



Mini Review

Role of NLRP3 inflammasomes in hepatic ischemia-reperfusion injury

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Increasing evidence indicates that inflammation plays an important role in the pathogenesis of hepatic ischemia-reperfusion (I/R) injury. However, it is still unclear how I/R stimuli induce inflammatory responses in the liver. NLRP3 is an intracellular pattern recognition receptor and a component of NLRP3 inflammasomes that can induce caspase-1 activation, and regulate the processing of a potent inflammatory cytokine, interleukin-1 β (IL-1 β). Several investigations have recently suggested that inflammatory responses are mediated through NLRP3 inflammasomes in hepatic I/R injury. On the other hand, we recently found that NLRP3 regulates neutrophil functions and contributes to hepatic I/R injury independently of inflammasomes. This review summarizes the basic information on NLRP3 inflammasomes and discusses inflammasome-dependent/independent roles of NLRP3 in the pathophysiology of hepatic I/R injury.

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Introduction

Hepatic ischemia-reperfusion (I/R) injury is a major cause of liver dysfunction and serious complications in hepatic surgery and liver transplantation. Systemic low-flow ischemia and hypoxia, such as trauma, hemorrhagic shock, sepsis, congestive heart failure, and respiratory failure, may also lead to hepatic I/R injury. In general, I/R injury results from a prolonged ischemic insult followed

by restoration of blood flow, causing “reperfusion injury”. Although hepatic I/R injury comprises complex pathological processes involving numerous cell types and molecular mediators, it is considered that the liver damage is caused by excessive inflammatory responses characterized by the release of inflammatory cytokines and chemokines that recruit circulating leukocytes, mainly neutrophils and macrophages, into ischemic tissues¹⁻³. In addition,



because Kupffer cells are located as resident macrophages in the liver, these resident cells may also be involved in inflammatory responses after hepatic I/R injury. However, it is still not known how I/R stimuli induce inflammatory responses in the liver.

Inflammation is defined as the process by which the body responds to an injury or infection, and it is triggered by the innate immune system. It is widely accepted that the innate immune system is considered as the first line of defense against infections. However, inflammatory responses also occur in the absence of infection and these are referred to as “sterile inflammation⁴.” Increasing evidence indicates that several types of sterile inflammation in diseases are mediated through newly discovered innate immune pathways known as NLRP3 inflammasomes⁴⁻⁸. NLRP3 inflammasomes are intracellular multiprotein complexes that serve as molecular platforms to induce caspase-1 activation and interleukin-1 β (IL-1 β) secretion, leading to inflammatory responses. Our group recently demonstrated the importance of NLRP3 inflammasomes in the sterile inflammatory responses of vascular injury^{7, 9}, atherosclerosis¹⁰, myocardial I/R injury^{11, 12}, chronic kidney disease¹³, and nanoparticle-triggered pregnancy complications¹⁴. Furthermore, NLRP3 inflammasomes have been implicated in the process of sterile inflammatory diseases such as gout, pseudogout, type 2 diabetes mellitus, metabolic syndrome, asbestosis, silicosis, and Alzheimer's disease^{5, 6}. The role of NLRP3 inflammasomes has been reported in hepatic I/R injury¹⁵. On the other hand, we recently demonstrated that NLRP3 regulates neutrophil functions and contributes to hepatic I/R injury independently of inflammasomes¹⁶. This review summarizes the basic information on NLRP3 inflammasomes and discusses inflammasome-dependent/independent roles of NLRP3 in the pathophysiology of hepatic I/R injury.

Innate immune pathway and pattern recognition receptors

The innate immune system is the first line of host defense against pathogens and is mediated by phagocytes, including macrophages and dendritic cells (DCs)¹⁷. The system consists of multiple families of germline-encoded pattern-recognition receptors (PRRs) that recognize microbial as well as non-microbial insults. PRRs of the innate immune system are divided into at least 4 distinct genetic families: the Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and nucleotide-binding oligomerization

domain, leucine-rich repeat-containing receptors (NLRs), also known as Nod-like receptors. PRRs recognize conserved motifs associated with microbial components, known as pathogen-associated molecular patterns (PAMPs), as well as endogenous danger signals, known as danger/damage-associated molecular patterns (DAMPs). Activation of these receptors leads to the production of inflammatory cytokines, which drive the inflammatory responses. Among them, NLR family proteins have been associated with large multiprotein complexes called inflammasomes, which induce caspase-1 activation and IL-1 β production^{5, 12, 18}. TLRs also play a critical role in IL-1 β production by inducing pro-IL-1 β synthesis. The pathophysiological role of TLRs in hepatic I/R injury is comprehensively reviewed elsewhere^{19, 20}. To date, little information is available on the role of RLRs and CLRs in hepatic I/R injury.

Inflammasomes

The inflammasome is a large multiprotein complex formed in response to DAMPs and PAMPs in the cytosol. Most inflammasomes contain a member of the NLR family proteins, the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and the inflammatory pro-caspase-1¹⁸. Upon activation, the NLR protein changes its conformation and associates with ASC, which recruits caspase-1 and then induces its activation. Because caspase-1 was previously known as an IL-1 β -converting enzyme (ICE), active caspase-1 subsequently cleaves pro-IL-1 β into its mature forms, which can be secreted. Inflammasomes are also involved in the processing of IL-18, another IL-1 family member. In addition to IL-1 β secretion, inflammasome activation induces caspase-1-dependent cell death, termed “pyroptosis.” Pyroptosis is a highly inflammatory form of cell death, characterized by both apoptosis (*e.g.*, DNA fragmentation) and necrosis (*e.g.*, cell swelling and rupture). To date, many types of inflammasomes have been described, each of which is generally named according to the specific NLR it contains. These include NLRP1 [NLR family, pyrin domain (PD) containing 1], NLRP3 (also known as NALP3), NLRP6, NLRP7, NLRP12, and NLRC4 [NLR family, caspase recruitment domain (CARD) domain containing 4]. Furthermore, two other inflammasomes that contain pyrin and HIN domain-containing protein (PYHIN) family proteins have been described: absence in melanoma 2 (AIM2) inflammasomes and IFN- γ -inducible protein 16 (IFI16) inflammasomes¹⁸.

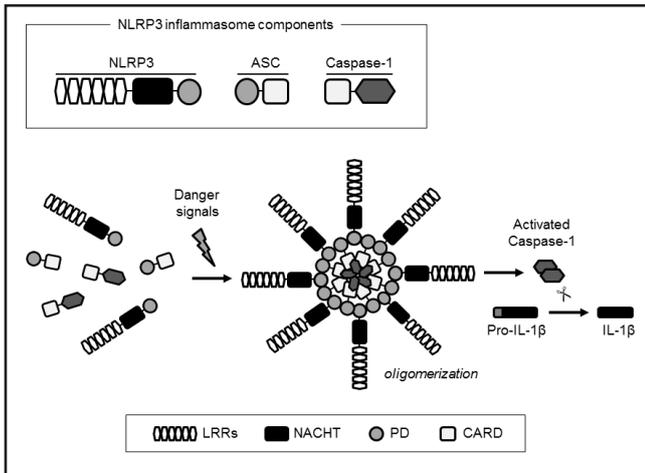


Fig.1 Activation model of NLRP3 inflammasomes

NLRP3 inflammasomes are formed by NLRP3, ASC, and caspase-1. PD-PD and CARD-CARD homotypic interactions are critical for the recruitment and activation of either ASC or caspase-1. LRRs of NLRP3 are considered to sense putative ligands, leading to the oligomerization of the NACHT domain and to initiate the formation of NLRP3 inflammasome assembly.

Among these inflammasomes, the NLRP3 inflammasomes are the most studied, and their activation has been linked to endogenous and exogenous danger signals⁴. NLRP3 inflammasomes are formed by NLRP3, ASC, and caspase-1 (Fig. 1). NLRP3 is composed of three domains: C-terminal leucine-rich repeats (LRRs), a central nucleotide domain termed the NACHT domain, and an N-terminal effector domain (pyrin domain, PD). ASC contains an N-terminal PD and a C-terminal CARD. Caspase-1 comprises CARD and a caspase domain. LRRs of NLRP3 are considered to sense putative ligands, leading to the oligomerization of the NACHT domain and initiating the formation of the NLRP3 inflammasome assembly. When activated, PD of NLRP3 homotypically interacts with that of ASC, after which CARD of ASC recruits and binds to pro-caspase-1. These interactions finally form the assembly of NLRP3 inflammasomes that leads to caspase-1 activation. Until now, three common pathways of NLRP3 inflammasome activation have been described^{5, 18}: (1) potassium efflux by channels or ionophoric compounds, (2) lysosomal destabilization and release of its enzymes, and (3) mitochondrial reactive oxygen species (ROS) generation (Fig. 2).

Endogenous danger signals that activate the NLRP3 inflammasomes include extracellular adenosine triphosphate (ATP)²¹, monosodium urate (MSU)²², calcium phosphate crystals¹⁰, cholesterol crystals²³, amyloid- β ²⁴,

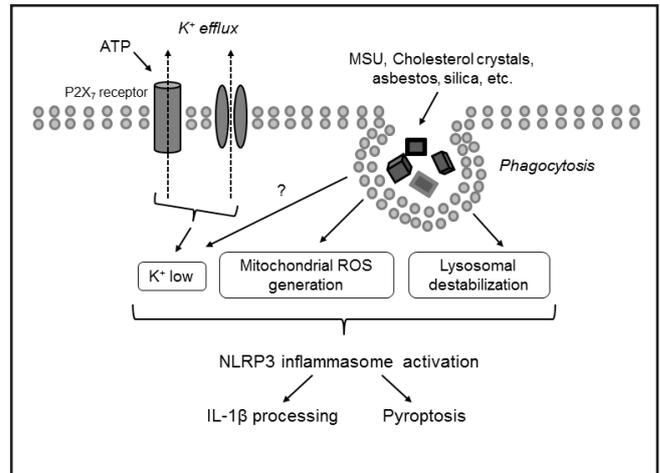


Fig.2 NLRP3 inflammasome activation pathways

Common pathways of NLRP3 inflammasome activation include (1) potassium efflux by channels or ionophoric compounds, (2) lysosomal destabilization and release of its enzymes, and (3) mitochondrial ROS generation. Activation of NLRP3 inflammasomes induces IL-1 β processing and caspase-1-dependent cell death (pyroptosis).

hyaluronan²⁵, and islet amyloid polypeptide (also known as amylin)²⁶, whereas exogenous activators include asbestos and silica²⁷. The NLRP3 inflammasome activation by these DAMPs triggers inflammatory responses that may contribute to the pathophysiology of various diseases. Importantly, however, IL-1 β secretion requires another signal: pro-IL-1 β synthesis at the transcriptional level. This first signal is thought to be mediated by a TLR-nuclear factor- κ B (NF- κ B) pathway¹². Thus, a system comprising pro-IL-1 β synthesis and inflammasome-mediated IL-1 β processing is believed to be necessary for the tight regulation of this potent inflammatory cytokine secretion.

Hepatic I/R injury and NLRP3 inflammasomes

Tissue I/R injury is characterized not only by the generation of ROS but also by the release of inflammatory cytokines and chemokines that recruit circulating leukocytes, mainly neutrophils and macrophages, into the injured tissue. Hence, one of the hallmarks of I/R injury is excessive inflammatory responses. In particular, one prominent and early mediator for inflammatory responses in tissue I/R injury is IL-1 β ²⁸; this suggests that NLRP3 inflammasomes play a substantial role in tissue I/R injury. We have previously shown that, using mice deficient in ASC and caspase-1, I/R stimuli markedly induce inflammatory responses in

the heart and that systemic deletion of inflammasome components, such as ASC and caspase-1, significantly reduces inflammatory responses such as inflammatory cell infiltration and cytokine expression, as well as subsequent myocardial injuries such as infarct development and cardiac remodeling¹¹. Furthermore, we revealed that inflammasome activation occurs in cardiac fibroblasts, but not in cardiomyocytes, during myocardial I/R injury and proposed cardiac fibroblasts as sentinel cells that can sense DAMPs and trigger initial inflammatory responses¹¹.

With respect to hepatic I/R injury, Zhu et al.²⁹ reported that, using a small hairpin RNA interference approach, the NLRP3 signaling is involved in hepatic I/R injury and NLRP3 gene silencing attenuates hepatic I/R injury by reducing inflammatory cytokines, including IL-1 β and tumor necrosis factor- α (TNF- α), as well as high mobility group box 1 (HMGB1) release. Recently, Kamo et al.¹⁵ showed that NLRP3 inflammasome activation mediated by ASC leads to IL-1 β production and subsequently induces HMGB1 induction, which triggers TLR4/NF- κ B-driven inflammatory responses (Fig. 3). In their study, lower levels of inflammatory responses and hepatic I/R injury were shown by ASC-deficient mice. Huang et al.³⁰ also reported that the livers of mice deficient in NLRP3 and caspase-1 are protected from hepatic I/R injury and that extracellular histones activate the NLRP3 inflammasomes as DAMPs during hepatic I/R (Fig. 3). Using mice deficient in NLRP3, ASC, and caspase-1, we also examined the effect of I/R on inflammation and injury in the liver, and surprisingly we found that NLRP3-deficient mice are markedly protected from hepatic I/R injury, but this protective effect is not detected in ASC- and caspase-1-deficient mice¹⁶. Although the reason for the discrepancy between our study and that by Kamo et al.¹⁵ is not known, the differences between these two studies are the hepatic I/R protocol used and the extent of injury³¹. Compared with our study, it is likely that the I/R protocol adopted by Kamo et al. induced excessive inflammation and injury in the liver. These data suggest that the contribution of the NLRP3 inflammasomes depends on the severity of liver injury and the extent of inflammatory responses. On this issue, Kamo and Weglinski commented that this conclusion requires testing of mice “under one roof” by the same microsurgeon and under identical experimental conditions because even minor differences in room temperature may significantly affect liver damage in this model of hepatic I/R injury³². They also suggested the idea that different TLRs and inflammasome components operate

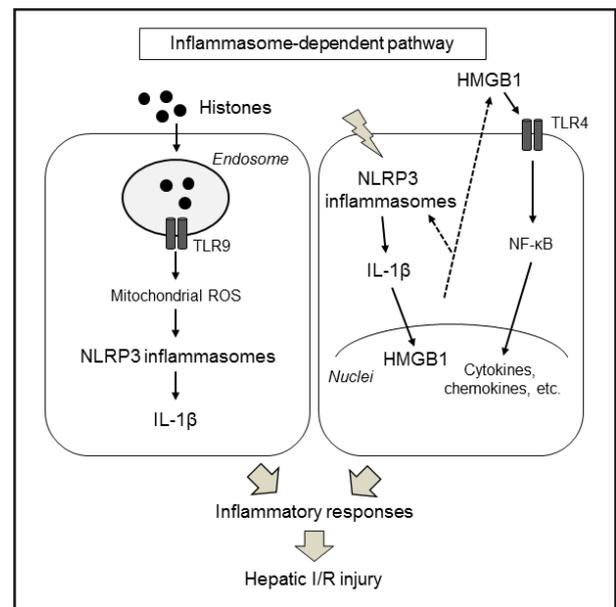


Fig.3 Role of NLRP3 inflammasomes in hepatic I/R injury

In the inflammasome-dependent pathway, extracellular histones and HMGB-1 may activate NLRP3 inflammasomes as DAMPs and induce inflammatory responses and subsequent I/R injury.

at distinct stages and in different cell types during hepatic I/R injury. More recently, although Kim et al.³³ reported that AIM2 inflammasomes might be involved in hepatic I/R injury, its role remains to be elucidated.

In addition to the role of NLRP3 inflammasomes, the role of IL-1 β in hepatic I/R injury is also controversial. Kato et al.³⁴ reported that no difference in liver injury was observed between wild-type and IL-1 receptor (IL-1R)-deficient mice after hepatic I/R injury, suggesting a limited role of IL-1 β in causing hepatic I/R injury. In contrast, Tan et al.³⁵ demonstrated that I/R upregulates hepatic IL-1 β and that hepatic I/R injury, liver inflammation, and neutrophil infiltration are attenuated in mice deficient in IL-1R1 or treated with the IL-1R antagonist Anakinra. Similarly, we observed that hepatic I/R injury is attenuated in IL-1 β -deficient mice¹⁶. Thus, further investigations are necessary to elucidate the precise role of NLRP3 inflammasomes and IL-1 β in the pathophysiology of hepatic I/R injury.

As a possible explanation for the mechanism by which NLRP3 deficiency attenuates hepatic I/R injury, we revealed a reduction in neutrophil infiltration, inflammatory cytokine expression, ROS production, and apoptotic cell death in I/R livers of NLRP3-deficient mice¹⁶. Furthermore, we verified that NLRP3-deficient neutrophils exhibited an impairment of chemokine-mediated signaling and functions, including

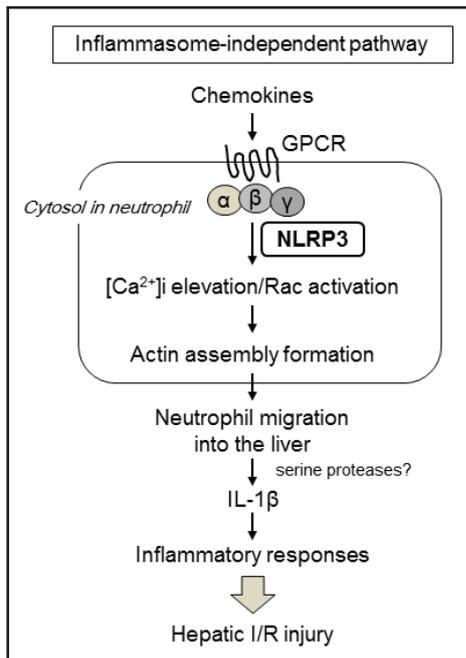


Fig.4 Inflammasome-independent role of NLRP3 in hepatic I/R injury

In the inflammasome-independent pathway, NLRP3 may regulate neutrophil functions, including activation of heterotrimeric G-proteins via GPCRs, $[Ca^{2+}]_i$ elevation, Rac activation, and actin assembly formation, and migration. IL-1 β processing by putative serine proteases derived from neutrophils may induce inflammatory responses and contribute to hepatic I/R injury.

activation of heterotrimeric G-proteins via G-protein-coupled receptors (GPCRs), $[Ca^{2+}]_i$ elevation, Rac activation, and actin assembly formation, and migration in response to chemokine CXCL1 (KC) (Fig. 4). It is generally accepted that neutrophils play a pivotal role in excessive inflammatory responses and resultant tissue injury during I/R in the liver³⁶. I/R stimuli trigger chemotactic signals (e.g., KC) from the parenchyma (e.g., hepatocytes), leading to the recruitment of neutrophils into the ischemic tissues. The recruited neutrophils are then activated, and they produce a large amount of ROS and inflammatory cytokines, which promote apoptotic cell death and tissue injury. Therefore, our findings on neutrophil function provide a molecular basis for the prevention of hepatic I/R injury observed in NLRP3-deficient mice.

The observation that hepatic I/R injury is reduced in mice deficient in NLRP3 and IL-1 β , but not in ASC and caspase-1, is rather puzzling. These data suggest that the

inflammasome-independent IL-1 β -driven inflammatory responses are important in hepatic I/R injury. In this regard, previous investigations reported that neutrophil-derived serine proteases, such as neutrophil elastase and proteinase 3, mainly induce IL-1 β processing and suggest a redundant or minor role of caspase-1 in neutrophil IL-1 β processing^{37, 38}. Guma et al.³⁹ reported that IL-1 β processing is observed in neutrophils isolated from caspase-1-deficient mice. Menzel et al.⁴⁰ observed no protective effects against liver injury and inflammatory responses during trauma and hemorrhagic shock in caspase-1-deficient mice. Taken together, the inflammasome-independent IL-1 β -driven inflammatory responses might be involved in hepatic I/R injury.

Previous studies suggest that NLRP3 inflammasomes are mainly activated in inflammatory cells such as macrophages and neutrophils. Our data on experiments with bone marrow transplantation indicate that NLRP3 not only in bone marrow-derived inflammatory cells but also in non-bone marrow-derived cells plays a role in hepatic I/R injury¹⁶. In this regard, Watanabe et al.⁴¹ reported that the inflammasome components, including NLRP3 and ASC are present in hepatic stellate cells and contribute to the progression of liver fibrosis. Consistent with this, we previously observed that inflammasome activation of cardiac fibroblasts plays an essential role in I/R injury and subsequent cardiac remodeling in the heart¹¹. Therefore, it is assumed that hepatic stellate cells participate in the development of hepatic I/R injury.

Another important issue to be discussed is the inflammasome-independent roles of inflammasome components. We observed that hepatic I/R injury is prevented in mice deficient only in NLRP3, but not in ASC and caspase-1¹⁶, suggesting an inflammasome-independent role of NLRP3 in hepatic I/R injury. In addition, we revealed that NLRP3 regulates neutrophil function without activation of NLRP3 inflammasomes. Consistent with our findings, several investigations suggest that each inflammasome-related molecule has a role independently of inflammasome activation. For example, Ippagunta et al.⁴² showed that ASC regulates the lymphocyte migration and antigen uptake by DCs via Dock2 expression and contributes to adaptive immune responses, independently of the inflammasomes. They further importantly noted that the loss of Dock2 expression was detected in several, but not all, ASC-deficient mouse lines⁴³. Therefore, caution should be taken when interpreting results from various different ASC-



deficient mice. Supporting our findings on hepatic I/R injury, Shigeoka et al.⁴⁴⁾ also showed that a reduction in renal I/R injury was observed in mice deficient in NLRP3, but not in those deficient in ASC or caspase-1, and suggested that an NLRP3-dependent, inflammasome-independent pathway contributes to the development of I/R injury in the kidney. At present, the inflammasome-independent role of inflammasome components is still not entirely clear and remains to be determined in the future.

Conclusion

Accumulating evidence suggests the essential role of NLRP3 inflammasomes in various sterile inflammatory diseases. Simultaneously, several investigations have recently reported a limited role of NLRP3 inflammasomes in such disease conditions. In hepatic I/R injury, there are somewhat inconsistent reports: NLRP3 inflammasome activation may mediate hepatic I/R injury and NLRP3 contributes to hepatic I/R injury independently of inflammasome activation (Fig. 3 and 4). In particular, as described in this review, an inflammasome-independent role of inflammasome components has attracted much attention. A better understanding of the role of inflammasomes and their component molecules will not only offer new therapeutic targets but also break new ground in the study of the role of inflammation in various types of sterile inflammatory diseases.

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Conflict of interests

None

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