Role of NLRP3 Inflammasomes in Atherosclerosis

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Inflammation with macrophage infiltration is a key feature of atherosclerosis. Although the mechanisms had been unclear, emerging evidence unveiled that NLRP3 inflammasomes, which regulate caspase-1 activation and subsequent processing of pro-IL-1β, trigger vascular wall inflammatory responses and lead to progression of atherosclerosis. NLRP3 inflammasomes are activated by various danger signals, such as cholesterol crystals, calcium phosphate crystals, and oxidized low-density lipoprotein in macrophages, to initiate inflammatory responses in the atherosclerotic lesion. Recent studies have further clarified the regulatory mechanisms and the potential therapeutic agents that target NLRP3 inflammasomes. In this study, we reviewed the present state of knowledge on the role of NLRP3 inflammasomes in the pathogenesis of atherosclerosis and discussed the therapeutic approaches that target NLRP3 inflammasomes.

Key words: Cholesterol, Cytokines, Inflammation, Interleukin-1, Leukocytes
inflammasome molecular complex in the cytosol and leads to inflammatory responses. Indeed, recent studies demonstrated that NLRP3 inflammasome-driven inflammatory responses contributed to the progression of atherosclerosis. This study highlighted the present state of knowledge on the role of NLRP3 inflammasomes in the pathogenesis of atherosclerosis.

**Inflammasomes**

An inflammasome is a cytoplasmic complex containing multiple proteins, which is formed in response to DAMPs or PAMPs, and serves as a molecular platform for activation of the cysteine protease caspase-1. It typically contains one NLR protein, an apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and the cysteine protease caspase-1. Since caspase-1 was originally identified as an IL-1 converting enzyme (ICE), inflammasome-mediated activation of caspase-1 promotes processing of the IL-1β precursor, a potent inflammatory cytokine, and induces mature IL-1β release to cause tissue inflammatory responses. Similar to IL-1β, caspase-1 can process pro-IL-18 into its mature form, which is another IL-1 family cytokine.

Several types of inflammasome complexes have been reported; among the NLRs, NLRP1, NLRP3, NLRC4, NLRP6, and NLRP12 can participate in the inflammasomes. Similar to NLRs, the pyrin and HIN domain-containing protein (PYHIN) family, including absence in melanoma 2 (AIM2) and IFN-γ-inducible protein 16 (IFI16), is also a component of inflammasomes. In general, inflammasomes are named according to the specific NLR that it contains; therefore, NLRP3 inflammasomes are composed of NLRP3, ASC, and caspase-1 (Fig. 1).

**NLRP3 Inflammasomes**

Unlike other inflammasomes, NLRP3 inflammasomes are activated by endogenous or exogenous DAMPs and are involved in the process of sterile inflammation. As described above, NLRP3 inflammasomes are composed of NLRP3, ASC, and caspase-1, which have conserved domains for homophilic interaction (Fig. 1). NLRP3 contains three domains: C-terminal leucine-rich repeats (LRRs), a central nucleotide domain termed as 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). Caspase-1 contains a CARD and catalytic domains (p10 and p20). When cells are stimulated by DAMPs, NLRP3 assembles by the NACHT domain to provide a scaffold for ASC oligomerization by their interaction between PYDs. In turn, because NLRP3 lacks CARD and cannot recruit caspase-1 except in the presence of ASC, the oligomerized ASC interacts with caspase-1 via CARD homophilic interaction and induces caspase-1 auto-activation to exert pro-inflammatory effects by its proteolytic activity. As mentioned above, caspase-1 also processes pro-IL-18 to its bioactive mature form. In addition, recent studies showed that caspase-1 cleaves gasermin D (GSDMD) to induce pyroptosis, which is an inflammatory programmed cell death accompanied by increased plasma membrane permeability. Although IL-1β has no signal sequence for exocytosis and the mechanism of its release is still unknown, this pyroptosis-mediated membrane permeabilization is critical for IL-1β release.

Although various endogenous and exogenous danger signals, such as extracellular adenosine triphosphate (ATP), monosodium urate (MSU) crystal, and silica, are known to activate NLRP3 inflammasomes, the precise mechanism of NLRP3 recognition of DAMPs remains unclear. Nevertheless, several common upstream pathways, including potassium (K+) efflux, generation of mitochondrial reactive oxygen species (ROS), and lysosomal destabilization, have been reported to be important for activation of NLRP3 inflammasomes. Of these, it has been well accepted that lysosomal destabilization and a subsequent cathepsin release is responsible for NLRP3 inflammasome activation by particulate matters. In addition, K+ efflux inhibition prevents NLRP3 inflammasome activation in response to most or all NLRP3 stimuli, including particulate matters, suggesting K+ efflux as a common trigger of its activation. The production and release of IL-1β is regulated in two-steps: Transcriptional synthesis of pro-IL-1β and proteolytic processing into a mature form by inflammasomes (Fig. 2). The transcriptional regulation of IL-1β mRNA is mediated by PRRs or cytokine receptors, including TLRs and IL-1 receptor (signal 1), and is known as priming. Apart from NF-kB-mediated mRNA induction of IL-1β and NLRP3, activation of these receptors has also been shown to prime the NLRP3 by posttranscriptional regulation, such as ubiquitination and deubiquitination. Thereafter, the accumulated pro-IL-1β in the cytosol is rapidly processed by activated caspase-1, which is activated by NLRP3 inflammasomes (signal 2). Thus, this two-step system is considered to be necessary for tight regulation of the potent inflammatory cytokine IL-1β to maintain inflammatory homeostasis.

At first, NLRP3, which is also known as CIAS1 gene that encodes the cryopyrin protein, was identified by a gain-of-function mutation in a gene that was
Fig. 1. Representative inflammasomes and their components
Several PRRs that recognize distinct DAMPs form the inflammasome complex, which serves as the molecular platform to activate caspase-1. NLRP3 inflammasomes are composed of NLRP3, ASC, and caspase-1. NLRP3 binds ASC via PYD-PYD interaction. Subsequently, ASC binds caspase-1 via CARD–CARD interaction. NLRC4 inflammasomes are composed of NLRC4 and caspase-1, whereas AIM2 inflammasomes are composed of AIM2, ASC, and caspase-1. ATP, adenosine triphosphate; CARD, caspase recruitment domain; DAMPs, damage/danger-associated molecular patterns; HIN, hematopoietic interferon-inducible protein with a 200-amino-acid repeat; LRR, leucine-rich repeats; MSU, monosodium urate; NACHT, found in NAIP, CITA, HET-E, and TP1; NLR, nucleotide-binding oligomerization domain-like receptor; PRRs, pattern recognition receptors; PYD, pyrin domain.

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Role of NLRP3 Inflammasomes in Atherosclerosis

Many clinical and experimental studies have associated with autoinflammatory syndrome, cryopyrin-associated periodic syndrome (CAPS). CAPS includes three different conditions, including familial cold autoinflammatory syndrome, Muckle–Wells syndrome, and chronic infantile neurological cutaneous and articular syndromes. Since CAPS is an extremely rare genetic disease, only a few studies on NLRP3 inflammasomes had been conducted. In 2006, however, uric acid crystals in gout were discovered to activate NLRP3 inflammasomes. This discovery motivated further studies on the association between NLRP3 inflammasomes and sterile inflammatory diseases. Indeed, other research workers and we have revealed a pivotal role of NLRP3 inflammasome in the pathophysiology of cardiovascular and renal diseases, including atherosclerosis, vascular injury, aortic aneurysm, myocardial infarction, chronic kidney disease, and acute kidney injury. Furthermore, in the last decade, this premise was further supported by studies that described NLRP3 inflammasomes as a key mediator of other diseases associated with sterile inflammation, including type 2 diabetes and metabolic syndrome.
showed that the presence of oxidized low-density lipoprotein (LDL), which exhibits highly atherogenic properties, can lead to cholesterol crystallization and activation of priming signals to induce NLRP3 and pro-IL-1β expressions; these indicated that oxidized LDL could sufficiently provide signal 1 and signal 2 to induce IL-1β release. According to a later study by Sheedy et al.39), incorporation of oxidized LDL via a scavenger receptor CD36 provoked intracellular cholesterol crystallization. Together with the findings that the lack of IL-1β decreased the severity of atherosclerosis in ApoE−/− mice36), which are frequently used as another atherosclerosis-prone model, these observations indicated that NLRP3 inflammasome-driven IL-1β release contributed to the progression of atherosclerosis. However, Menu et al.40) reported that neither NLRP3 and ASC nor caspase-1 deficiency could prevent macrophage infiltration and atherosclerosis in ApoE−/− mice. Using a similar experimental model, we observed that caspase-1 deficiency clearly reduced macrophages.

Fig. 2. Mechanisms of NLRP3 inflammasome-driven IL-1β release

IL-1β release is regulated in two-steps: Transcriptional synthesis of pro-IL-1β and proteolytic processing into its mature form by inflammasomes. The transcriptional regulation of IL-1β mRNA is mediated by TLRs and IL-1 receptor (signal 1), which also provides NLRP3 mRNA. Then, the NLRP3 inflammasome-activated caspase-1 processes accumulated pro-IL-1β and induces the release of IL-1β (signal 2). The common upstream pathways of NLRP3 inflammasomes include potassium efflux, generation of mitochondrial ROS, and lysosomal destabilization and leakage of cathepsin B. Activated caspase-1 also cleaves GSDMD, whose processed N-terminal fragment (GSDMD-N) increases plasma membrane permeability, resulting in pyroptosis. ROS, reactive oxygen species; TLRs, Toll-like receptors.
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Mechanisms of NLRP3 Inflammasome Activation in Atherosclerosis

The mechanism of cholesterol crystal-induced NLRP3 inflammasome activation has been described. Since vascular wall macrophage infiltration and development of atherosclerotic lesions in ApoE−/− mice11. The reason for this discrepancy is not entirely clear, but the more atherogenic diet used in that study, in comparison with our study, may have influenced the immune status and inflammatory responses. In addition to the studies mentioned above, several studies have described the role of NLRP3 inflammasomes in the progression of atherosclerosis (Table 1). It should be noted that the conventional caspase-1−/− mouse strain lacks both caspase-1 and caspase-11 because these genes were quite close in the genome to have been segregated.

| Reference          | Model     | Diet        | Deficient gene    | Lesion size |
|--------------------|-----------|-------------|-------------------|-------------|
| Duewell et al.     | Ldlr−/−   | WTD (0.15% Cho) | Nlrp3, Asc (BMT)  | decrease    |
| Menu et al.        | Apoe−/−   | HFD (1.25% Cho) | Nlrp3, Asc, Casp1/11 | no change  |
| Usui et al.        | Apoe−/−   | WTD (0.15% Cho) | Casp1/11         | decrease    |
| Gage et al.        | Apoe−/−   | HFD (1.5% Cho)  | Casp1/11         | decrease    |
| Hendrikx et al.    | Ldlr−/−   | HFD (0.2% Cho)  | Casp1/11 (BMT)    | decrease    |

Abbreviation: BMT, bone marrow transplant; Cho, cholesterol; HFD, high fat diet; WTD, western diet

Table 1. Effects of NLRP3 inflammasome deficiency on progression of atherosclerosis in mice

Regulation of NLRP3 Inflammasomes in Atherosclerosis

The negative regulation of NLRP3 inflammasomes is a potential therapeutic target for atherosclerosis. Recent studies have shown that autophagy, an intracellular degradation system to maintain cellular homeostasis, negatively regulated NLRP3 inflammasome activation through several mechanisms47, 48. Because mitochondrial ROS is important for NLRP3 inflammasome activation in response to many stimuli, clearance of damaged mitochondria by autophagy (mitophagy) can inhibit its activation49. Another study showed that autophagy was able to capture and degrade the assembled complex of NLRP3 inflammasomes via ubiquitination and modulate its activity48. Indeed, autophagy-defective Atg 5−/− mice developed accelerated atherosclerosis, accompanied by enhanced activation of NLRP3 inflammasomes50. Furthermore, because lysosome is an essential organelle for autolysosome formation and degradation of a particular matter, activation of lysosome biogenesis by overexpression of transcription factor EB ( TFEB) in macrophages has been shown to inhibit cholesterol crystal-induced NLRP3 inflammasome activation and attenuate the progression of atherosclerosis51.

Phosphorylation of protein kinase A (PKA) was reported to be a negative regulator of NLRP3 inflammasomes51. Transmembrane G protein-coupled recep-
circulatory injury mice model, we previously demonstrated that ASC was upregulated at the site of vascular injury and that its deficiency significantly attenuated IL-1 expression and neointimal formation after vascular injury. In addition, inflammasome activation in bone marrow-derived inflammatory cells was demonstrated to be essential for neointimal formation in bone marrow chimeric mice. More recently, we found that NLRP3 inflammasome activation by mitochondrial oxidative stress on adventitial macrophages led to the development of abdominal aortic aneurysm in ApoE−/− mice infused with angiotensin II. The importance of inflammasomes in adventitial macrophages was further supported by the finding that ASC was expressed in the cells of patients with abdominal aortic aneurysm. Therefore, these findings strongly suggested that NLRP3 inflammasomes were a key mediator of inflammation-related vascular diseases.

**Concluding Remarks**

Increasing evidence indicated the importance of NLRP3 inflammasomes in the pathophysiology of sterile inflammatory diseases. In the progression of atherosclerosis, cholesterol and/or calcium phosphate crystals activate NLRP3 inflammasomes and induce subsequent IL-1β release, leading to vascular inflammation. In a similar manner, using a wire-mediated vascular injury mice model, we previously demonstrated that ASC was upregulated at the site of vascular injury and that its deficiency significantly attenuated IL-1β expression and neointimal formation after vascular injury. In addition, inflammasome activation in bone marrow-derived inflammatory cells was demonstrated to be essential for neointimal formation in bone marrow chimeric mice. More recently, we found that NLRP3 inflammasome activation by mitochondrial oxidative stress on adventitial macrophages led to the development of abdominal aortic aneurysm in ApoE−/− mice infused with angiotensin II. The importance of inflammasomes in adventitial macrophages was further supported by the finding that ASC was expressed in the cells of patients with abdominal aortic aneurysm. Therefore, these findings strongly suggested that NLRP3 inflammasomes were a key mediator of inflammation-related vascular diseases. A better understanding of the molecular mechanisms underlying NLRP3 inflammasome activation and development of specific...
inhibitors will offer new therapeutic or preventive options and break new grounds for studying the role of inflammation in atherosclerosis as well as in other vascular diseases.

Conflict of Interest

None.

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