

Cardiovascular Pathobiology of Inflammasomes: Inflammatory Machinery and Beyond

Pin-Lan Li

Abstract

Significance: In response to infection or cellular stress, inflammasomes are assembled and activated to mediate host defense and to initiate or promote the development of different diseases, in particular, autoinflammatory diseases and chronic degenerative diseases. Understanding of inflammasomes and related physiological and pathological relevance to the cardiovascular system will open a new chapter on the pathogenesis of inflammation and related diseases and will help develop novel therapeutic strategies for prevention or treatment of cardiovascular diseases. **Recent Advances:** The inflammasome, in particular the nucleotide oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome, has been recently recognized as a fundamental mechanism to mediate or promote the pathogenesis of degenerative diseases. Some important mechanisms responsible for NLRP3 inflammasome activation have been proposed and many molecular targets associated with this inflammasome activation are shown to be the possible candidates of therapeutic targets for treatment of cardiovascular diseases. **Critical Issues:** The concepts that NLRP3 inflammasome activation occurs just in immune cells or phagocytes and that its role is only for the inflammatory progression of cardiovascular diseases are oversimplified. A large body of other cell types are capable of NLRP3 inflammasome activation, and many uncanonical effects of this inflammasome may also be implicated in the development of cardiovascular diseases, which are discussed in a great detail by this Forum. **Future Directions:** More mechanistic and translational studies will rapidly widen the horizon of knowledge on NLRP3 inflammasome activation and regulation, which may help develop novel effective therapeutic strategies to target this inflammasome for treatment or prevention of cardiovascular diseases. *Antioxid. Redox Signal.* 22, 1079–1083.

THE INFLAMMASOME HAS been recognized as the intracellular molecular machinery that switches on the inflammatory response in a variety of mammalian cells. In particular, the nucleotide oligomerization domain-like receptor protein with pyrin domain containing 3 (NLRP3) inflammasome is well characterized, which consists of a proteolytic complex formed by NLRP3, the adaptor protein, ASC, and caspase-1. Caspase-1 is activated when the inflammasome complex is formed, which produces active interleukin (IL)-1 and IL-18 by cleavage of their precursors. Recently, NLRP3 is found to detect endogenous stress-associated danger signals, such as excessive ATP, β -amyloid, cholesterol crystal, and homocysteine, to produce local tissue sterile inflammation. Many studies on NLRP3 inflammasomes have highlighted the implication of their formation and activation in the pathogenesis of autoinflammatory dis-

eases and various chronic metabolic or degenerative diseases, including diabetes mellitus, gout, silicosis, acute myocardial infarction, atherosclerosis, glomerular sclerosis, Alzheimer's disease, and liver cirrhosis. Recently, a new concept is emerging that activation of various inflammasomes is the root of or route to many chronic degenerative diseases. It is believed that inflammasomes will take center stage in pathogenesis of cardiovascular diseases, such as atherosclerosis, hypertension, vascular inflammation, cardiac remodeling, and associated end-stage organ diseases.

Pathogenic Role of Inflammasome Activation Not Only Due to Inflammation

Interestingly, recent studies have shown that the pathogenic role of inflammasome activation in diseases is not only

due to instigation of classical inflammatory responses but also due to its direct damaging actions on various cardiovascular cells. These direct actions may interfere with the synthesis of cell-specific proteins, activate the production of damage-associated molecular patterns (DAMPs), enhance cell membrane permeability or instability, and induce cell pyroptosis. It has been shown that there are more than 120 substrates for caspase-1 within different cells, which support the concept of the uncanonical or noninflammatory actions of inflammasome activation because many of these substrates are not related to classical inflammatory responses. This concept is particularly important because it may answer a long-lasting question of why the only use of anti-inflammatory therapy could not efficiently prevent or reverse the development of cardiovascular diseases, such as atherosclerosis, hypertension, or cardiac remodeling or failure. There is accumulating evidence that the noninflammatory actions of inflammasome activation, in particular NLRP3 inflammasome activation, concurrent with the innate immune response or inflammatory response, are implicated in the pathogenesis of many chronic metabolic and degenerative diseases, such as atherosclerosis, Alzheimer's diseases, diabetes mellitus, and some autoinflammatory diseases.

In the development of cardiovascular diseases, such as atherosclerosis, hypertension or arteritis, vascular dementia, and myocardial ischemia or remodeling, the well-known inflammatory actions are associated with the activation and recruitment of inflammatory cells such as macrophages and T cells and corresponding cytokine production and their actions. The activation of inflammasomes such as NLRP3 inflammasomes is known as the intracellular inflammatory machinery that mediates this typical inflammatory response. Many studies consider this inflammation-instigating action of inflammasome activation canonical or classical in the development of cardiovascular diseases such as atherosclerosis or arteritis. However, recent studies have indicated that inflammasome activation in cardiovascular tissues may produce a variety of injurious actions independent of typical inflammation, which are referred to as noncanonical effects of inflammasome activation.

In endothelial cells, this noncanonical effect of inflammasome activation may serve as a novel early mechanism that leads to endothelial dysfunction and injury, initiating atherogenesis and other vascular diseases. It has been demonstrated that different atherogenic factors, such as hypercholesterolemia, hyperhomocysteinemia, or cytokines, including death factors or adipokines, activated the NLRP3 inflammasome, increasing caspase-1 activity and IL-1 β production in cultured endothelial cells or the endothelial layer of the arterial wall. This indicates the inflammasome activation, which reduces nitric oxide (NO) bioavailability, leading to impaired endothelium-dependent vasodilation (EDVD). This impaired vasomotor response could be blocked by silencing or deletion of the *NLRP3* gene. In addition, activation of the NLRP3 inflammasome was found to decrease the junction protein level such as ZO-1 in endothelial cells, increasing endothelial permeability, which was also blocked by deletion of the *NLRP3* gene or caspase-1 inhibition. These effects of inflammasome activation are due to a direct effect on endothelial cells, but not through a classical inflammatory response (1, 9).

Inflammasome activation may directly facilitate the activation of tightly controlled myofibroblasts, which will result

in the continual synthesis of collagens and other extracellular matrix proteins, leading to fibrotic damage and ultimate fibrosis or sclerosis. This role of inflammasome activation may not be due to its typical inflammatory response, but to its direct action on myofibroblast proliferation, differentiation, and corresponding function changes. This uncanonical action of NLRP3 inflammasome activation together with its inflammation-instigating effect is now considered as a common thread or pathological process linking divergent fibrogenic diseases (2).

The role of inflammasome activation in cardiac injury also includes classical inflammatory and uncanonical noninflammatory effects depending upon its activation in different cell types of the heart. In fibroblasts, activation of the inflammasome stimulates myofibroblast differentiation and collagen synthesis, leading to tissue healing or fibrotic changes. In leukocytes, the inflammasome activation triggers and amplifies the inflammatory response and at the same time induces leukocyte cell death due to pyroptosis. In cardiomyocytes, inflammasome activation mainly enhances caspase-1 activity and leads to pyroptosis, but does not release mature IL-1 β . During inflammasome activation, however, cardiomyocytes that are very sensitive to IL-1 β will be easily damaged due to reduction of contractile function and apoptosis of cardiac myocytes. In addition to cardiac injury, the inflammasome activation has been reported to cause adverse cardiac remodeling and heart failure (HF), which may also be associated with its inflammatory and noninflammatory actions (7). In this regard, there are reports that the inflammasome activation is involved in the transdifferentiation events of resident cardiac fibroblasts, shifting to a profibrotic phenotype and thereby producing interstitial fibrosis. This effect of the inflammasome activation is independent from IL-1 β , IL-18, and even caspase-1, but linked to Smad signaling and myofibroblast differentiation, which occurs independently of the canonical action of inflammasome activation or caspase-1 activity (3).

Molecular Activation of Inflammasomes by PAMPs and DAMPs

It has been well known that both exogenous pathogen-associated molecular patterns (PAMPs) and endogenous danger signals or DAMPs are identified by the human innate immune system through pattern recognition receptors (PRRs) to produce a host defense reaction or an inflammatory response. Recent studies have extended the concept of PRRs to a variety of nonimmune cells, including cardiovascular cells. There are numerous reports that various PRRs are expressed not only in immune cells but also in other types of cells, such as endothelial cells, vascular smooth muscle cells, and cardiac myocytes from the cardiovascular system. It has been demonstrated that PRRs have three families of members that detect the extracellular danger signals, including C-type lectin receptors, toll-like receptors, and pentraxins, and two families of members that sense and respond to intracellular signals, namely the nucleotide-binding domain leucine-rich repeats (NLRs) and RIG-I-like receptors (RLRs).

Although activation of PRRs has been found to enhance recruitment of innate immune cells into local tissue, to initiate tissue repair, and to activate adaptive immune responses, PRRs activated different signaling pathways or cellular

activities and are often accompanied by excessive inflammation, which are importantly implicated in the development of various cardiovascular diseases (8). Among these PRRs, however, the NLR and RLRs are the major contributors to the activation of inflammasomes. In particular, the role of the NLR family of PRRs in inflammasome activation has been widely known. In particular, NLRP3 as an NLR subfamily member is well characterized to serve as a sensor to detect PAMPs or DAMPs within cells to form and activate inflammasomes. Activation of RLRs has also been reported to form and activate the inflammasome even without NLRP3 participation (1, 5, 7, 8).

Under diverse pathological conditions, three primary activating pathways of NLRP3 inflammasomes have been proposed originally, including ATP and potassium efflux, lysosome destabilization, and frustrated phagocytosis, as well as reactive oxygen species (ROS). ATP as an endogenous DAMP was first reported to induce the depletion of cytosolic K⁺ and thereby activate both NLRP3- and ASC-dependent caspase-1 to produce IL-1 β . This K⁺ efflux-induced NLRP3 inflammasome activation mainly responds to danger factors, such as ATP, nigericin, monosodium urate (MSU) crystals, and pore-forming toxins. The second NLRP3 inflammasome-activating pathway is due to lysosome destabilization and frustrated phagocytosis. It has been shown that in response to MSU crystals, cholesterol crystals, asbestos, silica, and aluminum salts, lysosome swelling and destabilization may occur leading to lysosomal rupture to release cathepsin-B (Cathp-B), which activates NLRP3 inflammasomes. Another pathway relates to the action of ROS produced upon stimulation by different PAMPs or DAMPs. The effects of ROS that activate NLRP3 inflammasomes are associated with two distinct proteins, namely NLRP3-thioredoxin-interacting protein (TXNIP) and mitochondrial antiviral signaling protein (MAVS). It has been demonstrated that TXNIP is able to bind to NLRP3 after dissociation from thioredoxin (TRX) when ROS are increased. TXNIP-NLRP3 binding causes the inflammasome formation and activation. NLRP3 also binds to MAVS to result in its oligomerization with ASC and caspase-1, forming a typical inflammasome complex and activating caspase-1 (1).

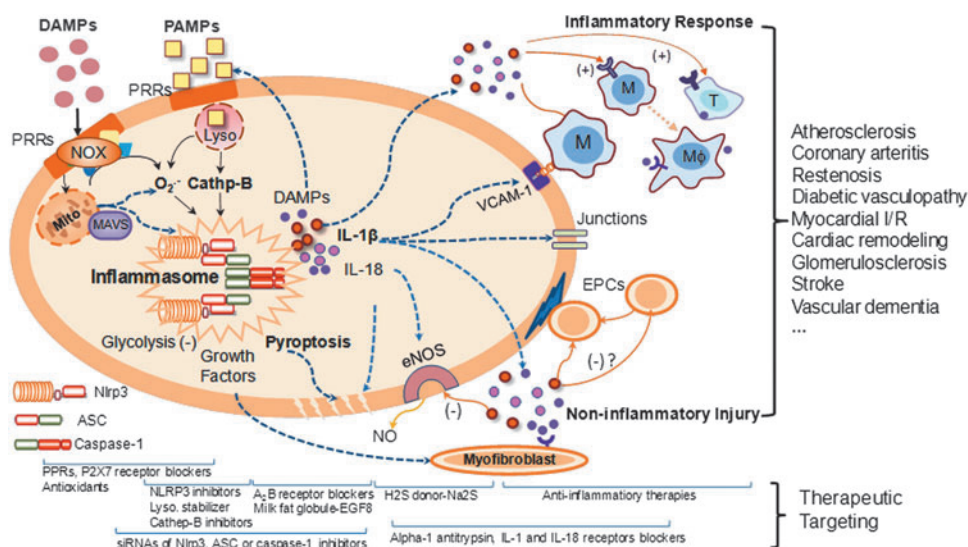
In addition to these primary pathways, recent studies have indicated that many other mechanisms may also be involved in the activation of inflammasomes. These mechanisms include the endoplasmic reticulum stress, deubiquitination, microRNA regulation, adenosine, and mitochondrial signaling proteins. However, these inflammasome-activating mechanisms are found to be more specific to certain cell types or to different individual danger factors. More studies are needed to further elucidate how they work as a common pathway in the inflammasome activation (1, 7). In this regard, some studies have shown that NLRP3 also localizes in the mitochondrial structure in some immune cells and, upon DAMP stimulation, these inflammasomes can be assembled and thus exert their effects there. In human cardiac fibroblasts, NLRP3 was found to be constitutively associated with mitochondria, where NLRP3 may regulate ROS production (3).

In addition, it has been shown that pyocyanin, a membrane-permeable pigment and toxin released by *Pseudomonas aeruginosa*, interacts with the mitochondrial respiratory chain in neutrophils, resulting in the release of ROS, the mitochondrial acid sphingomyelinase (ASM) activation, and the release of cytochrome c from mitochondria. Increased mitochondrial ceramide *via* ASM may trap proinflammatory molecules, preventing them from a proper interaction with inflammasome components and thus inhibiting the inflammasome activation (4). Such a negative regulatory mechanism was also shown to be the major role of another mitochondrial protein, the leucine-rich repeat FII-I-interacting protein 2 (LRRFIP2). It can bind to NLRP3 *via* its N terminus and exert its inhibitory activity on inflammasome activation (7).

Targeting NLRP3 Inflammasomes as a Therapeutic Strategy for Cardiovascular Diseases

Recently, anti-inflammatory drugs have been considered as a promising therapeutic strategy for prevention or treatment of cardiovascular diseases. Several candidates are at clinical trials for a possible anti-inflammatory therapy for cardiovascular diseases. Due to the complexity of inflammatory processes in these diseases, little success has been

FIG. 1. Activation of NLRP3 inflammasomes and associated cardiovascular diseases. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars



reached so far to make such type of cardiovascular therapy available clinically. Recent failures of GlaxoSmithKline's darapladib—an oral inhibitor of lipoprotein-associated phospholipase A2 (Lp-PLA2)—disappointed the scientific community, which may dim our hopes for anti-inflammatory drugs for treatment of cardiovascular diseases (6). Other types of anti-inflammatory drugs such as cox-1 and cox-2 inhibitors also failed to effectively prevent or treat cardiovascular diseases (1). It is increasingly believed that the commonly used anti-inflammatory therapies by targeting oxidative stress or those known inflammatory pathways or factors may only have partial effects on the pathogenic processes due to the inflammasome activation in cardiovascular tissues or cells, but its uncanonical or noninflammatory effects on cell function and associated injury are not targeted. A possible novel therapeutic target to treat or prevent cardiovascular diseases may be the NLRP3 inflammasome, which can eliminate the root of these diseases by a blockade of both the inflammatory response and uncanonical injurious actions on cell function and metabolism from activation of this inflammasome.

There is indeed increasing evidence that targeting the NLRP3 inflammasome and related signaling or regulatory mechanisms is beneficial for fibrotic diseases, including cardiovascular diseases such as atherosclerosis. Experimentally, the blockade of NLRP3 inflammasome activation or gene deletion of inflammasome molecules was shown to attenuate or abolish both inflammatory and noninflammatory effects of the NLRP3 inflammasome activation (1). Pirfenidone, as an antifibrotic drug, has been shown to inhibit NLRP3 activation and thereby decrease collagen synthesis in a model of cardiac fibrosis (2). In addition, IL-1 β blockers, such as anakinra, canakinumab, and riloncept, are now used for the clinical treatment of inflammasome-mediated diseases, in particular autoinflammatory diseases. These IL-1 β blockers were also tested in the animal model of myocardial ischemia and infarction, which was shown to reduce myocardial injury and decrease related adverse cardiac remodeling. Two small pilot clinical trials with anakinra (recombinant human IL-1Ra) have already demonstrated that anakinra at 100 mg daily for 14 days significantly lowered incidence of HF (5% vs. 30% of placebo). This therapeutic strategy with IL-1 β blockers has also been shown to improve cardiac function in patients with symptomatic HF.

Although there are no clinical trials yet to target inflammasome molecules directly, numerous preclinical studies have evaluated the beneficial effects of inflammasome inhibition in protection against cardiac diseases. A number of molecules that are involved in the inflammasome activation are targeted in different models of cardiovascular diseases, such as atherosclerosis, glomerular sclerosis, cardiac ischemic injury, stroke, and vascular dementia, including the use of NLRP3 inhibitors, the P2X7 receptor blockers, IL-1 and IL-18 receptor blockers, caspase-1 inhibitors, alpha-1 antitrypsin, adenosine A2B receptor blockers, miRNAs, H₂S donor Na₂S, lysosome stabilizer, Cathp-B inhibitors, and milk fat globule EGF-8 as an endogenous inhibitor of inflammasome-induced IL-1 β production (1, 5, 7).

Figure 1 summarizes the topics that this Forum discussed and reviewed. When cardiovascular cells, such as ECs,

SMCs, cardiac myocytes, or interstitial cells, are exposed to danger factors, including PAMPs or DAMPs, NLRP3 inflammasomes will be formed and activated *via* different mechanisms, such as redox signaling, lysosome permeability changes to release Cathp-B, and other pathways, where procaspase-1 is cleaved into active caspase-1, producing IL-1 β , IL-18, and other factors. Activation of caspase-1 and associated products or cellular changes can not only recruit and activate inflammatory cells (*e.g.*, M Φ and T cells) in local tissues to produce the inflammatory response but also directly induce a series of metabolic and functional disturbances in different cells, such as pyroptosis, impaired EDVD, enhanced monocyte adhesion, loss of junction proteins, myofibroblast differentiation, and disturbance of extracellular matrix metabolism. All these pathogenic pathways ultimately provoke devastating inflammatory and fibrotic changes in the arterial wall or myocardium, leading to different cardiovascular diseases. Based on the molecular mechanisms, activating inflammasomes, and the pathogenic pathways mediating the effects of inflammasome products, a number of therapeutic strategies being developed or tested currently are also listed in the diagram.

In perspective, all authors of this Forum believed that the concept of the inflammasome activation only as a mechanism for the inflammatory progression of cardiovascular diseases is oversimplified and that targeting the inflammasomes to block their role for the inflammatory response and to abolish many uncanonical effects on cell metabolism or activity will be a promising innovative therapeutic strategy for cardiovascular diseases.

Acknowledgment

Many studies cited in this editorial from our laboratory were supported by grants, HL57244, HL075316, DK54927, and HL091464, from National Institutes of Health.

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Address correspondence to:

Dr. Pin-Lan Li
Department of Pharmacology and Toxicology
School of Medicine
Virginia Commonwealth University
410 North 12th Street
P.O. Box 980613
Richmond, VA 23298

E-mail: pli@vcu.edu

Date of first submission to ARS Central, March 16, 2015;
 date of acceptance, March 16, 2015.

Abbreviations Used

ASM = acid sphingomyelinase
 Cathp-B = cathepsin-B
 DAMPs = damage-associated molecular patterns
 EDVD = endothelium-dependent vasodilation
 HF = heart failure
 IL = interleukin
 Lp-PLA2 = lipoprotein-associated phospholipase A2
 LRRFIP2 = leucine-rich repeat FII-I-interacting protein 2
 MAVS = mitochondrial antiviral signaling protein
 MSU = monosodium urate
 NLRP3 = nucleotide oligomerization domain-like receptor containing pyrin domain 3
 NLRs = nucleotide-binding domain leucine-rich repeats
 NO = nitric oxide
 NOX = NADPH oxidase
 PAMPs = pathogen-associated molecular patterns
 PRRs = pattern recognition receptors
 RLRs = RIG-I-like receptors
 ROS = reactive oxygen species
 TLRs = toll-like receptors
 TRX = thioredoxin
 TXNIP = thioredoxin-interacting protein