Mini Review

Role of the inflammasome in vascular injury and atherosclerosis

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A growing body of evidence indicates that inflammation plays a key role in the pathogenesis of vascular injury and atherosclerosis. However, it is still unclear how inflammatory responses are trigged by vascular injury and atherosclerosis in the vascular walls. The inflammasome is a large multiprotein complex that is formed in the cytosol in response to danger signals; it drives the proinflammatory cytokine interleukin (IL)-1β, which is a key mediator in the disease process of sterile inflammation. Since IL-1β is an early and prominent player in vascular injury and atherosclerosis, the inflammasome is one of the best potential candidates for the initial mediator in this vascular disease. Here, this review briefly describes the role of the inflammasome in the pathogenesis of vascular injury and atherosclerosis and discuss the potential of the inflammasome as a therapeutic target.

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Introduction

The vascular system in the body plays a critical role not only in the regulation of tissue homeostasis by distributing nutrients and oxygen but also in the development process of variety of diseases. The main disorder of the vascular system is atherosclerosis. In fact, ischemic heart disease due to the development of atherosclerosis is a leading cause of death in Japan and other western countries. Although the precise mechanism of atherosclerosis is not fully understood, vascular injury resulting from various pathological stimuli, such as mechanical force (shear stress), oxidized lipids and infectious agents, is thought to be the initial event that allows diffusion of lipids and inflammatory cells into the vascular walls, ultimately inducing progression of atherosclerosis[1]. In particular, more extensive vascular injury, such as the loss of endothelial cells leads to migration of medial vascular smooth muscle cells into the intima and their proliferation, resulting in neointimal formation. This resultant neointimal formation is the pathological basis of restenosis that occurs after revascularization procedures such as percutaneous coronary intervention (PCI). Therefore, vascular injury is believed to be a common initiating event in the development of atherosclerosis and restenosis after PCI. A better understanding of the molecular basis of vascular injury and atherosclerosis is an important issue that should be investigated.

Atherosclerosis is a slowly progressing chronic disease characterized by lipid retention and inflammation in the vascular walls. Atherosclerotic plaques are infiltrated by inflammatory cells, mainly macrophages, and the number of these cells is linked to disease severity. Inflammatory cytokines and chemokines are involved in all stages of the atherosclerosis process[2]. The inflammatory nature of atherosclerosis is evidenced by the association between cardiovascular events and serum levels of inflammatory markers, particularly C-reactive protein[3]. Similar to the atherosclerotic process, inflammation plays an important role in the process of restenosis after PCI. These findings indicate that inflammation is causally linked to vascular injury and atherosclerosis. However, it is not known how stimuli can trigger inflammatory responses in the vascular walls.

Recently, a newly discovered innate immunity pathway known as the "inflammasome" has attracted attention because it has been shown to be a key player in sterile inflammatory diseases such as gout, pseudogout, asbestosis, silicosis, Alzheimer's disease, atherosclerosis, and type 2 diabetes mellitus (T2DM; see review[4-6]). Our group recently found that the inflammasome-related adaptor molecule ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) is clearly upregulated at sites of vascular injury. We showed that ASC deficiency reduces infiltration of inflammatory cells and expression of inflammatory cytokines after vascular injury, thereby attenuating neointimal formation[7]. More recent studies suggest that cholesterol crystals can activate the inflammasome in macrophages and promote the development of atherosclerosis[8, 9]. Collectively, these findings suggest that the inflammasome acts as an initial sensor for sterile inflammation in the vascular walls. This review will focus on the role of the inflammasome in the pathogenesis of vascular injury and atherosclerosis and discuss the potential of the inflammasome as a therapeutic target.

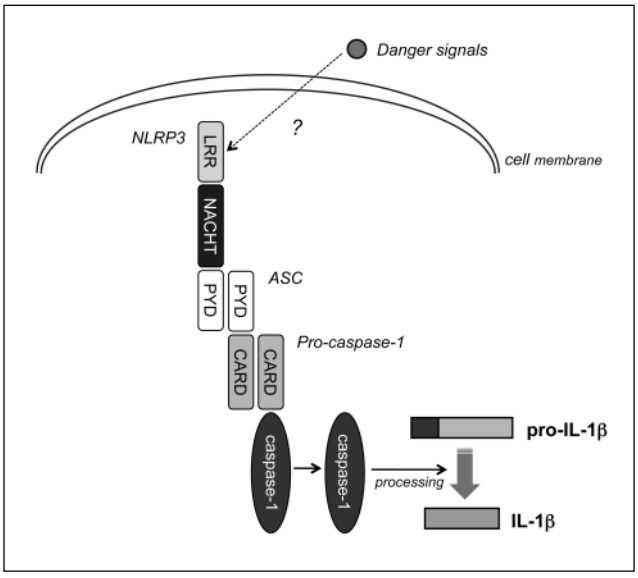

Fig.1 Model of NLRP3 inflammasome activation

The NLRP3 inflammasome is a prototype inflammasome. NLRP3 is composed of 3 domains: C-terminal leucine-rich repeats (LRRs); a central nucleotide domain termed the NACHT domain; and an N-terminal effector domain (pyrin domain [PYD]). ASC contains an N-terminal PYD and a C-terminal caspase recruitment domain (CARD). Pro-caspase-1 consists of a CARD followed by a caspase domain. The LRRs of NLRP3 are thought to sense putative ligands, leading to inflammasome assembly. After the LRRs of NLRP3 may recognize danger signals, the PYD of NLRP3 homotypically interacts with the PYD of ASC, and then the CARD of ASC recruits and binds pro-caspase-1. These interactions finally form the active NLRP3 inflammasome and activate caspase-1, which is essential for maturation and secretion of IL-1β and IL-18.

The inflammasome

1)The innate immune system

The innate immune system consists of multiple families of germ-line encoded pattern-recognition receptors (PRRs) that recognize microbial as well as non-microbial insults. The PRRs of the innate immune system can be divided into at least 4 distinct families: Toll-like receptors (TLRs), retinoic acid-inducible gene-I-like receptors (RLRs), C-type lectin receptors (CLRs), and the nucleotide-binding domain leucine-rich repeat containing receptors (NLRs; also known as Nod-like receptors)[4-6]. These receptors recognize conserved moieties associated with cellular damage or invading organisms. Activation of these receptors ultimately leads to the production of inflammatory cytokines, which drive the inflammatory response. NLR family proteins mainly participate in the formation of the inflammasome.

2)The inflammasome

The inflammasome is a large multiprotein complex formed in response to danger signals in the cytosol. The inflammasome contains NLRs associated with ASC, which recruits caspase-1 and induces its activation (Fig 1). Caspase-1 is an interleukin (IL)-1β-converting enzyme (ICE); it processes pro-IL-1β into mature IL-1β. In addition, activated caspase-1 can process pro-IL-18 and pro-IL-33 into mature-IL-18 and IL-33, respectively. Importantly,

recent evidences indicates that the inflammasome mediates a number of sterile inflammatory responses triggered by danger signals and tissue damage[4].

To date, 4 types of inflammasome complexes have been reported, each of which is named according to the specific PRR it contains: NALP1 inflammasome, NLRP3 (NALP3) inflammasome, NLRC4 (IPAF) inflammasome, and absence in melanoma 2 (AIM2) inflammasome[5, 6]. Of these, the NLRP3 inflammasome is the most studied. The NLRP3 inflammasome can recognize non-microbial danger signals leading to sterile inflammation in various types of the diseases. For instance, endogenous NLRP3 activators include ATP[10], monosodium urate (MSU)[11], calcium pyrophosphate dihydrate (CPPD), cholesterol crystals[8, 9], amyloid-β[12], hyaluronan[13], and possibly glucose[14], whereas exogenous activators include asbestos and silica[15]. The NLRP3 inflammasome leads to production of IL-1β, a potent proinflammatory cytokine, and induces inflammatory responses. Recently, AIM2, which is not structurally related to the NLR family, was shown to recognize cytoplasmic double-stranded DNA (dsDNA) and to form the AIM2 inflammasome[16-18]. Therefore, the AIM2 inflammasome may also play a role in sterile inflammation in response to cell death. However, whether AIM2 can recognize other sterile danger signals is not known.

The inflammasome in vascular injury

A growing body of evidence indicates that inflammatory cytokines and chemokines play causal roles in neointimal formation after vascular injury[19]. Of these, IL-1β is a prominent and early mediator of neointimal formation after vascular injury. We therefore hypothesized that the inflammasome mediates early inflammatory responses after vascular injury and acts as an initial sensor for sterile inflammation in the vascular walls. Using a wire-mediated vascular injury model, we showed that ASC is upregulated at the site of vascular injury and that ASC deficiency reduces IL-1β expression and neointimal formation after injury. Furthermore, the absence of ASC specifically in bone marrow cells attenuates neointimal formation. Our data suggest that inflammasome activation in bone marrow-derived cells is essential for the development of neointimal formation after vascular injury. Although our studies have not identified the initial sensor(s) of vascular injury, recent investigations suggest that NLRP3 can act as a PRR for non-microbial danger signals, leading to inflammasome formation and sterile inflammatory responses. Further investigations are necessary to elucidate the precise mechanism of inflammasome activation after vascular injury.

Currently, 3 types of mechanical injury models in mice are widely used to examine vascular remodeling after vascular injury: wire-mediated endovascular injury, perivascular cuff-replacement, and flow-restriction by ligation[20]. Of these, wire-mediated endovascular injury causes loss of endothelium and disrupts the internal elastic lamina, resulting in robust neointimal formation[21-23]. Since the endothelium is not denuded in other vascular injury models (perivascular cuff-replacement and flow-restriction by ligation), we believe that the wire-mediated vascular injury most closely mimics revascularization procedures and is most suitable to investigate restenosis process after PCI. Therefore, to investigate the role of the inflammasome as described above, we used a wire-mediated vascular injury model.

Interestingly, the contribution of bone marrow-derived cells to neointimal formation markedly differs among wire-mediated vascular injury models and other models[24]. These data suggest that the model of injury might influence the contribution of the inflammasome to vascular injury. Thus, the role of the inflammasome must be examined in other vascular injury models.

The inflammasome in atherosclerosis

Many clinical and experimental studies have shown the importance of IL-1β in the pathogenesis of atherosclerosis and demonstrated that IL-1β acts as a pro-atherogenic cytokine[2, 25]. These studies suggest that the inflammasome is involved in the development of atherosclerosis. Recently, Duewell and colleagues[9] demonstrated that cholesterol crystals activate the NLRP3 inflammasome; this results in cleavage and secretion of IL-1β in mouse macrophages *in vitro*. The authors showed that lysosomal damage- and cathepsin-mediated IL-1β activation. To investigate the contribution of bone marrow-derived cells (e.g., macrophages), the authors produced several types of bone marrow transplanted mice (LDL receptor-deficient mice). Bone marrow in these mice was transplanted with NLRP3-, ASC-, or IL-1α/β-deficient bone marrow cells. The absence of these inflammasome-related molecules specifically in bone marrow cells reduced the plaque size of atherosclerosis. Similarly, Rajamaki et al.[8] reported that the NLRP3 inflammasome is activated by cholesterol crystals through

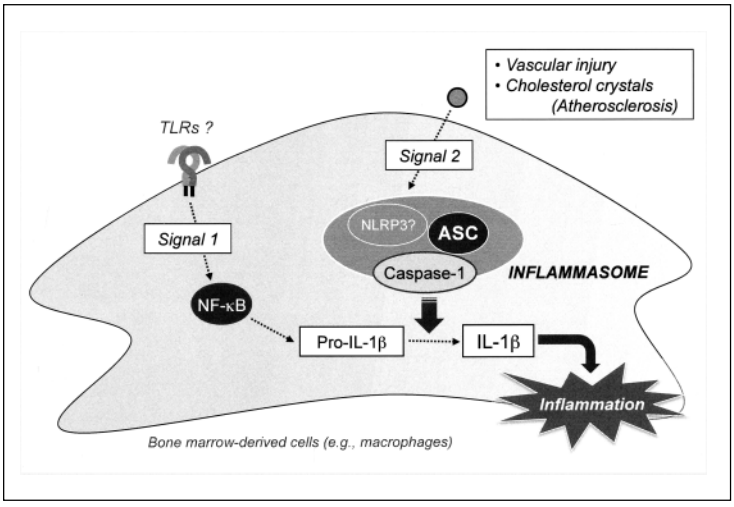

Fig.2 Proposed mechanisms of inflammasome activation in vascular injury and atherosclerosis

Vascular injury and cholesterol crystals induce inflammasome activation in bone marrow-derived cells (e.g., macrophages). Its activation stimulates processing of pro-IL-1β to mature IL-1β, resulting in inflammation. In addition to this inflammasome signal (signal 2), another signal is needed to induce pro-IL-1β. The first signal (signal 1) might initiate with TLRs and subsequently transmit through the NF-κB pathway. The system comprising these 2 signals is believed to be necessary for tight regulation of the potent proinflammatory cytokine IL-1β.

cathepsin B and potassium efflux in human macrophages. The findings reported by Duewell et al.[9] and Rjamaki et al.[8] strongly suggest that cholesterol crystals act as danger signals in inflammasome activation. More recently, however, Menu et al.[26] generated apolipoprotein E (apoE)/NLRP3, apoE/ASC, and apoE/caspase-1 double-deficient mice. There were no significant differences in plaque size or macrophage infiltration between double-deficient mice and apoE single-deficient mice. The authors concluded that atherosclerosis in apoE-deficient mice can progress independently of the NLRP3 inflammasome. In contrast, we produced a similar experimental model (apoE/caspase-1 double-deficient mice), and observed that deficiency of caspase-1 resulted in reduction of the atherosclerotic plaque areas (*Usui and Takahashi*, manuscript under preparation). One difference between our study and Menu's study is diet. The diet used in the Menu study contained more amount of cholesterol than that present in the diet used in our study, suggesting that the diet can influence immune status and inflammatory responses. Therefore, both the diet and the disease models should be considered when interpreting findings.

Clinical perspectives

The findings described in this article suggest that the inflammasome is a potential target for prevention and treatment of vascular injury and atherosclerosis. Unfortunately, compounds that can specifically modulate inflammasome activation are currently unavailable. However, 3 types of IL-1 inhibitors were developed to inhibit processes downstream of inflammasome activation: anakinra (Kineret™), a recombinant IL-1 receptor antagonist (IL-1RA); rilonacept (IL-1Trap/Arcalyst™), a cytokine trap; and canakinumab, a fully humanized monoclonal anti-IL-1β antibody[27]. These IL-1 inhibitors are now being clinically tested and used in patients with genetic dysregulation of the NLRP3 inflammasome, such as those with cryopyrin-associated periodic syndromes (CAPS) and familial Mediterranean fever (FMF). Of these, clinical use of anakinra in patients with CAPS was the most studied. The first trial reported by Hawkins et al.[28] described that treatment with anakinra resulted in clinical remission and improved inflammatory markers, such as C-reactive protein (CRP) and serum amyloid A, in Muckle-Wells syndrome (one form of CAPS). After this trial, similar effectiveness of anakinra therapy with a variety of doses and treatment regimens in CAPS patients has been demonstrated[29-32]. Rilonacept and canakinumab have also been demonstrated to be effective in patients with CAPS[33-35]. Some of these agents are also effective in patients with sterile inflammatory diseases, such as gout, pseudogout, and T2DM[36-38]. Moreover, small molecule inhibitors targeting caspase-1 have also been developed and are currently being tested[27].

Conclusion

Several investigations have demonstrated that inflammation is a major contributing factor to the pathogenesis of vascular injury and atherosclerosis. In addition, the inflammasome, a novel innate immune pathway, is likely a causal mediator for various types of sterile inflammation. Our group extended these findings when we revealed a causal link between the inflammasome and neointimal for-

mation after vascular injury. In addition, several groups have shown that inflammasome activation can be directly induced by cholesterol crystals and that activation is implicated in the development of atherosclerosis[8, 9]. IL-1β secretion depends on 2 signals (Fig.2). Signal 1 induces pro-IL-1β. Signal 2 activates the inflammasome. The first signal might be initially provided by TLRs and subsequently by the NF-κB pathway. The system comprising these 2 signals is believed to be necessary for the tight regulation of the potent proinflammatory cytokine IL-1β. The inflammasome is a crucial mediator of the pathogenesis of vascular injury and atherosclerosis. Therefore, an understanding of the molecular basis of inflammasome activation and the development of inflammasome inhibitors will lead to new therapeutic options and novel developments in the study of inflammation in cardiovascular disease.

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Conflict of Interest

None

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