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REVIEW

# Negative regulators and their mechanisms in NLRP3 inflammasome activation and signaling

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**Inflammasomes are cytosolic multiprotein complexes that cause the release of biologically active interleukin-1β. The best-characterized inflammasome is the NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 or Nod-like receptor protein 3) inflammasome. The NLRP3 inflammasome forms an assembly consisting of the ASC (apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain) adaptor protein and the effector, caspase-1 (cysteine-dependent aspartate-directed protease-1). Numerous agents and ligands derived from pathogens, modified self-cells and the environment induce NLRP3 inflammasome complex formation. NLRP3 inflammasome activation is tightly controlled at the transcriptional and post-translational levels to prevent unwanted excessive inflammation. Recent studies have highlighted the roles and mechanisms of several negative regulators that inhibit the assembly of NLRP3 inflammasome complexes and suppress inflammatory responses. The identification and characterization of new players in the regulation of NLRP3 inflammasome may lead to the development of inflammasome-targeting therapeutics against various inflammatory diseases related to NLRP3 inflammasome-associated pathogenesis.**
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## INTRODUCTION

Prevention of many chronic degenerative diseases and suppression of infectious diseases require balanced and appropriate control of inflammation.[1,2] Inflammatory responses are triggered by the innate immune recognition of a variety of foreign pathogenic or threatening signals.[2,3] Activation of inflammatory caspases (for example, caspase-1) results in the specialized secretion of both interleukin (IL)-1β and IL-18.[2] Uncontrolled IL-1β secretion is associated with widespread tissue damage and is involved in the pathogenesis of numerous acute and chronic inflammatory human diseases.[4–6] Cytosolic protein complexes, termed inflammasomes, are critically involved in the maturation and secretion of IL-1β and IL-18.[3,7] Among several inflammasome complexes, the NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 or Nod-like receptor protein 3) inflammasome is the best characterized with respect to its pathophysiological roles and clinical implications.[3,7]

Numerous pathogen-associated molecule pattern or damage-associated molecule pattern signals have been reported to activate NLRP3 inflammasome complexes.[2,4,7] Upon activation, NLRP3 undergoes oligomerization and recruits the ASC adaptor protein (apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain) and the inflammatory caspase-1, and this complex activates the proteolytic cleavage of pro-IL-1β and pro-IL-18.[7,8] NLRP3 inflammasome activation is an essential mechanism of the innate host defense system that protects against numerous microbial infections, including influenza A viral infection.[9] However, its uncontrolled activation of aberrant NLRP3 inflammasome may inevitably lead to pathologies related to autoinflammatory diseases such as cryopyrin-associated periodic syndromes (CAPS), and autoimmune diseases, including gout, rheumatoid arthritis and lupus.[10,11] Thus, tight regulation of NLRP3 inflammasome complex activation at the transcriptional and post-translational levels is required to prevent the onset and progression of various inflammatory and metabolic diseases, such as type 2 diabetes and atherosclerosis.[4,5,12]

NLRP3 inflammasome complex activation has been shown to involve a two-step process: priming and assembly.[12] Recent studies have revealed several regulators and their molecular mechanisms that exert tight control of NLRP3 inflammasome complexes at both the priming and activation steps.[3,5] Several endogenous and microbial-driven molecules are involved in switching off NLRP3 inflammasome activation.[6] Adaptive immune activation by CD4(+) regulatory T cells has a role in attenuating excessive tissue damage.[13] Autophagy has a critical role in regulating inflammasome activation,[14] and this intracellular catabolic pathway has been reviewed extensively in several articles. In contrast, this review focuses on other regulatory aspects of NLRP3 inflammasome activation. We discuss the accumulating data regarding a variety of agents and drugs that function in the regulation of NLRP3 inflammasome activation; such agents show promise as new therapeutics for human inflammatory diseases related

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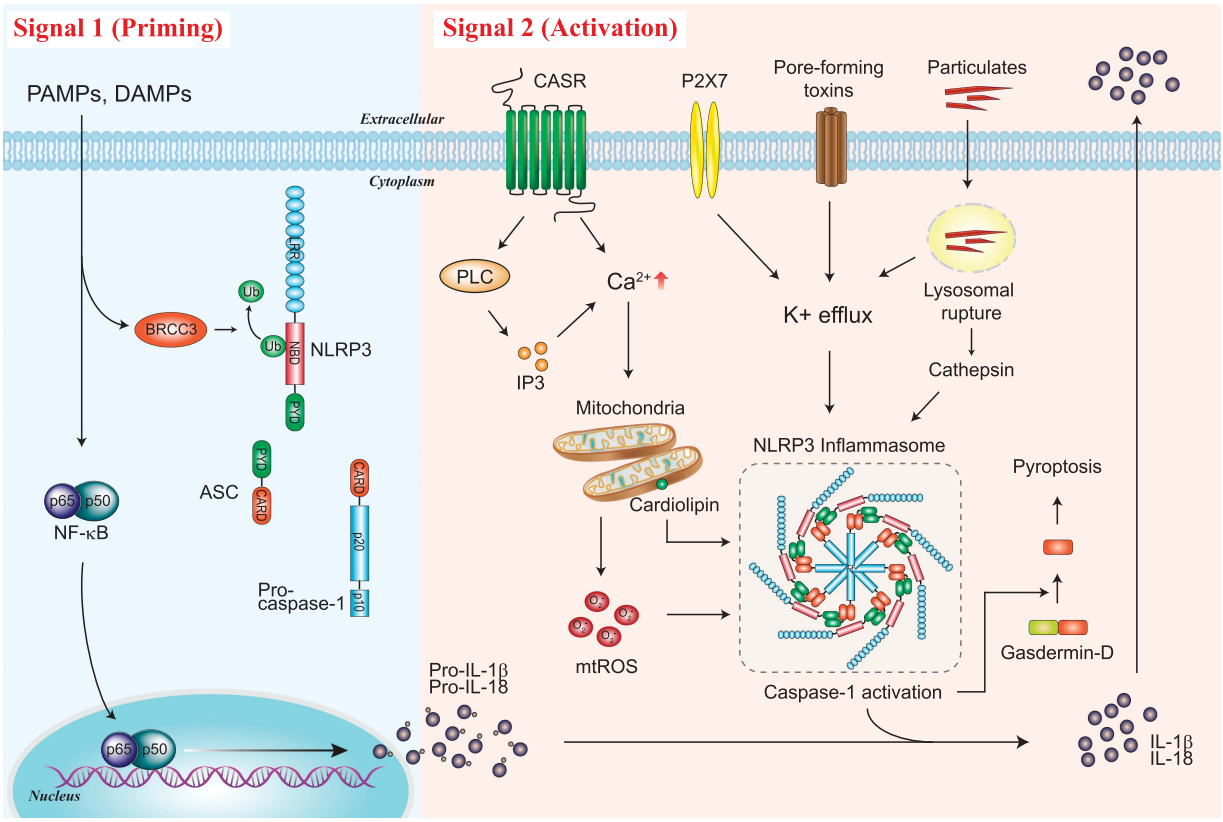


**Figure 1** Two signals are required to activate NLRP3 inflammasome. Various PAMPs and DAMPs stimulate NF-κB translocation into the nucleus to transcript pro-IL-1β and pro-IL-18. In addition, the enzyme BRCC3 activates NLRP3 through deubiquitination (signal 1). Signal 2 induces the oligomerizaiton of NLRP3 and assembly. P2X7, pore-forming toxins, and particulates trigger $K^+$ efflux to activate NLRP3 inflammasome complex. Furthermore, mitochondrial cardiolipin and ROS are initiator of NLRP3 inflammasome activation. CASR activates NLRP3 inflammasome through increased intracellular $Ca^{2+}$ level. Finally, active caspase-1 produce IL-1β and IL-18 by active caspase-1. CASR, calcium-sensing receptor; DAMP, damage-associated molecule pattern signal; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecule pattern.

to inflammasome pathogenesis. In addition, this review highlights the recently identified negative regulators and pathways by which NLRP3 inflammasome activation is finely tuned, and we discuss the relevance of the roles of negative regulators in the modulation of inflammasome-related inflammatory diseases.

## OVERVIEW OF NLRP3 INFLAMMASOME COMPLEX ACTIVATION

The NLRP3 inflammasome mediates the maturation of pro-inflammatory cytokines IL-1β and IL-18 in response to various pathogen-associated molecule patterns or cellular damage-associated molecule patterns (Figure 1).[4,5,7] Inflammasome activation triggers the assembly of inflammasome complexes, which involves NLRP3 oligomerization followed by the clustering of ASC adaptor protein and activating caspase-1 to induce the proteolytic cleavage and maturation of IL-1β and IL-18.[3,12] It is generally accepted that NLRP3 inflammasome activation occurs in two steps: priming and activation.[3,12] The stimulation of pattern recognition receptors, such as Toll-like receptor 4, initiates the nuclear factor-κB signaling pathway; this is important for the transcription of the NLRP3 protein and the precursor of IL-1β, which is not typically expressed in naive cells.[2,12] In addition, non-transcriptional priming of NLRP3 occurs via the deubiquitination of NLRP3 through mitochondrial reactive oxygen species (ROS)-dependent mechanisms. Inflammasome assembly is followed by the formation of ASC specks, that is, the clustering of ASC filaments into macromolecular aggregates.[3,12]

Recent studies have revealed that NLRP3 deubiquitination by the deubiquitinating enzyme BRCC3 is also required for the activation of NLRP3 priming.[15] Moreover, inhibition of deubiquitination affects ASC speck formation to suppress caspase-1 activation.[16] Recent studies have revealed that the mitochondrial-specific phospholipid cardiolipin is required for oxazolidinone antibiotic-induced NLRP3 inflammasome activation through an ROS-independent mechanism and direct binding to NLRP3, which could serve as a mitochondrial docking site for NLRP3 inflammasome assembly.[17] In addition, epidermal fatty acid-binding protein, a major lipid carrier in macrophages and keratinocytes, was found to be essential for triggering high-fat diet-induced NLRP3 inflammasome activation and upregulation of IL-1β and IL-18 in skin tissues.[18] Furthermore, calcium-sensing receptor activates the NLRP3 inflammasome through increased intracellular $Ca^{2+}$ and decreased cyclic AMP (cAMP) and phospholipase C, which catalyzes inositol-1,4,5-trisphosphate production.[19]

Several expert reviews have described the mechanisms of NLRP3 inflammasome activation, including the mechanisms involving mitochondrial ROS production, potassium efflux and lysosomal destabilization.[20,21] Thus, this current review only briefly mentions the established mechanisms of NLRP3 inflammasome complex

activation, instead of focusing on several newly reported factors/mechanisms involved in inflammasome activation.

## NEGATIVE REGULATORS OF NLRP3 INFLAMMASOME COMPLEXES

Recent studies have revealed the physiological molecules and mechanisms are required for limiting NLRP3 inflammasome activation. Here we discuss some of the recently identified molecules and compounds that interfere with NLRP3 activation and their mechanisms of action. Many of the negative regulators do not directly target NLRP3, but rather molecules/components downstream of the receptor to inhibit other inflammasome receptor complexes.[22,23]

### Negative regulatory molecules involved in NLRP3 inflammasome activation

*IL-18 binding protein, type I interferons.* IL-18 binding protein (IL-18BP), a natural antagonist of IL-18, promotes osteoblast differentiation, and may inhibit the expression of NLRP3 in osteoblasts.[24] In female osteoporotic subjects, serum IL-18BP levels are decreased while serum IL-18 levels are increased, suggesting a role for IL-18BP as a new therapeutic agent to treat postmenopausal osteoporosis.[24] Another study showed that type I interferons (IFNs) function in suppressing NLRP3 and NLRP1 inflammasome activities through the transcription factor STAT1, although the molecular mechanisms remain unclear.[25] During infection with *Mycobacterium tuberculosis*, type I IFNs display major suppressive effects on the production of IL-1α and IL-1β in innate immune cells and *in vivo*. The interplay between type I IFN and IL-1 cytokines, and the molecular mechanisms by which type I IFN regulates inflammasome activation, were summarized in a recent review by Labzin *et al.*[26] Type I IFN-mediated regulation of the inflammasome is mediated through the nitrosylation of NLRP3 and the expression of key interferon-stimulated genes, including IL-10, 25-hydroxycholesterolase and a suppressor of cytokine signaling.[26] As IL-1α and IL-1β have essential roles in host resistance to *M. tuberculosis*, this type 1 IFN-induced decrease in NLRP3 inflammasome response might be detrimental to the human host infected with tuberculosis.[27]

*Heat shock protein, TRIM30 and sirtuin 3.* A recent study showed a novel role and regulatory mechanism for heat shock transcription factor 1 (HSF1)–β-catenin signaling in NLRP3 inflammasome activation during sterile inflammatory injury in the liver.[28] In ischemia/reperfusion-stressed liver, myeloid-specific HSF1 inhibits liver inflammation by activating β-catenin signaling to regulate liver inflammatory responses.[28] Importantly, the HSF1–β-catenin axis is required for inhibiting XBP1-dependent NLRP3/caspase-1 activation and suppressing IL-1β production in macrophages.[28]

Sirtuin 3, a NAD(+)-dependent deacetylase, is required for inhibiting NLRP3 inflammasome activation and production of IL-1β and IL-18 to ameliorate kidney dysfunction and renal tubular cell injury in acute kidney injury models.[29] Previous studies revealed that TRIM30 (tripartite interaction motif-containing-30: a RING domain-containing TRIM protein) functions to suppress NLRP3 inflammasome activation by modulating ROS generation in response to NLRP3 inflammasome stimulators.[30]

A recent study revealed that comparative gene identification-58 (an important cofactor for adipose triglyceride lipase), which functions in the activation of peroxisome proliferator-activated receptors (PPARs) and intracellular fat hydrolysis, are required for regulating high-fat diet-induced NLRP3 inflammasome activation by maintaining PPAR-γ signaling and mitochondrial homeostasis.[31]

*Nuclear receptors.* Recent studies showed that orphan nuclear receptor small heterodimer partner (SHP) has an essential role in regulating second signal activation of the NLRP3 inflammasome complex. SHP binds to NLRP3 and competitively inhibits the interaction between NLRP3 and ASC on the mitochondria. SHP deficiency led to excessive inflammatory and pathological responses in various inflammatory diseases related to NLRP3 inflammasome activation, including acute kidney necrosis and gout models.[32] Another recent study showed that the activation of cannabinoid receptor 2 with HU 308, a selective cannabinoid receptor 2 agonist, led to amelioration of dextrate sulfate sodium-induced colitis *in vivo* and an inhibition of NLRP3 inflammasome activation through autophagy activation in macrophages.[33]

*Adaptive immune cytokine IFN-γ, nitric oxide and carbon monoxide.* An important adaptive immune cytokine, IFN-γ, exerts a regulatory effect on IL-1 production and immunopathological responses through generation of nitric oxide, which inhibits the NLRP3 inflammasome assembly via nitrosylation of the NLRP3 protein.[34] CD40, a costimulatory molecule, exhibits an inhibitory function in ATP-Toll-like receptor 4-mediated inflammasome activation in microglia.[35] IFN-γ-mediated CD40 expression leads to decreased production of IL-1β and suppresses the neurotoxic inflammasome activation required for pathogenic Th17 polarization.[35] These findings corroborate results from another study, which found that increased nitric oxide in TXNIP-deficient mice exerted a negative effect on excessive inflammatory responses and inflammasome activation during endotoxic shock through induction of *S*-nitrosylation of NLRP3.[36] In addition, the regulatory function of nitric oxide is mediated through stabilization of mitochondria, as iNOS deficiency led to an increased accumulation of dysfunctional mitochondria during NLRP3 inflammasome activation.[37]

Carbon monoxide produced by heme catabolism functions in the inhibition of NLRP3 inflammasome-mediated immune responses, including caspase-1 activation and the release of IL-1β and IL-18 in macrophages, presumably due to the carbon monoxide effects on the prevention of mitochondrial dysfunction.[38]

*MicroRNA regulation of NLRP3 inflammasome activation.* MicroRNAs (miRNAs), small noncoding RNAs of ~22 nucleotides in length, have a role as post-transcriptional regulators of gene expression by targeting mRNAs. miRNAs generally promote degradation of target mRNA or translational repression by binding to complementary sequences within the 3′-untranslated regions of target mRNAs.[39]

Previous studies have suggested that miR-223 has a role in decreasing NLRP3 protein expression, thus inhibiting IL-1β production from the inflammasome.[40] There is a lineage-specific pattern of miR-223 expression, and the expression of miR-223 was increased during granulocytic differentiation.[41] In addition, miR-223 is a key regulator of the differentiation of intestinal macrophages and dendritic cells, as its deficiency leads to severe colitis.[42] In endothelial cells, sterol regulatory element-binding protein 2-induced miR-92a is required for NLRP3 inflammasome activation through targeting of sirtuin 1, Krüppel-like factor 2 and Krüppel-like factor 4. Thus, antisense miR-92a is involved in inhibiting inflammasomes, conferring beneficial effects on vasodilation and atherogenesis.[43]

Recent studies have shown that miR-146a deficiency is linked to increased M1 macrophage activation, concomitant with the upregulation of pro-inflammatory cytokines IL-1β and IL-18 in macrophages from diabetic miR-146a-deficient mice.[44] However, this study did not characterize the exact miRNA targeted by miR-146a during

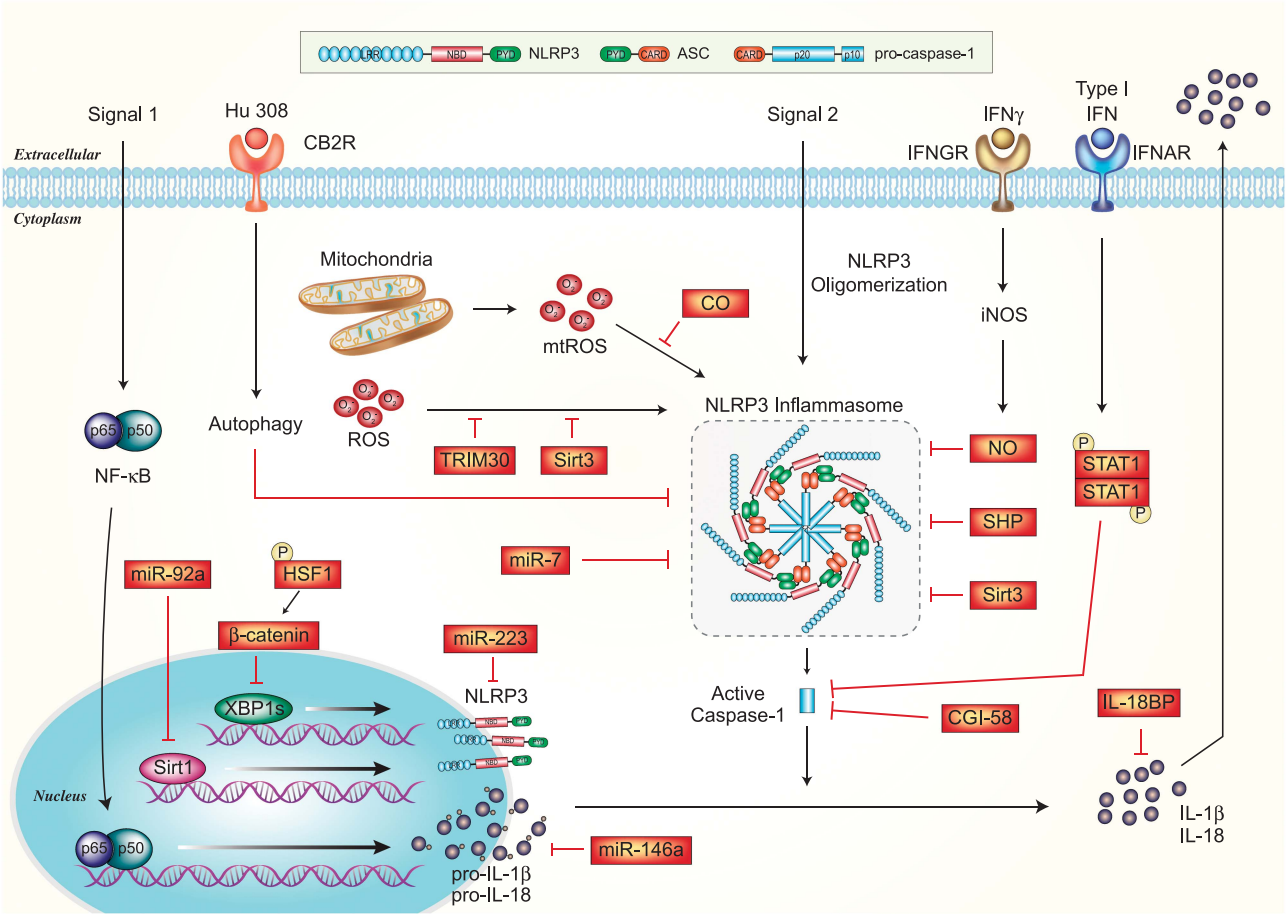


**Figure 2** Negative molecules of NLRP3 inflammasome activation. IL-18BP inhibits NLRP3 inflammasome activation as a natural antagonist of IL-18. Type I IFNs regulate transcription factor STAT1 and IFN-γ generates NO to inhibit NLRP3 inflammasome assembly. In addition, comparative gene identification-58 (CGI-58) activates PPARs to regulate NLRP3 inflammasome activation through maintaining PPAR-γ signaling.

inflammasome activation.[44] Interestingly, miR-7 targets NLRP3, thus inhibiting microglial NLRP3 inflammasome activation.[45] Thus, increased understanding of the effects of miR-7 on the amelioration of NLRP3 inflammasome-mediated neuroinflammation might be useful for developing novel therapeutics against Parkinson's disease.[45] These data suggest that various miRNAs are involved in regulating NLRP3 inflammasome complex activation. Future studies are required to clarify the roles of the complex miRNA networks in fine-tuning NLRP3 inflammasome activation and their relevance to a variety of human inflammatory diseases (Figure 2).

### Modulation of the signaling pathways involved in NLRP3 inflammasome activation

*Regulation of oxidative stress and mitochondrial function.* Accumulating evidence suggests that the modulation of ROS generation and mitochondrial function are important for regulating NLRP3 inflammasome activation.[46] In endothelial cells, mitochondrial fission by dynamin-related protein 1 action and increased oxidative stress participate in NLRP3 inflammasome activation by palmitate.[47] A beneficial function of a natural triterpenoid corosolic acid in endothelium is the inhibition of mitochondrial fission via the increased dynamin-related protein 1 phosphorylation at Ser637 and the activation of AMP-activated protein kinase (AMPK) activity. In addition, corosolic acid functions in the inhibition of NADPH oxidase 2 activation, which contributes to palmitate-induced NLRP3 induction.[47] Treatment of podocytes with antioxidants reduced the levels of miR-377, which has an important role in glomerular podocyte oxidative stress, thus inhibiting fructose-induced podocyte injury by attenuating ROS-mediated NLRP3 inflammasome activation.[48]

*AMPK signaling activation.* Several reports have emphasized the roles of AMPK in modulating NLRP3 inflammasome activation. Earlier studies showed that AMPK activation by the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside or constitutively active α1 subunit of AMPK led to the inhibition of the NLRP3–ASC inflammasome complex activation and IL-1β production induced by the saturated fatty acid palmitate.[49] In addition, patients with type 2 diabetes showed increased NLRP3 inflammasome activation and maturation of IL-1β, which were significantly reduced by treatment with the antidiabetic drug metformin through AMPK activation.[50]

AMPK activation via metformin treatment led to attenuation of hyperalgesia associated with NLRP3 inflammasome activation and increased levels of IL-1β and IL-18 in mice; furthermore, it improved the clinical symptoms in patients with fibromyalgia, a chronic pain disease.[51] In addition, AMPK activation limited monosodium urate crystal-induced inflammasome activation, as colchicine (which is used in gout therapeutics) increases the phosphorylation of AMPKα and inhibits the activation of caspase-1 and the release of IL-1β during inflammasome activation.[52]

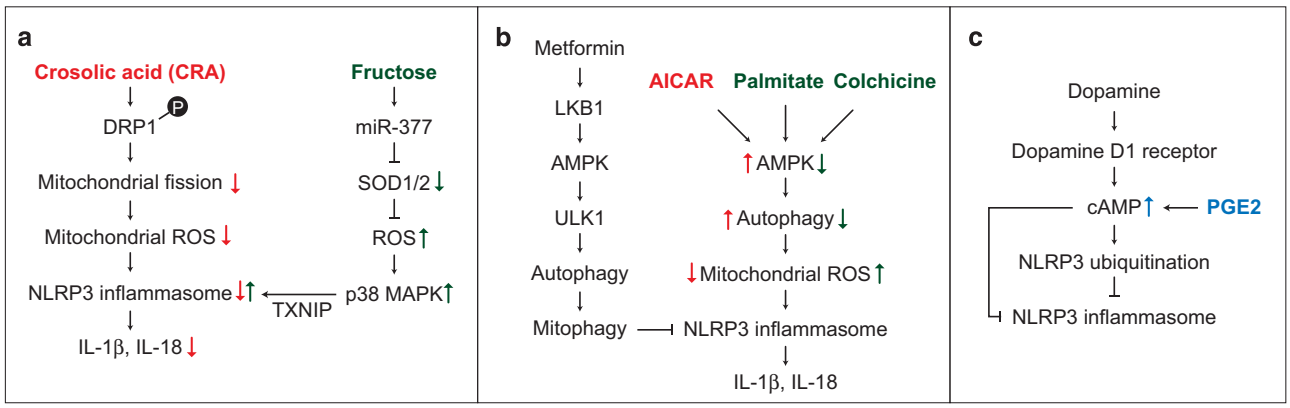


**Figure 3** Signaling pathways involved in modulation of NLRP3 inflammasome activation. (**a**) Regulation of oxidative stress and mitochondrial function. Corosolic acid (CRA) phosphorylates dynamin-related protein 1 (Drp1) to decrease mitochondrial fission and ROS. It inhibits NLRP3 inflammasome activation. In addition, fructose-induced miR-377 activates $O_2^-$/p38 MAPK/TXNIP/NLRP3 inflammasome pathway in podocyte. P, phosphorylation. (**b**) AMPK signaling activation. Metformin induces autophagy through LKB-1/AMPK/ULK1 signaling and autophagy regulates mitochondria homeostasis by removing old or damaged mitochondria. 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) inhibits palmitate-induced NLRP3 inflammasome but colchicine activates NLRP3 inflammasome through phosphorylation of AMPK. (**c**) cAMP signaling. cAMP acts as negative regulator of NLRP3 inflammasome via directly binding to NLRP3. Furthermore, dopamine and dopamine D1 receptor induce cAMP-mediated NLRP3 ubiquitination. PGE2 increases cellular cAMP to control NLRP3 inflammasome in human macrophages. Thus, cAMP has a role in maintenance of inflammation.

*cAMP signaling.* An earlier study showed an inhibitory effect of cAMP on NLRP3 inflammasome assembly through direct interaction with NLRP3.[19] Increasing cAMP was beneficial to ameliorate the uncontrolled secretion of mature IL-1β from peripheral blood mononuclear cells in patients with CAPS.[19] PGE2 is involved in inhibiting NLRP3 inflammasome activation in human macrophages through its receptor subtype 4 (EP4) and increased intracellular cAMP.[53] Importantly, high doses of PGE2 led to an inhibition of constitutive IL-1β secretion from peripheral blood mononuclear cells in patients with CAPS.[53] The inhibition of endogenous PGE2 production achieved by blocking cytosolic phospholipase A2α or cyclooxygenase 2 resulted in increased activation of the NLRP3 inflammasome complex.[53] One study showed that the neurotransmitter dopamine and dopamine D1 receptor signaling can inhibit systemic or peripheral inflammation by regulating NLRP3 inflammasome activation and that dopamine-induced NLRP3 degradation and ubiquitination was mediated through cAMP.[54] Thus, cAMP and PGE2 might have a role in maintaining homeostasis during inflammation induced by NLRP3 inflammasome activation (Figure 3).

**Post-translational modification in NLRP3 inflammasome complex**

*Ubiquitination and deubiquitination of NLRP3 inflammasome complex.* Endogenous deubiquitinating enzyme BRCC3 is required for the activation of the NLRP3 inflammasome complex through the deubiquitination of NLRP3.[15] A small-molecule inhibitor of this deubiquitinating enzyme (G5) efficiently suppresses NLRP3 inflammasome activation by inhibiting the deubiquitination of NLRP3.[15] A recent study identified a mechanism by which lipopolysaccharide priming increases NLRP3 protein levels. The F box protein, F-box O3, which is induced by lipopolysaccharide stimulation, is required for the ubiquitination and degradation of another F box protein, F-box L2 (FBXL2). FBXL2 interacts with and targets NLRP3 Lys-689 for ubiquitin ligation and degradation. The study also showed that a small-molecule inhibitor of F-box O3 suppresses NLRP3 protein levels and the release of IL-1β by restoring FBXL2 levels.[55] In addition, A20, a well-known deubiquitinating enzyme of nuclear factor-κB signaling, has a restricting role on NLRP3 activity by inhibiting pro-IL-1β-associated ubiquitination, which is dependent on RIPK3.[22]

HOIL-1L (heme-oxidized IRP2 ubiquitin ligase 1), a component of the linear ubiquitination assembly complex (LUBAC), was found to be critical for the linear ubiquitination of ASC and the assembly of the NLRP3/ASC inflammasome complex.[56] A recent study showed that SHANK-associated RH domain-interacting protein, a component of LUBAC, is essential for the optimal activation of NLRP3 inflammasome complex.[57] Interestingly, a recent finding showed that *Shigella flexneri* E3 ligase effectors, IpaH1.4 and IpaH2.5, interact with LUBAC subunits HOIL-1L and HOIL-1-interacting protein (HOIP), leading to proteasome-dependent degradation of HOIL-1-interacting protein. Further studies are urgently required to identify negative regulators/pathways for the regulation LUBAC machinery involved in NLRP3 inflammasome activation (Figure 4).

*Regulation of phosphorylation in the NLRP3 inflammasome complex.* Recent evidence has shown that basal IκB kinase α (IKKα) activity appears to be important in the inhibition of inflammasome activation. IKKα inhibits caspase recruitment domain-containing ASC-dependent inflammasomes by interacting with the ASC adaptor and phosphorylating its Ser293 residue.[23] Upon NLRP3 activation, IKKα kinase activity can be inhibited by the phosphatase PP2A (and presumably by other phosphatases), which releases ASC to promote NLRP3 inflammasome assembly. Indeed, IKKα functions as a negative regulator in activating not only NLRP3 but also NLRC4 and AIM2 inflammasomes.[23] In addition, protein kinase A, which is induced by PGE2, is critically involved in the inhibition of NLRP3 inflammasome activation through phosphorylation of the cytoplasmic receptor NLRP3 and the attenuation of its ATPase function. Mutations in the regions adjacent to the protein kinase A-mediated phosphorylation site at NLRP3 Ser295 are critically involved in the autoinflammatory disease, CAPS.[10]

Numerous studies have revealed a genetic and functional association of protein tyrosine phosphatase non-receptor 22 (PTPN22) in the pathogenesis of a variety of inflammatory disorders, such as Crohn's disease, rheumatoid arthritis and type I diabetes,[58] all of which are related to dysregulated inflammasome function.[11] A recent study revealed a critical function of PTPN22 in the robust activation of NLRP3 inflammasome and IL-1β secretion. PTPN22 interactions

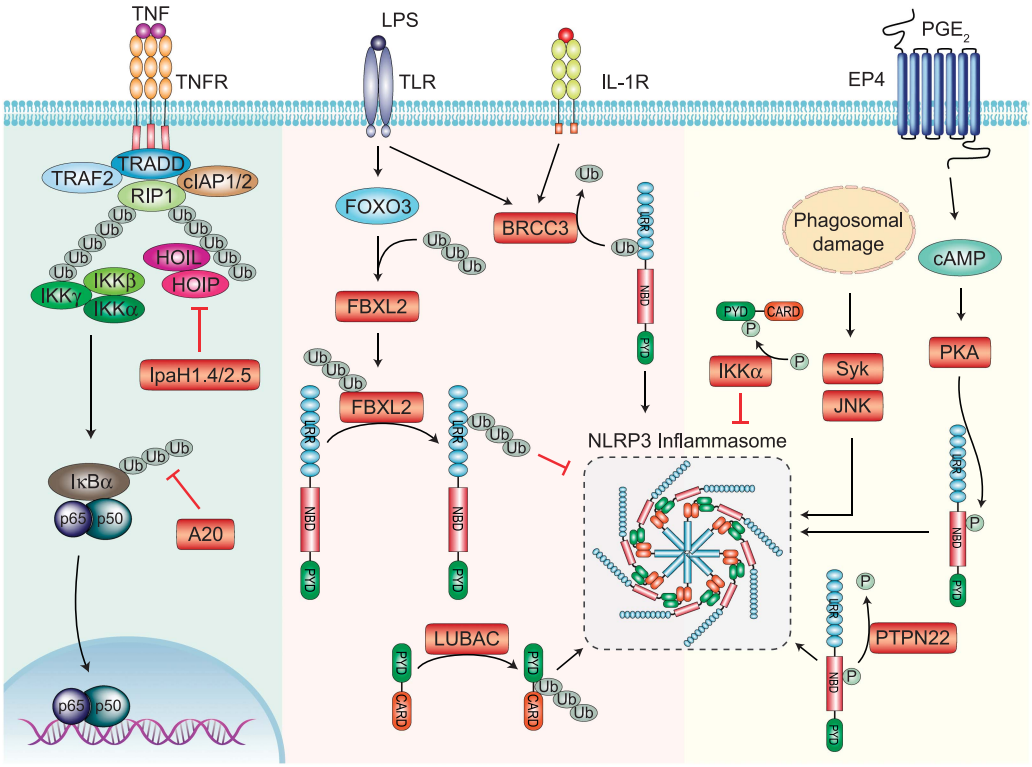


**Figure 4** Post-translational modulation of NLRP3 inflammasome complex. Deubiquitinating enzyme BRCC3 activates NLRP3 inflammasome but G5 has inhibitory role of NLRP3 inflammasome activation as inhibitor of deubiquitinating enzyme. On the other hand, F-box O3 degrades FBXL2 through ubiquitination, and FBXL2 interacts with NLRP3 to regulate its activation. In addition, A20 suppresses the NLRP3 inflammasome activation through pro-IL-1β-associated ubiquitination. Furthermore, HOIL-1L and SHANK-associated RH domain-interacting protein (SHARPIN), components of LUBAC, regulate NLRP3 inflammasome activation. IpaH1.4 and IpaH2.5, E3 ligase effectors, interacts with NLRP3 and induce the degradation of HOIL-1-interacting protein (HOIP). IKKα interacts with ASC and phosphorylates residue Ser293 of ASC to inhibit NLRP3 inflammasome activation. IKKα acts as a negative regulator in the activation of NLRC4 and AIM2. Protein kinase A (PKA) also suppresses NLRP3 activation through phosphorylation of NLRP3. In addition, PTPN22 directly interacts with NLRP3, leading to dephosphorylation of NLRP3. Both Syk and Jnk phosphorylate ASC and contribute to the activation of NLRP3 and caspase-1.

directly with NLRP3 and dephosphorylates NLRP3 at Tyr861 leading to NLRP3 inflammasome activation.[59] It was noted that patients with inflammatory bowel disease who carried the gain-of-function variants of PTPN22 showed increased IL-1β levels in intestinal biopsy and serum samples. Thus, tyrosine phosphorylation at NLRP3 (by an undetermined kinase) is an important regulatory mechanism for inhibiting aberrant NLRP3 inflammasome assembly.[59] Syk and Jnk are additional important kinases that contribute to the activation of caspase-1 related to the NLRP3 and AIM2 inflammasomes.[60] Both Syk and Jnk are involved in the phosphorylation of ASC and in the formation of ASC specks, but they do not affect the interaction of ASC with NLRP3 during inflammasome activation.[60]

*Compounds that regulate NLRP3 inflammasome activation.* Accumulating evidence has identified various compounds that influence NLRP3 inflammasome activation to modulate inflammatory disease pathogenicity (Table 1). MCC950 inhibits the auto-activation of the inflammasome caused by mutated NLRP3 and blocks the formation of NLRP3-induced ASC specks, which were not observed with other inflammasome stimuli.[61] Although the mechanism by which MCC950 acts is not clearly defined, MCC950 may act directly on NLRP3 rather than other inflammasome complexes.[61] In addition, MCC950 effectively ameliorates the pathological responses to experimental autoimmune encephalomyelitis and improves the survival rate in a mouse model of CAPS, suggesting a potential therapeutic effect for NLRP3-associated inflammatory and autoimmune diseases.[61] Cinnamaldehyde and allopurinol, which possess antioxidative and anti-inflammatory activities, were beneficial in relieving cardiac inflammation and fibrosis in fructose-induced cardiac injury related to NLRP3 inflammasome activation.[62] In addition, the widely used antidiabetic drug metformin and the sirtuin 1 activator resveratrol were preventive the NLRP3 inflammasome activation in adipose tissue dysfunction models.[63] Resveratrol also inhibits pyroptosis in macrophages by maintaining mitochondrial integrity and autophagy activation.[64] Withaferin A, a withanolide purified from *Withania somnifera*, acts on the inhibition of *Helicobacter pylori*-induced IL-1β production (which is associated with the development of gastric cancer) in bone marrow-derived dendritic cells.[65]

Interestingly, a widely used antidepressant, fluoxetine, significantly suppresses ROS–PKR (double-stranded RNA-dependent protein kinase)–NLRP3 signaling pathways in macrophages and microglia.[66] Furthermore, fluoxetine suppresses NLRP3 inflammasome activation in macrophages and the hippocampus in chronic mild-stress mice, suggesting a promising therapeutic role for treatment in NLRP3 inflammasome-associated depression.[66] Isoliquiritigenin, a flavonoid derived from *Glycyrrhiza uralensis*, has an activity that suppresses NLRP3 inflammasome activation to inhibit diet-induced obesity, insulin resistance and adipose tissue inflammation.[67] Polyenylpyrroles from soil *ascomycete Gymnoascus reessii* function as anti-inflammatory

**Table 1 Inhibitory compounds of NLRP3 inflammasome complex**

| *No.* | *Name* | *Host/cell type* | *Effects* | *Dosage* | *References* |
|---|---|---|---|---|---|
| 1 | MCC950 | C57BL6/BMDMs | NLRP3-specific ASC oligomerization | 3 mg $kg^{-1}$ (i.v.), 20 mg $kg^{-1}$ (OA)/7.5 nM ($IC_{50}$) | 61 |
| 2 | Cinnamaldehyde | Rat | CD36-mediated TLR4/6-IRAK4/1 signaling | 20, 40 and 80 mg $kg^{-1}$ (drinking water) | 62 |
| 3 | HCAG | RAW 264.7 cells/ J774A.1 cells | ATP-mediated phosphorylation of AKT and PKC-α/δ | ~100 μM | 69 |
| 4 | Allopurinol | Rat | CD36-mediated TLR4/6-IRAK4/1 pathway | 5 mg $kg^{-1}$ | 62 |
| 5 | Metformin | ICR male mice/3T3-L1 adipocytes | Drp1-mediated mitochondrial fission and ER stress | 200 mg $kg^{-1}$ (OA)/1 mM | 63 |
| 6 | Resveratrol | ICR male mice/3T3-L1 adipocytes | Drp1-mediated mitochondrial fission and ER stress | 50 mg $kg^{-1}$ (OA)/10 μM | 63 |
| 7 | Withaferin A | BMDCs | *Helicobacter pylori*-induced IL-1β production | 100, 250 and 500 nM | 65 |
| 8 | Fluoxetine | BMDMs/RAW 264.7 cells/microglia | ROS–PKR–NLRP3 pathway | 10 μM | 66 |
| 9 | Isoliquiritigenin | C57BL/6 BMDMs | TLR4/MD2–NLRP3 pathway | HFD (60% fat)-ILG (0.5% w/w)/10 μM | 67 |
| 10 | Polyenylpyrroles (auxarconjugatin A and 12E-isorumbrin ) | RAW 264.7 cells/ J774A.1 cells | ROS–MAPK–NLRP3 pathway | 20 μM | 68 |
| 11 | GW501516 | C57BL/6J | mRNA expression of pro-inflammatory cytokines and NLRP3, NLRP6 and NLRP10 | 3 mg $kg^{-1}$ (OA) | 70 |
| 12 | α-Linolenic acid metabolites (13-(S)-HPOTrE and 13-(S)-HOTrE | RAW 264.7 cells | Inactivation of NLRP3 inflammasome complex activation and the PPAR-γ pathway | 1, 50 and 100 μM | 71 |

Abbreviations: BMDMs, bone marrow-derived macrophages; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; HCAG, 4-hydroxycinnamaldehyde-galactosamine; HFD, high-fat diet; $IC_{50}$, the half maximal inhibitory concentration; ILG, isoliquiritigenin; i.v., intravenous; PPAR-γ, peroxisome proliferator-activated receptor-γ; OA, oral administration.

agents to ameliorate NLRP3 inflammasome-related diseases through ROS- and MAPK-dependent pathways.[68]

The bioactive compound cinnamaldehyde derivatives function in renoprotective effects in a mouse model of lipopolysaccharide-induced renal inflammation by inhibiting NLRP3 inflammasome activation through ATP-mediated phosphorylation of AKT and PKC-α/δ.[69] In addition, PPAR-δ agonist ameliorates liver steatosis and inhibits the mRNA expression of pro-inflammatory cytokines and NLRP3, NLRP6 and NLRP10. Although this report did not interpret the inhibitory effects of PPAR-δ agonist on the inflammasome complex assembly, PPAR-δ agonist might function as a potential treatment of non-alcoholic fatty liver diseases.[70] In addition, α-linolenic acid (ω-3 polyunsaturated fatty acid) inhibits the inflammatory responses *in vitro and in vivo* through inactivation of the NLRP3 inflammasome complex and the PPAR-γ pathway.[71]

Studies on the effects of currently used drugs on the modulation of NLRP3 inflammasome activation are needed to determine their potential adverse effects on pathological responses during metabolic and inflammatory diseases. For example, statins, which are used widely for reducing lipid levels, are detrimental in the aggravation of insulin resistance in adipose tissue and adipocytes through an NLRP3/caspase-1-dependent mechanism, thus contributing to the development of type 2 diabetes.[72]

## CONCLUDING REMARKS

Inflammasome research produces new insights into the molecular mechanisms of the NLRP3 inflammasome complex and its role in diverse human disorders. Although many current studies have focused on the activation mechanisms leading to inflammatory mediator maturation, new issues have arisen that highlight the importance of maintaining physiological homeostasis to prevent harmful and excessive inflammasome activation. The identification of caspase recruitment domain-containing regulators and pyrin domain-only proteins has provided novel insights into the regulation of inflammasome complex platform assembly. Future studies of additional pyrin domain- and caspase recruitment domain-containing inhibitors will increase our understanding of the regulation of canonical inflammasome assembly. Emerging evidence suggests that a variety of signaling molecules have negative regulatory roles in the transcriptional activation of NLRP3 and the assembly of the inflammasome complex. In addition, several intracellular signaling pathways function as a gear for preventing the potentially harmful activation of the NLRP3 inflammasome complex and the maintenance of homeostasis. Recent studies on the post-translational regulation of inflammasome complexes highlight the importance of investigating protein modifications of the components of the NLRP3 inflammasome complex. Rapidly growing advances related to the compounds regulating inflammasome complexes will lead to the development of therapeutics targeting diverse inflammatory and immune diseases related to the pathogenesis of the NLRP3 inflammasome. This research will help to direct drug-repurposing toward the search for novel therapeutics that target the control of NLRP3 inflammasome activation and inflammatory pathologies in inflammasome-related disorders. Many challenges remain for elucidating the exact mechanisms by which the agents/compounds regulate the activation of the NLRP3 inflammasome complex in these contexts.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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