

Novel Insights Into the NLRP3 Inflammasome in Atherosclerosis

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Atherosclerosis underlies the leading cause of death in industrialized countries.¹ Atherosclerosis is a maladaptive, nonresolving chronic inflammatory disease that occurs predominantly at sites of disturbed laminar flow, notably, arterial branch points and bifurcations, of large and medium-sized arteries.¹ Atherosclerosis is initiated by subendothelial buildup of apolipoprotein B–containing lipoproteins (in particular LDL [low-density lipoprotein]).¹ LDL, particularly following oxidation, has properties of damage-associated molecular patterns² and thereby provokes the activation of endothelial cells (ECs)³ and ensuing recruitment of blood-borne monocytes to the lesion.⁴ The Ly6C^{hi} subpopulation of monocytes differentiates into macrophages.⁴ Serving to protect the organism against tissue damage, macrophages have developed plasticity to tailor the inflammatory responses. In short, macrophages have the capacity to trigger inflammation when necessary and to quell the inflammatory response when it is no longer required. In the atherosclerotic milieu, macrophages are predominantly polarized to an inflammatory phenotype.⁴ Lipid-loaded cells known as foam cells constitute a hallmark of atherosclerotic lesion, which can be detected at very early stages of atherosclerosis.⁵ The causal role for metabolic stress factors such as LDL, particularly oxidized LDL (oxLDL) and cholesterol, in promoting vascular inflammation and atherosclerosis has long been appreciated.⁶ However, the precise mechanism whereby metabolic stress elicits the proinflammatory status and induces atherogenesis remained unclear. When resolution of inflammation goes awry, nonresolving inflammation characterizes the atherosclerotic lesions.⁷ The mechanisms behind the nonresolving nature of

vascular inflammation in the atherosclerotic lesion remain to be entirely defined. The inflammatory environment incurs apoptosis of intimal cells. Defective efferocytosis, a process by which phagocytic cells (predominantly macrophages) engulf the dead cells, promotes the formation of the necrotic core.² Immune responses are further amplified by relentless production of damage-associated molecular patterns from necrotic cells in the advanced lesions.^{5,7}

Innate immunity plays crucial roles in plaque initiation, progression, and rupture.⁵ It has been well established that the inflammasomes constitute an important component of innate immunity. The term *inflammasome* was first coined by Jurg Tschopp and his colleagues in 2002 to explain the mechanism for caspase-1 activation and interleukin-1 β processing.⁸ To date, diverse inflammasomes have been discovered. Among the various inflammasomes identified, the nucleotide-binding oligomerization domain, leucine-rich repeat–containing receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome is best characterized.⁸ The groundbreaking findings that the NLRP3 inflammasome is a key player in orchestrating lipid-driven vascular inflammation and plays a fundamental role in the initiation and progression of atherogenesis⁹ are transforming the field of atherosclerosis. The focus of this review is on our current understanding of the role, regulation, and mechanisms of NLRP3 inflammasome activation in atherosclerosis. We will also discuss the therapeutic potential of NLRP3 inflammasome intervention.

Two-Step Activation Model of the NLRP3 Inflammasome

Inflammation is a protective response to infection and injury, which is mounted by the innate immune system. Innate immunity response depends on the recognition of pathogen-associated molecular patterns and damage-associated molecular patterns through the pattern-recognition receptors, such as the Toll-like receptors and NLRs.¹⁰ The NLRs are now recognized as the key sensors of pathogens and danger signals. Engagement of the NLRs elicits downstream signaling cascades and leads to the production of proinflammatory cytokines and type I interferons.¹⁰ The core components of the inflammasomes include the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), a zymogen

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pro-caspase-1 and an NLR family member, such as the best-demonstrated NLRP3.⁸ The NLR is engaged to dictate the assembly of the inflammasomes in response to pathogen-associated molecular patterns or damage-associated molecular patterns.⁸ The NLRP3 inflammasome is a multimeric protein complex that is composed of NLRP3, ASC, and pro-caspase-1.⁸ The NLRP3 contains the N-terminal pyrin domain responsible for recruitment of ASC, the central nucleotide-binding oligomerization domain, and the C-terminal leucine-rich repeat.¹⁰ The nucleotide-binding oligomerization domain enables the activation of the signaling complex via oligomerization, whereas leucine-rich repeat is thought to function in ligand sensing and autoregulation.⁸ NEK7, a serine and threonine kinase that is involved in mitosis, can directly bind NLRP3 and control NLRP3 oligomerization.¹¹ NEK7 appears to be a new component of the NLRP3 complex.¹¹

Basal levels of NLRP3 are inadequate for efficient inflammasome formation.¹² Meanwhile, NLRP3 is kept in an inactive ubiquitinated state until a priming signal provokes its deubiquitination.¹³ It has been generally accepted that the activation of the NLRP3 inflammasome requires 2 signals: a priming and an activation signal (Figure 1). A priming signal induces NF κ B-dependent transcriptional up-regulation of NLRP3¹² and the deubiquitination of NLRP3 by the Lys63-specific deubiquitinase BRCC3.¹³ As the second step in the activation of the NLRP3 inflammasome, an activation signal triggers the assembly and activation of the inflammasome, culminating in the activation of caspase-1.⁸ In brief, primed NLRP3 undergoes oligomerization in response to the activation signal. Oligomerized NLRP3 serves as a scaffold to recruit ASC through the pyrin domain–pyrin domain interactions,¹⁴ leading to the generation of long ASC filaments, the latter of which recruit pro-caspase-1. The close proximity of pro-caspase-1 protein then induces autoproteolytic maturation of pro-caspase-1 into active caspase-1.¹⁴

Because of the vast number of structurally dissimilar agonists for the NLRP3 inflammasome,⁸ it seems unlikely that these agonists induce the activation of the inflammasome by directly binding to NLRP3. A favorite theory is that NLRP3 responds to a common cellular event that can be initiated by the diverse NLRP3 activators. However, despite years of research, no unified mechanism underlying NLRP3 inflammasome activation has been recognized. To date, different mechanisms behind inflammasome activation have been put forward, involving potassium efflux, calcium influx, mitochondrial dysfunction, the generation of mitochondrial reactive oxygen species (ROS), the release of mitochondrial DNA or cardiolipin, and lysosomal destabilization and rupture.^{15–19} Notably, a recent study suggested that diverse NLRP3 stimuli can induce the disassembly of the trans-Golgi network to the dispersed trans-Golgi network (dTGN).²⁰ NLRP3 is then recruited to the dTGN through an interaction between a

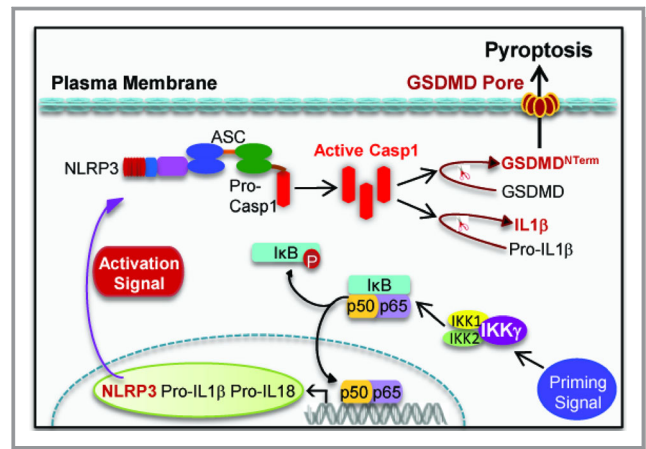


Figure 1. Two-step activation model of the NLRP3 inflammasome. Activation of the NLRP3 inflammasome requires 2 signals: a priming signal and an activation signal. In unstimulated cells, the transcription factor NF κ B is sequestered in the cytoplasm by the I κ B family members. I κ B degradation is a prerequisite for NF κ B activation, which is initiated upon phosphorylation by the I κ B kinase (IKK) complex in response to priming signal. The IKK complex consists of 2 catalytic subunits (IKK1 and IKK2) and a regulatory subunit IKK γ . As the second step in inflammasome activation, an activation signal triggers the assembly and activation of the inflammasome, culminating in the activation of caspase-1 and the production of interleukin-1 β , interleukin-18 and the GSDMD^{NTerm}. ASC indicates apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; GSDMD, gasdermin D; GSDMD^{NTerm}, N-terminal GSDMD; IL18, interleukin-18; IL1 β , interleukin-1 β ; I κ B, inhibitor of kappa B; NF κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3; Pro-Casp1, pro-caspase-1.

polybasic region within the NLRP3 and phosphatidylinositol-4-phosphate on the dTGN. The dTGN functions as a scaffold for NLRP3 aggregation, which is essential for ASC polymerization and ensuing activation of the downstream signaling cascade. The recruitment of NLRP3 to dTGN is thought to be a common event that is required for NLRP3 aggregation and activation in response to different activators.²⁰

Role for the NLRP3 Inflammasome in Atherosclerosis

The components of the NLRP3 inflammasome are dominantly expressed in macrophages and foam cells within human carotid atherosclerotic plaques.²¹ As such, the majority of studies on the NLRP3 inflammasome were carried out in macrophages. Intriguingly, NLRP3 inflammasome components are also expressed in ECs.²² In resting cells, endogenous NLRP3 in macrophages and ECs is expressed at low levels. In stark contrast, human atherosclerotic plaques have dramatically elevated NLRP3 inflammasome components (including

activated caspase-1) when compared with healthy counterparts.²³

Accumulated evidence reveals a causative role for the NLRP3 inflammasome in the initiation and progression of atherosclerosis, although the studies on the role of the NLRP3 inflammasome in this disease have yielded mixed results.^{9,24} Remarkably, the impact of inflammasome activation in atherosclerosis is consistent with the earlier findings that genetic depletion of interleukin-1 β or interleukin-1 receptor (interleukin-1R) attenuates atherosclerosis in hypercholesterolemic mice.^{25,26} Genetic ablation of interleukin-1R antagonist, an endogenous competitive inhibitor of interleukin-1R that can block interleukin-1 α and interleukin-1 β responses, aggravates atherogenesis in atherosclerosis-prone mice.²⁷

Consistent with this proposition, LDL receptor-deficient (Ldlr^{-/-}) mice transplanted with bone marrow from mice deficient in NLRP3, ASC, or interleukin-1 β , respectively, had significantly reduced aortic lesion size and serum interleukin-18 levels.⁹ While genetic ablation of caspase-1 under an ApoE (apolipoprotein E)-deficient background ameliorated atherosclerosis,²⁸ reconstitution of Ldlr^{-/-} mice with caspase-1^{-/-} bone marrow significantly thwarted the development of atherosclerotic lesions in comparison with the control bone marrow.²⁹ A recent seminal study indicated that a Western diet induces functional reprogramming of myeloid cells, incites trained immunity, and instigates systemic inflammation in a mouse model of atherosclerosis.³⁰ This study provided further evidence showing that the NLRP3 inflammasome pathway mediates the trained immunity in the context of Western diet feeding and its deleterious effects on inflammatory diseases such as atherosclerosis.³⁰

The major factors that activate the NLRP3 inflammasome in the atherosclerotic scenario has been identified recently (Figure 2). Hypoxia prevails in the atherosclerotic lesion.³¹ Hypoxia favors plaque angiogenesis,^{31,32} promotes foam cell formation,³³ and contributes to the formation of the plaque necrotic core.³⁴ It has long been hypothesized that hypoxia may conspire with inflammation to exacerbate atherosclerosis. In support of this notion, a recent study established a direct link between hypoxia and the NLRP3 inflammasome.³⁵ In addition to stabilization of interleukin-1 β protein by restricting its autophagic degradation, hypoxia increased NLRP3 expression and activation in human macrophages and in the plaques.³⁵ This study provided strong evidence that low oxygen tension exacerbates atherosclerosis by aggravating inflammation.

NLRP3 can sense various types of the metabolic stress molecules, as exemplified by cholesterol crystals (CCs)⁹ and oxLDL.^{9,36} We will discuss how CCs and oxLDL drive atherosclerosis later. Different metabolites contribute to the activation of the NLRP3 inflammasome. For instance, the best-known NLRP3 activator ATP can be released from dying/

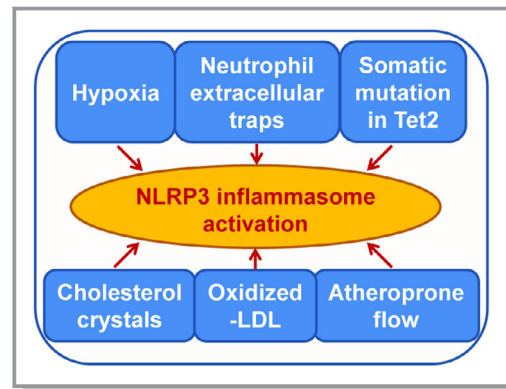


Figure 2. Signals involved in NLRP3 inflammasome activation in atherosclerosis. The activation of the NLRP3 inflammasome drives the initiation and progression of atherosclerosis. Shown are the key stimuli identified to date that induce priming and activating of the NLRP3 inflammasome in the atherosclerotic milieu. LDL indicates low-density lipoprotein; Tet2, tet methylcytosine dioxygenase 2.

dead cells into the atherosclerotic necrotic core,³⁷ where it efficiently activates the NLRP3 inflammasome through the engagement of its cognate receptor P2X7R.³⁸ As expected, P2X7R was found to be abundantly expressed in the atherosclerotic plaques.³⁸ Moreover, deletion of P2X7R in Ldlr^{-/-} mice led to declined lesional inflammasome activation and reduced atherosclerotic plaque size.³⁸ These findings implicate lesional ATP in NLRP3 inflammasome activation. Additionally, calcium phosphate crystals were found to participate in the activation of caspase-1 and the production of active interleukin-1 β in atherosclerotic lesions.³⁹

The oscillatory shear stress likely represents one of the initial triggers for NLRP3 inflammasome activation in atherogenesis.²² Strikingly, this stimulus acts as both priming and activating signals in NLRP3 inflammasome activation in ECs.²² In addition, neutrophil extracellular traps (NETs) can induce the activation of the NLRP3 inflammasome in macrophages.⁴⁰

Regulation and Mechanisms of CCs-Triggered NLRP3 Inflammasome Activation

The NLRP3 inflammasome links arterial deposition of lipids and lipoproteins to the inflammatory responses driving the initiation and progression of atherosclerosis. The consumption of high-fat, high-cholesterol diets underlies the culprit of hypercholesterolemia, particularly in individuals with genetic predisposition. Cholesterol is an extremely important lipid that has crucial roles in membrane structure; it is essential for maintaining membrane permeability and cell signaling.⁴¹ Mammalian cells cannot degrade cholesterol; elimination of excessive cellular cholesterol is primarily mediated by HDL

(high-density lipoprotein).⁴¹ Disruption in cholesterol homeostasis leads to CC buildup within the necrotic core causing plaque rupture. The advent of CCs in the atherosclerotic plaques was once considered a late characteristic of atherosclerosis. However, emerging evidence pointed out that the formation of small CCs occurs even at early stage of atherosclerotic lesions.⁹ A plaque containing abundant CCs is a hallmark of vulnerable plaques. CCs may induce cell death and perforate the fibrous cap.⁴² Thus, developing agents that dissolve CCs is expected to offer an alternative approach to stabilize vulnerable plaques. Besides their mechanical and toxic effect, CCs induce arterial wall injury through triggering inflammation.

Atherosclerosis begins with deposition of cholesterol into the arterial wall via LDL particles.⁴³ Once LDL is oxidized in the subendothelial region of arterial wall, it can be assimilated by lesion macrophages.⁴² The lesional macrophages can promote reverse cholesterol transport by producing nascent HDL.⁴⁴ Impairment of this pathway facilitates the formation of foam cell and CCs.⁴² Cholesterol within LDL exists in the esterified form.⁴² Reverse cholesterol transport requires the conversion of esterified cholesterol to free cholesterol (FRC) for it to be mobilized by transporters ATP-binding cassette A-1 and G1 (ABCA-1 and ABCG-1).⁴⁵ ABCA1 and ABCG1 transport FRC out of cells to HDL, promoting cholesterol efflux from macrophages onto HDL particles or onto apolipoprotein A1.⁴⁵ Through this process, cholesterol is transported from peripheral tissues back to the liver, followed by excretion into bile and feces.⁴⁶ It is known that HDL can partially dissolve CCs.⁴⁷ Deficiency of ABCA1/G1 in myeloid cells induces significant accumulation of cholesterol in macrophages and ensuing NLRP3 inflammasome activation.⁴⁸ Mechanistically, myeloid ABCA1/G1 deficiency increases the expression of *Nlrp3* and pro-interleukin-1 β mRNA likely through a Toll-like receptor 4-mediated priming effect, and elicits a membrane cholesterol sensing mechanism triggering noncanonical NLRP3 inflammasome activation.⁴⁸ It is known that noncanonical inflammasome activation induces caspase-11 cleavage, leading to the activation of the NLRP3 inflammasome.⁴⁹ Indeed, myeloid *Abca1/g1* deficiency enhanced caspase-11 cleavage in Ly6G⁺ neutrophils and Ly6G⁻CD11b⁺ macrophages.⁴⁸ Consistent with the observations made in mouse atherosclerosis model, the patients of Tangier disease with mutations in ABCA1 have higher plasma interleukin-1 β , suggestive of conservation of cholesterol-mediated inflammasome activation in humans.⁴⁸

With the progression of atherosclerosis, there is an increase in local and systemic inflammation that can induce HDL dysfunction, as primarily characterized by reduced cholesterol efflux capacity of HDL.⁵⁰ HDL dysfunction causes FRC accumulation in the cell membrane and extracellular space, which leads to CC formation and cell death.⁵¹ In addition to HDL dysfunction, imbalance between esterified

cholesterol and FRC contributes to FRC buildup within foam cells.⁵⁰ It is known that cholesterol ester hydrolase convert esterified cholesterol to FRC, while acyl-coenzyme A cholesterol acyltransferase 1 (ACAT1) converts FRC to esterified cholesterol.⁵¹ Acyltransferase 1 inhibition gives rise to liquid crystalline and cholesterol monohydrate crystals formation in macrophages' plasma membrane bilayer.⁵¹ These membrane cholesterol domains serve as the platforms for CC formation.⁵¹ Notably, cells with rich cholesterol membranes including dying foam cells are thought to be the additional sources of FRC.⁵² The release of cellular contents from dying cells favors CC formation by means of inducing local physical change.⁵³

Cholesterol efflux restrains the production of inflammatory mediators in macrophages.⁵⁴ Defective cholesterol efflux promotes monocyte and neutrophil production in the bone marrow and the spleen.⁵⁵ Mouse and human studies revealed that excessive production of inflammatory cells under hypercholesterolemic conditions have a causal role in promoting atherosclerotic cardiovascular disease (CVD).⁵⁵ Macrophages in atherosclerotic plaques may be derived from blood-borne monocytes, which are produced in the bone marrow and the spleen.⁵⁶ As a systemic consequence of inflammasome activation in myeloid cells, neutrophil activation and recruitment into the plaques ensue, leading to the formation of NETs in early atherosclerotic lesions.⁴⁸

CCs, which are the major driver of atherosclerosis, are now regarded as the most important trigger for NLRP3 inflammasome activation.⁹ CCs provide both priming and activating signals for the NLRP3 inflammasome. Importantly, HDL can blunt NLRP3 inflammasome activation and interleukin-1 β production incited by CCs in cultured macrophages and in mouse peritonitis model.⁴⁷ Although more work is needed to unveil the precise mechanism of action, HDL appears to function by antagonizing the transcription of NLRP3 and interleukin-1 β , the activation of caspase-1, and lysosomal damage imposed by CCs.⁴⁷ These findings highlight the significance of HDL in the regulation of the NLRP3 inflammasome. Further study is required to explore the impact of HDL on NLRP3 priming.

Extensive studies have been conducted to uncover the mechanisms whereby CCs activate the NLRP3 inflammasome. As discussed below, CCs initiate inflammation via triggering the activation of the NLRP3 inflammasome through multiple mechanisms (Figure 3).

CCs prime macrophage NLRP3 inflammasome in atherosclerosis through inciting the formation of NETs, a process known as NETosis.⁴⁰ NETosis has been implicated in atherothrombosis.⁵⁷ NETs are large extracellular weblike structures containing DNA, histones, proteases, and myeloperoxidase,⁵⁸ which have been discovered in atherosclerotic plaques.⁵⁹ There is a potential link between the NLRP3

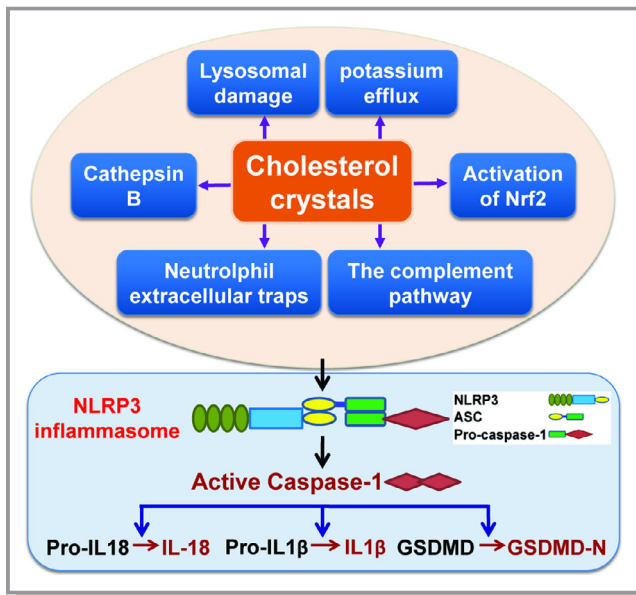


Figure 3. Mechanisms underlying the activation of the NLRP3 inflammasome by cholesterol crystals. The causative role for cholesterol in vascular inflammation and atherosclerosis is undeniable, yet the precise mechanism by which cholesterol elicits the inflammatory response and dictates atherogenesis remained mysterious. The newly identified mechanisms whereby cholesterol crystals license the activation of the NLRP3 inflammasome are summarized. GSDMD-N indicates the amino terminus of GSDMD; IL-18, interleukin-18; IL1 β , interleukin-1 β ; Nrf2, nuclear factor E2-related factor 2.

inflammasome and plaque NETs. A recent study indicated that CCs promote the release of NETs by neutrophils, which further triggers the activation of the NLRP3 inflammasome in lesional macrophages.⁴⁰ The mechanism whereby NETs activate the NLRP3 inflammasome needs to be delineated in the future. CCs induce NETosis likely through inflammasome activation.⁶⁰ However, the finding that the NETs cause the activation of the NLRP3 inflammasome is under debate.⁶¹ Further uncovering the mechanisms linking CCs to NETs' release may lead to the development of novel therapeutics specifically targeting atherosclerotic inflammation.⁶⁰

It is well demonstrated that CCs fuel NLRP3 inflammasome activation by inducing lysosomal damage. CC uptake or formation in macrophages induces lysosomal damage and ensuing NLRP3 inflammasome activation, leading to the production of atherogenic cytokines interleukin-1 β and interleukin-18.^{9,36} CCs can be degraded in macrophage lysosomes.⁶² Inefficient solubilization of CCs causes lysosomal dysfunction,⁶² which may interfere with autophagy.⁶³ Autophagy has been reported to restrain the activity of the NLRP3 inflammasome by promoting their degradation in lysosomes.⁶⁴ Macrophage autophagy plays an atheroprotective role by abrogating NLRP3 inflammasome activation incited by CCs. Defective autophagy potentiates CCs-mediated

inflammasome activation in macrophages.⁶³ Consistent with this proposition, macrophages loaded with atherogenic lipids showed an increase in the autophagy chaperone p62, a response that is caused predominantly by disruption of the autophagy-lysosome system,⁶⁵ boosting the formation of the NLRP3 inflammasome in atherosclerotic macrophages.⁶⁵ ApoE^{-/-} mice with autophagy deficiency in macrophages exhibited enhanced inflammasome activation in macrophages and enlarged atherosclerotic plaques with increased the content of CCs.⁶³ Further support for this proposition comes from a gain-of-function study carried out by forced expression of the transcription factor EB, which is known as the master regulator of lysosomal biogenesis that elicits the expression of lysosomal and autophagy genes in response to lysosomal stress.⁶⁶ As expected, overexpression of transcription factor EB was shown to rescue CC-mediated lysosomal dysfunction and to block interleukin-1 β secretion.⁶⁶ Additionally, cathepsin B and potassium efflux are required for CC-triggered activation of the NLRP3 inflammasome.⁶⁷

CCs can trigger the activation of the NLRP3 inflammasome through a mechanism associated with the oxidative stress-responsive transcription factor Nrf2.⁶⁸ Mechanistically, CCs trigger Nrf2-dependent interleukin-1 β expression in macrophages in a manner that is dependent on NLRP3 inflammasome-mediated caspase-1 activation.⁶⁸ Importantly, the NLRP3 inflammasome integrates inflammation and oxidative stress, both of which are known to contribute significantly to atherogenesis.

CCs trigger NLRP3 inflammasome activation by increasing CD36 expression.⁶⁹ Elevated CD36 levels potentiate oxLDL uptake into macrophages,⁶⁹ promoting intracellular cholesterol crystallization and NLRP3 inflammasome activation.³⁶ In addition, macrophages can be efficiently primed by oxLDL for CC-mediated NLRP3 inflammasome activation.⁹ These results demonstrate the dual role of oxLDL as NLRP3 priming and activating signals.

CCs can elicit the activation of the NLRP3 inflammasome by their potential to activate the complement pathways, specifically the C5a and the C5aR pathways,⁷⁰ which can be engaged to activate the NF κ B pathway.⁷⁰ C5a is important for reactive oxygen species production and caspase-1 activation. Thus, the activated complement factors provide the priming signal for the NLRP3 inflammasome and also promote CC phagocytosis.⁷⁰

Role and Mechanism for Shear Stress in Regulating Endothelial NLRP3 Inflammasome

ECs are considered as the atypical immune cells.⁷¹ ECs express pattern-recognition receptors such as Toll-like receptors and CD36 scavenger receptor, engagement of which leads to the activation of NF κ B signaling.⁷¹ ECs also express NLRs such as

NLRP3.⁷² Recent studies demonstrated that the activation of the NLRP3 inflammasome in ECs occurs in response to diverse insults such as disturbed flow.²² Mimicking disturbed flow and oscillatory shear stress substantially augments the production of active caspase-1 and interleukin-1 β in ECs.²² In line with the in vitro finding, the activation of the NLRP3 inflammasome is evident in mouse aortic arch, as evidenced by elevated levels of active caspase-1 and interleukin-1 β . A study showed that macrophage-derived microparticles increase the expression of cell adhesion molecules in ECs via the activation of endothelial NLRP3 inflammasome.⁷³ In addition to provoking the processing of caspase-1 and proinflammatory cytokines, activated caspase-1 triggers pyroptosis, the inflammatory cell death that appears to precipitate the development of atherosclerosis.^{22,72}

In contrast to the well-defined model for inflammasome activation in macrophages, much less is known regarding the 2-step mechanism underlying NLRP3 inflammasome activation in ECs. SREBP2 (Sterol regulatory element-binding protein 2) was identified as a potent activator of the NLRP3 inflammasome in ECs. SREBP2 is known as a master regulator in cholesterol biosynthesis.⁷² In support of the above finding, SREBP2 exacerbates the initiation and progression of atherosclerosis. Intriguingly, disturbed flow-activated SREBP2 can induce the NLRP3 inflammasome via transactivation of NLRP3 in ECs.²² Taken together, SREBP2 is involved in both priming and activation of the NLRP3 inflammasome. A recent study pointed out that SREBP2 is a transcription factor that regulates tumor necrosis factor- α receptor-associated factor (TRAF)-interacting protein with a forkhead-associated domain (TIFA).⁷⁴ TIFA was shown to activate NF κ B signaling, at least partly, through interacting with tumor necrosis factor- α receptor-associated factor 2 or tumor necrosis factor- α receptor-associated factor 6.⁷⁵ Remarkably, TIFA is involved in the induction of inflammasome components through the SREBP2-TIFA-NF κ B axis.⁷⁴ Notably, oxidative and/or inflammatory stimuli, including oscillatory shear stress, tumor necrosis factor- α and oxLDL, elicit a robust activation of Akt, which can target TIFA for phosphorylation. Phosphorylated TIFA undergoes oligomerization and subsequently interacts with caspase-1, thereby potentiating the assembly and activation of the NLRP3 inflammasome. Collectively, TIFA is involved in both priming and activation of the NLRP3 inflammasome in ECs.⁷⁴

Role and Mechanism for the NLRP3 Inflammasome in Tet2 Somatic Mutation-Driven Atherosclerosis

Although the contribution of hypercholesterolemia to atherosclerosis is undeniable, the impact of hypercholesterolemia on cardiovascular risk, however, was found to

reduce gradually with aging.⁷⁶ Recent studies showed that most individuals at low cardiovascular risk were affected with substantial subclinical atherosclerosis.⁷⁷ Furthermore, emerging evidence suggests that unidentified age-related factors contribute to the development of atherosclerosis.⁷⁸ Accumulated evidence emphasizes that somatic mutations in hematopoietic cells may drive atherosclerotic CVD.⁷⁹ The somatic mutation may confer a competitive advantage to the cell, leading to clonal expansion,⁸⁰ which is particularly true for the hematopoietic stem/progenitor cell. Somatic mutation-driven clonal hematopoiesis, whose role in the development of atherosclerosis is poorly defined, is common in the elderly population.⁸¹ The impact of somatic mutation-driven clonal hematopoiesis on atherosclerosis is attracting increasing attention. Most somatic mutations associated with clonal hematopoiesis occur in 4 genes: tet methylcytosine dioxygenase 2 (TET2), DNA methyltransferase 3 α (DNMT3A), additional sex combs like 1 and Janus kinase 2.⁸² However, their relevance in CVD remained unexplored until a recent discovery demonstrating the causative role of somatic mutation-driven clonal hematopoiesis in exacerbating atherosclerosis.^{83,84}

Ldlr^{-/-} mice acquired a small number of Tet2^{+/-}, Tet2^{-/-}, or myeloid cell-specific Tet2^{-/-} cells through competitive bone marrow transplantation, which largely recapitulates the scenario of clonal hematopoiesis linked to somatic mutations in human hematopoietic Tet2. These cells expanded progressively in bone marrow with a preferential differentiation into the Ly6C^{hi} monocyte population.⁸⁴ The clonal hematopoiesis robustly accelerated atherosclerosis.⁸⁴ These findings were independently replicated in mice with full hematopoietic ablation of Tet2.⁸³ Mechanistically, somatic mutation or defect of Tet2 leads to the overproduction of proinflammatory cytokines (particularly interleukin-1 β) in macrophages.⁸³⁻⁸⁵ Tet2 is known as a DNA demethylating enzyme that enhances transcriptional activation by catalyzing the oxidation of 5-methylcytosine in DNA to 5-hydroxymethylcytosine.⁸⁴ Intriguingly, Tet2 suppresses pro-interleukin-1 β transcription through nucleating histone deacetylases to its promoter region, a function that is independent of Tet2 catalytic activity.⁸⁴ More importantly, Tet2 deficiency promotes NLRP3 inflammasome activation, facilitating the processing of pro-interleukin-1 β .⁸⁴ In support of this finding, pharmacologic blockade of the NLRP3 inflammasome significantly ameliorated atherosclerosis development incited by somatic mutation in Tet2. These observations highlight the fundamental importance of the NLRP3 inflammasome in mediating Tet2 mutation-driven atherosclerosis.⁸⁴ Excessive interleukin-1 β production by Tet2-deficient cells promotes the expression of P-selectin and the activation of ECs in the plaque, leading to increased monocyte recruitment to the lesion.⁸⁴ Since interleukin-1 β is known to stimulate its own expression,⁴³ it

is likely that overproduction of interleukin-1 β by Tet2-deficient cells promotes further expression of interleukin-1 β in both Tet2-deficient and wild-type cells. This finding demonstrated that the recruitment of small number of Tet2-mutant cells in the plaque may be adequate to fuel atherosclerosis by promoting NLRP3/pro-interleukin-1 β -driven vascular inflammation.⁸⁴ It is reasonable to speculate that individuals carrying somatic mutations in Tet2 may respond much more favorably than general population to NLRP3/interleukin-1 β -targeted therapeutic, which will furnish the basis for a personalized therapy for atherosclerotic CVD patients with somatic mutations in Tet2.

Mechanism for the NLRP3 Inflammasome to Drive Atherosclerosis

Since the NLRP3 inflammasome plays critical roles in the initiation and progression of atherosclerosis, it is important to unveil how NLRP3 inflammasome activation drives atherogenesis. The link between NLRP3 inflammasome activation and atherosclerosis remains to be completely elucidated. It has been well established that NLRP3 inflammasome activation results in the maturation of interleukin-1 β and interleukin-18, both of which are considered to be the major contributors of atherogenesis.^{86,87} Both cytokines have been documented to be highly expressed in human atherosclerotic plaques compared with normal arteries and positively correlated to disease severity.^{88,89} Interleukin-1 β deficiency ameliorates atherosclerosis in ApoE^{-/-} mice,²⁵ while atherosclerotic lesion size in mice with partial deletion of interleukin-1R antagonist (interleukin-1Ra) (interleukin-1Ra^{+/-}/ApoE^{-/-}) is significantly elevated compared with that in interleukin-1Ra^{+/+}/ApoE^{-/-} mice.⁹⁰ Notably, interleukin-1Ra is the structural homologue of interleukin-1 that competes with interleukin-1 for binding to the interleukin-1R but fails to initiate interleukin-1R activation.⁹¹ Likewise, genetic ablation of interleukin-18 mitigated the development of atherosclerosis, whereas administration of recombinant interleukin-18 significantly increased the size of the atherosclerotic lesions in hypercholesterolemic mice.^{86,92} These findings indicate that interleukin-18 plays a proatherogenic role in the development of atherosclerosis.

In addition to inducing the production of interleukin-1 β and interleukin-18, the activation of the NLRP3 inflammasome boosts the migratory capacity of macrophages and augments lipids deposition in lysosomes in macrophages.⁹³ These events facilitate entry of macrophages into the arterial wall, stimulate foam cell formation, and ultimately promote atherosclerosis.⁹³ The major role and mechanism for interleukin-1 β to drive vascular inflammation and atherosclerosis are outlined in Figure 4.

Interleukin-1 β is a primordial proinflammatory cytokine⁹⁴ and has been shown to be involved in a broad spectrum of inflammatory disorders.⁹⁵ The experimental and clinical evidence demonstrates interleukin-1 β as both a local vascular and systemic contributor to atherogenesis.⁴³ Numerous studies support causality of interleukin-1 β in coronary heart disease. Interleukin-1 β exerts its functions through the autocrine, paracrine, or endocrine mechanisms.⁹⁴

Interleukin-1 β is the apical proinflammatory mediator and among the most powerful inducers of innate immunity.⁹⁵ Interleukin-1 β induces its own gene expression in various cell types that are major driver of atherogenesis, an amplification loop termed *autoinduction*.⁴³ Also, interleukin-1 β triggers and/or amplifies endothelial dysfunction.⁴³ Interleukin-1 β stimulates the expression of adhesion molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Interleukin-1 β incites the expression of chemokines such as monocyte chemoattractant protein-1.⁴³ Accordingly, interleukin-1 β promotes leukocyte adhesion to vascular ECs and leads to procoagulant activity and recruitment of leukocytes to the lesions.⁹⁶ Interleukin-1 β also boosts the recruitment of neutrophils to atherosclerotic lesions.⁹⁷ Neutrophils are present in all stages of atherosclerotic plaque promoting atherogenesis and plaque rupture.⁹⁸ Interleukin-1 β stimulates the production of NETs in a vicious circle.⁹⁹

Interleukin-1 β lies in the upstream in the pathway and is central in shaping the proinflammatory response by strongly inducing diverse cells, including smooth muscle cells to elaborate secondary inflammatory mediators involving interleukin-6.^{43,100} Interleukin-6 acts as the primary cytokine to incite the acute phase response with hepatic production of C-

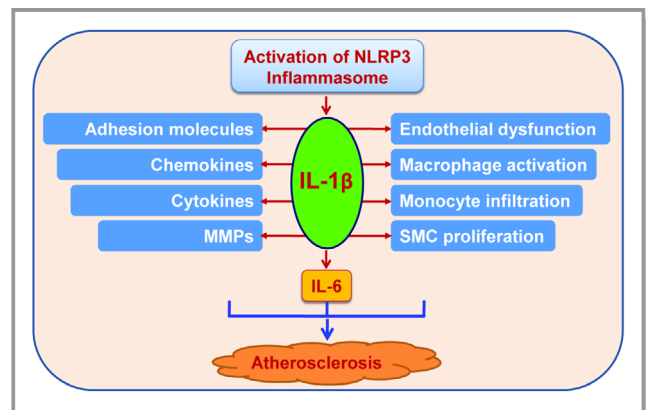


Figure 4. Selected functions of interleukin-1 β linked to atherosclerosis. Interleukin-1 β acts on different cell types and organs, including those involved in atherosclerosis such as endothelial cells, vascular smooth muscle cells, macrophages and the liver. Interleukin-1 β exerts diverse biochemical and biological functions shown in autocrine, paracrine, and endocrine manners. IL-1 β indicates interleukin-1 β ; IL-6, interleukin-6; MMPs, matrix metalloproteinases; SMC, smooth muscle cell.

reactive protein, a risk marker for atherothrombosis.¹⁰⁰ Accordingly, interleukin-1 β is critical for the activation of the humoral arm of innate immunity. Interleukin-1 β also has multiple effects on smooth muscle cells.⁴³ For example, interleukin-1 β can induce the production of platelet-derived growth factor, a crucial growth factor that can stimulate the proliferation of smooth muscle cells.⁴³

NLRP3 inflammasome activation causes pyroptosis, a proinflammatory programmed cell death that stimulates the pathological ion efflux and release of inflammatory substances, further amplifying local inflammation.¹⁰¹ Recent studies demonstrated that mature caspase-1 mediates proteolytic cleavage of gasdermin D, which in turn triggers pyroptosis through formation of membrane pores.¹⁰² Therefore, caspase-1-dependent pyroptosis may very well participate in atherosclerosis.

Targeting the NLRP3 Inflammasome for Treatment of Atherosclerotic Disease

Elevated levels of blood cholesterol, more precisely LDL cholesterol, account for the major risk factor and have been causally linked to the occurrence of atherogenesis.¹⁰³ To date, LDL cholesterol-lowering statins remain the mainstay for treatment of atherosclerosis. However, atherosclerotic plaques still undergo progression to a great extent in a large proportion of individuals whose blood cholesterol levels dramatically decline upon treatment with cholesterol-lowering drugs.¹⁰⁴ Thus, a large burden of residual disorder in individuals treated with statins demonstrates an unmet need for new therapies.⁴⁶ Given that atherosclerosis is a chronic inflammatory disorder, various clinical trials are currently being conducted to assess anti-inflammatory agents for atherosclerosis treatment. Both LDL cholesterol and C-reactive protein should be measured to evaluate an individual's residual cholesterol or inflammatory risk for recurrent cardiovascular events. The patients will definitely benefit from personalized biomarker-based therapeutic approach.

Numerous studies have established the critical role for the NLRP3 inflammasome in driving atherogenesis.^{9,72} From a translational point of view, the NLRP3 inflammasome is an attractive drug target for atherosclerosis. Indeed, targeting the NLRP3 inflammasome or its products in susceptible patients is emerging as an important topic in atherosclerosis field. Because of its well-demonstrated causality in atherosclerosis,¹⁰⁵ interleukin-1 β has been selected as a valuable therapeutic target for atherosclerosis. Canakinumab is a human monoclonal antibody that can antagonize the interaction of interleukin-1 β with interleukin-1R by binding interleukin-1 β , which blunts subsequent proinflammatory signaling events.¹⁰⁵ The CANTOS (Canakinumab Anti-inflammatory Thrombosis

Outcomes Study) trial evaluated the impact of canakinumab on the occurrence/recurrence of cardiovascular events among 17 200 patients with stable coronary artery disease.¹⁰⁵ Compared with placebo, canakinumab treatment reduced the risk of major adverse cardiovascular events (including myocardial infarction, stroke, and cardiovascular death).¹⁰⁵ Notably, administration of canakinumab significantly reduces plasma inflammatory markers (such as C-reactive protein and interleukin-6) without affecting the levels of LDL cholesterol or HDL cholesterol.¹⁰⁵ Statin-treated patients with residual inflammatory risk will benefit from canakinumab administration. Importantly, canakinumab selectively inhibits interleukin-1 β , while leaving other interleukin-1 family members unaffected. In this regard, long-term treatment with canakinumab might be safer compared with treatment with anakinra, a recombinant interleukin-1R antagonist.

The data from the CANTOS trial provide strong support for the critical role of the NLRP3 inflammasome in the initiation and progression of atherosclerosis. However, caution should still be used since targeting inflammatory pathway systemically may bear the risk of interfering with immune homeostasis. Direct targeting the NLRP3 inflammasome is expected to mitigate local inflammatory responses through the retardation of maturation and secretion of interleukin-1 β and interleukin-18 as well as by the suppression of pyroptotic cell death. It is tempting to hypothesize that direct intervention of NLRP3 inflammasome function may be beneficial for reducing cardiovascular risk. For instance, arglabin, a natural product that has an anti-inflammatory capacity, restrains atherosclerotic development in atherosclerosis-prone mice.¹⁰⁶ Mechanistically, arglabin impedes the activation of the NLRP3 inflammasome in macrophages.¹⁰⁶ Consistent with this observation, arglabin administration led to the reduction of plasma interleukin-1 β levels.¹⁰⁶ This study indicated that inhibition of the NLRP3 inflammasome potently restricts the initiation and progression of atherosclerosis, making the NLRP3 inflammasome a promising therapeutic target for the treatment of atherosclerosis. In addition, remarkable attempts were made with atorvastatin¹⁰⁷ and colchicine¹⁰⁸ to restrain atherosclerosis by targeting the NLRP3 inflammasome. There is a considerable interest in developing small molecule inhibitors for the NLRP3 inflammasome.¹⁰⁹ MCC950, a potent and selective inhibitor of the NLRP3 inflammasome,¹¹⁰ dramatically prevents the production of mature interleukin-1 β upon exposure of macrophages and dendritic cells to lipopolysaccharide and CCs.¹¹⁰ Strikingly, this work showed that MCC950 ameliorates the development of atherosclerotic lesions,¹¹⁰ highlighting the therapeutic potential of these small molecule inhibitors for CVD.

NLRP3 inflammasome becomes activated in response to several key triggers (in particular CCs) that have been well characterized to fuel the development of atherosclerosis. An

alternative strategy for the treatment of atherosclerosis is to remove the atherogenic NLRP3 inflammasome triggers. Importantly, animal studies showed that 2-hydroxypropyl- β -cyclodextrin, a cholesterol-solubilizing substance, blunts vascular CCs deposition, mitigates atherosclerotic development and promotes the regression of atherosclerosis.¹¹¹ In addition to removing CCs, 2-hydroxypropyl- β -cyclodextrin induces cholesterol efflux and reverse cholesterol transport.¹¹¹ These findings highlight that removing the trigger of the NLRP3 inflammasome is a valuable therapeutic approach to intervention of atherosclerosis development. Targeting the NLRP3 inflammasome will usher in a new era of anti-inflammatory therapies for atherosclerosis.

Concluding Remarks and Future Perspectives

Years of studies have brought rapid progress in our understanding of NLRP3 inflammasome biology. While some old mysteries remain unsolved, these developments have raised new questions. Unraveling the regulation and molecular mechanisms responsible for NLRP3 inflammasome activation is critical for improving our understanding of the pathogenesis of atherosclerosis. To provide reliable therapeutic strategies for atherosclerosis that target the NLRP3 inflammasome, extensive studies need to delineate the molecular mechanisms for this inflammasome. Obviously, our understanding of NLRP3 inflammasome activation needs to be integrated with information for the molecular structure of the NLRP3 inflammasome. These advances and questions set the stage for future studies to achieve unprecedented understanding of the NLRP3 inflammasome at a structural and biochemical level. We have just begun to understand the negative regulators and their mechanisms that finely control and prevent excessive inflammasome activation (for a detailed review, see Broz and Dixit¹¹²). Extensive analysis of the negative regulators and signals should help us manipulate NLRP3 inflammasome activation therapeutically. Identifying and characterizing specific binding partners modulating NLRP3 inflammasome activation in vitro and in vivo may be interesting and challenging.

These advances in the NLRP3 inflammasome are transforming the field of atherosclerosis. How will this newly obtained knowledge be translated into treatment for atherosclerosis? CCs license the activation of the NLRP3 inflammasome and thereby trigger vascular inflammation, governing the initiation and progression of atherosclerosis. Understanding how CCs and other danger signals can induce the activation of the NLRP3 inflammasome remains a topic of considerable interest. Given that the formation of CCs occurs even at early stage of atherosclerotic lesions, CCs are now

regarded as one of the initiating inflammatory insults for atherosclerosis. In the future, targeting cholesterol crystallization is a promising therapeutic approach for atherosclerosis and is worthwhile to investigate. We are entering an exciting era in which we may reap therapeutic benefits from mechanistic findings in the NLRP3 inflammasome and inflammatory pathways in atherosclerosis. However, the extrapolation of discoveries from a mouse model to human disease always requires caution. In addition, it will be important to define the interplay between NLRP3 inflammasome signaling with other inflammatory pathways involved in atherosclerotic development.

Somatic mutation in TET2 is the first to be causally linked to atherosclerotic CVD.^{83,84} It has been appreciated that somatic mutations of additional genes, as exemplified by DNA methyltransferase 3 α , additional sex combs like 1, and Janus kinase 2, may be implicated in atherosclerosis.⁸³ Based upon their unique functions and underlying mechanisms, it is plausible to speculate that these mutated genes may differentially impact atherosclerotic CVD. Future studies will need to uncover the contribution of somatic mutations in these genes to atherosclerotic CVD.

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Disclosures

None.

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