Inflammation and Tissue Remodeling as Potential Therapeutic Targets

Review

Role of NLRP3 Inflammasome in Cardiac Inflammation and Remodeling after Myocardial Infarction

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An accumulating body of evidence indicates that inflammation plays a crucial role in the pathophysiology of myocardial infarction (MI). Nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome is an intracellular multiprotein complex that regulates caspase-1 activation and the subsequent processing of the potent inflammatory cytokine interleukin (IL)-1β as well as triggering inflammatory cell death pyroptosis. We and other investigators demonstrated that deficiency of the NLRP3 inflammasome components reduces inflammation and improves cardiac dysfunction and remodeling in rodent models of MI. Therefore, the regulation of NLRP3 inflammasome has been regarded as a potential therapeutic target for MI. Furthermore, a recent Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial revealed the efficacy of IL-1β inhibition in preventing recurrent cardiovascular events in patients with MI. This review focuses on the role of NLRP3 inflammasome in the process of cardiac inflammation and remodeling after MI, and discusses its potential as a therapeutic target for the prevention and treatment of MI.

Key words: cardiomycocyte; cytokine; interleukin-1; macrophage; fibroblast

1. INTRODUCTION

Myocardial infarction (MI) is serious disease condition in the developed world, and is projected to be the future leading cause of death worldwide.1 MI is accompanied by inflammatory responses, such as the infiltration of inflammatory cells and the release of inflammatory cytokines/chemokines. Because these inflammatory responses lead to myocardial damage and remodeling, which in turn influence a patient’s conditions and mortality, inflammation is regarded to be a potential therapeutic target. In the hospital, acute MI patients are usually treated with thrombolytic/fibrinolytic therapy or percutaneous coronary intervention (PCI) to restore the disrupted coronary blood flow. Indeed, the myocardial reperfusion therapy has been proven effective for reducing the size of the infarcted myocardium and improving the clinical outcome in acute MI patients; however, it has also been shown to potentiate myocardial damage, known as myocardial “isch-eemia–reperfusion (I/R) injury,” and paradoxically decreases the beneficial effects of the reperfusion therapy.2 Furthermore, myocardial I/R also enhances inflammatory responses, which contribute to further development of the damage and remodeling. Although certain levels of inflammation are prerequisite for inducing myocardial healing and appropriate remodeling after MI, excessive inflammation enhances myocardial damage and inappropriate remodeling. Thus, a better understanding of the underlying mechanisms of the inflammation in MI will be important to the process of identifying new therapeutic strategies for the treatment of MI.

Inflammation in MI occurs in the absence of infectious agents, and has therefore been termed “sterile inflammation.”3,4) Similar to pathogen-induced inflammation, the hallmarks of sterile inflammation are the infiltration of inflammatory/innate immune cells, such as macrophages and neutrophils, and the release of inflammatory cytokines and chemokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Recently, sterile inflammation has received considerable attention because it has been shown to be involved in various types of diseases, and to contribute to the disease pathogenesis.3,5) Furthermore, recent evidence indicates that several types of sterile inflammation are mediated through an intracellular large multi-protein complex, known as a nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome.3,4) We have recently demonstrated that the NLRP3 inflammasome is a critical mediator in sterile inflammation and the development of cardiovascular diseases, including neointimal formation after vascular injury, atherosclerosis, abdominal aortic aneurysm, and myocardial I/R injury.6–9) This review focuses on the role of NLRP3 inflammasome in cardiac inflammation and remodeling after MI, and discusses its potential as a therapeutic target for MI.

2. NLRP3 INFLAMMASOME

Inflammasome is a cytosolic large multiprotein complex that serves as a molecular platform to activate the cysteine protease caspase-1.3,10) The inflammasome is formed and activated in response to various danger signals, and is involved in both host defense and sterile inflammation. There have been many reported danger signals that are generally classified into pathogen-associated molecular patterns (PAMPs)
derived during infection, as well as danger/damage-associated molecular patterns (DAMPs) derived during cellular stress and damage. The PAMPs and DAMPs are recognized by pattern recognition receptors (PRRs) expressed in mainly innate immune cells, including monocytes/macrophages, neutrophils, and dendritic cells. Although several types of inflammasome complexes have been described so far, NLRP3 inflammasome is most extensively studied and known to be activated in response to DAMPs, indicating that NLRP3 inflammasome is a key mediator for the development of sterile inflammation. Indeed, we and other research groups have demonstrated that NLRP3 inflammasome is involved in a wide variety of diseases, such as gout, type 2 diabetes mellitus, Alzheimer’s disease, and cardiovascular and renal diseases.5,11)

NLRP3 inflammasome is composed of NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and the cysteine protease pro-caspase-1 (Fig. 1). NLRP3 contains leucine-rich repeats (LRRs), NACHT domain, and pyrin domain (PYD). ASC contains PYD and caspase recruitment domain (CARD). Pro-caspase-1 contains CARD and catalytic domains (p10 and p20). The LRRs of NLRP3 are thought to sense putative ligands, leading to the oligomerization of the NACHT domain and the triggering the formation of NLRP3 inflammasome. Then, the PYD of NLRP3 interacts with the PYD of ASC, after which the CARD of ASC recruits and binds to the CARD of pro-caspase-1. These interactions finally form the NLRP3 inflammasome that triggers self-cleavage of pro-caspase-1 into active caspase-1.

Because caspase-1 is known to be an IL-1β converting enzyme, NLRP3 inflammasome-driven caspase-1 activation induces the processing of pro-IL-1β into its mature form, and the release of this potent inflammatory cytokine IL-1β causes tissue inflammation and damage (Fig. 2). Similar to IL-1β processing, activated caspase-1 also induces the maturation of another IL-1 family cytokine IL-18. Furthermore, activation of caspase-1 also triggers a process of inflammation-related cell death, termed as “pyroptosis.” Pyroptosis is characterized by pore formation in the plasma membrane, cell swelling, and membrane rapture, with consequent release of the intracellular content. Recently, gasdermin D (GSDMD) has been identified as a responsible mediator for pyroptosis.12–14) Caspase-1 enzymatically cleaves GSDMD, and its cleaved N-terminus forms pores on the cell membrane, resulting in pyroptosis. Although IL-1β has no signal sequence for exocytosis and the

Fig. 1. Structure of NLRP3 Inflammasome Components and Their Assembly

NLRP3 inflammasome is composed of NLRP3, ASC, and pro-caspase-1. NLRP3 contains LRRs, NACHT domain, and PYD. ASC contains PYD and CARD. Pro-caspase-1 contains CARD and catalytic domains (p10 and p20). The LRRs of NLRP3 are thought to sense putative ligands, and lead to the oligomerization of the NACHT domain and the triggering of the formation of NLRP3 inflammasome. Then, PYD of NLRP3 interacts with the PYD of ASC, after which the CARD of ASC recruits and binds to the CARD of pro-caspase-1. These interactions finally form the NLRP3 inflammasome that triggers self-cleavage of pro-caspase-1 into active caspase-1.

Fig. 2. Mechanisms of NLRP3 Inflammasome-Driven IL-1β Release and Pyroptosis

A two-signal model is proposed for NLRP3 inflammasome-driven IL-1β release. The first/priming signal provides the expression of pro-IL-1β and NLRP3 by TLR or cytokine receptor-mediated NF-κB signaling (signal 1). The priming signal also licenses NLRP3 expression by its transcriptional induction or de-ubiquitination mechanism. The second signal provides NLRP3 inflammasome activation, which induces the processing of pro-IL-1β into its mature form (signal 2). Furthermore, NLRP3 inflammasome activation cleaves GSDMD and its cleaved N-terminus forms pores on the cell membrane, resulting in pyroptosis. The common upstream pathways for NLRP3 inflammasome activation include K+ efflux, generation of mitochondrial ROS, and lysosomal rupture-induced cathepsin release, and these pathways induce the interaction of Nek7 with NLRP3.
3. NLRP3 INFLAMMASOME IN MI

3.1. Role of NLRP3 Inflammasome in MI

Numerous inflammatory mediators have been shown to be involved in the development of MI, and IL-1β is one of the most prominent mediators of inflammation in MI. Indeed, experimental studies using animal models of myocardial I/R injury and non-perfused MI suggest the importance of IL-1β in cardiac inflammation and remodeling after MI. These findings prompted us to investigate the role of NLRP3 inflammasome in MI. In 2011, using a mouse model of myocardial I/R injury, we revealed that NLRP3 inflammasome mediates sterile inflammation in MI according to the following observations: 1) ASC was clearly expressed at the mouse I/R myocardium and the human MI tissue of autopsy cases; 2) ASC and caspase-1 deficiency reduced inflammatory responses such as inflammatory cell infiltration and cytokine expression, and subsequent injuries such as infarct development, myocardial fibrosis, and dysfunction in a mouse model of myocardial I/R injury. At that time, we could not determine whether NLRP3 is responsible for this inflammasome activation because NLRP3-deficient mice were unavailable in our laboratory. However, this was the first report describing the role of the inflammasome in cardiac inflammation and remodeling after MI. Shortly thereafter, Mezzaroma et al. examined the role of NLRP3 using a mouse model of non-reperfused MI, and showed that the inhibition of NLRP3 (cryopyrin) by small interfering RNA prevented inflammasome activation and cardiac cell death, resulting in ameliorating myocardial remodeling after MI. These two reports clearly demonstrate that NLRP3 inflammasome plays an important role in the development of MI.

3.2. Distinctive Role of Cardiac Fibroblasts and Cardiomyocytes

Inflammatory cells, especially macrophages, express the components of NLRP3 inflammasomes and are the main cellular source of NLRP3 inflammasome-driven IL-1β. Indeed, macrophages can produce a large amount of IL-1β in response to NLRP3 inflammasome activators, such as ATP and nigericin. In a mouse myocardial I/R injury model, however, bone marrow transplantation experiments unexpectedly showed that NLRP3 inflammasome, not only in bone marrow-derived inflammatory cells but also in cardiac resident cells, contributes to the development of MI. Furthermore, myocardial damage by I/R is observed prior to the infiltration of inflammatory cells. These findings suggest that...
cardiac resident cells, but not infiltrated inflammatory cells, are important at the early stage of myocardial I/R injury. To investigate the role of the cardiac resident cells, we cultured murine neonatal cardiomyocytes and cardiac fibroblasts in vitro and showed that the production of IL-1β in response to NLRP3 inflammasome activators is detected only in cardiac fibroblasts, but not in cardiomyocytes, although NLRP3 inflammasome components are expressed in both cell types.

The mammalian heart is composed of many types of cells, the most abundant being cardiomyocytes, cardiac fibroblasts, and vascular cells. Cardiac fibroblasts comprise more than half of the cells in the heart and are responsible for the processes associated with myocardial fibrosis and remodeling. In addition to these roles, our data suggest that cardiac fibroblasts can sense DAMPs and produce IL-1β by NLRP3 inflammasome activation. Consistently, Sandanger et al. reported that cardiac fibroblasts are responsible for NLRP3 inflammasome-driven IL-1β production in MI. In contrast, the activation of NLRP3 inflammasome in cardiomyocytes leads to caspase-1-dependent cell death, pyroptosis, but not IL-1β release. Therefore, NLRP3 inflammasome activation drives IL-1β release in cardiac fibroblasts and pyroptosis in cardiomyocytes, which promotes cardiac inflammation and remodeling in MI. Vascular cells, especially endothelial cells also have the NLRP3 inflammasome components and produce IL-1β in response to DAMPs; however, their role in MI remains to be determined. Taken together, the role of NLRP3 inflammasome is cell-type-specific in the pathophysiology of MI.

3.3. Mechanisms of NLRP3 Inflammasome Activation during MI Although the molecular mechanism underlying NLRP3 inflammasome activation in MI has not been completely understood, several common pathways, as described above, seem to be involved. We previously showed that hypoxia/reoxygenation stimuli activates NLRP3 inflammasome by ROS generation and K+ efflux in cardiac fibroblasts. Extracellular ATP is known as a DAMP and induces K+ efflux via the P2X7 purinergic receptor, leading to the activation of NLRP3 inflammasome. Given the fact that extracellular ATP stimulates NLRP3 inflammasome activation in cardiomyocytes and cardiac fibroblasts in vitro, extracellular ATP released from injured tissues of MI acts as DAMPs and induces K+ efflux-mediated NLRP3 inflammasome activation in MI. However, the precise mechanisms underlying NLRP3 inflammasome activation in MI remain to be elucidated.

3.4. Role of NLRP3 Inflammasome-Driven IL-18 IL-18 production is also regulated by the NLRP3 inflammasome. Our previous report showed that IL-18 is produced during myocardial I/R and this production is suppressed by ASC deficiency, suggesting the role of NLRP3 inflammasome-driven IL-18 in MI. Indeed, Venkatachalam et al. reported that treatment with a neutralizing antibody against IL-18 ameliorates myocardial I/R injury. However, at present, the precise role of NLRP3 inflammasome-driven IL-18 in MI has not been largely understood.

4. NLRP3 INFLAMMASOME AS A THERAPEUTIC TARGET FOR MI NLRP3 inflammasome and IL-1β are potential therapeutic targets for MI. Accordingly, a number of compounds and factors have been reported to inhibit the activation of NLRP3 inflammasome. Some of these are also shown to exert the favorable effects in animal models of MI. Unfortunately, however, no selective NLRP3 inhibitor is clinically available at present. Instead, anakinra, a recombinant IL-1 receptor antagonist, and canakinumab, a humanized monoclonal anti-IL-1β antibody, are being explored in clinical trials for patients with MI. Several clinical trials using anakinra have been reported for patients with MI, but these trials were conducted in a relatively small number of patients, and the results are controversial (VCU-ART and VCU-ART2; MRC-ILA-Heart Study). A recent review of the literature nicely described recent clinical trials on IL-1 inhibition in MI patients.

In 2017, the results of a phase III Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) trial were reported. Canakinumab was approved for treating cryopyrin-associated periodic syndrome (CAPS), a gain-of-function mutations in NLRP3 gene encoding cryopyrin, and other inflammatory disorders, including refractory familial Mediterranean fever (FMF), TNF receptor-associated periodic syndrome (TRAPS), and hyper immunoglobulin D (IgD) syndrome (HIDS) in Japan. In the CANTOS trial, 10,061 patients with previous MI and elevated levels of high sensitive C-reactive protein (hsCRP) (≥2 mg/L) were enrolled and randomized to receive canakinumab (50, 150, or 300 mg, every 3-months subcutaneous injection) or a placebo, and followed up studies on these patients were conducted for a median of 3.7 years. The result of this trial revealed that IL-1β inhibition with canakinumab (150-mg group) results in a significantly lower incidence of recurrent cardiovascular events than in those patients given the placebo, independent of lipid-lowering. However, this significant benefit was not observed in the 50- and 300-mg group. On the other hand, there was no significant difference in all-cause mortality between the canakinumab and placebo groups. The results of this CANTOS trial provided the evidence that therapies targeting NLRP3 inflammasome and IL-1β may effective in MI; however, because of the expensive cost and the ongoing need to administer the drug, it is unlikely that canakinumab will be widely used for the prevention of recurrent MI. Therefore, we need to develop other less expensive and more widely available therapies to block the NLRP3 inflammasome/IL-1β pathway for the prevention and treatment of MI. One candidate is colchicine, which is used for the treatment of gout and FMF. Colchicine has recently been shown to inhibit NLRP3 inflammasome assembly by preventing microtubule-mediated shutting of mitochondrial-associated ASC to NLRP3 in the endoplasmic reticulum. Although recent experimental and clinical studies have suggested the potential efficacy of colchicine in MI further large scale trials are needed to confirm this efficacy.

5. CONCLUSION

Increasing evidence indicates that inflammation is a major contributing factor to the development of MI. We and other investigators have demonstrated that NLRP3 inflammasome-driven IL-1β is a key mediator of sterile inflammation in MI. Furthermore, a recent CANTOS trial showed the efficacy of IL-1β inhibition in the secondary prevention of cardiovascular events after previous MI patients. These findings indicate
that the therapies targeting NLRP3 inflammasome may be an effective strategy for the prevention and treatment of MI. However, inflammation in MI has been shown to be both detrimental and beneficial in the process of myocardial injury and remodeling. Thus, further investigations are necessary to elucidate the exact role of NLRP3 inflammasome in its therapeutic potential in MI. A better understanding of the mechanism underlying inflammation and the development of specific NLRP3 inflammasome inhibitors will break new ground for research on sterile inflammation and offer new therapeutic options for the treatment of MI.

Conflict of Interest  The author declares no conflict of interest.

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