NLRP3 Inflammasome as a Novel Player in Myocardial Infarction
Masafumi TAKAHASHI, MD

SUMMARY

Inflammasomes are multiple protein complexes that serve as molecular platforms to activate caspase-1 and regulate maturation of a potent proinflammatory cytokine, interleukin (IL)-1β, as well as proinflammatory cell death, pyroptosis. Although several types of inflammasomes have been reported so far, recent investigations indicate that the NLRP3 inflammasome recognizes non-microbial danger signals and leads to sterile inflammatory responses in various disease conditions. Sterile inflammatory responses are also implicated in the development of myocardial infarction (MI). In particular, IL-1β is an early and prominent mediator of inflammatory responses in MI, suggesting the pathophysiologic role of NLRP3 inflammasomes in MI. This review highlights the current state of knowledge regarding the role of NLRP3 inflammasomes in MI. (Int Heart J 2014; 55: 101-105)

Key words: Cytokine, Inflammation, Interleukin, Reperfusion

Myocardial infarction (MI) is a common disease condition and has been predicted to become the leading cause of death worldwide in the future. MI is defined as myocardial cell death due to prolonged ischemia. The major functional consequence of MI is a progressive deterioration of pump function in the left ventricle, which results in heart failure. In addition, MI frequently induces electrical instability and fatal arrhythmias. The primary underlying cause of MI is atherosclerosis of the coronary arteries, where atherosclerotic plaques become unstable, rupture, and facilitate thrombus formation, resulting in the occlusion of the coronary artery, thus leading to MI. In the case of acute MI, successful reperfusion and revascularization procedures such as thrombolytic/thrombolysis therapy and percutaneous coronary intervention can effectively decrease the infarct size in the myocardium and improve the clinical outcome. However, the reperfusion may paradoxically lead to exacerbated and accelerated injury in the myocardium, called myocardial ischemia-reperfusion (I/R) injury. Increasing evidence indicates that MI is accompanied by inflammatory responses that lead to leukocyte accumulation and subsequent myocardial damage, healing, and scar formation. Accumulated leukocytes, such as neutrophils and macrophages, at the site of MI, release inflammatory cytokines/chemokines and proteinases that may further exacerbate the inflammatory responses and promote myocardial damage and remodeling after MI.

Inflammation is defined as the process by which the body responds to an injury or infection and is triggered by components of the innate immune system, such as neutrophils and macrophages. Although the innate immune system has been recognized as the first line of defense against foreign pathogens, inflammatory responses also occur in the absence of infection. This type of inflammation is referred to as “sterile inflammation” and has received considerable attention. Recent evidence suggests that newly discovered innate immune pathways known as the “inflammasomes” functions as key players in the processes of sterile inflammation in diseases, such as gout, pseudogout, type 2 diabetes mellitus, metabolic syndrome, atherosclerosis, asbestosis, silicosis, and Alzheimer’s disease (see reviews4-7). Our group also recently demonstrated the importance of inflammasomes in neointimal formation after vascular injury and atherosclerosis. Furthermore, we have revealed that sterile inflammatory responses are mediated through inflammasomes in myocardial I/R injury. These findings suggest that inflammasomes are initial sensors for sterile inflammatory responses in the pathophysiology of MI. This review highlights the current state of knowledge regarding the role of inflammasomes in MI and myocardial I/R injury.

Innate immune system and pattern recognition receptors: Inflammation is initiated by the innate immune system, which consists of multiple families of germ-line encoded pattern-recognition receptors (PRRs). Increasing evidence indicates that the PRRs recognize conserved motifs associated with microbiological components, known as pathogen-associated molecular patterns (PAMPs), as well as endogenous danger signals, known as danger/damage-associated molecular patterns (DAMPs). In particular, inflammatory responses triggered by DAMPs have received considerable attention as the underlying mechanism in sterile inflammation. To date, PRRs are divided into at least 4 distinct families: Toll-like receptors (TLRs), retinoic acid-inducible gene-I-like receptors (RLRs), C-type lectin receptors

From the 1 Division of Inflammation Research, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Japan.
1 Address for correspondence: Masafumi Takahashi, MD, PhD Division of Inflammation Research Center for Molecular Medicine Jichi Medical University 3311-1 Yakushiji, Shimotsuke Tochigi 329-0498, Japan. E-mail: masafumi2@jichi.ac.jp
Received for publication December 31, 2013. Revised and accepted January 14, 2014.
Released advance online J-STAGE March 14, 2014.
All rights are reserved to the International Heart Journal Association.

101
danger signals and induce sterile inflammatory responses is the most extensively studied and has been shown to recognize putative ligands, leading to an assembly of the inflammasome. Common upstream mechanisms implicated in NLRP3 inflammasome activation include potassium efflux, lysosomal destabilization, and mitochondrial ROS generation. When activated, the PD of NLRP3 homotypically interacts with that of ASC, after which the CARD of ASC recruits and binds to caspase-1. These interactions finally form the NLRP3 inflammasome assembly that leads to caspase-1 activation. Because caspase-1 is known as an IL-1β-converting enzyme, its activation processes pro-IL-1β into its biologically active mature form; activated caspase-1 can also process pro-IL-18 into its mature form. In addition to the release of IL-1β, inflammasome activation induces caspase-1-dependent cell death, termed pyroptosis. Pyroptosis is a highly inflammatory form of cell death, characterized by both apoptosis (such as DNA fragmentation) and necrosis (such as cell swelling and rupture). Endogenous danger signals that activate the NLRP3 inflammasome include extracellular adenosine triphosphate (ATP), monosodium urate, calcium phosphate crystals, cholesterol crystals, amyloid-β, hyaluronan, and islet amyloid polypeptide (also known as amylin), whereas exogenous activators include asbestos and silica. The NLRP3 inflammasome activation by these danger signals induces IL-1β release and subsequent inflammatory responses that contribute to the development of various diseases. However, the induction of IL-1β release requires another signal, that is, the transcriptional induction of pro-IL-1β. Hence, a system comprising pro-IL-1β induction and inflammasome-mediated IL-1β processing is believed to be necessary for the tight regulation of this potent inflammatory cytokine.

**NLRP3 inflammasome and myocardial infarction (MI):** Inflammation is a key process involved in mediating myocardial damage and repair after MI. Previous investigations have demonstrated that interventions targeted for inflammatory responses improve MI. Among numerous inflammatory mediators involved, IL-1β is an early and prominent mediator for inflammatory responses in MI. Animal experiments showed that a neutralizing antibody against IL-1β and Anakinra, a recombinant IL-1 receptor antagonist (IL-1RA), exerted beneficial effects on acute MI. Furthermore, inflammatory responses can occur more aggressively in myocardial I/R compared with permanent MI. These findings allow us to assume that inflammasomes may play a substantial role in the pathophysiology of myocardial I/R injury. ASC is clearly expressed in human myocardial tissues of MI.

To further investigate the pathophysiological role of inflammasomes, we used ASC-knockout (KO) and caspase-1-KO mice and applied myocardial I/R, induced by occlusion of the left anterior descending artery, followed by reperfusion. Similar to the human MI tissue, ASC expression was detected primarily in infiltrated neutrophils and macrophages, but it was also expressed in vascular cells and cardiac resident fibroblasts in myocardial I/R. ASC-KO or caspase-1-KO mice exhibited a significant decline of inflammatory responses, such as inflammatory cell infiltration and cytokine/chemokine expression. These mice also showed a significant reduction of infarct size, myocardial fibrosis, and left ventricular dysfunction after MI. Bone marrow transplantation experiments unexpectedly showed that the reduction in infarct size that occurs after myocardial I/R in ASC-KO non-bone marrow cells is similar to that observed in ASC-KO bone marrow cells. This finding sug-
gests the importance of inflammasomes not only in bone marrow-derived inflammatory cells but also in resident myocardial cells such as cardiomyocytes and cardiac fibroblasts. This idea is further supported by the observations that myocardial damage after I/R can be detected in the early stages before inflammatory cell infiltration.\(^\text{10}\) Furthermore, in vitro experiments have determined that cardiac fibroblasts, but not cardiomyocytes, are responsible for inflammasome activation, even though ASC is expressed in both cell types.

Although the molecular mechanism of inflammasome activation in myocardial I/R injury has not been completely understood, findings show that its activation could be mediated through ROS and potassium efflux.\(^\text{10}\) This study clearly demonstrates that inflammasome activation of cardiac fibroblasts is essential for inflammatory responses and injury after myocardial I/R. This study also highlighted a novel role of cardiac fibroblasts; although cardiac fibroblasts comprise two-thirds of the cell population in the heart, they have not attracted much attention except in relation to processes associated with myocardial fibrosis and remodeling.\(^\text{30,31}\) It is now evident that cardiac fibroblasts have a crucial role not only in the myocardial fibrosis process but also in cardiac disease development due to the production of autocrine/paracrine factors (eg, cytokines). Thus, this study revealed that cardiac fibroblasts could act as "sentinel" cells that sense danger signals and interact with other cells such as cardiomyocytes, vascular cells, and inflammatory cells in a paracrine manner.

To induce the release of IL-1β from cardiac fibroblasts, myocardial I/R triggers two signals. The first signal provides the transcription of pro-IL-1β by the TLR-nuclear factor-κB (NF-κB) pathway, and the second signal provides processing of pro-IL-1β to its mature form by the inflammasome (Figure 2). The released IL-1β from cardiac fibroblasts induces the initial inflammatory response and the release of cytokines/chemokines, which recruit and activate inflammatory cells such as monocytes/macrophages and neutrophils to the ischemic myocardium. Activation of the infiltrated inflammatory cells enhances the inflammatory responses and myocardial injury. However, our study has not identified the PRRs responsible for myocardial I/R injury.

The NLRP3 inflammasome recognizes various danger signals and induces sterile inflammatory responses;\(^\text{46}\) therefore, it is a highly plausible candidate for PRR in MI. Supporting this assumption, using a murine model of permanent MI, Mezzaroma, et al\(^\text{32}\) reported that inhibition of NLRP3 (cryopyrin) and the P2X7 receptor by small interfering RNA or a pharmacological inhibitor prevented inflammasome activation and cardiac cell death, resulting in ameliorating myocardial remodeling after MI. P2X7, is the purinergic receptor channel that is activated by extracellular ATP released from injured cells, leading to potassium efflux and subsequent NLRP3 inflammasome activation.\(^\text{33}\) They also showed that cardiomyocytes formed NLRP3 inflammasome assembly and that its activation ultimately induced caspase-1-dependent cardiomyocyte cell death, known as pyroptosis, but not IL-1β release (Figure 3). The pathophysiologic role of the NLRP3 inflammasome was also confirmed by Sandanger, et al\(^\text{35}\) who reported that the NLRP3 inflammasome was predominantly upregulated in the cardiac fibroblasts of the ischemic myocardium in murine and rat permanent MI models. They further showed that cardiac fibroblasts secreted IL-1β in response to ATP, which is released extracellularly by tissue damage during MI. In addition, NLRP3-KO mice preserved myocardial function and reduced infarct size after myocardial I/R in ex vivo Langendorff-perfused murine hearts. Taken together, it is likely that cardiomyocytes and cardiac fibroblasts function in different roles, which ultimately contribute to cardiac inflammation and remodeling in MI (Figure 3).

The investigations on the NLRP3 inflammasome in MI and myocardial I/R injury raise interesting unsolved questions.

---

Figure 2. Role of NLRP3 inflammasome in myocardial I/R and MI. Myocardial I/R and/or MI activate the NLRP3 inflammasome through ROS generation and/or potassium efflux. In this process, extracellular ATP and/or other danger signals may be involved. Activation of the NLRP3 inflammasome induces IL-1β release and pyroptosis from cardiac fibroblasts and cardiomyocytes, respectively, resulting in cardiac inflammation and remodeling.

Figure 3. Role of TLR and inflammasomes in IL-1β release during myocardial I/R injury. To induce IL-1β release from cardiac fibroblasts after myocardial I/R, two signals are required. The first signal (signal 1) may initially be provided by the TLR-NF-κB pathway, and it induces pro-IL-1β synthesis in the cytosol. The second signal (signal 2) is the NLRP3 inflammasome pathway, which processes pro-IL-1β to its biologically active mature form, leading to IL-1β release and inflammatory responses.
In the study by Sandanger, et al., myocardial dysfunction and injury in response to I/R was significantly improved in ex vivo Langendorff-perfused hearts isolated from NLRP3-KO mice compared with hearts isolated from wild-type and ASC-KO mice, suggesting different roles of NLRP3 and ASC in terms of myocardial damage after I/R. Similarly, Shigeoka, et al. showed that renal I/R injury was reduced in NLRP3-KO mice, but not in ASC-KO mice, and concluded that NLRP3 contributes to the development of renal I/R injury independent of the inflammasome. We have also recently observed that hepatic I/R injury was significantly ameliorated in NLRP3-KO mice, but not in ASC-KO mice. These findings indicate that the inflammasome components, such as NLRP3 and ASC, can function independently of the inflammasome and that NLRP3 and ASC may have cell-intrinsic roles in different cell types such as cardiac fibroblasts, cardiomyocytes, and leukocytes. Thus, the contribution of NLRP3 and ASC should be determined in genetically modified animals in a tissue-specific manner. Furthermore, although NLRP3 participates in one inflammasome (NLRP3 inflammasome), ASC can participate in other inflammasomes (eg, NLRP3, NLRP1, NLRP6, and Aim2 inflammasomes), suggesting the possibility of functional redundancy in other inflammasomes.

There are several issues to be discussed. First, discrepancy in the findings in two studies using a similar ex vivo Langendorff-perfused heart model should be noted. Inconsistent with the report by Sandanger, et al., Zaubier, et al. showed no improvement in either myocardial function or cell death in response to I/R in Langendorff-perfused hearts isolated from NLRP3-KO mice, and pointed out that the ex vivo model of Sandanger, et al. was suboptimal in terms of physiological performance. Taken together, the contribution of the inflammasome may vary depending on the experimental model and conditions, such as the extent of stress and status of inflammatory responses. Second, because inflammation also plays a critical role in the process of cardiac healing after MI, inhibition of inflammation may influence cardiac rupture and aneurysm formation. An initial study by Hwang, et al. suggested that neutralization of IL-1β reduced collagen accumulation in the heart after experimental MI and increased the occurrence of cardiac rupture. However, impaired cardiac healing and increased rupture after MI were not observed in the studies using genetic deletion of IL-1 receptor or pharmacological blockades (Anakinra or IL-1 trap [rilonacept]). In addition, recent investigations using IL-1β antibodies specifically developed for in vivo use also showed a significant inhibition of cardiac enlargement and dysfunction after MI. At present, the discrepancy between the study by Hwang, et al. and other studies is likely related to differences in the nature of the neutralizing antibody used. In terms of the inflammasome, there is no report describing the relationship between the increased risk of cardiac rupture or aneurysm and genetic deletion of the inflammasome components so far.

Closing remarks: A growing body of evidence indicates that the NLRP3 inflammasome-driven inflammatory responses contribute to the pathophysiology of MI. The investigations also suggest that targeting the NLRP3 inflammasome or IL-1β may be a potential and effective therapeutic strategy to treat MI. Unfortunately, compounds that can specifically inhibit NLRP3 inflammasome activation are currently unavailable, although several types of IL-1 blockades are available: Anakinra (Kineret™), a recombinant IL-1RA; rilonacept (Arcalyst™), a cytokine trap; canakinumab (Irais™), a humanized monoclonal anti-IL-1β antibody; and gevokizumab (XOMA052), a humanized monoclonal anti-IL-1β antibody. A recent literature review revealed that several human clinical trials using IL-1 blockades have been conducted in patients with MI (MRC-ILA-HEART study, VCU-ART study, and CANTOS study). However, inflammatory responses in MI have been shown to be both detrimental and beneficial in the process of myocardial damage and remodeling. In addition, because the function of the NLRP3 inflammasome may be different in various cell types and tissues, conditional gene-modified mice will have to be developed. Thus, further investigations are necessary to understand the precise role of the NLRP3 inflammasome and its therapeutic potential in MI. A better understanding of the mechanism that underlies sterile inflammation and the development of specific inhibitors will not only offer new therapeutic modalities but also break new ground for studying the pathophysiological role of inflammation in MI.

References

17. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflam-


