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The NLRP6 inflammasome


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Summary

The NOD-like receptor family pyrin domain containing 6 (NLRP6), a member of the NOD-like receptor (NLR) family, acts as a cytosolic innate immune sensor that recognizes microbe-associated molecular patterns. In some circumstances upon activation, NLRP6 recruits the adaptor apoptosis-associated speck-like protein (ASC) and the inflammatory caspase-1 or caspase-11 to form an inflammasome, which mediates the maturation and secretion of the pro-inflammatory cytokines IL-18 and IL-1 β . In other contexts, NLRP6 can exert its function in an inflammasome-independent manner. Tight regulation of the NLRP6 inflammasome is critical in maintaining tissue homeostasis, while improper inflammasome activation may contribute to the development of multiple diseases. In intestinal epithelial cells, the NLRP6 inflammasome is suggested to play a role in regulating gut microbiome composition, goblet cell function and related susceptibility to gastrointestinal inflammatory, infectious and neoplastic diseases. Additionally, NLRP6 may regulate extra-intestinal diseases. In this review, we summarize current knowledge on the NLRP6 inflammasome and its activation and regulation patterns, as well as its effector functions contributing to disease modulation. We discuss current challenges in NLRP6 research and future prospects in harnessing its function into potential human interventions.

Keywords: caspase; inflammasome; innate immune receptors; microbiome; NLRP6; pattern recognition receptors.

INTRODUCTION

Viruses and bacteria infecting or colonizing mammalian organisms induce host signalling, mediated by physical contact, or by toxin, microbial nucleic acid, metabolite or foreign protein section, which result in a variety of host responses to such infection. These so-called microbe-

associated molecular patterns (MAMPs) can be sensed by host pattern recognition receptors (PRRs), which initiate innate immune responses.¹⁻⁵ Apart from MAMPs, sterile stressors associated with host cell damage such as ATP or heat-shock proteins, termed damage-associated molecular patterns (DAMPs), are likewise sensed by PRRs.⁶ PRRs are localized either extracellularly like the Toll-like

Abbreviations: AMP, Antimicrobial peptide; ASC, Apoptosis-associated speck-like protein; Asp-Glu-Ala-His, RNA helicase DEAH; ATP, Adenosine triphosphate; CRH, Corticotropin-releasing hormone; DAMPs, Damage-associated molecular patterns; Dhx15, DEAH (Asp-Glu-Ala-His) box helicase 15; DSS, Dextran sulphate sodium; HSCT, Haematopoietic stem cell transplantation; IEC, Mammalian intestinal epithelial cell; IL, Interleukin; KO, Knockout; LPS, Lipopolysaccharide; LRR, Leucine-rich repeat; LTA, Lipoteichoic acid; MAMPs, Microbe-associated molecular patterns; MAPK, Mitogen-activated protein kinase; NAFLD, Non-alcoholic fatty liver disease; NBD, Nucleotide-binding domain; NF- κ B, 'kappa-light-chain-enhancer' of activated B cells; NLR, NOD-like receptor; NLRP6, NOD-like receptor family pyrin domain containing 6; NLRs, Nucleotide oligomerization domain (NOD)-like receptors; PPAR- γ , Peroxisome proliferator-activated receptor γ ; PRRs, Pattern recognition receptors; PYD, Pyrin domain; TLR, Toll-like receptors; TNF- α , Tumour necrosis factor α ; WT, Wild type

receptors (TLR) or intracellularly like the nucleotide oligomerization domain (NOD)-like receptors (NLRs), enabling the cell to sense both extra- and intracellular stressors and infections. A subset of these NLRs is able to build multiprotein complexes with apoptosis-associated speck-like protein (ASC) and caspase-1 or caspase-11^{7,8} upon DAMP or MAMP-mediated activation, termed inflammasomes. Through inflammasome assembly, the associated caspase gets activated and cleaves the inactive precursors of interleukin (IL)-1 β or IL-18, respectively, into the mature cytokine, thereby inducing an inflammatory immune response in reacting to infection or cellular damage.^{8,9} Alternatively, the activated caspase processes gasdermin D, consequently resulting in pore formation in the membrane of affected cells, coupled with a cytokine release, collectively driving pyroptosis, a form of programmed cell death.^{10,11}

NLRP6 (originally termed PYPAF5) belongs to the NLR family and together with NLRP1, NLRP3, NLRP7 and NLRC4 constitutes a capacity to build a fully operational inflammasome.⁹ Consequently, conventional *Nlrp6* deficiency in mice is accompanied by reduced serum IL-18 levels in steady-state conditions and decreased IL-18 levels in colon and serum in mice upon induction of chemical-induced colitis and colitis-associated tumorigenesis.^{12,13} While in vitro experiments demonstrate an activation of IL-1 β via the NLRP6 inflammasome,¹⁴ mature IL-1 β protein level remains unaltered or even increased in *Nlrp6* KO mice.¹³ Of note, hepatic NLRP3 is upregulated in *Nlrp6*-deficient mice,¹⁵ suggesting a compensatory mechanism of NLRP3 activation upon NLRP6 deficiency, potentially explaining the unexpected surge in IL-1 β levels in this setting. Like other NLR family members, NLRP6 is composed of three domains. The N-terminus consists of a pyrin domain (PYD) and is considered the essential element for inflammasome assembly as it interacts with ASC.¹⁶ The nucleotide-binding domain (NBD) builds the central module of NLRP6 and is followed by the C-terminal leucine-rich repeat (LRR) domain, which senses DAMPs and MAMPs.^{9,17} The NLRP6 protein is mainly expressed in the lung and liver but is highest at the intestine,^{12,18} making the gastrointestinal tract the most widely studied organ for the investigation of the NLRP6 inflammasome in health and disease. Interestingly, in human periodontium and gingiva, NLRP6 seems to also impact on the homeostasis of the oral cavity.^{19,20}

A tight regulation of inflammasomes in general is of special organismal importance, as its chronic and unbalanced activation manifests in several inflammatory and metabolic diseases, for example familial Mediterranean fever, which is caused by mutations in the pyrin-coding gene *MEFV*, or cryopyrin-associated periodic syndrome, which is ascribed to point mutations in *Nlrp3* gene.²¹ Unlike the NLRP3 inflammasome, the cell-specific role of

its close relative NLRP6 in health and disease is not fully elucidated to date. In this review, we summarize recent insights related to assembly and activation mechanisms of NLRP6 and the NLRP6 inflammasome, as well its pleiotropic roles in contributing to tissue homeostasis and disease in multiple organs.

NLRP6 INFLAMMASOME ASSEMBLY, REGULATION AND EFFECTOR FUNCTION

Nlrp6 expression is reported to be regulated at the transcriptional and post-translational level, which, in turn, is mainly controlled by upstream microbial and metabolic stimuli (Figure 1). In vitro studies on Caco-2 cells demonstrate that the *Nlrp6* promoter region contains binding sites for the transcription factor peroxisome proliferator-activated receptor γ (PPAR- γ), commonly known to be involved in metabolic regulation.²² Furthermore, expression of *Nlrp6* is induced by the PPAR- γ agonist rosiglitazone.²³ In addition, an increase in PPAR- γ protein levels during embryonic development is accompanied by an increase in *Nlrp6* mRNA transcription.²³ Moreover, inflammatory signals such as tumour necrosis factor- α (TNF- α) or viral stimuli are able to induce the transcription of *Nlrp6*, at least in mouse embryonic fibroblasts.²⁴ Beyond transcription, *Nlrp6* was shown to be regulated by miRNA-331-3p in haemin-treated BV2 cells.²⁵ Once translated, NLRP6 activity is tightly regulated, which requires specific triggers to induce an assembly of a bona fide NLRP6 inflammasome. Upon activation, NLRP6 assembles with ASC, leading to caspase-1 and nuclear factor 'kappa-light-chain-enhancer' of activated B cell (NF- κ B) activation in vitro.¹⁴ A recent cryo-electron microscopy study revealed the structural mechanism of NLRP6 inflammasome assembly.¹⁶ The PYD domain of NLRP6 forms filamentous structures through self-assembly, followed by conformational changes, which enable the recruitment of the adaptor ASC via PYD-PYD binding. This assembling process is strengthened by the NBD domain of NLRP6.

The activation of NLRP6 inflammasome can be regulated by different activators or inhibitors (Figure 1). As very recently described, the deubiquitinase Cyld regulates the function of the NLRP6 inflammasome and prevents excessive inflammation via the production of IL-18. Cyld cleaves the K63-linked ubiquitination of NLRP6, thereby inhibiting NLRP6's ability to interact with ASC.²⁶ Among others, bacteria of different taxa were shown to regulate NLRP6 inflammasome formation.²⁷⁻²⁹ In line, the microbial ligands lipopolysaccharide (LPS), MDP, iE-DAP and Pam3CSK4 have been reported to induce NLRP6 activation in human periodontal ligament cells.¹⁹ Additionally, lipoteichoic acid (LTA), a surface-associated adhesion amphiphile of Gram-positive bacteria, induces the formation of NLRP6 inflammasome, and interestingly, in this

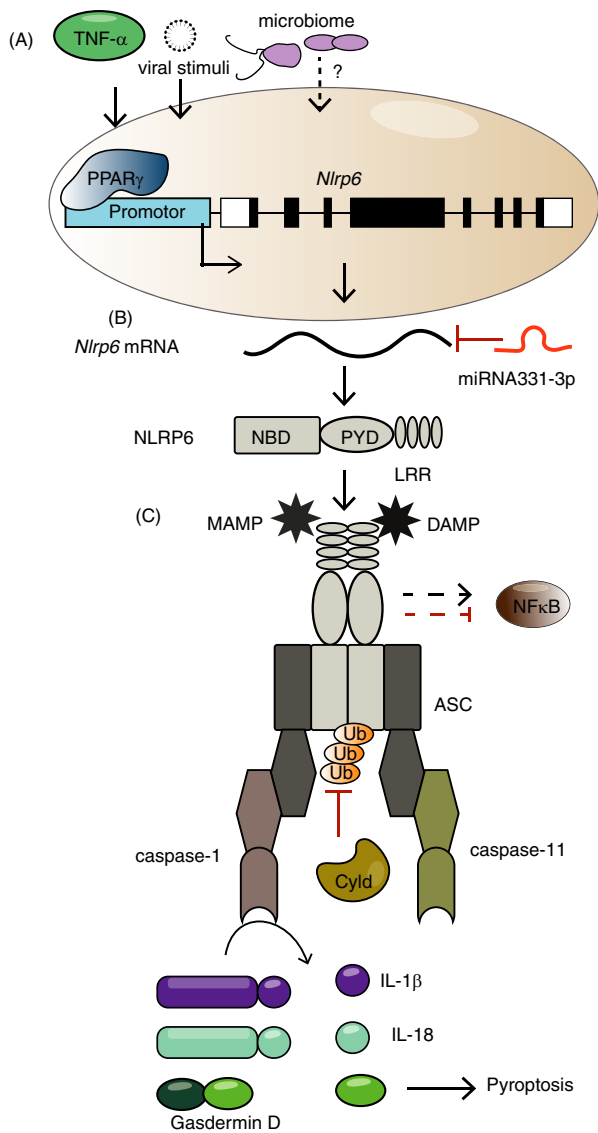


FIGURE 1. Regulation of *Nlrp6* expression and NLRP6 inflammasome assembly. (A) Tumour necrosis factor- α (TNF- α), viral stimuli and elusive microbiome-mediated signalling can induce the transcription of *Nlrp6*. The transcription factor peroxisome proliferator-activated receptor- γ (PPAR γ) binds to the promoter region of *Nlrp6* and induces its transcription. (B) The miRNA 331-3p inhibits translation of the *Nlrp6* transcript. (C) Microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs) bind to the C-terminal leucine-rich repeats (LRR) of the NLRP6 protein to enable a bona fide inflammasome assembly. However, these MAMPs and DAMPs remain mostly unidentified to date. The NLRP6 protein further contains a nucleotide-binding domain (NBD) and a pyrin domain (PYD), which interact with the ASC adaptor. The NLRP6 inflammasome activates caspase-1 or caspase-11 and cleaves the proIL-1 β and proIL-18 precursors into the mature cytokines. Gasdermin D is cleaved and drives pyroptosis. NLRP6 inhibits or increases NF- κ B activity, probably in a cell- and context-specific manner. The deubiquitinase Cyld inhibits NLRP6 inflammasome formation

case NLRP6 activates the non-canonical pathway involving caspase-11 *in vivo*.²⁷ Furthermore, the microbial metabolites taurine, histamine and spermine have been shown to modulate the immune responses by regulating NLRP6 inflammasome assembly.²⁹ Beyond bacterial products, NLRP6 also functions as a viral sensor by interacting with the adenosine triphosphate (ATP)-dependent RNA helicase DEAH (Asp-Glu-Ala-His) box helicase 15 (Dhx15).²⁴ Full-length NLRP6, but not NLRP3, is required for binding the viral protein Dhx15.²⁴

Upon NLRP6 inflammasome assembly, it induces inflammatory responses via the cleavage of proIL-1 β and proIL-18 into their mature forms. Other downstream signalling pathways can also be induced by the NLRP6 inflammasome. Chen et al show that alkali-burn injury induces NLRP3 and NLRP6 inflammasome formation, whereby NLRP3 is activated by NF- κ B, while NLRP6 dampens NF- κ B activity, demonstrating the opposing roles of these two inflammasomes in this context.³⁰ In human periodontal ligament cells, NLRP6 inhibits NF- κ B and ERK signalling, thereby dampening the release of IL-6 and TNF- α .¹⁹ Additionally, NLRP6 inhibits the activation of NF- κ B signalling pathway and p38 mitogen-activated protein kinase (MAPK) signalling pathway after allogeneic haematopoietic stem cell transplantation-induced liver damage in mice. Following haematopoietic stem cell transplantation in *Nlrp6* knockout (KO) mice, an overcompensatory NLRP3 expression is accompanied by exacerbated liver damage, suggesting an opposing role of the NLRP6 and NLRP3 inflammasome in this context.¹⁵ Interestingly, *Nlrp6*-deficient mice are highly resistant to infections with the facultative intracellular pathogens *Listeria monocytogenes* and *Salmonella typhimurium* and the extracellular pathogen *Escherichia coli*, indicating that NLRP6 might dampen the inflammatory response and acts as a counterpart to the other PRRs by downregulating the TLR-mediated NF- κ B and MAPK signalling.³¹ Collectively, several independent studies demonstrate an interaction of the NLRP6 inflammasome with NF- κ B signalling pathways, driving inflammatory or anti-inflammatory functions at different physiological contexts. To better delineate these partly opposing results, a cell-specific role of NLRP6 needs to be further investigated in future studies.

ROLE OF NLRP6 IN HEALTH AND DISEASE

NLRP6 in the gut

The mammalian intestinal mucosa and lumen contain trillions of microorganisms, including mostly not only bacteria but also fungi, virus and parasites, which have coevolved with the host and are collectively termed the microbiome. Regulating host-microbe symbiosis is critically important in enabling critical functions conferred by

the microbes, while avoiding pathogenic invasion into the sterile host or elicitation of uncontrolled inflammation against non-invading resident microbes. Such a fine and tightly regulated balance is achieved by an induction of host immune tolerance to innocuous stimuli during steady state, coupled with an ability to induce a potent immune response against invading pathogens or pathobionts.³² The high expression of *Nlrp6* in the mammalian intestinal epithelial cells (IECs), including both enterocytes and goblet cells,^{12,33} positions it as a potential culprit regulator of these interactions (Figure 2). Indeed, in one vivarium, *Nlrp6* deletion in mice led to faecal microbiota shifts towards higher abundance of the bacterial family Prevotellaceae and phyla TM7.¹² This altered microbiome was transferrable to wild-type (WT) mice and associated with higher susceptibility to dextran sulphate sodium (DSS)-induced colitis.¹² In another vivarium, NLRP6 was important in controlling the abundance of the colitis-promoting pathobiont *Akkermansia muciniphila*, thus protecting IL-10 KO mice from developing spontaneous colitis.³⁴ The aberrant gut microbial community in *Nlrp6* inflammasome-deficient mice also promotes the susceptibility to colitis-induced carcinogenesis through the activation of IL-6 signalling in IEC.³⁵ Importantly, the impact of NLRP6 on gut microbiota composition is community-dependent and manifests itself upon exposure to a diverse enough microbiome configuration. As such, microbiome-deficient *Nlrp6*-deficient mice transferred with microbiome configurations from a high barrier facility, in which community diversity was highly limited and pathobionts that were absent failed to induce a state of dysbiosis in recipient *Nlrp6*-deficient mice. In contrast, transfer of a diverse microbiome configuration led to development of a marked dysbiosis, suggesting that the absence of *Nlrp6*, coupled with environmental microbial diversity, mutually engage in promoting dysbiosis.^{36–38} These observations provide a framework for vivarium specificity of microbiome alterations that spans beyond that of NLRP6, and mirror human interindividual microbiome variability driving distinct disease features even in the presence of identical host genetic susceptibilities.³⁹ From a research perspective, this highlights the importance of combining littermate-controlled experimental design together with faecal microbiome transfer into germ-free mice (or microbiome-depleted mice) in studying microbiome modulatory roles of this and other NLRs.⁴⁰

The molecular mechanism by which NLRP6-associated signalling modulates the gut microbiome is just in the beginning to be revealed. As mentioned above, commensal-derived metabolites (including taurine, histamine and spermine) modulate the gut epithelial NLRP6 inflammasome and IL-18 secretion, and subsequently impact the expression of downstream antimicrobial peptide (AMP) genes in the gut, thus shaping the host–microbiome interface and determining susceptibility to DSS-induced

colitis.²⁹ A mechanism for this modulation may involve metabolite-induced morphological alterations of IEC intercellular connections and cellular morphology.⁴¹ In addition to commensal-associated modulators, NLRP6 can directly bind and be activated by LTA derived from Gram-positive bacteria, that is *Listeria monocytogenes*.²⁷ Sensing of cytosolic LTA by NLRP6 in macrophages results in the activation of caspase-11 and caspase-1 via the adaptor ASC, which in turn leads to IL-1 β and IL-18 maturation and enhances Gram-positive pathogen infection in vivo. Additionally, the Gram-negative bacterial ligand LPS binds directly to NLRP6 monomer, and triggers its oligomerization and recruitment of ASC, thus initiating the assembly of NLRP6 inflammasome, suggesting that NLRP6 might also act as a potential LPS sensor.⁴² Moreover, the dietary component flavone is suggested to be another modulator of NLRP6 signalling pathway, and exerts its anti-inflammatory effect by alteration of gut microbiome downstream of the inflammasome-independent NLRP6 signalling.⁴³ As mentioned above, the deubiquitinase Cylid inhibits NLRP6 protein interactions, which tightly regulates NLRP6-mediated IL-18 secretion and limits severe intestinal inflammation in mice upon *Citrobacter Rodentium* infection,²⁶ providing insights into possible mechanisms promoting negative regulation of the NLRP6 inflammasome. Corticotropin-releasing hormone (CRH), a stress hormone released during a water-avoidance stress model in mice, also exerts inhibiting effects on *Nlrp6* expression and induces gut dysbiosis, which consequently mediates the development of enteritis in mice.⁴⁴

The NLRP6 inflammasome is also expressed in intestinal goblet cells and thus impacts on mucus secretion by goblet cells. One suggested mechanism to this effect involves autophagy, a critical process for exocytosis of goblet cell mucin granule.³³ *Nlrp6*-deficient mice feature impaired autophagy leading to altered secretion of mucus granules in goblet cells, thereby contributing to susceptibility to *Citrobacter Rodentium* infection.³³ A second mechanism involves sentinel goblet cells situated at the entrance to the colonic crypt, which may sense non-specific TLR ligands and trigger intracellular NLRP6 activation, subsequently inducing mucin exocytosis to expel bacteria.⁴⁵ As above, in some vivaria, only excess administration of LPS enables this NLRP6 goblet cell function.⁴⁶

It should be noted that, in addition to IEC, NLRP6 may also play a role in regulating intestinal homeostasis through its functions in other intestinal cell types. For example, upon DSS-induced intestinal injury, an upregulated expression of *Nlrp6* in infiltrating Ly6C^{hi} inflammatory monocytes is critical in controlling bacteria-driven inflammation, mediated through induction of TNF- α production and IL-18 signalling.⁴⁷ Likewise, the activity of NLRP6 in colonic myofibroblast⁴⁸ and haematopoietic cells¹³ is suggested to be important in controlling the epithelial proliferation and self-renewal upon chronic

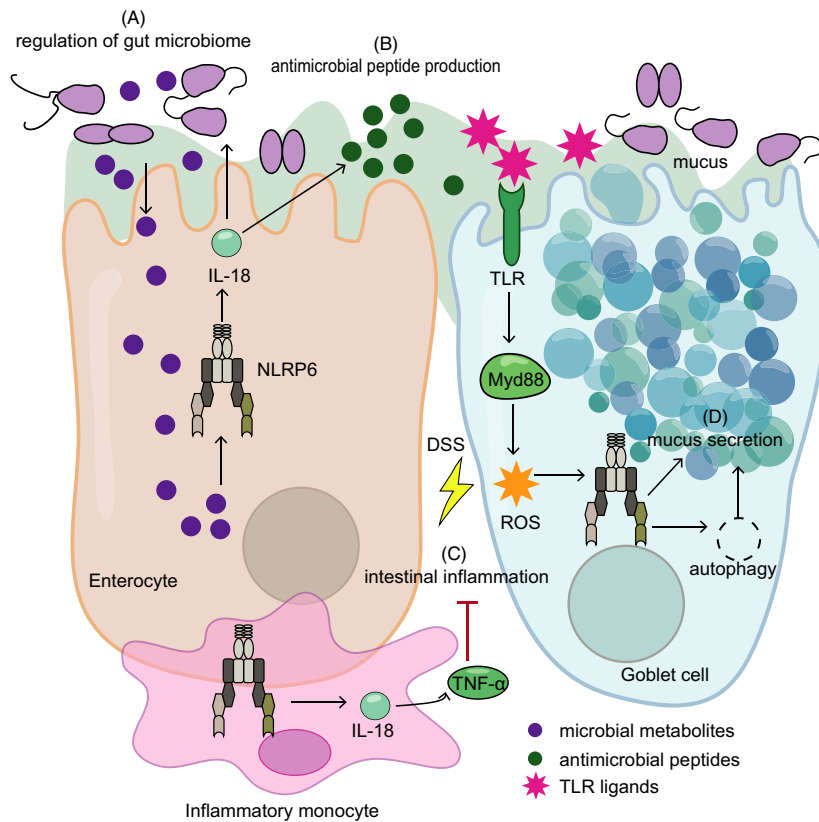


FIGURE 2. NLRP6 in the gut. In intestinal enterocytes, NLRP6 inflammasome can be modulated by commensal-derived metabolites (eg taurine, spermine and histamine), resulting in regulation of mature IL-18 production and of gut microbiome composition in a vivarium-specific and community-dependent manner (A), as well as the generation of unique antimicrobial peptide repertoires (B). The shaping of gut microbiome is suggested, in turn, to contribute to the susceptibility to dextran sulphate sodium (DSS)-induced intestinal inflammation (C). Upon DSS-induced intestinal injury, the upregulated *Nlrp6* expression in infiltrating inflammatory monocytes also reduces susceptibility to DSS colitis, mediated by IL-18-dependent tumour necrosis factor- α (TNF- α) production. In goblet cells, NLRP6 inflammasome activation impacts the secretion of mucus (D) through two suggested mechanisms. The first involves regulation of autophagy, which controls exocytosis of goblet cell mucin granule, while the second involves sensing of Toll-like receptor (TLR) ligands by TLRs, triggering Myd88-dependent reactive oxygen species (ROS) production, which induces Muc2 exocytosis

injury. In experimental mouse models of allogeneic bone marrow transplantation, host NLRP6 exacerbates the severity and mortality of gastrointestinal graft-versus-host disease, independent of microbial dysbiosis. This phenotype relies on the expression of *Nlrp6* in host non-haematopoietic compartment, and in part stems from signalling through the intestinal metabolite taurine.⁴⁹ Furthermore, NLRP6 can modulate host response to bacterial infection in other inflammasome- and microbiome-independent manners and physiological contexts. For example, *Nlrp6*-deficient mice are less susceptible to several Gram-positive and Gram-negative bacterial pathogens, along with enhanced recruitment of monocytes and neutrophils.³¹ In this context, NLRP6 plays the detrimental role by negatively regulating the activation of NF- κ B and MAPK signalling.³¹ In addition to the aforementioned antibacterial immunity, NLRP6 is critical in controlling the enteric virus infection when mice are challenged with encephalomyocarditis virus or murine norovirus 1.²⁴

Furthermore, upregulation of *Nlrp6* expression (and also *Pycard*, *Caspase-1* and *IL18*) in rat intestine at late fetal development²³ may point towards a potential role in intestinal organogenesis, which merits further mechanistic studies. In summary, NLRP6 emerges as an important player in gut homeostasis and interactions with commensal bacteria, viruses and pathogens. This highlights NLRP6 as a potential future exploitable checkpoint, whose manipulations may be harnessed to control infectious, inflammatory and potentially metabolic disease. However, the role of NLRP6 at the cellular and molecular level needs to be investigated in more depth to develop such adequate therapeutic strategies.

NLRP6 in the liver

At the hepatic sinusoids, the oxygen-rich blood of the hepatic artery is mixed with the less oxygenated but nutrient-rich portal blood returning from the intestines.

The portal venous blood also contains ample antigens and microbial products of digested food and the intestinal microbiome, thereby constantly challenging the liver as the 'first-pass organ'.⁵⁰ This influx bears pathophysiological relevance. In obesity, for example, an altered release of DAMPs and MAMPs by a dysbiotic gut microbiome composition⁵¹ is delivered to the liver through the portal circulation, where it creates a state of chronic low-grade inflammation contributing to the development of non-alcoholic fatty liver disease (NAFLD).^{52,53} NLRP6 modulates these interactions in mice, through regulation of gut microbiota leading to enhanced influx of TLR4 and TLR9 ligands from the intestine into the liver upon *Nlrp6* deficiency.⁵² Other NLRP6 hepatic effects may be independent of its impact on the gut microbiome, as is described below (Figure 3).

Indeed, when investigating the expression patterns of PRRs in human and murine solid organs, a relatively high expression level of *Nlrp6* mRNA was found not only in the murine intestine but also in the liver when compared to the spleen.^{12,18} Likewise, NLRP6 protein expression is

most dominant in the intestine, followed by the liver.²⁴ Importantly, *Nlrp6* expression is decreased in human biopsies from fibrotic and cirrhotic livers when compared to healthy controls,⁵⁴ suggesting a putative role of NLRP6 in human liver disease. Indeed, Zhu and colleagues⁵⁴ identified a protective in vitro function of NLRP6 in the hepatic stellate cell line LX-2, in which it inhibited cell proliferation and reduced collagen expression. On a molecular level, NLRP6 inhibited TGF- β -mediated phosphorylation of Smad2/3 in a PPM1A-dependent manner, suggesting that it builds a complex with PPM1A to exert its inhibitory effect on TGF- β .⁵⁴ Interestingly, overexpression of *Nlrp6* in gingival fibroblasts induces pyroptosis,²⁰ suggesting a potential pan-fibroblast activity across organs. Hepatocytes treated with either palmitic acid or LPS featured an increase in *Nlrp3*, *Nlrp10* and *Nlrp6* mRNA levels,⁵⁵ suggesting an additional hepatocyte-specific role of NLRP6 in these conditions. Intriguingly, deep RNA sequencing and single-molecule transcript imaging demonstrated that *Nlrp6* mRNA level is more abundant in the nucleus than the cytoplasm in murine liver, which

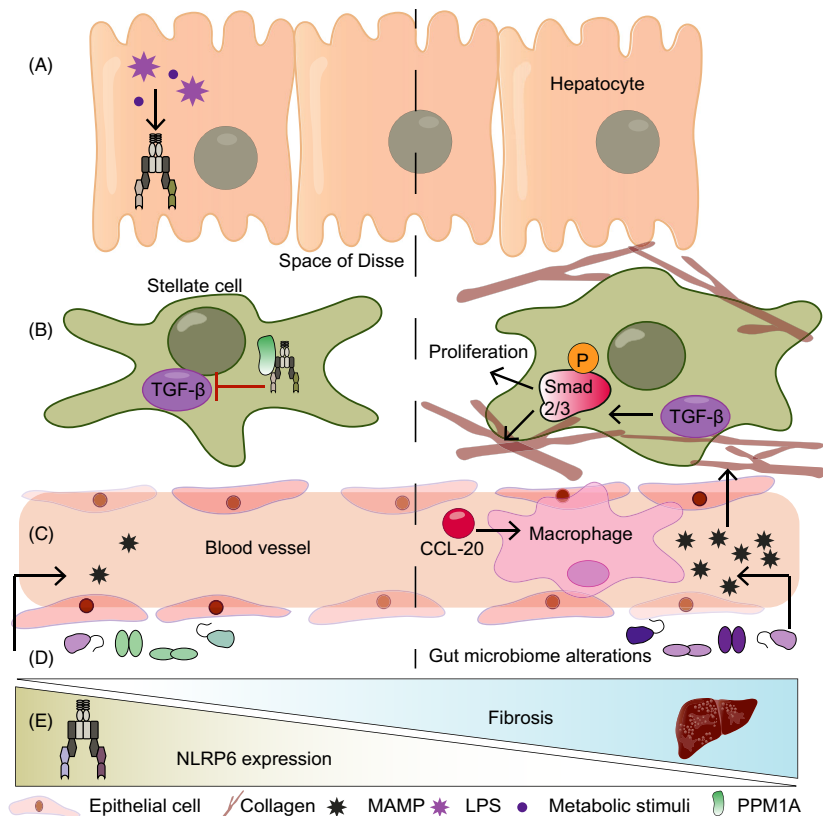


FIGURE 3. NLRP6 in the liver. (A) Stimulation of hepatocytes with LPS or palmitic acid is accompanied by increased *Nlrp6* expression. (B) In hepatic stellate cells, NLRP6 interacts with PPM1A to inhibit transforming growth factor- β (TGF- β). This, in turn, leads to reduced phosphorylation of Smad2/3 and reduced proliferation and collagen expression. (C) NLRP6 prevents the infiltration of macrophages into the liver by reducing C-C chemokine ligand 20 (CCL20) levels. (D) Whole-body *Nlrp6* deletion leads to a dysregulated microbiota, which increases the influx of TLR ligands in the circulation, thereby promoting fibrosis. (E) In fibrotic livers, *Nlrp6* expression is decreased, the functional implications of which remain unknown to date

might play a role in buffering the cytosolic *Nlrp6* gene expression noise.⁵⁶

Following the induction of liver damage secondary to allogenic haematopoietic stem cell transplantation (HSCT), hepatic *Nlrp6* expression is increased in mice, while *Nlrp6*-deficient mice manifest more severe liver damage after HSCT compared to controls. This exacerbated liver damage is coupled with higher levels of TGF- β , suggesting a protective role of NLRP6 in the liver via regulation of TGF- β .¹⁵ However, the lack of a conditional *Nlrp6* KO models precludes the identification of a cell-specific role of NLRP6 in this model. In an alcoholic hepatitis model in mice, *Nlrp6* expression is downregulated compared with controls, while NLRP6 is suggested to prevent the infiltration of macrophages into the liver by reducing CCL20 levels, thereby putatively impacts disease severity. In agreement, a viral overexpression of *Nlrp6* repressed the development of fibrosis in an alcoholic hepatitis model.⁵⁷ Taken together, a protective role of NLRP6 in the liver is suggested across different disease models. In contrast, parasitic-induced liver fibrosis by *Schistosoma mansoni* infection in whole body of *Nlrp6*-deficient mice is associated with a reduced macrophage and neutrophil infiltration, coupled with abrogated cytokine release (eg CCL2, CCL3, IL-10 and IL-5) and collagen deposition.⁵⁸ Cell-specific depletion of *Nlrp6* may clarify these apparent contradictory NLRP6-driven innate immune responses noted in different liver disease models. Of note, in whole body of *Nlrp6*-depleted mice, secondary effects induced by *Nlrp6*-deficiency, such as compositional changes of the gut microbiome, cannot be excluded at present as potential indirect contributors to the observed phenotypes and may impact the discrepancies noted between different models induced at different vivaria.

NLRP6 in other organs

The function of the NLRP6 inflammasome, and implications on organ-specific diseases, is increasingly investigated in tissues beyond the gut and liver, including, but not limited to, the central nervous system, lung and kidney. For example, in a rat model of middle cerebral artery occlusion/reperfusion, an upregulated brain expression of *Nlrp6* aggravates cerebral ischaemic and reperfusion injury and worsens neurological functions, indicating a potential neuroprotective role of NLRP6.⁵⁹ In this regard, a recent in vitro study showed that upon oxygen–glucose deprivation, NLRP6 inflammasome assembly in astrocytes induces the production of the pro-inflammatory cytokines IL-1 β and IL-8, coupled with promotion of pyroptosis.⁶⁰ Likewise in another mouse model of intracerebral haemorrhage, *Nlrp6* downregulation targeted by a micro-RNA miR-331-3p dampens the inflammatory responses and restores the neurological function after cerebral haemorrhage.²⁵ In contrast, NLRP6 plays a protective role

in promoting the recovery after peripheral nerve injury, suggestively via dampening the MAPK signalling pathway in an inflammasome-independent manner.⁶¹

In the lung, *Nlrp6*-deficient mice are more resistant to pulmonary *Staphylococcus aureus* infection compared with WT counterparts, and this protective effect is mediated by enhanced neutrophil recruitment and neutrophil-associated bacterial clearance following infection.²⁸ *Nlrp6* is also expressed in the tubular epithelium of healthy murine and human kidneys, and its expression is reduced upon acute kidney injury induced by folate acid or cisplatin overdose, in both human or mouse models.⁶² Furthermore, deficiency of *Nlrp6* in mice aggravates the severity of nephrotoxic acute kidney injury, characterized by enhanced apoptotic and pro-inflammatory responses in the tubular cells, suggesting a nephroprotective role of NLRP6 in acute kidney injury.⁶² Taken together, the complicated and multifaceted functions of NLRP6 in regulating disease associated with a multitude of organs warrant a continued thorough investigation.

CHALLENGES AND UNKNOWNNS IN NLRP6 INFLAMMASOME RESEARCH

The above-mentioned pleiotropic and multifaceted functions of the NLRP6 inflammasome make it an enigmatic but intriguing NLR, along with many unknowns and challenges remaining to be investigated. First, the sterile and non-sterile ligands of NLRP6, and their mechanisms of activity, have only partially been deciphered to date. Despite the revealed NLRP6 activators such as commensal-associated metabolites, bacterial ligands LTA, LPS and viral RNA,^{24,27,29,42} the manners and mechanisms by which other pathogenic stimuli derived from fungi, parasites and host-derived activating signals (eg DAMPs) bind and trigger NLRP6 signalling remain to be explored. This would likely necessitate unravelling of the Cryo-EM atomic-resolution structure of NLRP6 ligand binding. Second, identification of negative regulators of NLRP6 inflammasome in the context of different diseases is an equally important and fascinating topic. Given the intimate interaction between NLRP6 and the gut microbiome,¹² it is yet to be determined how various microbial activators and inhibitors counteract to fine-tune the activation of the NLRP6 inflammasome and therefore maintain gut homeostasis, especially at the human setting. Third, more researches are warranted in identifying the downstream effectors of NLRP6 inflammasome activation beyond secretion of pro-inflammatory cytokines, including but not limited to induction of pyroptosis, regulation of intestinal barrier function, modulation of adaptive immunity and orchestration of cell metabolism. Moreover, given inconsistent results regarding the role of NLRP6 in regulating gut microbiome composition across different vivaria, future studies investigating its role in

modulating gastrointestinal and extra-gastrointestinal diseases will require proper littermate-controlled and microbial-transfer strategies. However, in all the above-mentioned strategies, deciphering a cell-specific role of NLRP6 is of essential importance as it could help to delineate opposing and complementary cellular functions, while decoding the roles of elusive NLRP6-modulated pathways. Last but not least, the role of NLRP6 inflammasome in the context of human health and disease is still poorly understood, as the majority of studies to date are conducted in murine models. To date, several inhibitors of NLRP3 inflammasome activation (eg MCC950, CY-09) have been recognized and applied as promising interventions in different disease models,⁶³ while similar therapeutic applications have not been identified to date for the NLRP6 inflammasome. It is imperative that future research will focus on translating the NLRP6 inflammasome biology towards such identification and application of potential therapeutic targets as disease treatment in human NLRP6-driven disease.

CONCLUDING REMARKS

Recent advances in revealing the biology and functions of NLRP6, in the context of inflammasome formation or non-inflammasome functions, and its connection to health and disease have greatly enhanced our understandings of how NLRP6 impacts the mammalian host. However, conflicting observations in different studies suggest that NLRP6 can harness a context-specific pro-inflammatory or anti-inflammatory innate immune activation. Variable microbiome configurations can further impact NLRP6-mediated modulation of mammalian health and disease traits. We strongly believe that utilization of such inherent variability may facilitate achieving a mechanistic understanding of NLRP6 interactions with the host and its microbiome, thereby enabling deciphering molecular and cell-specific mechanism of NLRP6 regulation and effector function. Unveiling of such personalized structural and molecular considerations governing the modulation of NLRP6 may enable to identify new regulatory checkpoints, to be further developed as potential therapeutics of NLRP6-associated disease.

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CONFLICT OF INTEREST

EE is a salaried scientific consultant for DayTwo and BiomX. DZ and LK have nothing to declare.

AUTHOR CONTRIBUTION

All authors performed an extensive literature research, contributed substantially to discussion of the content, and wrote and edited the manuscript.

DATA AVAILABILITY STATEMENT

There are no raw data generated in this manuscript.

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