

# The NLRP3 inflammasome in viral infection (Review)

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**Abstract.** The interplay between pathogen and host determines the immune response during viral infection. The Nod-like receptor (NLR) protein 3 inflammasome is a multi-protein complex that induces the activation of inflammatory caspases and the release of IL-1 $\beta$ , which play an important role in the innate immune responses. In the present review, the mechanisms of the NLR family pyrin domain containing 3 inflammasome activation and its dysregulation in viral infection were addressed.

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## 1. Introduction

Inflammasomes are a group of multiprotein complexes that recognize both extracellular and cytoplasmic intracellular pathogens and danger signals. The assembly of the specific inflammasomes is induced by various pattern-recognition receptors (PRRs) in response to pathogen associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), and results in the induction of pro-inflammatory cytokines, such as IL-1 $\beta$  and IL-18 (1-3). In addition, inflammasomes have been proposed to regulate other key events in inflammation and tissue repair, such as pyroptosis, a highly inflammatory form of programmed cell death. Therefore,

the inflammasomes play an important role in a variety of human pathophysiological processes, including antimicrobial response and development of metabolic syndromes, cancer, autoimmune and neurodegenerative diseases (4).

To date, the following five types of PRRs have been identified: Toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors, C-type lectin receptors (CLRs), DNA sensors [DNA-dependent activator of IFN-regulatory factors and absent in melanoma 2], and the nucleotide-binding domain (NBD), leucine-rich repeat-containing (LRR) proteins (NLRs) (1,2). Several studies have demonstrated that the NLR family includes NLR proteins (NLRP) 1, 2, 3, 6, 12, the NLR family caspase activation and recruitment domain (CARD), and the nucleotide-binding oligomerization domain-containing protein 2 (5-9). Among them, the NLRP3 inflammasome can be activated by a wide variety of stimuli, including DAMPs, PAMPs and bacterial toxins. In the present review, the biological process of the NLRP3 inflammasome assembly, its activation, and the recent developments of its involvement in viral infection are reviewed. The findings highlight the research challenges and future directions on this scientific field.

## 2. The NLRP3 inflammasome

**NLR structure.** NLRs consist of three separate domains, including an N-terminal pyrin domain (PYD), a central NBD, and a C-terminal LRR (Fig. 1). The N terminal domain is a caspase recruitment domain, a CARD, or a baculovirus inhibitory repeat domain, which has been applied as a structural subclassification of the NLR family (1,10). NLRP3 (NLR family, pyrin domain containing 3), which is also called cryopyrin, NALP3, CIAS1, CLR1.1, and PYPAF1 contains a PYD, a central NACHT (nucleotide-binding domain or NAIP, CIITA, HET-E and TPI) and a C-terminal LRR, which lacks CARD and cannot recruit pro-caspase-1 in the absence of the adaptor molecule caspase recruitment domain (ASC) (1). The NACHT domain is primarily responsible for dNTPase activity and oligomerization, while the PYD domain mediates downstream signaling of homotypic protein-protein interactions. LRR has been involved in ligand-sensing and autoregulation. All three domains are involved in the protein interaction networks (7).

**NLRP3 complex.** In addition to the inflammasome sensor molecules, ASCs are present that connect caspase 1 in the NLRP3 inflammasome complex. ASC contains two

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death-fold domains: One PYD and one CARD. The interaction between the upstream inflammasome sensor molecules triggers ASC assembly via the pyrin domain into a large protein complex (11,12). ASC brings procaspase 1 into close proximity with CARD, which initiates caspase 1 self-cleavage and activation (13,14).

**Caspase 1.** The cysteine protease caspase 1 is a key player of the inflammatory response. Procaspace-1 recruitment to the ASC speck enables its dimerization and autoactivation (1). The CARD domain is separated by a CARD domain linker from the C-terminal catalytic domain, containing large (p20) and small (p10) subunits (15). A cleavage product, p33 (CARD + p20), is generated by the interdomain linker between p20 and p10. It is confirmed that the active species of caspase 1 in macrophages is a transient tetramer composed of p33 and p10 subunits (p33/p10). The complex removes the CARD domain by self-cleavage, leading to the release of p20/p10 and the loss of enzymatic activity (15).

**IL-1 $\beta$  and IL-18.** Active caspase 1 further processes pro-IL-1 $\beta$  and pro-IL-18 into their mature forms. Serine proteinases including cathepsin G, elastase, and proteinase 3 can cleave pro-IL-1 $\beta$  except for caspase-1 (16). Furthermore, mast cell chymase is able to cleave pro-IL-18 (17). IL-1 family cytokines are key initiators and regulators of immune responses and inflammation. IL-1 $\beta$  and IL-18 are significant cytokines that regulate innate and adaptive immune responses (18). IL-18 plays a major role in the induction of the Th1 response, while IL-1 $\beta$  contributes to the T-helper 17 response (19). In various cases, the secretion of IL-1 cytokines is closely linked to cell death. Previous studies have suggested that cell lysis is the primary release mechanism for IL-1 $\beta$  and IL-18 (19,20). However, recently, a common secretory pathway in the absence of cell death and cell lysis was identified, which depended on membrane permeability (20). The secretion of IL-1 $\beta$  and IL-18 indicates the important role of the NLRP3 inflammasome in inflammatory and infectious diseases.

Except for the cytokine release, inflammasomes also trigger pyroptosis, which is induced by inflammatory caspases (murine caspase 1 and caspase 11 or human caspase 4 and caspase 5) (21). Pyroptosis is a necrotic form of infected cell death that is different from classical apoptosis or necrosis and is characterized by cell swelling, osmotic lysis and the subsequent release of intracellular content (21). Gasdermin D is required for the induction of pyroptosis and its N-terminal fragment indicates the intrinsic pyroptosis-inducing activity (22).

### 3. Activation and regulation of the NLRP3 inflammasome

NLRP3 is known to respond to a variety of stimuli, including PAMPs, DAMPs and bacterial toxins. These PAMPs include fungi, such as *Candida albicans* and *Saccharomyces cerevisiae* (23); bacteria, including *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* and *Neisseria gonorrhoeae* (24-26); and viruses, such as Sendai, influenza, encephalomyocarditis viruses and adenovirus (7,27-29). In addition, several host-derived molecules, which are indicative of cellular injury or stress can also activate the NLRP3

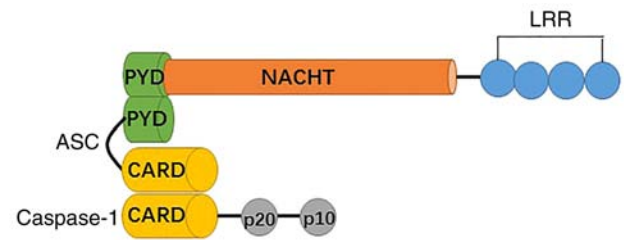


Figure 1. Structure of the NLRP3 inflammasome. The NLRs consist of three separate domains, including an N-terminal PYD, a central NACHT and a C-terminal LRR. The ASC contains two death-fold domains: One PYD and one CARD, connecting caspase1 with the NLRP3 inflammasome complex. NLRP3, NLR family, pyrin domain containing 3; NLR, Nod-like receptor; PYD, pyrin domain; LRR, leucine-rich repeat; ASC, adaptor molecule caspase recruitment domain; CARD, caspase activation and recruitment domain.

inflammasome, including ATP released from necrotic cells, hyaluronan, glucose, monosodium urate, myeloid- $\beta$ , skin irritants, imidazoquinoline compounds, silica, asbestos and alum (Fig. 2) (30). In certain cases, the inflammasome is activated by individual microbial components, such as, the  $\alpha$ -toxin of *S. aureus* (24,31-33).

**Canonical activation.** Despite these findings, a universal activation mechanism for NLRP3 has not been reported. Extracellular ATP stimulates the P2X purinoceptor 7, triggering efflux of potassium and inducing the progressive recruitment of the membrane pore pannexin-1 (34). Under the second model, phagocytes engulf the inflammasomal activators, such as silica, monosodium urate, amyloid- $\beta$ , asbestos, and alum, leading to lysosomal damage. The cytosolic release of lysosomal contents is sensed by the NLRP3 inflammasome (35,36). At last, nearly all NLRP3 agonists induce the generation of reactive oxygen species (ROS) (37-39). ROS blockade by chemical scavengers inhibit NLRP3 inflammasome activation (23,40,41). In addition, mitochondrial dysfunction induces changes in intracellular calcium levels, and the release of oxidized mitochondrial DNA can lead to NLRP3 activation (42).

**ROS.** Inflammasome activation can be triggered by ROS; however, the exact mechanism remains unclear. A nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is involved in the process, since NLRP3 inflammasome is inhibited upon suppression of NADPH oxidase common p22 subunit (39). A recent study suggests that thioredoxin-interacting protein (TXNIP/VDUP1) is implicated in NLRP3 activation. Inflammasome activators induce the ROS-dependent TXNIP dissociation from thioredoxin. TXNIP further binds to NLRP3 and its deficiency impairs activation of the inflammasome and subsequent secretion of IL-1 $\beta$  (30).

**Oxidized mitochondrial DNA (mtDNA).** The oxidized mtDNA from stressed mitochondria is also suggested to be the cytoplasmic activator of the NLRP3 inflammasome. A previous study conducted by Shimada *et al* (43) revealed that oxidized mtDNA could bind directly and activate the NLRP3 inflammasome, while macrophages lacking mtDNA severely reduced

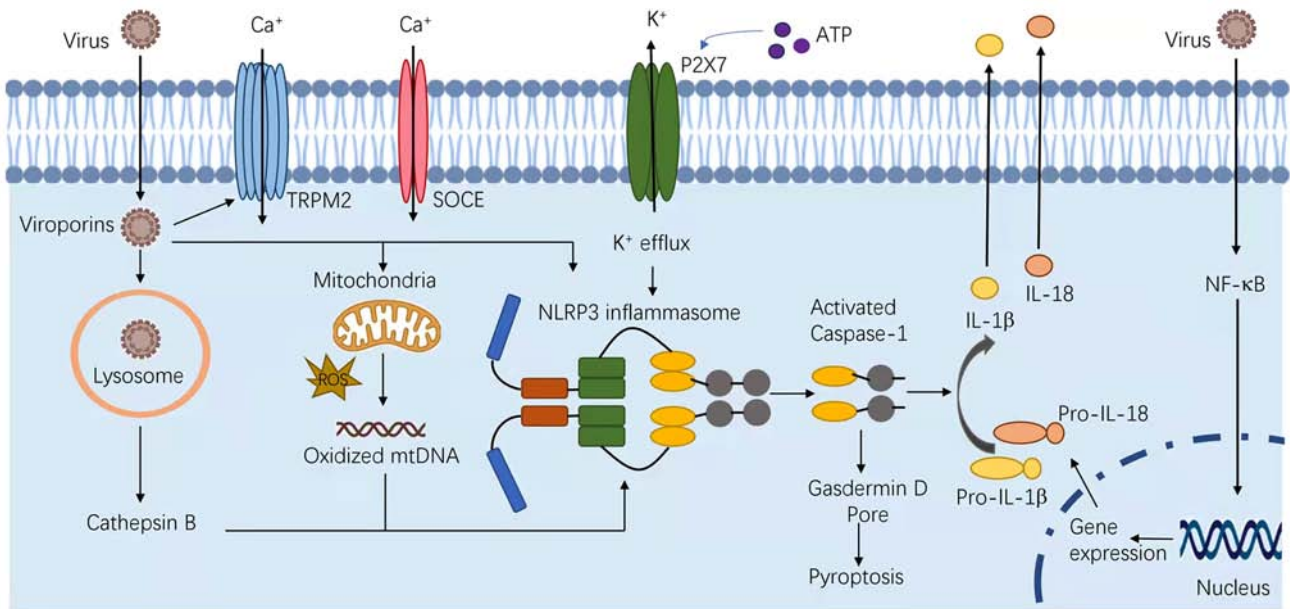


Figure 2. NLRP3 inflammasome activation is involved in viral infection. NLRP3 is known to respond to a variety of stimuli, including viral infection and ATP. Viral infections trigger the generation of reactive oxygen species, promote potassium efflux, and the release of lysosomal cathepsin B into the cytoplasm, which causes the activation of the NLRP3 inflammasome. Caspase 1 clustering induces autoactivation and processes pro-IL-1 $\beta$  and pro-IL-18 into their mature forms. Alternatively, NLRP3 inflammasomes may trigger pyroptosis, which is an inflammatory form of programmed cell death. NLRP3, NLR family, pyrin domain containing 3.

IL-1 $\beta$  production. In addition, ROS may also be of mitochondrial origin. However, whether ROS or oxidized mtDNA acts as a direct activator of the NLRP3 inflammasome or as its cofactor remains unknown.

**Non-canonical activation.** In complement with the canonical inflammasome pathway, the non-canonical pathway is mediated by murine caspase 11 and caspase 4 or caspase 5 in human cells in response to Gram-negative bacteria (44-47). Lipopolysaccharide (LPS, a component of the Gram-negative bacterial cell wall) was shown to induce the activation of caspase 11 in mice and of caspase 4 or caspase 5 in human cells, which was previously attributed to the LPS receptor TLR4 (45-47). However, recent studies have shown that host cells have developed TLR4-independent mechanisms to recognize the cytoplasmic LPS. It was found that TLR4(-/-) mice primed with the TLR3 agonist polyinosinic: Polycytidylic acid induced pro-caspase-11 expression, which could possibly explain this discrepancy (45-47). The activation of caspases 4, 5 and 11 initiates pyroptosis similarly to caspase 1, which is not responsible for cleaving pro-IL-1 $\beta$  or pro-IL-18 (44,48). Upon activation, these caspases also promote the assembly of the NLRP3 inflammasome (44,49,50).

In the absence of microbial stimulation, exposure to TNF- $\alpha$  significantly promotes ATP or silica-mediated caspase 1 activation and IL-1 secretion in macrophages and dendritic cells (51). Signals provided by NF- $\kappa$ B activators are not sufficient for NLRP3 activation (52). Certain studies have shown that NIMA-related kinase 7 is an important component of the NLRP3 complex, which is required for NLRP3 activation, responding to both canonical and non-canonical stimuli (53-55). The kinase spleen tyrosine kinase (SYK) is essential for NLRP3 inflammasome activation (23,41).

**Positive regulation.** Over the past decade, numerous studies have uncovered the role and regulation of inflammasomes during certain pathophysiological processes. The regulatory mechanisms include transcriptional, post-transcriptional regulation, post-translational modifications and regulatory proteins that target the receptors, ASC or the caspases. Furthermore, several other studies have highlighted the microbial evolution of inflammasome inhibitors.

The integration with PRRs or cytokine receptors highly influences the NLRP3 inflammasome activation and pro-IL-1 $\beta$  availability (51,52). In response to PRR stimulation, NLRP3 deubiquitylation, which is mediated by the K63-specific deubiquitinase BRCA1-BRCA2-containing complex subunit 3, also induces inflammasome signaling (56,57). The double-stranded RNA-dependent protein kinase R or eukaryotic translation initiation factor 2- $\alpha$  kinase 2 was shown to control NLRP3 inflammasome activity (58). Selective activation of the NLRP3 inflammasome was promoted by guanylate binding protein 5, in responses to pathogenic and soluble bacteria but not in response to crystalline inflammasome priming substances (59). These data suggest that small heterodimer partner is a key mediator in controlling NLRP3 inflammasome activation via mechanisms that interact with NLRP3 and maintain mitochondrial homeostasis (60).

The SYK and JNK enzymes phosphorylate the inflammasome adaptor ASC, which further contributes to the activation of caspase 1 (61). It has also been shown that SYK promotes NLRP3-dependent caspase 1 activation during infections with *Candida albicans* (23). The TGF $\beta$ -activated kinase 1 (TAK1 or MAP3K7)-JNK pathway was activated by ruptured lysosomes and promoted NLRP3 inflammasome activation, through the oligomerization of an adapter protein and apoptosis-associated speck-like protein including ASC (62).

**Negative regulation.** A number of proteins containing a CARD or a PYD have been proposed to inhibit inflammasome activity by blocking inflammasome component recruitment. Certain CARD-only proteins (COPs), such as human CARD16 (also known as COP or PSEUDO-ICE), CARD17 (also known as INCA), and CARD18 (also known as ICEBERG), are highly similar to the CARD of caspase 1 (63-65). They are suggested to suppress inflammasome activation by sequestering caspase 1. In addition, they may also regulate other signaling pathways. For instance, CARD16 and CARD18 activate receptor-interacting serine/threonine protein kinase 2 (63-65). Caspase 12 is an inhibitor of caspase 1 and its overexpression appears to abrogate caspase 1 activity (66).

PYD-only proteins (POPs), including pyrin, POP1 (PYDC1), POP2 (PYDC2) and POP3 (PYDC5), regulate inflammasome signaling at the molecular level of the PYD-PYD interaction (67-70). It was shown that binding of POP1 to ASC inhibited ASC-dependent inflammasome assembly by preventing inflammasome nucleation, and consequently reducing IL-1 $\beta$  release. Although mice do not have the ortholog of POP1, its transgenic expression can protect them from PAMP-triggered inflammation. Moreover, POP1 was regulated by TLR and IL-1 receptor signaling; therefore, it was proposed that POP1 would provide a regulatory feedback loop and shut down excessive inflammatory responses (70). An additional member of POPs, POP2, binds to ASC and PAN1, and inhibits the formation of cryopyrin and inflammasomes (68). In addition, it also inhibits NF- $\kappa$ B activation (67). The other PYD-only protein, POP3 was identified to compete with ASC for recruitment of liver regeneration-associated proteins, further reducing inflammasome activation (69).

The kinase I $\kappa$ B kinase- $\alpha$ , is involved in the negative regulation of ASC by controlling its subcellular localization in resting macrophages; however, it is not clear whether this process is associated with ASC phosphorylation (71).

#### 4. Viral infection and NLRP3 inflammasome

The NLRP3 inflammasome is critical in the host anti-viral immune function. Previous studies have shown that infection from both RNA and DNA viruses can induce activation of the NLRP3 inflammasome and promote the secretion of IL-1 $\beta$  and IL-18 (72), such as Rift Valley fever virus, encephalomyocarditis virus (EMCV), foot-and-mouth disease virus, Mayaro virus (MAYV), hepatitis B and C viruses, Zika virus (ZIKV), H7N9 influenza A virus, Dengue virus and varicella-zoster virus (72-80). When viruses invade their hosts, the NLRP3 inflammasome can be activated by sensing viral components, including RNA, DNA and proteins. However, the mechanisms of the NLRP3 inflammasome activation during a viral infection are still debatable.

Viroporins are a group of low-molecular-weight proteins, which are important for innate immune responses, notably for the activation of the NLRP3 inflammasome, such as P7 viroporin of the hepatitis C virus, viroporin 2B of EMCV, and envelope (E) protein of severe acute respiratory syndrome coronavirus viroporin 3a (72,81-83). During the infection, viroporins can increase the cell membrane permeability, facilitate viral invasion, and cause imbalance of ion concentration levels, such as Ca<sup>2+</sup>, which in turn produces ion fluxes

and promotes the activation of NLRP3 (84). Angiotensin converting enzyme 2, which is expressed in injured type II alveolar epithelial cells may lead to NLRP3 activation during coronavirus disease (COVID)-19 infection (85).

A subsequent study revealed that certain infections from RNA viruses could cause potassium efflux, which is a trigger for the activation of the NLRP3 inflammasome (86). Infection by murine hepatitis viral strain-3 can induce the quick release of ROS, which may act as a trigger for NLRP3 inflammasome activation (87). ROS also plays an important role in human immunodeficiency virus (HIV)-1 infection. HIV-1 will promote ROS production and induce inflammasome activation (88). MAYV infection can induce ROS release and potassium efflux, which subsequently triggers the activation of the NLRP3 inflammasome. Human parainfluenza virus type 3 infection can induce NLRP3 inflammasome activation via TLR2 activation and potassium efflux (89). The M2 and PB1-F2 proteins of the influenza virus can activate the NLRP3 inflammasome by regulating intracellular ionic and mitochondrial ROS production. During this process, oxidized DNA plays an important role and influenza viruses can induce oxidized DNA release (90). Influenza viral infection will also induce lysosomal damage through the lysosomal pathway, further activating NLRP3 (29). Adenoviral infections release lysosomal cathepsin B into the cytoplasm, which can cause the activation of the NLRP3 inflammasome (91).

The RNase L system can sense double-stranded RNAs and generate RNA cleavage products to activate the NLRP3 inflammasome. DExD/H-box helicase and the mitochondria-associated molecule mitochondrial antiviral-signaling protein (MAVS) participate in this process (92). MAVS can recruit NLRP3 to the mitochondria and promote the secretion of IL-1 $\beta$  (93). DEAD/H-box RNA helicases (DDX), DDX33 and DDX19A act as RNA sensors involved in NLRP3 inflammasome activation (94). The RNA helicase RIG-I acts as a sensor to certain RNA viruses, such as vesicular stomatitis and influenza viruses, through the NF- $\kappa$ B pathway to activate the NLRP3 inflammasome (29). Certain RNA viruses activate NLRP3 via the RIP1-receptor-interacting serine/threonine-protein kinase 3 (RIP3)-DRP1 pathway. The RIP1-RIP3 complex is assembled, which induces mitochondrial damage and activates NLRP3 (95).

Viruses have also evolved multiple mechanisms to evade the host immune response. Certain viral proteins can suppress the NLRP3 inflammasome-associated immune response, such as the V protein of measles virus. Sendai virus can inhibit activation of the NLRP3 inflammasome and reduce secretion of IL-1 $\beta$  by interacting with NLRP3 (96,97). The influenza A viral NS1 protein suppresses ASC ubiquitination and speck formation via the RIG-I/type I IFN pathway to inhibit NLRP3 inflammasome activation and reduce the production of IL-1 $\beta$  (98,99). The PB1-F2 protein of avian influenza A suppresses NLRP3 inflammasome-dependent IL-1 $\beta$  secretion by targeting the MAVS-NLRP3 interaction (100). RNA I of adenovirus VA inhibits the activation of the NLRP3 inflammasome by suppressing ASC phosphorylation and oligomerization (101). The non-structural protein NS3 of ZIKV reduces NLRP3-mediated IL-1 $\beta$  secretion (102). An Epstein-Barr virus microRNA (miR) can inhibit the NLRP3 inflammasome by targeting the miR-223 site (103). Enterovirus 71 can cleave NLRP3 by activation of proteases 2A and 3C, which will inhibit NLRP3 and IL-1 $\beta$



secretion (104). Hepatitis B viral infection can inhibit NF- $\kappa$ B phosphorylation and ROS production. Hepatitis B e-antigen suppresses the activation of NLRP3 and IL-1 $\beta$  production (105). Herpes simplex virus 1 has evolved mechanisms to block the NLRP3 activity (106).

## 5. Conclusion

The NLRP3 inflammasome has been widely studied in innate immune responses. However, the exact mechanisms of NLRP3 inflammasome activation and regulation remain unclear, notably during the viral infection process. In the present review, the current knowledge of the NLRP3 assembly process and its regulatory mechanisms during viral infection were summarized. The findings indicate that viruses can evade the host immune response by inhibiting the NLRP3 cytokine storm. As a result, it is of value to elucidate the immunological balance and the underlying mechanism of this process. Certain studies have supported the hypothesis that targeting the NLRP3 pathway can be used in the development of therapeutic strategies for viral infection, notably for COVID-19. The development of antiviral therapies requires additional studies that will explore the molecular pathogenesis of NLRP3 inflammasome activation in response to viral infection.

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## Availability of data and materials

Not applicable.

## Authors' contributions

QZ and HC conceived and designed the study. QZ, CH and QL collected data. QZ, CH and QL prepared the draft of the manuscript. HC reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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