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Review Article

Pyrin Domain (PYD)-containing Inflammasome in Innate Immunity

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Inflammasome is a cytosolic multiprotein complex to activate caspase-1 leading to the subsequent processing of inactive pro-interleukin-1-beta (Pro-IL-1β) into its active interleukin-1 beta (IL-1β) in response to pathogen- or danger-associated molecular pattern. In recent years, a huge progress has been made to identify inflammasome component as a molecular platform to recruit and activate caspase-1. Nucleotide-binding oligomerization domain-like receptor (NLR) family proteins such as NLRP1, NLRP3 or interleukin-1β-converting enzyme (ICE)-protease activating factor (IPAF) have been first characterized to form inflammasome complex to induce caspase-1 activation. More recently, non-NLR type, pyrin-domain (PYD)-containing proteins such as pyrin or absent in melanoma2 (AIM2) were also proposed to form caspase-1-activating inflammasome machinery with apoptosis-associated speck-like protein containing a CARD (ASC), an essential adaptor molecule. Inflammasome pathways were shown to be crucial for protecting host organisms against diverse pathogen infections, but accumulating evidences also suggest that excessive activation of inflammasome/caspase-1 might be related to the pathogenesis of inflammation-related diseases. Indeed, mutations in NLRP3 or pyrin are closely associated with autoinflammatory diseases such as familial Mediterranean fever (FMF) syndrome or Muckle-Wells syndrome (MWS), indicating that the regulation of caspase-1 activity by inflammasome is a central process in these hereditary inflammatory disorders. Here, recent advances on the molecular mechanism of caspase-1 activation by PYD-containing inflammasomes are summarized and discussed.

Key Words: Inflammasome, Caspase-1, Interleukin-1-β (IL-1β), Pyrin-domain (PYD), NLR, Autoinflammatory disease

Pattern-recognition receptors

Innate immune system is highly conserved among species from plants to mammals and offers the first line of host defense against invading pathogens. Upon microbial infection, innate immune system recognizes the invasion of microbes by germline-encoded pathogen-recognition or pattern-recognition receptors (PRRs) (1) (Fig. 1). As far, four different PRR families have been identified such as Toll-like receptor (TLR), retinol-inducible gene-I (RIG-I)-like receptor (RLR), C-type lectin receptor (CLR) and nucleotide-binding oligomerization domain (NOD)-like receptor (NLR). These PRRs are predominantly expressed in innate immune cells including macrophages or dendritic cells, where they sense a unique pattern of microbial component, called pathogen-associated molecular pattern (PAMP).

The best-characterized PRR is a membrane-spanning Toll-like receptor (TLR) family, localized on the plasma or endosomal membrane. TLRs contain outward leucine-rich repeat (LRR) region, which specifically bind to PAMP, and cytoplasmic Toll/interleukin-1 receptor homology (TIR)

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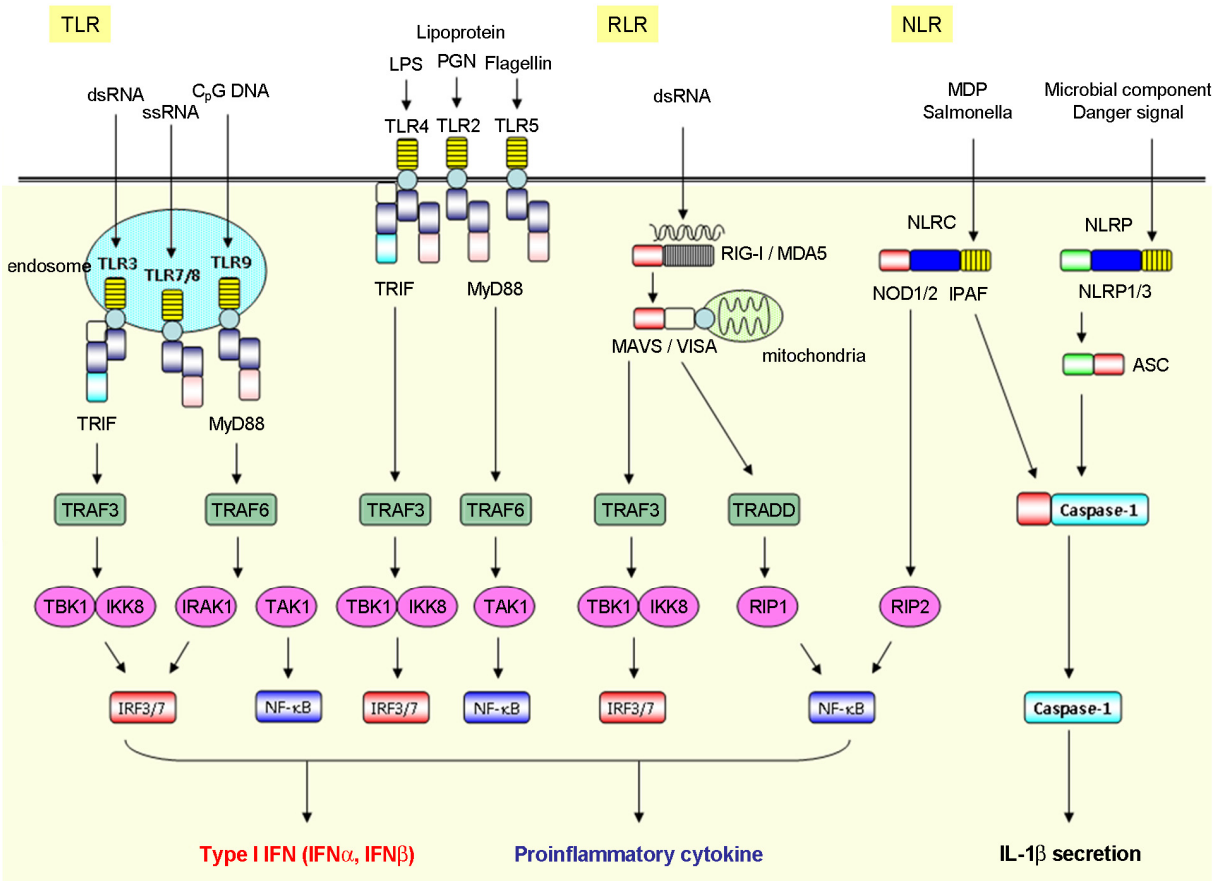

Figure 1. Pattern-recognition receptor (PRR) signaling pathways. Three classes of PRR families, such as TLRs, RLRs and NLRs, sense microbial products or endogenous danger signals to trigger downstream signaling pathways, resulting in the production of type I IFN, proinflammatory cytokines and the activation of caspase-1.

domain for protein-protein interaction. To date, 10 human and 12 mouse TLR members have been identified, and each TLR detects a specific microbial moiety such as viral single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA), unmethylated CpG DNA motifs and bacterial lipopolysaccharide (LPS), lipoprotein, lipopeptide or peptidoglycan (PGN) through its LRR motif (2).

Upon engagement of specific ligand, TLRs trigger a set of downstream signaling pathways via recruiting TIR domain-containing adaptor molecules such as myeloid differentiation primary response gene 88 (MyD88), MyD88-adaptor-like (MAL/TIRAP), TIR-domain-containing adaptor protein-inducing interferon-β (TRIF/TICAM1) and TRIF-related adaptor molecule (TRAM/TICAM2) (3, 4). These diverse TLR-mediated signaling cascades ultimately activate two transcription factors such as interferon regulatory factor (IRF) and nuclear factor-kappa B (NF-κB), leading to the induction of type I interferon (IFN) including IFNα or IFNβ, proinflammatory cytokines and the maturation of dendritic cell (1, 4).

Unlike TLRs, the other PRRs, including RLR and NLR family, are cytoplasmic receptors for sensing an intracellular PAMP (1, 5). While endosomal TLRs recognize microbial nucleic acids, such as dsRNA (TLR3), ssRNA (TLR7/8) and unmethylated CpG DNA (TLR9), RLR families, RIG-I and melanoma differentiation-associated gene 5 (MDA5), are specialized in detecting intracellular viral dsRNA through their RNA helicase domain (6). Upon sensing cytoplasmic dsRNA, RLRs interact with mitochondrial antiviral signaling protein (MAVS), also known as interferon-beta promoter stimulator 1 (IPS-1), Cardif and virus-induced signaling adaptor (VISA), through caspase recruitment domain

(CARD) homotypic interaction (1). Association of RIG-I with MAVS then triggers a series of downstream signaling cascade leading to the induction of antiviral type I IFN and proinflammatory cytokines as similar to TLR-mediated signaling pathways (7).

On the other hand, the main function of NLRs, another type of intracellular PRR family, has been considered to activate caspase-1 in response to PAMP or danger-associated molecular pattern (DAMP) (8). Although it is not clarified in detail how NLRs sense PAMP or DAMP, the engagement of pathogenic moiety to NLRs might trigger the formation of molecular complex comprising of NLR, apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase-1, resulting in the activation of caspase-1. This molecular assembly to activate caspase-1 is termed as inflammasome complex (9). NLR contains N-terminal CARD, pyrin domain (PYD) or baculovirus inhibitor of apoptosis repeat (BIR), followed by central NAIP, CIITA, HET-E, TP-1 (NACHT) domain and C-terminal LRR motif (5). In addition to NLR family, another PYD-containing proteins such as pyrin and absent in melanoma2 (AIM2) were also identified to assemble inflammasome complex with ASC, resulting in the activation of caspase-1 (10~14). Here, we will describe the molecular function of PYD-containing inflammasomes in innate immune system.

Caspase-1: Inflammatory caspase

Caspase-1, also known as interleukin-1β-converting enzyme (ICE), belongs to a cysteine protease caspase family, which mainly orchestrate the molecular event of cellular apoptosis or inflammation. To date, 14 caspases have been identified in human and mouse, and they are classified into two subgroups according to their function as inflammatory (Group I) or apoptotic (Group II or III) caspases (15). Inflammatory caspases include caspase-1, -4 and -5 in human and caspase-1, -11 and -12 in mouse, and they are clustered on human chromosome 11q22 and mouse chromosome 9A1 (16). Caspase-1 is responsible for processing pro-interleukin 1β (pro-IL-1β), pro-IL-18 and pro-IL-33 into their matured form, but the physiological functions of the other inflammatory caspases except caspase-1 are poorly characterized yet.

The significance of caspase-1 in innate immune protection was highlighted using caspase-1 knockout mice study. For example, deficiency of caspase-1 conferred resistance to lipopolysaccharide (LPS)-induced shock, whereas mice lacking both IL-1β and IL-18 are still susceptible (17, 18). Recent studies also demonstrated that caspase-1-deficient mice are more susceptible to the infection of bacterial pathogen such as *Francisella tularensis* and *Salmonella enterica* than wild type mice (19, 20). These results collectively indicate that caspase-1 is crucial component in regulating endotoxin-induced inflammation and clearance of intracellular bacterial pathogens.

In addition to the maturation of IL-1β, caspase-1 seems to regulate more noncanonical processes contributing into innate immune responses, based on the phenotypic differences between caspase-1 and IL-1β or IL-18 knockout mice studies. Caspase-1 might regulate the unconventional secretion of leaderless proinflammatory proteins such as IL-1β and high-mobility group box 1 (HMGB1), although the molecular mechanism remains largely unknown (18, 21). Furthermore, caspase-1 induces pyroptotic cell death in macrophages and dendritic cells in response to microbial infection, probably to clear the infected cells (21). Further study will be needed to clarify the molecular mechanism by which caspase-1 regulates these noncanonical functions.

Like all other apical caspases, caspase-1 is synthesized as an inactive zymogen, which contains N-terminal CARD domain, followed by p20 and p10 domain. To gain its full activity, procaspase-1 has to be recruited to molecular platform and auto-processed by proximity model into heterotetrameric structure of p20 and p10 (22, 23). Attempts to find a CARD-containing protein, which specifically binds to CARD of procaspase-1, first uncovered two proteins, which are able to interact with procaspase-1 to induce its processing, namely ASC and ICE-protease activating factor (IPAF/NLRC4) (24, 25).

ASC: a key adaptor molecule for caspase-1 activation and pyroptotic cell death

ASC is a bipartite protein containing N-terminal PYD and C-terminal CARD, which engages procaspase-1 via CARD homotypic interaction. Interestingly, overexpression or enforced oligomerization of ASC is sufficient to activate caspase-1 and the subsequent maturation of IL-1β, indicating that the oligomerization of ASC is a crucial event to induce the activation of caspase-1 (24, 26, 27). In a physiological condition, ASC requires an additional PYD-containing protein for triggering the oligomerization of ASC (Fig. 2). Protein data base search to find a PYD-containing protein to engage and oligomerize ASC sheds light on the identification of caspase-1-activating molecular machinery such as NLRP3, pyrin and AIM2, all of which are able to interact with ASC through PYD-PYD homotypic interaction to assemble inflammasome complex (Fig. 2). Moreover, accumulating ASC-deficient mice studies confirmed that ASC is an essential molecular adaptor protein to activate caspase-1 in response to a variety of stimuli (19, 28~32).

PYD belongs to death domain superfamily, which is characterized by 6 α-helix bundle and plays an important role in the assembly of apoptotic and inflammatory proteins (33). Based on the nuclear magnetic resonance (NMR) structural analysis on the PYD of NLRP1 and ASC, PYD has a unique long loop region between H2 and H3 helices or at H3, which may determine specificity of PYD (33~35). Although the structural mode of PYD-PYD interaction is not characterized yet, structural and mutational analysis on the PYD of ASC revealed that charged and hydrophobic

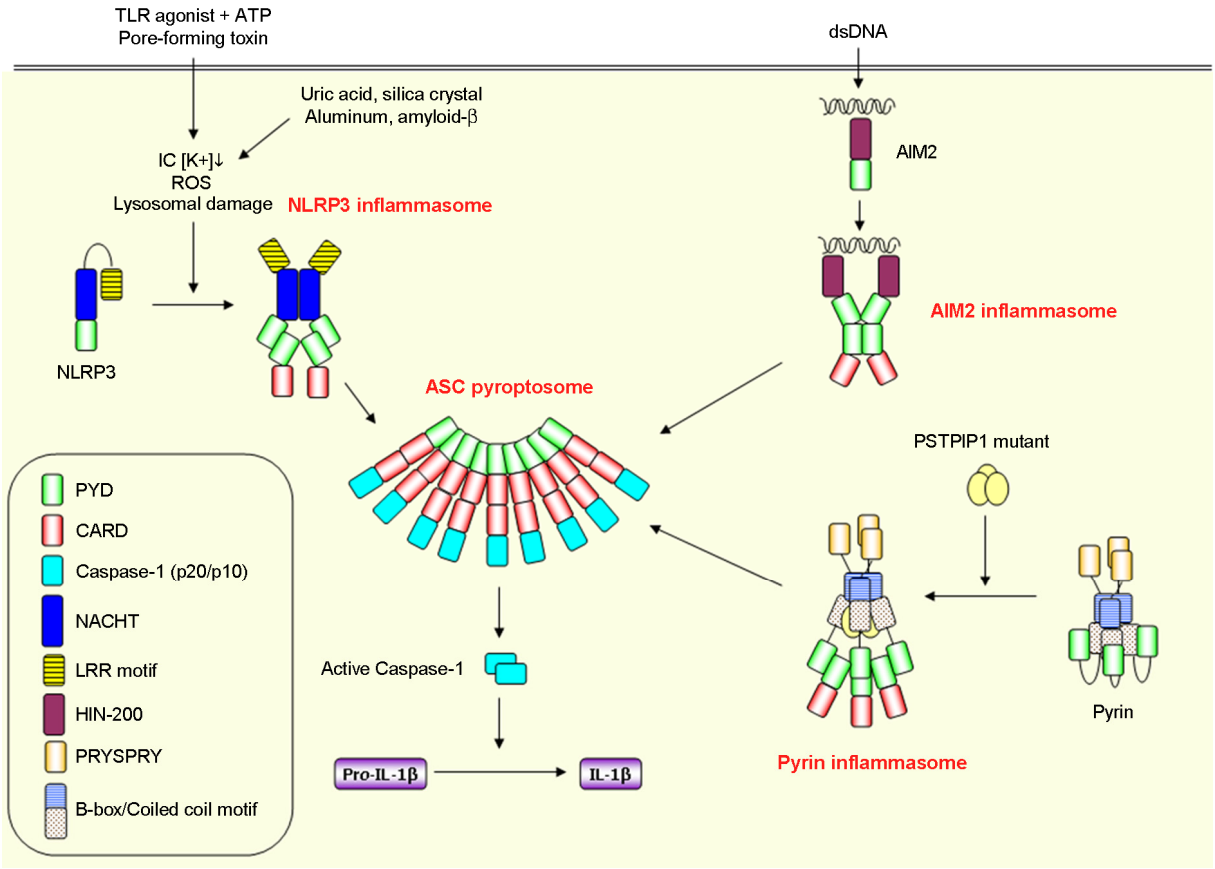

Figure 2. PYD-containing inflammasome pathways. Inactive PYD-containing inflammasome components are activated by sensing diverse microbial infection or danger signals (NLRP3), cytoplasmic dsDNA (AIM2) and autoinflammatory disease-associated PSTPIP1 mutant (pyrin) to trigger the formation of ASC pyroptosome, resulting in the activation of caspase-1.

residue in H2 and H3 plays a critical role in homotypic or heterotypic PYD interaction (36).

A number of PYD-containing proteins to associate with ASC have been reported to activate or inhibit caspase-1 activity suggesting ASC is a hub molecule in the regulation of caspase-1/inflammasome pathway. While PYD-containing proteins equipped with self-oligomerizing domain generally activates caspase-1, cytosolic or viral PYD-only protein such as PYD-only protein 1 (POP1) or POP2 and M13L has been shown to inhibit ASC-mediated caspase-1 or NF-κB activation through PYD-PYD interaction (37~39).

ASC is highly oligomerized into form ASC pyroptosome (Fig. 2), a supramolecular assembly of ASC, responding to a variety of proinflammatory stimuli. Interestingly, the macrophages containing ASC pyroptosome rapidly undergo caspase-1-dependent inflammatory cell death, called pyroptosis (27, 40). Indeed, overexpression or oligomerization of ASC is already known to induce apoptosis although detailed molecular mechanism is not fully understood (41, 42). ASC, also known as target of methylation-induced silencing (TMS1), is frequently silenced by abberant methylation in several tumor cell types (43, 44) and induced in a p53-dependent manner (42). It will be thus of interest to investigate the role of ASC or inflammasome-mediated cell death in developing these tumors.

NLR subfamily

To date, more than 20 NLR members have been identified and grouped into five different subfamilies mainly based on its N-terminal moiety, which are NLRA (CIITA), NLRB (NAIP), 5 NLRC, 14 NLRP and NLRX (45). Among NLRC subfamilies, which contain N-terminal CARD, only IPAF (NLRC4) has been clearly shown to assemble inflammasome complex leading to the activation of caspase-1 and the maturation of IL-1β upon infection with Gram-negative bacterial pathogen such as *Salmonella typhimurium* and *Shigella flexneri* (28, 46~48). IPAF could associate directly with procaspase-1 through direct CARD-CARD interaction. However, it is poorly understood whether ASC is required for IPAF inflammasome-induced caspase-1 activation, because ASC-deficient mice also showed a defect in caspase-1 activation in response to *S. typhimurium* infection (28).

Contrary to ICE-protease activating factor (IPAF), two NLRC proteins, NOD1 (NLRC1) and NOD2 (NLRC2), have been shown to activate NF-κB signaling by recognizing the specific motif of bacterial peptidoglycans, indicating that NLRC might cooperate with TLR to defend host cells against bacterial infections (49). Intriguingly, another NLRC subfamily protein, NLRC5 was recently proposed to inhibit not only NF-κB signaling via interacting with IKKα and IKKβ, but type I IFN signaling via associating with RIG-I and MDA5, suggesting that NLRC5 might regulate TLR- or RLR-mediated innate immune signaling pathways (50). However, deficiency of NLRC5 failed to confer the differences in the secretion of proinflammatory cytokine or type I IFN upon viral or bacterial infections (51). As NLRC5 was rather proposed to activate NLRP3 inflammasome (52), further study will be needed to elucidate the physiological function of NLRC5 in innate immune system.

NLRP subfamilies, which contain N-terminal PYD, has been widely regarded as a major inflammasome component to activate caspase-1 by way of association with ASC. NLRP1 (also known as NALP1, CARD7) is the first proposed inflammasome, which forms complex with caspase-1, caspase-5, ASC and cardinal (9), but its detailed molecular mechanism to activate caspase-1 is still poorly understood. NLRP1 was demonstrated to activate caspase-1 in cell free system upon stimulation with muramyl dipeptide (MDP) and ATP (53), and mouse *Nlrp1b* was shown to play a crucial role in *Bacillus anthracis* lethal toxin-induced caspase-1 activation and macrophage cell death in mouse (54). However, physiological role of human NLRP1 to activate caspase-1 is not characterized yet. Nevertheless, genetic variations in NLRP1 are associated with vitiligo-associated autoimmune or autoinflammatory diseases indicating NLRP1 may play a crucial role in the pathogenesis of these inflammatory disorders (55).

NLRP3 inflammasome

NLRP3 (previously known as NALP3, cryopyrin or PYPAF1) is currently the most well-defined inflammasome to activate caspase-1 in an ASC-dependent manner upon stimulation with a variety of microbial infection and host-derived danger signal (29~32, 56~58). Interest has been initially focused on the protective role of NLRP3 inflammasome to recognize PAMPs derived from invading microbial pathogens including bacteria producing pore-forming toxin, viruses and fungi (49). Although NLRP3-deficient mice exhibited a defect in caspase-1 activation and IL-1β secretion upon infection with the pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, influenza virus, adenovirus and *Candida albicans*, it remains to be determined how NLRP3 senses these wide range of pathogen-derived signals. Instead, emerging evidences have proposed that NLRP3 is also the crucial player to trigger sterile inflammatory responses in response to a variety of host-derived signals from tissue injury or metabolic stress (5, 59). NLRP3 inflammasome is obviously responsible for detecting endogenous indicators of cellular danger signal such as extracellular ATP, monosodium uric acid (MSU), amyloid β, silica, asbestos and cholesterol crystal, leading to the secretion of IL-1β (30, 56, 57, 60). Reduced inflammation was observed in NLRP3-deficient mice responding to a variety of sterile stimuli, proposing that NLRP3 inflammasome might be the potential target for treating chronic sterile inflammatory diseases.

Although the activation mechanism of NLRP3 inflammasome is not fully characterized yet, several models have been proposed to stimulate NLRP3 in response to diverse PAMPs or DAMPs. First model involves the intracellular level of potassium. Infections with bacteria secreting pore-forming toxins such as listeriolycin, pneumolysin or streptolysin O have been shown to activate NLRP3 inflammasome (5). Pore formation might trigger both the entry of unknown ligands for NLRP3 and the efflux of potassium. Interestingly, extracellular ATP or nigericin has been well known to stimulate NLRP3 inflammasome through potassium efflux. Although it remains to be determined how potassium efflux affects the NLRP3 inflammasome, potassium efflux seems also critical for ASC pyroptosome formation or AIM2 inflammasome upon infected with *Francisella tularensis* (27, 61). These observations thus suggest that potassium efflux might be the common event to trigger inflammasome activation. The second model is mediated by lysosomal destabilization. Endogenous particulates such as MSU, silica, alum or cholesterol crystal, are likely to cause lysosomal rupture leading to the cytoplasmic release of lysosomal contents including cathepsin B that might activate NLRP3 inflammasome (58, 60). Seemingly, inhibitor of cathepsin B, partially abrogated NLRP3-mediated IL-1β secretion upon stimulated with silica, but not with ATP, indicating that the release of cathepsin B is responsible for lysosomal destabilization-mediated NLRP3 inflammasome activation (58). Further study will be needed to clarify how lysosomal destabilization triggers the activation of NLRP3 inflammasome. Next model for activation of NLRP3 is mediated by the intracellular ROS, produced responding to infection or tissue injury. A wide range of PAMPs or DAMPs have been shown to induce the production of ROS in an NADPH oxidase (NOX)-dependent or mitochondria-dependent manner (5, 62). It remains poorly defined how ROS affects NLRP3 inflammasome, but recent study proposed that thioredoxin-interacting protein (TXNIP) is released from thioredoxin upon oxidative stress and interacts with NLRP3 leading to formation of NLRP3 inflammasome (63). It will be of interest to investigate whether TXNIP could affect other inflammasome component.

Furthermore, mutations in NLRP3 are more frequently found in the patients of hereditary periodic fever syndrome, featured by elevated level of IL-1β, proposing that disease-associated mutants of NLRP3 might be the constitutive activator of caspase-1 leading to these disorders such as Muckle-Wells Syndrome (MWS), familial cold autoinflammatory syndrome (FCAS) and neonatal-onset multisystem inflammatory disease (NOMID). Disease-associated mutations in NLRP3 might disrupt a potent inhibitory effect of LRR motif on the inflammasome assembly, it is still

unknown how these mutations are associated with these autoinflammatory disorders. Nevertheless, knock-in mice harboring Cryopyrin Associated Periodic Syndromes (CAPS)-associated mutations in NLRP3 exhibited an increase in proinflammatory cytokines including IL-1β and an inflammasome-dependent systemic inflammation (64, 65). Furthermore, mutations in NLRP3 might be involved in the susceptibility for more common autoinflammatory disease, Crohn's disease (66). These observations will give a new insight that constitutive assembly of NLRP3 or other inflammasome may be associated with chronic auto-inflammatory diseases.

Pyrin inflammasome in autoinflammatory syndrome

MEFV gene, which encodes pyrin (marenostrin), was identified by positional cloning as a susceptible gene for familial Mediterranean fever (FMF), a recessively inherited autoinflammatory disorder (67, 68). Pyrin contains N-terminal PYD, followed by B-box domain, coiled-coil domain and SPRY (also known as B30.2) domain. As far, more than 80 mutations, in pyrin have been associated with the phenotype of FMF (69), however, the molecular mechanism of pyrin mutation contributing into this periodic fever syndrome is poorly understood.

Pyrin belongs to a tripartite motif (TRIM)-containing protein superfamily, which are induced by type I or II IFN to regulate host innate immune response against viral infection (70). TRIM family proteins are featured by a unique N-terminal RING/B-box/coiled-coil (RBCC) motif comprising really-interesting-new-gene (RING) domain, B-box domain and coiled-coil domain, followed by diverse C-terminal domain as one or combinations of 10 distinct domains such as SPRY domain and C-terminal subgroup one signature (COS) domain (70). Numerous TRIM members including TRIM5α were shown to interfere with the life cycle of infected HIV at different stages (71, 72). Interestingly, some TRIM proteins were recently shown to regulate TLR- or RLR-mediated anti-viral innate immune responses in a positive or negative way. C-terminal SPRY domain of TRIM25 binds to CARD of RIG-I, which induces ubiquitination of RIG-I, leading to the induction of type I IFN signaling (73). Similarly, TRIM56 associates with stimulator of interferon genes (STING) to induce ubiquitination of STING, consequently resulting in the production of type I IFN in response to cytoplasmic dsDNA (74). On the contrary, mouse TRIM30α and TRIM27 were reported to inhibit NF-κB- or IRF-mediated proinflammatory gene expression through the interaction with TAK1 and IKKs (75, 76). These evidences suggest that TRIM family, induced by IFN, could play a key role not only in restricting a retroviral progression but in regulating PRR-mediated innate immune responses as a positive or negative feedback mechanism.

Pyrin (TRIM20) is the only TRIM member containing N-terminal PYD instead of RING domain. Using a yeast two-hybrid screening, ASC was identified to interact with pyrin through PYD-PYD homotypic interaction (77). However, the physiological function of pyrin in ASC/inflammasome signaling has been debated. Pyrin was first proposed to reduce NLRP3-mediated apoptosis and NF-κB activation via disrupting the interaction between ASC and NLRP3 in a transfection experiment (78). In consistent with this observation, mouse macrophages expressing truncated pyrin exhibited an increased IL-1β secretion upon stimulated with LPS, suggesting that pyrin might act as a negative regulator of caspase-1 activation by way of sequestering of ASC (79). The inhibitory role of pyrin in inflammasome formation was further supported by the following studies demonstrating that pyrin directly interacts with caspase-1, IL-1β and NLRP3 to inhibit caspase-1 activation and knockdown of pyrin leads to a slight increase in IL-1β secretion in THP-1 cells stimulated with LPS or MSU (80, 81).

On the contrary, a characteristic speck-like structure of ASC was clearly observed by the coexpression of pyrin, indicating that pyrin is able to oligomerize ASC (77, 79, 82). In a HEK293T cell-based reconstitution system, pyrin was not likely to compete with NLRP3 or caspase-1 for the binding to ASC, instead pyrin forms a NLRP3-independent molecular assembly with ASC and procaspase-1 leading to

caspase-1 activation, proposing pyrin inflammasome hypothesis (82). This pyrin inflammasome model is consistent with the previous report demonstrating both pyrin and NLRP3 enhance IL-1β secretion in the presence of ASC (83). Seshadri *et al*. also showed that overexpression of pyrin in HEK293 or THP-1 cells increases caspase-1 activation and IL-1β secretion in an ASC-dependent manner, and knockdown of pyrin suppresses IL-1β release from LPS-stimulated THP-1 and peripheral blood mononuclear cells (84). Notably, upregulated endogenous pyrin by retroviral infection is correlated with robust caspase-1 activation and IL-1β secretion in THP-1 cells (10). This observation also revealed that autoinflammatory Pyogenic sterile Arthritis, Pyoderma gangrenosum, and Acne (PAPA) syndrome-associated mutants of Proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) interact with B-box domain of pyrin, and this engagement exposes PYD of pyrin, which then bind to PYD of ASC, resulting in the activation of caspase-1 (10). These results raise the possibility that pyrin is a bona fide inflammasome component to activate caspase-1 in an ASC-dependent manner.

Considering that mouse pyrin lacks the C-terminal SPRY domain, the most susceptible region for disease-associated mutations in the FMF patients, the above discrepancy might be arisen from the difference in the presence of SPRY domain. To give a molecular insight on the physiological role of pyrin, Chae *et al*. recently developed a knockin mouse model to express chimeric pyrin containing C-terminal human FMF-associated SPRY domain (85). This study clearly demonstrated that knockin mice harboring chimeric pyrin spontaneously developed inflammatory phenotypes in ASC- and IL-1β-dependent way, supporting that pyrin could assemble inflammasome complex. However, it remains to be characterized how the FMF-associated mutations enable pyrin to form inflammasome machinery.

Single nucleotide missense mutations in the FMF patients are more frequently found in SPRY domain indicating this domain may exert a critical role in the pathogenesis of FMF, but it is largely unknown how these disease-associated mutations cause FMF. Considering pyrin is an antiviral TRIM family member, it is also possible that pyrin might act as a sensor for viral infection through SPRY domain. Further study on the physiological ligand for pyrin will give an important insight into the molecular mechanism of pyrin inflammasome complex.

AIM2 inflammasome: Molecular sensor for cytosolic double-stranded DNA

Microbial nucleic acid is an important molecular pattern for host innate immune system to distinguish nonself pathogenic moiety from self upon microbial infection (86). Bacterial DNA was, indeed, shown to induce type I IFN response more than 25 years ago, proposing that microbial DNA might be immunostimulatory (87). Krieg *et al*. also demonstrated that unmethylated CpG dinucleotides in bacterial DNA, but not methylated CpG motif in vertebrate DNA, trigger immune responses to produce proinflammatory cytokines or type I IFN (88). In 2000, TLR9, which are present within endosomal space, was identified as the first receptor for bacterial immunostimulatory DNA containing unmethylated CpG motif (89). Host DNA is normally sequestered inside nucleus or mitochondria, but it can be released into cytosol from damaged host cell. Similar to the microbial DNA, cytosolic dsDNA from host damaged cells was also demonstrated to activate innate immune systems in a TLR9-independent pathway, indicating that the immune systems are stimulated not only by foreign DNA but by host unnecessary DNA (90, 91).

In recent five years, TLR9-independent cytoplasmic DNA sensors mediating type I IFN production or caspase-1 activation have been extensively identified. DNA-dependent activator of Interferon-regulatory factor (DAI), also known as DLM-1 or Z DNA binding protein-1 (ZBP1), was first proposed to recognize cytosolic dsDNA and induce type I IFN production via recruiting Tank-binding kinase-1 (TBK1) and interferon regulatory factor 3 (IRF3) (92). However, MEF cells lacking DAI were still responsive to cytoplasmic dsDNA to induce type I IFN response, suggesting that DAI is not the only dsDNA sensor (93). STING (also known as mitochondrial mediator of IRF3 activation [MITA] or Endoplasmic Reticulum Interferon

Stimulator [ERIS]) was then proposed as a crucial adaptor protein mediating type I IFN response upon cytoplasmic DNA or RNA (94, 95). It is now evident that STING is essential for cytosolic DNA-mediated IFN response and for host defense against HSV-1 or VSV infection (96), but STING does not directly bind to cytoplasmic nucleic acids. It remains to be characterized how upstream DNA sensors regulates STING mediating IFN response. In addition, both HMGB proteins and interferon, gamma-inducible protein 16 (IFI16) were also identified to sense cytoplasmic dsDNA leading to type I IFN signaling in a STING-dependent manner (97, 98). These findings indicate that several cytoplasmic DNA sensors are responsible for type I IFN response upon virus infection or host DNA release. It will be of interest to clarify how the DNA-sensing sentinels are orchestrated and involved in the autoimmune diseases such as systemic lupus erythematosus.

Cytoplasmic dsDNA was also shown to induce caspase-1 activation in an ASC-dependent, but NLRP3-independent manner, indicating that unknown dsDNA sensor might be present to mediate inflammasome pathway (56, 99). In 2009, AIM2 was identified as a cytoplasmic dsDNA-sensing inflammasome to activate caspase-1 through assembling ASC (11~14). AIM2 belongs to a hematopoietic inducible nuclear protein (HIN)-200 family protein, which is comprised of N-terminal PYD and C-terminal HIN-200 domain containing two oligonucleotide/oligosaccharide-binding fold (100). As far, 4 human and 5 mouse HIN-200 members were cloned and characterized (101). Among them, only AIM2 is a cytosolic protein and able to associate with ASC through PYD-PYD interaction (11, 12). AIM2 was also shown to bind to dsDNA via HIN-200 domain and this engagement unexpectedly induced the oligomerization of AIM2, suggesting that dsDNA could act as a platform for inflammasome formation (11). Notably, cytosolic DNA derived from virus infection or host damaged cell is the first identified ligand to interact directly with the inflammasome component.

Cytosolic dsDNA-mediated caspase-1 activation was completely abrogated in AIM2-deficient macrophages (61, 102). Furthermore, mice lacking AIM2 exhibited a defect in innate immune responses including the activation of inflammasome and the production of IL-18 in response to infection of *Francisella tularensis* or vaccinia virus (61, 102, 103). AIM2 is thus essential for host protection against DNA-releasing pathogen through recognizing directly bacterial DNA, which triggers the activation of inflammasome. Interestingly, AIM2-lacking macrophages produced more type I IFN upon infected with *F. tularensis*, indicating that cytosolic DNA sensing by AIM2 is not involved in the production of type I IFN (61). Nevertheless, IRF3- or IFNAR1-deficient macrophages demonstrated a defect in caspase-1 activation by *F. tularensis* infection (61), suggesting that type I IFN is required for efficient inflammasome activation against *F. tularensis* infection, consistent with the previous report (104). Further study will be needed to describe how IFN enhances the inflammasome activity in response to bacterial infection.

AIM2 was also demonstrated to induce caspase-1-dependent pyroptotic cell death upon stimulation with cytosolic DNA (11). Considering AIM2 gene is known as a tumor suppressor gene and frequently silenced by DNA methylation, frame shift and missense mutation in many tumors, inactivation of AIM2 inflammasome-mediated pyroptotic pathway may play a crucial role in developing these tumors (11, 105). Mouse HIN-200 member, p202 was also shown to inhibit cytosolic DNA-induced caspase-1 and -3 activation supporting HIN-200 family is a major PRR to regulate cytoplasmic DNA-mediated immune response (14). The discovery of PYD-containing AIM2 inflammasome may contribute important insights into the role of dangerous cytosolic DNA in autoimmune disease or cancer.

Concluding Remarks

Inflammasome complex is increasingly recognized as a central guardian against microbial infection or endogenous danger signal. ASC is definitely a key adaptor molecule to assemble inflammasome complex via recruiting PYD-containing molecule as described above. PYD of ASC is needed for triggering or blocking the formation of ASC pyroptosome leading to the processing and activation of

caspase-1. Dysregulation of caspase-1 is closely associated with several autoinflammatory disorders and chronic inflammatory diseases. Hence, in addition to the production of type I IFN and proinflammatory cytokine through TLR- and RLR-mediated signaling pathway, the assembly of inflammasome complex to regulate caspase-1 activity is an essential part to keep cell integrity in response to invading pathogen or host damage.

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