to a phosphomimetic aspartate (S5D) completely abolishes NLRP3-dependent ASC speck formation.[126] However, mutation of the other 2 serine sites had no effect on NLRP3 activity.[126] Furthermore, phosphorylation of S5 disrupts the electrostatic interaction between the PYDs of NLRP3 and ASC,[126] suggesting that phosphorylation of S5 prevents activation of the NLRP3 inflammasome at steady state. Dephosphorylation of S5 requires phosphatase 2A, leading to the PYD–PYD interaction between NLRP3 and ASC.[126] Nitrosylation of NLRP3 has also been shown to regulate the NLRP3 inflammasome negatively. NLRP3-mediated IL-1$\beta$ release and speck formation are inhibited in the presence of NO donor S-nitroso-*N*-acetylpenicillamine or S-nitrosoglutathione, which stabilizes damaged mitochondria and provides protection against LPS-induced endotoxemia.[127] Furthermore, it was reported that thiol nitrosylation of NLRP3 resulted in inhibition of IL-1$\beta$-mediated pathology in the context of *M. tuberculosis* infection.[128] An inhibitory property of the NO donor, S-nitroso-*N*-acetylpenicillamine, toward the NLRP3 inflammasome has also been reported.[129] A plethora of other signaling components and cytokines have been shown to regulate negatively the expression or activation of the NLRP3 inflammasome, including CARD9, type I IFNs and IL-10.[130–132] These diverse, yet specific, modifications target the NLRP3 inflammasome at various checkpoints, indicating the importance of a tight regulation of this sensor of general stress and damage.

## 4 | NONCANONICAL NLRP3 INFLAMMASOME

The noncanonical NLRP3 inflammasome pathway is defined by a requirement for caspase-11 in the activation of the NLRP3 inflammasome complex[133] (Fig. 2B). Gram-negative bacteria, including *Citrobacter rodentium*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella enterica* serovar Typhimurium, *Legionella pneumophila*, and *Yersinia pseudotuberculosis*, are the major activators of the noncanonical NLRP3 inflammasome pathway.[133–136] Caspase-11, a murine inflammatory caspase, senses LPS in the cytoplasm.[134,137,138] The human analogs of caspase-11, -4, and -5 can likewise sense LPS[138] and initiate activation of the NLRP3 inflammasome and secretion of IL-1$\beta$ and IL-18.[139–141] Caspase-4, -5, and -11 bind to the lipid A portion of LPS with high specificity and affinity via their CARD.[138] Caspase-11 can also be activated by transfection or electroporation of LPS into macrophages.[133,134,137] Activated caspase-11 cannot directly proteolytically process pro-IL-1$\beta$ and pro-IL-18 but can induce pyroptosis without caspase-1.[133] Pyroptosis mediated by caspase-11 provides effective host defense against cytosolic Gram-negative bacteria in vivo.[135,142] The enzymatic activity of caspase-11, along with that of caspase-1, synergistically restricts the growth of intracellular Gram-negative bacteria in macrophages, intestinal epithelial cells, and fibroblasts.[143–147]

The mechanisms regulating the activation of the noncanonical NLRP3 inflammasome are still being deciphered. LPS that has gained entry into the cytoplasm activates inflammatory caspases,[133–135,148] allowing them to cleave and release directly the N-terminal fragment of gasdermin D to drive pyroptosis (further discussed in GASDERMIN below)[14–16] (Fig. 2B). The gasdermin D N-terminal fragment also has a role in activating the NLRP3 inflammasome via an undefined mechanism.[14–16] Activation of caspase-11 leads to a drop in intracellular potassium levels sufficient to activate NLRP3,[149] highlighting that the noncanonical pathway might also have a dependency on potassium efflux. A further study has shed light on this issue and found that LPS-induced activation of caspase-11 leads to caspase-11-dependent cleavage of the large pore channel pannexin-1.[150] As a result, ATP is released from the cell, activating the purinergic receptor P2X7R to drive pyroptosis.[150] The opening of the pannexin-1 pore also generates potassium efflux that induces IL-1$\beta$ release,[150] providing an explanation for how caspase-11 might activate the NLRP3 inflammasome to mediate secretion of IL-1$\beta$. However, it remains unclear how the caspase-11–pannexin-1–P2X7R model leads to pyroptosis and how this pathway amalgamates with the caspase-11–gasdermin D model.

Earlier studies have identified a role for type I IFN signaling in the activation of the noncanonical NLRP3 inflammasome[151–153] (Fig. 2B). Recognition of Gram-negative bacterial LPS by TLR4 initiates TRIF-mediated type I IFN signaling, leading to increased expression of caspase-11 and NLRP3.[151–153] A further study has shown that the complement-related peptidase carboxypeptidase B1 induces the expression of caspase-4, -5, and -11 downstream of the TLR4 and type I IFN signaling pathway.[154] Type I IFNs induce the expression of IFN-inducible GTPases, including GBPs and IRGs. GBPs are 65–67 kDa proteins conserved across species, with 7 GBPs in humans and 11 GBPs in mice.[155] In the context of inflammasome biology, GBPs encoded on mouse chromosome 3 (GBP1, -2, -3, -5, and -7) are required for activation of the noncanonical NLRP3 inflammasome induced by *S.* Typhimurium and *L. pneumophila*.[156,157] GBPs are recruited to the vacuole surrounding the intracellular bacterial pathogens, where they facilitate lysis of the vacuolar membrane such that LPS is leaked into the cytoplasm for detection by caspase-11.[156] GBPs also direct IRGB10 to the cell membrane of Gram-negative bacteria to induce bacteriolysis, thereby increasing the cytosolic accessibility of LPS for detection by caspase-11.[158] There is also evidence to suggest that GBPs encoded on mouse chromosome 3 facilitate activation of both noncanonical and canonical inflammasomes in macrophages infected with *Chlamydia muridarum*.[159] In this case, GBPs bound neither the vacuole surrounding *C. muridarum* nor the pathogens,[159] suggesting an alternative mechanism of GBPs in the regulation of inflammasome activity.

Recent studies have suggested that caspase-11 can recognize ligands other than LPS. Caspase-11 binds to host-derived oxidized phospholipids (oxAPC) in activated DCs.[160] LPS and oxAPC elicit caspase-11-induced inflammasome speck formation and IL-1$\beta$ release, but not pyroptosis, in DCs.[160] In addition, caspase-11 contributes to host resistance to the fungal pathogen *Aspergillus fumigatus* in vivo,[161] revealing a biologic role for caspase-11 in host defense against pathogens other than Gram-negative bacteria. Overall, substantial advances have

been made in our understanding of the noncanonical NLRP3 inflammasome. It is likely that caspase-11 has the potential to recognize additional stimuli. Therefore, further studies are required to provide a more complete picture of the role of caspase-11 in infection and sterile inflammation.

## 5 | GASDERMIN

The molecular mechanisms of how pyroptosis is executed following inflammasome activation are being revealed.[162] The current view is that inflammatory caspases cleave the pore-forming protein gasdermin D, yielding a 31 kDa N-terminal fragment that drives pyroptosis[14–16] (Fig. 2B). The N-terminal fragment also engages activation of the NLRP3 inflammasome via an undefined mechanism.[14–16] Various studies have since identified a capacity for gasdermin D to oligomerize on the cell membrane.[17–21] The N-terminal fragment associates with the inner leaflet of the host cell membrane, where it forms pores of 10–21 nm in diameter.[17–21] These pores and/or the physical rupture of the cell allow passive release of proteolytically processed IL-1$\beta$ and IL-18 into the extracellular space.[14–16] It is worthy to note that in some cases, release of IL-1$\beta$ could occur before pyroptosis or even in the absence of pyroptosis.[163,164]

The N-terminal fragment of gasdermin D also binds to lipids on bacterial cell membranes, creating pores on the bacterial cell membrane as a mechanism to exert antimicrobial activity.[17,19,20] Previous studies have argued that the primary bactericidal mechanism of pyroptosis is its ability to liberate whole bacteria or bacteria entrapped within pore-induced intracellular traps, such that these entities are phagocytosed and killed by neutrophils.[143,165] It would be important to investigate whether direct gasdermin D-induced killing of bacteria is a natural phenomenon that is executed within the host cell or outside of the cell.

Gasdermin D might also have functions in inhibiting apoptosis. Canonical activation of the inflammasome induces apoptosis in the absence of gasdermin D.[16] It has also been suggested that in the absence of gasdermin D, induction of apoptosis requires caspase-1-mediated activation of caspase-3 and -7.[166] Site-specific cleavage of gasdermin D has been suggested to dictate the functionality of gasdermin D in pyroptosis and apoptosis. A recent study has shown that inhibition of serine peptidases DPP8 and DPP9 leads to activation of caspase-1, cleavage of gasdermin D, and pyroptosis.[167] However, in response to classic apoptotic signals, activated caspase-3 and -7 cleave gasdermin D at residue D87, a different site to that cleaved by inflammatory caspases.[166] The resultant p45 gasdermin D fragment is inactive and cannot engage pyroptosis, allowing apoptosis to ensue.[166]

A related gasdermin member (gasdermin E) can also mediate cross-regulation between pyroptosis and apoptosis. HeLa cells expressing high levels of gasdermin E and stimulated with TNF or chemotherapeutic agents undergo caspase-3-mediated cleavage of gasdermin E at its linker region, generating an N-terminal fragment that perforates membrane and induces pyroptosis.[168] By contrast, HeLa cells expressing low levels of gasdermin E display apoptotic characteristics before pyroptotic morphology and lysis. Therefore, it is possible that a high-level expression of gasdermin E is required to enforce pyroptosis and over-ride the execution of apoptosis. These studies further highlight complex crosstalks between pyroptosis and apoptosis. The physiologic effects of gasdermin E have been elucidated in a mouse model. Wild-type mice injected with the chemotherapeutic drugs cisplatin, 5-fluorouracil, or bleomycin are susceptible to severe inflammation, tissue damage, infiltration of inflammatory cells, loss of crypts in the small intestine, and weight loss.[168] However, mice lacking gasdermin E are protected from these adverse effects, suggesting that gasdermin E-mediated pyroptosis has a vital role in chemotherapy drug-induced toxicity.[168] These findings highlight the importance of gasdermin proteins in inflammation and immunity and identify new therapeutic targets for the treatment of inflammasome-associated diseases.

## 6 | NAIP-NLRC4 INFLAMMASOME

The NLRC4 inflammasome is activated by cytosolic flagellin[169–173] and by both the basal body rod[174,175] and needle[176,177] proteins of the T3SS of many Gram-negative bacteria (Fig. 3). Other studies had shown the role of a related NLR family member (Naip5) in sensing flagellin[172,178–181] and instigating host response to the intracellular pathogen *L. pneumophila*.[182–185] Further studies have clarified the relationship between Naip proteins and NLRC4, showing that Naip proteins dictate the ligand specificity of the NLRC4 inflammasome.[186–189] Of the 7 Naip proteins encoded by the mouse genome,[190] Naip1 and Naip2 recognize the needle and rod protein components of bacterial T3SS, respectively,[175–177,186,187] whereas both Naip5 and Naip6 recognize flagellin.[180,186,187] Analysis of chimeric Naip proteins mapped the ligand-binding site to the NBD[191] rather than the LRR domain, which was thought to be responsible for ligand recognition in most NLR family proteins. The functions of the remaining murine Naip proteins remain uncharacterized. Unlike mice, humans encode a single NAIP protein, which was initially thought to be functionally similar to murine Naip1 in that it binds directly to the needle protein of bacterial T3SS to activate the NLRC4 inflammasome.[176,177] However, a later study has shown that human NAIP might also recognize flagellin,[192] highlighting the functional versatility of human NAIP compared with its murine counterparts (Fig. 3).

NLRC4 consists of an N-terminal CARD, a centrally located NBD, and a C-terminal LRR domain.[193] The CARD of NLRC4 can directly interact with the CARD of procaspase-1, generating an NLRC4–caspase-1 inflammasome complex that is sufficient to induce pyroptosis and processing of pro-IL-1$\beta$ and -IL-18 in the absence of ASC.[8,9,174,194–197] However, the NLRC4–ASC–caspase-1 inflammasome has also been observed endogenously in macrophages,[10] highlighting the compositional diversity of the NLRC4 inflammasome (Fig. 3). In this case, ASC enhances proteolytic cleavage and release of IL-1$\beta$ and IL-18.[8–10,196–199]

The structures of monomeric or inactive NLRC4,[200] and NLRC4 complexed with either flagellin and Naip5[201] or with T3SS rod protein and Naip2,[202,203] have been determined by crystallography and cryogenic electron microscopy. The crystal structure of inactive mouse

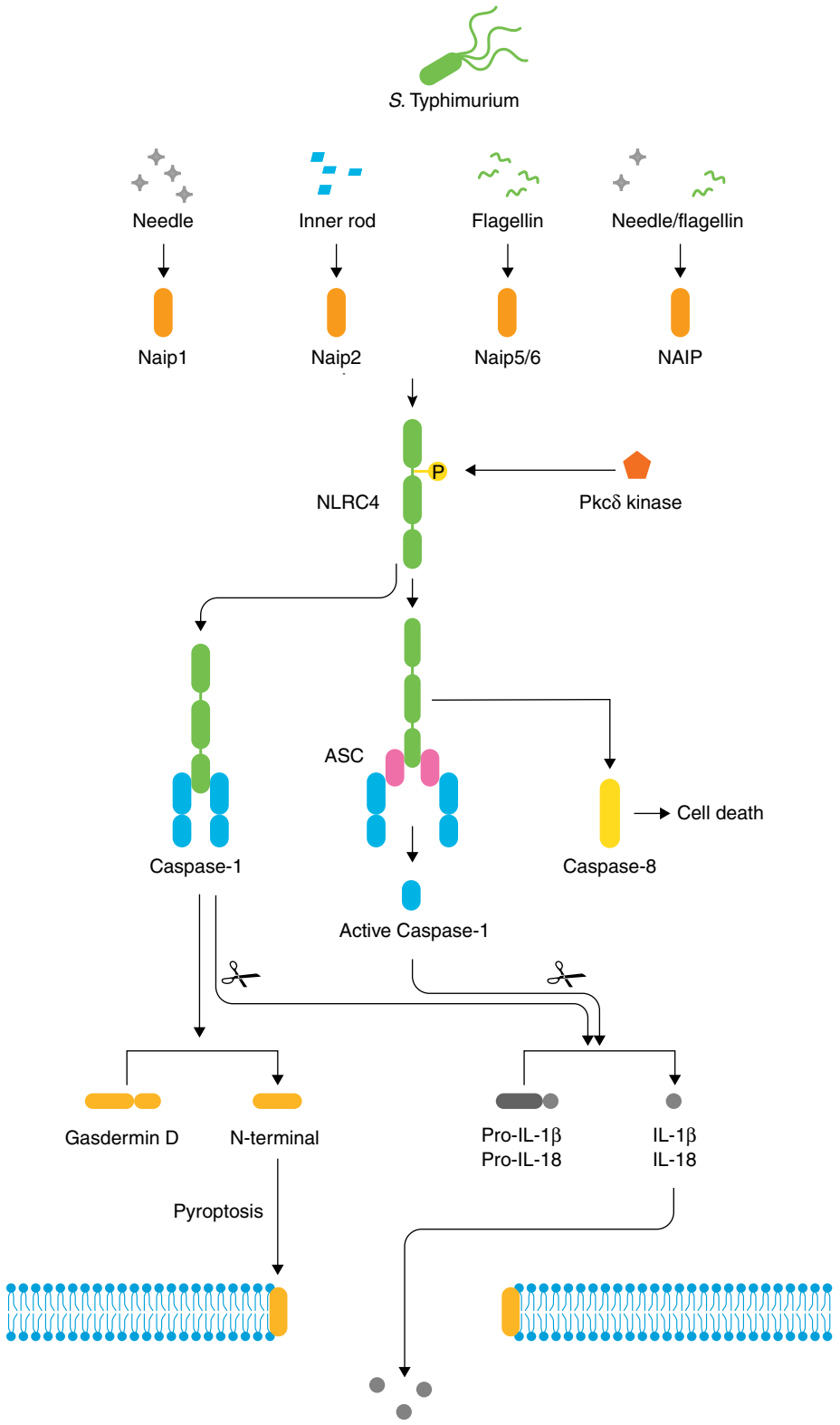


**FIGURE 3** **NAIP–NLRC4 inflammasome.** Pathogenic bacteria, such as *S.* Typhimurium, deliver effector proteins via the T3SS. This process leads to the injection of T3SS needle protein, inner rod protein, and flagellin into the cytoplasm for detection by mouse Naips or human NAIP.[176,177,186,187,192] Mouse Naips or human NAIP interact with NLRC4, inducing a conformational change in NLRC4 that catalyzes the recruitment of additional NLRC4 proteins to form a disc structure.[201–203] Phosphorylation of NLRC4 by the kinase Pkc$\delta$ downstream of ligand–NAIP interaction may contribute to full activation of the inflammasome.[204,205] The NAIP–NLRC4 complex assembles a NAIP–NLRC4–caspase-1 inflammasome or a NAIP–NLRC4–ASC–caspase-1 inflammasome.[9,10,169–171,194,196,197] The NAIP–NLRC4–caspase-1 inflammasome cleaves gasdermin D, unleashing the N-terminal fragment of gasdermin D that mediates pyroptosis. Both the NAIP–NLRC4–caspase-1 and NAIP–NLRC4–ASC–caspase-1 inflammasomes induce caspase-1-dependent proteolytic cleavage of pro-IL-1$\beta$ and -IL-18. The NAIP–NLRC4 inflammasome also induces caspase-8-dependent cell death[87,212,226,227]

NLRC4 showed that the LRR domain sterically occludes the NBD,[200] revealing an autoinhibitory function of the LRR domain. By contrast, the active structures of NLRC4, in complex with either Naip2[202,203] or Naip5,[201] showed that NLRC4 undergoes a conformational change, exposing an oligomerization surface to which inactive NLRC4 subunits can be recruited and bind to form a "disc" structure.

Phosphorylation of NLRC4 at residue S533 by the kinase PKC$\delta$ was initially found to be critical for activation of the NLRC4 inflammasome in macrophages in response to *S.* Typhimurium infection or flagellin[204] (Fig. 3). A further study found that phosphorylation of NLRC4 at residue S533 occurs independently of activation of Naip5 by flagellin.[205] This finding suggests that phosphorylation of NLRC4 at residue S533 and binding of Naip5 to its cognate ligand are unrelated events. A further study argued that activation of the NAIP–NLRC4 inflammasome in macrophages infected with either *S.* Typhimurium or *Shigella flexneri* was independent of PKC$\delta$.[175]

The complexity of the mechanisms regulating activation of the NAIP–NLRC4 inflammasome was addressed further. In the context of infection with *S.* Typhimurium, both NLRC4 and NLRP3 are activated in a dynamic manner,[9] with both NLRs colocalizing within a single inflammasome complex in macrophages.[10] To clarify the relationship between phosphorylation of NLRC4 at residue S533 and activation of the inflammasome, Qu and colleagues[206] mutated S533 to an alanine (S533A) and assessed the effect of this modification on NLRC4 inflammasome activity. The S533A mutation abolished phosphorylation of NLRC4 at this residue but only delayed activation of caspase-1 in response to *S.* Typhimurium infection.[206] However, genetic deletion of NLRP3 further reduced caspase-1 activation and cleavage of pro-IL-1$\beta$ in macrophages carrying the NLRC4 S533A mutation and infected with *S.* Typhimurium or transfected with flagellin compared with macrophages carrying the NLRC4 S533A mutation alone.[206] These results indicate that recruitment of NLRP3 to the NLRC4 inflammasome enhances caspase-1 activation, highlighting the dynamic interplay that exists among NLRs within the inflammasome. The cooperativity between NLRC4 and other NLRs in inflammasome activation has also been observed in contexts other than infection. For instance, mouse macrophages exposed to hyperosmotic stress produce IL-1$\beta$ in a manner dependent on both NLRC4 and NLRP3 inflammasome activity.[207] Likewise, NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes in response to the DAMP lysophosphatidylcholine.[62] Finally, in a rodent model of stroke, the NLRC4 and AIM2 inflammasomes contribute to ischemic brain injury in a manner that required ASC but not NLRP3.[208]

The biologic effect of NAIP–NLRC4 inflammasome activation varies with cell type. Unlike macrophages, which undergo rapid pyroptosis following activation of the NAIP–NLRC4 inflammasome, neutrophils release IL-1$\beta$ without undergoing pyroptosis.[209] It is possible that the pyroptosis-resistant phenotype of neutrophils enables killing of bacteria that have avoided macrophage-mediated bactericidal activity. The interplay between cell types driven by the NAIP–NLRC4 inflammasome can also be observed between DCs and T cells. NLRC4-driven IL-1$\beta$ and IL-18 production in DCs infected with *S.* Typhimurium and other gram-negative bacteria can stimulate the production of IFN-$\gamma$ in noncognate memory CD8$^+$ T cells.[210]

In the gastrointestinal tract, activation of the NAIP–NLRC4 inflammasome in intestinal epithelial cells exposed to *S.* Typhimurium or flagellin leads to cell expulsion from the epithelium.[211,212] A recent study has demonstrated that selective activation of the NAIP–NLRC4 inflammasome in intestinal epithelial cells stimulated cell expulsion and release of eicosanoids and cytokines in a manner that did not absolutely require caspase-1 or gasdermin D.[212] Instead, caspase-8 was able to compensate in caspase-1-deficient mice and cause cell expulsion of intestinal epithelial cells infected with *S.* Typhimurium.[212] This NLRC4-driven response provides host protection against invading pathogens, such as *S.* Typhimurium, by shedding infected cells into the intestinal lumen.

NLRC4 has also been shown to facilitate discrimination between commensal and pathogenic bacteria in the intestine by producing IL-1$\beta$ in response to *S.* Typhimurium and *Pseudomonas aeruginosa* but not commensal bacteria.[213] Indeed, expression of NLRC4 in the intestinal epithelial cells of mice protects against infection by the enteric pathogen *C. rodentium*.[214] A further interesting biologic effect of the NAIP–NLRC4 inflammasome is its ability to sustain the level of the growth hormone insulin-like growth factor 1 in white adipose tissue to promote the protective effect of a commensal *E. coli* O21:H$^+$ strain against muscle wasting induced by the pathogenic bacteria *Burkholderia thailandensis*.[215] However, uncontrolled activation of the NAIP–NLRC4 inflammasome is detrimental and leads to systemic inflammation and rapid sepsis-like death in mice infected with an *E. coli* pathobiont.[216] Likewise, systemic activation of the NLRC4 inflammasome by flagellin leads to rapid production of inflammatory lipid eicosanoids, loss of vascular fluid, and death in mice.[217]

As the NAIP–NLRC4 inflammasome is activated in response to specific bacterial ligands in the context of infection, it is possible that NAIP and NLRC4 may be activated differentially in response to other contextual cues.[218–221] Indeed, mouse Naips have been proposed to have NLRC4-independent functions.[219] In this case, Naips might suppress the transcription factor STAT3 and cell proliferation in the intestinal epithelium in response to carcinogen exposure.[219] On the other hand, NLRC4 might function independently of Naips or the inflammasome during colorectal tumorigenesis through a mechanism that targets inhibition of apoptosis.[218] Furthermore, NLRC4 mediates the production of cytokines and chemokines in tumor-associated macrophages and induces the generation of protective T cell responses independently of the inflammasome in response to melanoma tumor growth.[221] Finally, the nucleotide metabolites adenine and $N^4$-acetylcytidine have recently been shown to activate the NLRC4 inflammasome in elderly individuals and contribute to the development of arterial hypertension.[222] Additional studies are required to understand more fully the distinct molecular mechanisms governing NAIP and NLRC4 activation in conditions other than infectious diseases.

Several disease-association mutations of NLRC4 have been identified in humans and mice. Identification of these mutations has contributed to our understanding of the molecular mechanisms regulating activation of the NLRC4 inflammasome. Several gain-of-function missense mutations in the highly conserved NBD of NLRC4 have been observed, including V341A,[223] T337S,[224] and H443P.[225] Macrophages

carrying each of these NLRC4 mutants undergo spontaneous inflammasome formation, constitutive activation of caspase-1, and pyroptosis and produce increased levels of IL-1$\beta$ and IL-18.[223–225] Collectively, these studies highlight the importance of the LRR domain in holding NLRC4 in an autoinhibited state and indicate that mutations in the NBD that interrupt LRR domain binding lead to constitutive activation of NLRC4. Furthermore, a recent study has shown that NLRC4, carrying the H443P mutation, induces spontaneous caspase-8-dependent apoptosis in human lung epithelial cells,[226] confirming a role of NLRC4 in a nonpyroptotic cell death pathway. This finding is in line with an earlier study that identified human proteasome protein Sug1 as a binding partner of NLRC4 that potentiates a caspase-8 dependent cell death pathway by ubiquitinating NLRC4.[227]

Indeed, the NLRC4 mutations mentioned previously cause severe autoinflammatory syndromes when present in humans. Carriers of NLRC4 mutant H443P develop a familial cold autoinflammatory syndrome.[225] Likewise, 2 activating missense mutations in NLRC4 (T337S and V341A) led to the development of severe enterocolitis[223] and recurrent macrophage activation syndrome,[224] respectively. All 3 cases were characterized by constitutive caspase-1 activation and by release of IL-1$\beta$ and IL-18, indicating that the NLRC4 inflammasome was hyperactive in these patients. Of translational importance is that a patient suffering from a life-threatening macrophage activation syndrome caused by the NLRC4 mutation V341A was treated with recombinant human IL-18-binding proteins.[228] Within 4 d of the first treatment, the patient's overall demeanor improved, and the patient remains well after 11 mo of combined IL-1$\beta$ and IL-18 blockade.[228] This case report provides an elegant example of how investigations into the molecular mechanisms controlling activation of NLRC4 could lead to therapeutic success in the treatment of human diseases.

## 7 | AIM2 INFLAMMASOME

AIM2 is a cytoplasmic innate immune sensor of dsDNA, capable of forming an inflammasome complex with ASC and procaspase-1[229–232] (Fig. 4). AIM2 consists of a C-terminal dsDNA-binding HIN-200 domain and an N-terminal PYD. The dsDNA recognized by AIM2 is not sequence specific but must be at least 80 bp in length.[233] The crystal structure of AIM2 provides insight into the mechanism of activation of AIM2. In an inactive conformation, the HIN-200 domain of AIM2 interacts with the PYD in an autoinhibitory fashion.[234] The release of the AIM2 PYD upon binding dsDNA allows for oligomerization of AIM2 along the DNA[235] and interaction with the PYD of ASC.[233] Others have found that the PYD facilitates self-oligomerization of AIM2 and induces activation of the AIM2 inflammasome.[236,237] Whereas AIM2 is constitutively expressed and maintained in an autoinhibitory conformation in resting cells,[238] other host-specific mechanisms exist to ensure tight regulation of the AIM2 inflammasome. AIM2 can be tagged for degradation by the regulatory protein TRIM11 and the autophagy-associated protein p62.[239,240] Furthermore, several proteins that inhibit activation of the AIM2 inflammasome are also produced by the cell, including the PYD-containing proteins POP1[241] and POP3[242] in humans and the HIN domain-containing protein p202 in mice[232,243–246] (Fig. 4).

The AIM2 inflammasome provides immune surveillance for a subset of bacteria, including *Francisella tularensis*,[247,248] *Streptococcus pneumoniae*,[249,250] *Mycobacterium* species,[251–253] *L. monocytogenes*,[254–258] *C. muridarum*,[159] *Porphyromonas gingivalis*,[259] and *Brucella abortus*,[260,261] and the dsDNA viruses CMV and vaccinia virus.[248] The AIM2 inflammasome is also activated upon infection with the malaria parasite *Plasmodium*[262] and the fungal pathogen *A. fumigatus*.[109]

The mechanism of AIM2 activation and its dependency on type I IFNs differ depending on the pathogen encountered by the cell. Infection by murine CMV is sufficient to directly activate the AIM2 inflammasome in macrophages without a requirement for type I IFN signaling, a phenotype that is recapitulated by activation of the AIM2 inflammasome via transfection of naked dsDNA into the cytoplasm of macrophages.[263,264] It is likely that viral DNA is directly exposed to AIM2 in the cytoplasm during infection with murine CMV. By contrast, type I IFN signaling is important for AIM2 inflammasome activation in response to intracellular bacterial pathogens.[159,250,264–268]

The molecular mechanism underlying the differential requirement for IFN signaling between bacteria and viruses has begun to be unraveled (Fig. 4). In *Francisella* infection, a small quantity of bacterial DNA is released that is sufficient to activate the DNA sensors cGAS, STING, and IFI204 and promote type I IFN production but not sufficient to activate AIM2.[238,264,268,269] Type I IFNs released from cells bind the type I IFN receptor subunits IFNAR1 and IFNAR2 in an autocrine or paracrine manner, promoting signal transduction via the IFN-stimulated gene factor 3 (composed of subunits STAT1, STAT2, and IRF9) to up-regulate the production of hundreds of IFN-inducible genes. Of these families of genes, the genes encoding GBP members GBP2 and GBP5 and IRG member IRGB10 are up-regulated by the type I IFN pathway via the transcription factor IRF1.[158,264,268] GBPs and IRGB10 actively attack and lyse bacterial cells, releasing bacterial DNA into the cytoplasm to activate the AIM2 inflammasome[158,264,268] (Fig. 4). It is currently unclear why DNA released upon initial infection can activate the cGAS and STING signaling axis but not AIM2. It is possible that the small amount of DNA released initially is not sufficient to activate AIM2 but is sufficient to up-regulate IFN-inducible and host defense proteins to orchestrate cell-autonomous immunity and to avoid premature cell death. However, if a cell is unable to control bacterial replication, then a larger amount of DNA is liberated by GBPs and IRGB10 to a level sufficient to trigger activation of the AIM2 inflammasome, resulting in pyroptotic demise of the cell and removal of a replicative niche that supports further bacterial growth.

Activation of the AIM2 inflammasome can be inhibited by certain pathogens. For example, the human bacterial pathogen *Mycobacterium tuberculosis* but not the nonvirulent species *Mycobacterium smegmatis* inhibits the production of IFN-$\beta$ and thereby, prevents activation of the AIM2 inflammasome.[267] *L. pneumophila* encodes the effector protein SdhA, which maintains the integrity of the *Legionella*-containing vacuole[270] and therefore, prevents release of PAMPs, such as dsDNA, into the cytoplasm for sensing by the AIM2

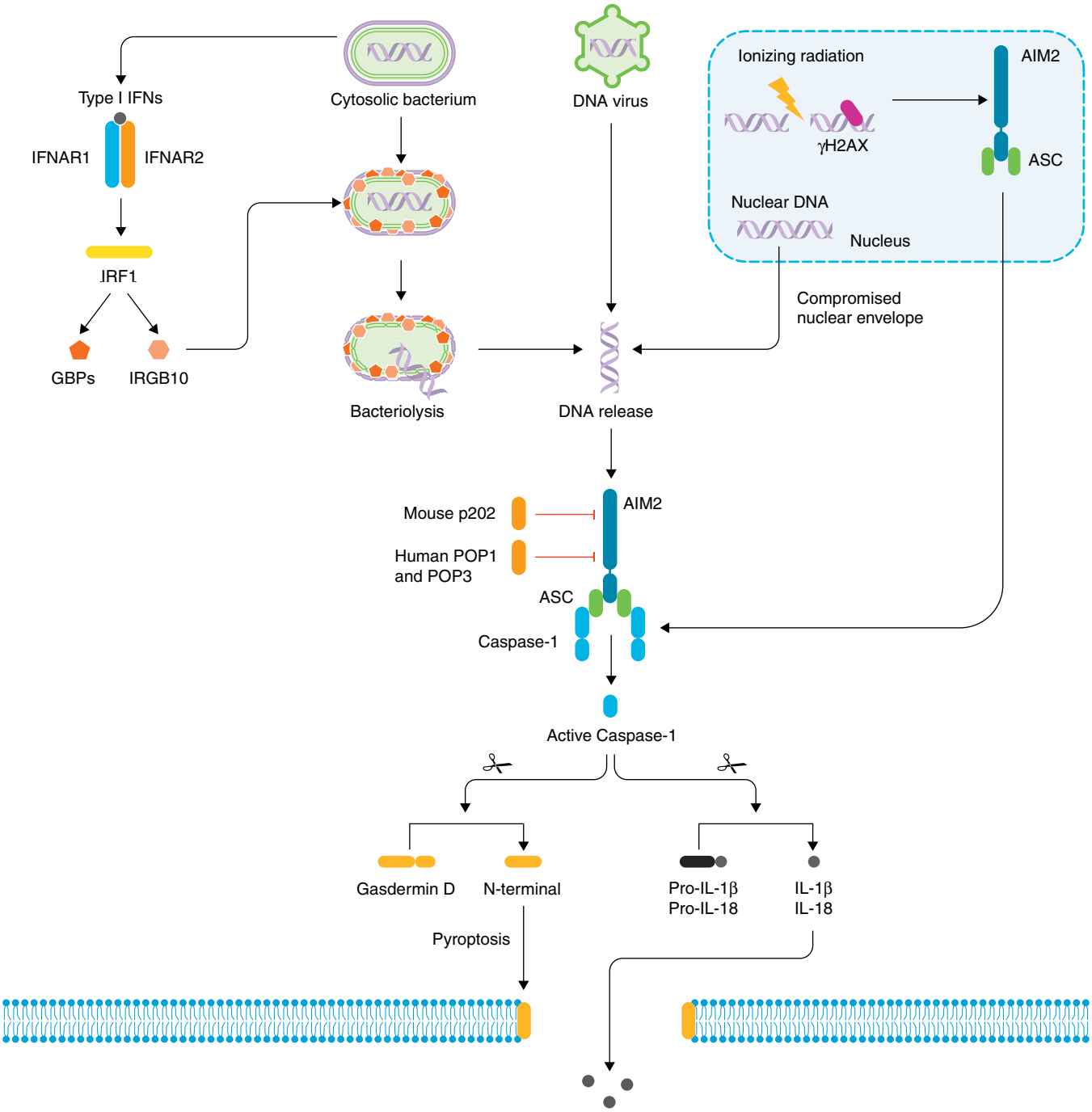


**FIGURE 4 AIM2 inflammasome.** Certain cytosolic bacteria and DNA viruses and mammalian DNA can activate the AIM2 inflammasome.[238,247,248,263] The cytosolic bacterium *Francisella novicida* induces the production of type I IFNs,[265] activating the type I IFN signaling pathway via the transcription factor IRF1.[264] IRF1 induces up-regulation of IFIs GBPs and IRGB10,[158,264] where these proteins directly attack the bacterial membrane of *F. novicida*.[158,264,268] The ruptured bacteria release DNA into the cytoplasm. The DNA virus mouse CMV activates AIM2 independently of type I IFN signaling. Nuclear DNA from host cells that are exposed to ionizing radiation undergo dsDNA breakage. AIM2 and ASC redistribute to the nucleus, where the 2 inflammasome proteins localize to dsDNA breaks and $\gamma$-H2AX.[285] The AIM2–ASC complex moves to the cytoplasm to assemble an inflammasome complex.[285] Damage to the nuclear envelope caused by the HIV protease inhibitor Nelfinavir mediates the release of nuclear DNA.[286] AIM2 is normally maintained in an autoinhibitory state via interaction between its HIN-200 domain and the PYD.[233] On recognition of DNA, AIM2 assembles an inflammasome complex. AIM2 can be inhibited by a mouse p202, a bipartite protein containing 2 HIN domains that interact with the HIN domain of AIM2.[232,245] AIM2 can also be inhibited by human POP1 and POP3[241,242]

inflammasome. The viral pathogen human CMV produces a tegument protein pUL83 that directly interacts with AIM2 to prevent inflammasome activation.[271] Interestingly, *F. novicida* encodes a Cas9-dependent clustered regularly interspaced short palindromic repeats-Cas system that enhances envelope integrity,[272] thereby promoting evasion of an AIM2 inflammasome immune response by potentially resisting GBP- and IRGB10-mediated bacteriolysis. The prevalence of AIM2 inhibitory mechanisms used by pathogens to escape innate

immune recognition highlights the importance of the AIM2 inflammasome in sensing PAMPs and clearance of pathogens.

Although best known for its function in providing protection against infection, AIM2 also plays a critical role in maintaining intestinal homeostasis.[273] Reduced expression and mutation of the AIM2 gene have been observed in the tumors of colorectal cancer patients.[274–277] Loss of AIM2 has been implicated in the development of colorectal cancer and is associated with poor survival in patients with colorectal cancer.[277] Mice deficient in AIM2 are hypersensitive to colorectal cancer in an azoxymethane and DSS model compared with wild-type mice.[278,279] Unlike its role in providing protection against infection, AIM2 suppresses colon tumorigenesis independently of inflammasome activity; instead, AIM2 interferes with DNA–protein kinase-dependent AKT activation in intestinal epithelial cells[278] and prevents Wnt signaling and proliferation in intestinal stem cells.[279] A further study has confirmed a role for AIM2 inhibiting the PI3K–AKT pathway in the human colorectal cancer cell line HCT116.[280] In the DSS-induced acute colitis model, AIM2-deficient mice are highly susceptible in a manner that is intricately linked to dysbiosis of the gut microbiota.[281,282] Interestingly, transfer of gut microbiota from AIM2-deficient mice to wild-type mice conferred higher susceptibility to colitis, whereas treatment with antibiotics completely attenuated the differences between wild-type and AIM2-deficient mice.[281] It has been proposed that the AIM2 inflammasome-mediated release of IL-18 triggers the up-regulation of the IL-22-binding protein and antimicrobial peptides by intestinal epithelial cells,[281,282] thereby modulating the composition of the gut microbiota.

Whereas AIM2 is beneficial in the context of infection and intestinal homeostasis and protective in colorectal cancer, activation of the AIM2 inflammasome can also cause damage to host cells. For instance, the accumulation of self-DNA in the cytoplasm of keratinocytes in psoriasis lesions[283] or in the joints of chronic polyarthritic mice[284] triggers AIM2 inflammasome activation, leading to the activation of caspase-1 and release of proinflammatory cytokines and inflammation. Furthermore, mice deficient in AIM2 are protected from the radiation-induced gastrointestinal syndrome and hematopoietic failure.[285] Importantly, AIM2 relocates to the nucleus, where it colocalizes with the marker of dsDNA breaks, $\gamma$-H2AX, and assembles an inflammasome complex in macrophages exposed to ionizing radiation[285] (Fig. 4). Additionally, disruption of the nuclear envelope by an HIV aspartyl protease inhibitor, Nelfinavir, was found to activate the AIM2 inflammasome by releasing DNA into the cytosol.[286] A further study has shown that dsDNA, released from intestinal cells damaged by chemotherapy treatment, activates the AIM2 inflammasome in immune cells, leading to production of IL-1$\beta$ and IL-18 and diarrhea.[287] In this case, abrogation of AIM2 signaling significantly reduced the incidence of diarrhea without affecting the anticancer effect of the chemotherapeutic.[287]

A detrimental role of AIM2 in metastatic skin cancer has also been proposed. A study has suggested that knockdown of AIM2 resulted in reduced cancer cell viability, suppression of growth, and vascularization of xenografts in vivo and the onset of apoptosis.[288] DNA released from damaged or dying cells during infection could also lead to activation of the AIM2 inflammasome. For example, host-derived DNA molecules are liberated from virus-infected cells and accumulate in the lungs following infection by the RNA virus IAV. A study has shown that that AIM2-deficient mice infected with IAV exhibited attenuated lung injury and significantly improved survival compared with wild-type mice.[289] Another study, however, has reported that AIM2-deficient mice had increased susceptibility to IAV infection, owing to increased numbers of infiltrating leukocytes and an exaggerated immune response.[290] The strikingly different host susceptibility to infection by IAV observed in these 2 studies could be owing to the different source and infection dose of the virus, as well as differences in the microbiota composition at different animal facilities. Nevertheless, these findings collectively demonstrate that AIM2 can also function as a sensor of DAMPs in that it activates the inflammasome in response to self-DNA; however, activation of the AIM2 inflammasome in many of these cases leads to excessive cell death and tissue injury rather than a protective inflammatory response.

## 8 | PYRIN INFLAMMASOME

Pyrin, encoded by the *MEFV* gene, is a member of the TRIM family. Missense mutations in MEFV are associated with the development of an autoinflammatory condition in humans, known as FMF.[291,292] Human Pyrin carries a PYD, 2 B-boxes and a coiled-coil domain, and a B30.2 (also known as the SPRY) domain. FMF-associated mutations are frequently found in the C-terminal B30.2 domain of human Pyrin.[291,292] Mouse Pyrin does not have a B30.2 domain. To study the physiologic effect of Pyrin and FMF-associated mutations, a mouse model of FMF was generated by knocking in the B30.2 domain of human Pyrin carrying 1 of 3 FMF-associated mutations into mice.[293] Introduction of the B30.2 domain carrying these FMF mutations in mice led to the development of spontaneous inflammation.[293] Importantly, deletion of the gene encoding ASC or IL-1R in these mouse strains abolishes their inflammatory phenotype,[293] providing robust genetic evidence to suggest that dysregulated Pyrin activity is linked to inflammasome-mediated pathology.

Earlier studies had suggested that Pyrin interacts with components of the NLRP3 inflammasome and that Pyrin inhibits the activation of caspase-1, NF-$\kappa$B, and apoptosis.[38,294–296] Further in vitro evidence demonstrates that Pyrin is an inflammasome-activating sensor (Fig. 5). Genetic silencing of Pyrin using small interfering RNA reduced inflammasome activation in THP-1 cells infected with *Burkholderia cenocepacia*.[297] Genetic deletion of Pyrin also abolished inflammasome assembly, caspase-1 activation, and IL-1$\beta$ and IL-18 secretion.[298] The activator of Pyrin was later identified as Rho-inactivating toxins produced by bacteria (Fig. 5), including those from *B. cenocepacia*, *Clostridium difficile*, *Clostridium botulinum*, *Vibrio parahemolyticus*, and *Histophilus somni*.[298] These toxins inactivate RhoA at the GTPase switch-I region, leading to activation of the Pyrin inflammasome.[298] In addition, the pertussis toxin was found to engage activation of the Pyrin inflammasome in a manner dependent on the ADP-ribosyltransferase activity of the toxin.[299] Furthermore, Pyrin contributed to pertussis toxin-induced neutrophil adhesion to

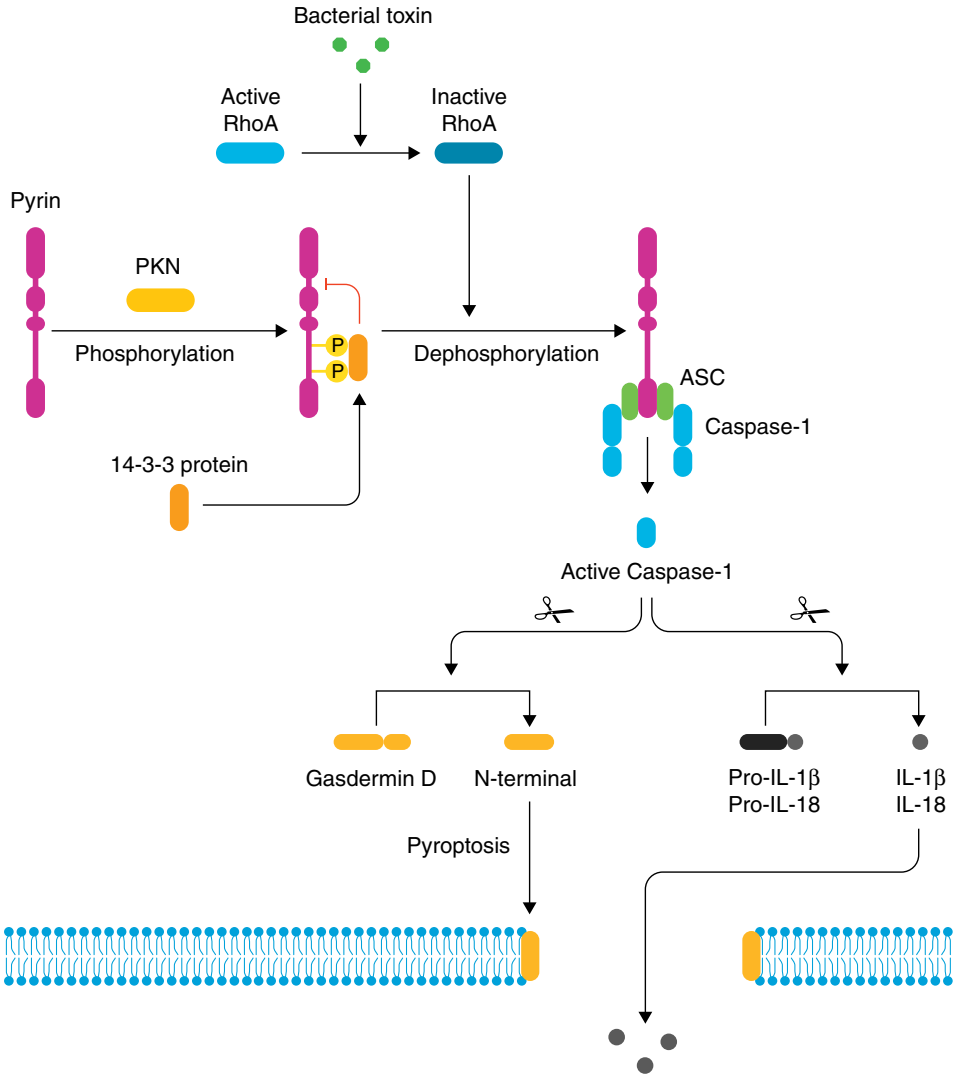


**FIGURE 5** **Pyrin inflammasome.** During homeostasis, Pyrin is phosphorylated by RhoA effectors and serine-threonine kinases PKN1 and PKN2.[259] Phosphorylated Pyrin is bound by 14-3-3 proteins, inducing inhibition of Pyrin.[302,303] Certain bacteria secrete toxins that inactivate RhoA, a process that mediates dephosphorylation of Pyrin.[298–300] Mutations in certain sites of Pyrin also lead to dephosphorylation of Pyrin.[304,305] This dephosphorylation event triggers Pyrin dissociation from 14-3-3 proteins, unleashing active Pyrin that is then capable of assembling an inflammasome complex[298]

cerebral capillaries and the induction of experimental autoimmune encephalomyelitis in mice.[299]

The expression of Pyrin is up-regulated following TLR priming of mouse BMDMs.[300] Whereas not necessary, TLR-dependent priming enhances activation of the Pyrin inflammasome by the *C. difficile* toxins TcdA and TcdB.[300] The role of Pyrin inflammasome in the host defense against infection has been demonstrated in a mouse model of lethal *B. cenocepacia* infection. Mice lacking Pyrin or ASC develop a milder form of lung inflammation in response to *B. cenocepacia* infection compared with wild-type mice.[298] Furthermore, *B. cenocepacia*, lacking the Type VI effector deamidase TecA and therefore, unable to modify Rho GTPases to activate the Pyrin inflammasome, induces lethality in wild-type mice more rapidly than did a wild-type *B. cenocepacia* strain.[301] These findings collectively demonstrate that the Pyrin inflammasome mediates recognition of Rho deamidase activity of TecA in *B. cenocepacia* to drive protective responses in the host.

Human Pyrin was previously found to interact with 2 isoforms of the regulatory proteins, known as 14-3-3$\varepsilon$ and 14-3-3$\tau$.[302] A further study has shown that Pyrin interacts with all 7 isoforms of 14-3-3 proteins in mouse BMDMs.[303] Phosphorylation of Pyrin at sites S208, S209, and S242 is necessary to mediate interaction with 14-3-3 proteins.[302] Further mechanistic studies have unraveled the biologic significance of the interaction between Pyrin and 14-3-3 proteins. A mutation in the *MEFV* gene that causes a S242R substitution abolishes inhibitory binding of 14-3-3 proteins to Pyrin.[304] This mutation was found in patients with a newly described autoinflammatory condition called PAAND.[304] The S242R mutation promotes excessive ASC speck formation and activation of caspase-1 and secretion of IL-1$\beta$ in 293T cells or in THP-1 monocytic cells.[304] Likewise, monocytes from patients with PAAND carrying the S242R mutation undergo spontaneous inflammasome activation in response to LPS stimulation.[304]

Studies in the murine system have revealed that mouse Pyrin can be phosphorylated on S205 and S241—2 sites corresponding to S208 and S242 of human Pyrin, respectively[303] (Fig. 5). The 2 serines are dephosphorylated in response to stimulation by Rho-inactivating toxins and infection with *B. cenocepacia*, releasing Pyrin from 14-3-3 proteins.[304] A further study has demonstrated that Pyrin is phosphorylated by the RhoA effector serine-threonine kinases PKN1 and PKN2.[305] Phosphorylation of human Pyrin at sites S208 and S242 by PKN1 or PKN2 promotes interaction between Pyrin and 14-3-3$\varepsilon$ or 14-3-3$\tau$ proteins, thereby maintaining inhibition of the Pyrin inflammasome in unstimulated cells.[305] The presence of FMF- or PAAND-associated mutations in Pyrin impairs binding of Pyrin with 14-3-3 or PKN proteins.[304,305] These findings provide an additional mechanistic insight into why patients carrying these mutations exhibit uncontrolled inflammasome responses.

Pharmacological drugs that target the microtubule, including colchicine, vinblastine, paclitaxel, BAL27862, nocodazole, ABT-751, CA4P, and CYT997, have all been reported to inhibit assembly and activation of the Pyrin inflammasome induced by TcdA or TcdB.[300,303,305] However, these microtubule inhibitors do not interfere with the dephosphorylation of Pyrin or dissociation of 14-3-3 proteins,[303] suggesting that Pyrin dephosphorylation acts upstream of microtubule activities. Human PBMCs carrying FMF-associated mutations in Pyrin secrete IL-1$\beta$ and IL-18 in response to stimulation with TcdA, even in the presence of colchicine treatment,[300] suggesting that Pyrin, harboring FMF-associated mutations, bypasses the requirement for microtubules in the activation of the inflammasome.

The role of the cytoskeleton in the activation of the Pyrin inflammasome has also been observed in a mouse model of systemic autoinflammatory disease. Mice carrying an inactivating mutation in the actin-depolymerizing cofactor WD repeat-containing protein 1 develop a systemic autoinflammatory disease that can be delayed by genetic deletion of Pyrin, ASC, caspase-1, or IL-18.[306] This finding suggests that dysregulated actin dynamics may be sensed by the Pyrin inflammasome, which could lead to inflammation and tissue damage. In support of this idea, a previous study has shown that Pyrin polarizes at the leading edge of migrating human monocytes, where it colocalizes with polymerizing actin.[307]

The relationship between Pyrin and other components of the cell has been identified. Human Pyrin was found to associate with the autophagy-related proteins ULK1, Beclin 1, and ATL16L1.[308] The same study also reported that Pyrin, but not Pyrin carrying FMF-associated mutations on the B30.2 domain, mediates recruitment of ULK1 to the NLRP3 inflammasome, where this complex is degraded by the autophagy machinery.[308] These observations indicate that under some conditions, Pyrin may negatively regulate activation of other inflammasomes.[38,294–296]

## 10 | CONCLUSIONS

Studies from the last 5 yr have propelled our understanding of inflammasome structures and signaling pathways. There is now clear evidence to conclude that inflammasome sensors can either directly interact with their ligands or sense general physiologic aberrations caused by PAMPs and DAMPs. Biochemical and structural studies have identified new components and regulators of the inflammasome. These studies provide molecular insights into how inflammasome sensors recognize PAMPs and DAMPs, assemble into macromolecular complexes, and induce downstream signaling events. Detection of the pathogen by inflammasome sensors heavily depends on accessibility to PAMPs. PAMPs can either be injected into the host cytoplasm via pathogen-associated secretion systems or exposed via active host-mediated processes. Binding between a single entity of ligand and an inflammasome sensor can induce a cascade of oligomerization events, culminating in inflammation and cell death, which define the physiologic outcomes in the host. These outcomes are either beneficial, such as immunity to infection, or detrimental, including development of inflammatory, metabolic, and neurologic diseases and cancer. Therefore, further studies unraveling the molecular basis of inflammasome activation at a structural and biochemical level would inform translational studies. These investigations would lead to the identification of novel inhibitors used to treat the cryopyrin-associated autoinflammatory syndrome, recurrent macrophage activation syndrome, FMF, and other clinical manifestations associated with dysregulated inflammasome signaling.

## AUTHORSHIP

A.M., J.A.H., and S.M.M. contributed to the writing of this review.

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## DISCLOSURES

The authors declare no conflicts of interest.

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