



## REVIEW

# Inflammasomes and Atherosclerosis

S. Vallurupalli, MD<sup>1,2</sup>, Yao Dai, MD, PhD<sup>1,2</sup> and J. L. Mehta, MD, PhD<sup>1,2</sup>

<sup>1</sup>Division of Cardiology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

<sup>2</sup>Central Arkansas Veterans Healthcare System, Little Rock, AR, USA

## Abstract

Inflammation plays an important role in atherosclerosis. Inflammasomes play a crucial role in innate immunity, which mediates the body's response to various pathogens. Of the different types of inflammasomes, NLRP3 has been implicated in atherosclerosis through the production of proinflammatory cytokines, IL-1 $\beta$  and IL-18. This review describes the role of the NLRP3 inflammasome in atherosclerosis and discusses potential therapeutic targets in the inflammasome pathway.

**Keywords:** inflammasomes; NLRP3; caspase 1; interleukins; atherosclerosis

## Introduction

Atherosclerosis is an inflammatory disorder. Inflammation plays a major role in the initiation and progression of atherosclerosis and leads to potentially life-threatening clinical manifestations via plaque rupture [1]. Inflammasomes represent a distinct form of innate immunity. These cytosolic protein complexes are formed in response to offending proteins and lead to the production of proinflammatory enzymes such as caspase 1, resulting in activation of proinflammatory cytokines such as IL-1 $\beta$  and IL-18; their activation results in cellular apoptosis [2]. Since the discovery of inflammasomes in 2002, there has been an explosion in our knowledge of the importance of inflammasomes in chronic inflammatory diseases, especially atherosclerosis.

The goal of the current review is to present the current evidence for the role of inflammasomes, especially NLRP3, in atherosclerosis.

## Overview of Inflammation in Atherosclerosis

Inflammation plays an important role in all phases of atherosclerosis – from initiation to its clinical manifestations such as plaque rupture [1, 3, 4]. When the normal endothelium is exposed to an atherogenic milieu, there is production of reactive oxygen species and reduced availability of nitric oxide. This results in expression of cell adhesion molecules, such as vascular cell adhesion molecule 1 and selectins, which recruit blood monocytes and lymphocytes into the atherosclerotic plaque. Selectins and integrins mediate increased adhesion of leukocytes to the activated endothelial cells. Under the influence of chemoattractant proteins such as monocyte chemoattractant protein 1 and macrophage colony stimulating factor, the adherent monocytes migrate into the intima and transform into macrophages. These macrophages transform into foam cells by uptake of modified LDL particles such as oxidized LDL (ox-LDL). T lymphocytes that are recruited into the plaque secrete cytokines and growth factors that promote the migration and proliferation of smooth muscle cells (SMCs). Repeated cycles

---

**Correspondence:** S. Vallurupalli, MD, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA, E-mail: Svallurupalli@uams.edu

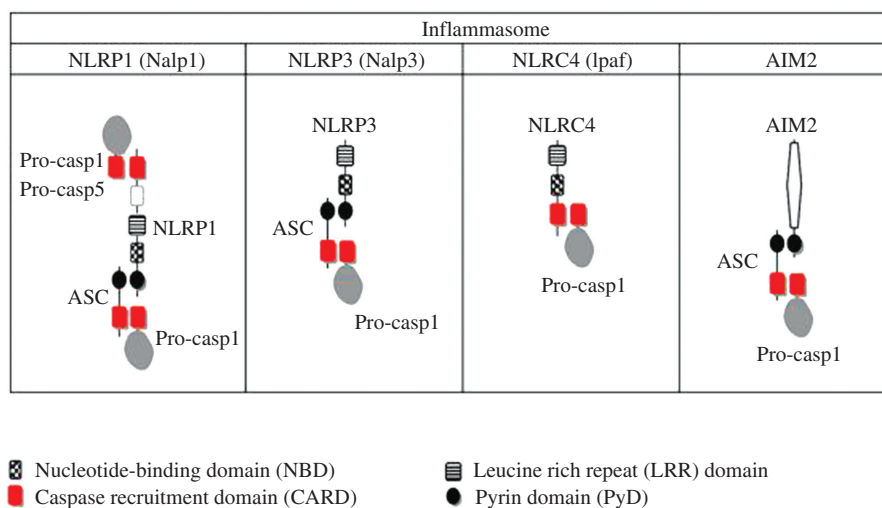
of apoptosis and cell death lead to accumulation of cellular debris with enlargement of the lipid core, and the atheromatous plaque grows. Medial SMCs and foam cells secrete matrix metalloproteinases, which degrade elastin and collagen. This leads to weakening of the plaque, especially at its shoulders, which makes it prone to rupture. When the plaque ruptures, the presence of tissue factor promotes local thrombus formation and leads to the clinical presentation of acute coronary syndromes. This simplified explanation of the atherosclerotic process underscores the vital role played by inflammation. Current thinking suggests that atherosclerosis is a result of such “sterile” inflammation.

## Overview of Inflammasomes

Inflammation is mediated by two major pathways: innate and adaptive immunity. Innate immunity provides a “fast and blunt” response to proinflammatory stimuli, while the response is “slower and more precise” with adaptive immunity. Inflammasomes are multimeric cytosolic proteins that assemble when molecular patterns that are perceived as a threat are recognized by germline-encoded pattern recognition receptors (PRRs) [5]. These molecular patterns could be either associated with pathogens (pathogen-associated molecular patterns) or proteins associated with cell damage (damage-associated molecular patterns) [6]. PRRs are expressed both in the cell membrane (e.g., Toll-like receptors and

C-type lectin receptors) and in the cytoplasm (e.g., Nod-like receptors) [7].

Figure 1 describes the basic structure of the four well-described inflammasomes (NLRP1, NLRP3, NLRC4, and AIM2). The multimer consists of three basic structural units: (1) a PRR (pathogen-associated molecular pattern or damage-associated molecular pattern), (2) an effector (IL-1 $\beta$ - and IL-18-processing system, i.e., caspase 1), and (3) a coupling protein (the adaptor protein apoptosis-associated speck-like protein containing a carboxy-terminal caspase activation and recruitment domain [CARD], ASC) [9]. ASC is common to all inflammasomes (except NLRP4, which recruits procaspase 1 using CARD alone) and contains two death-fold domains – a pyrin domain and a CARD. The pyrin domain interacts with PRRs and triggers the formation of large ASC multimers. CARD (present both in ASC and in NLRC4 and NLRP1, where it directly recruits procaspases 1 and 5) initiates the recruitment of procaspase 1 to the inflammasome and mediates its cleavage to caspase 1. This oligomerization requires ATP and low intracellular potassium concentrations [10]. The end product, caspase 1, is a cysteine protease that mediates a form of cell death called “pyroptosis” and proteolytically cleaves pro-IL forms to active IL-1 $\beta$  and IL-18 [5]. A comprehensive description of these inflammasomes is available elsewhere. Of these, NLRP3 will form the basis of this review because of its role in atherosclerosis.



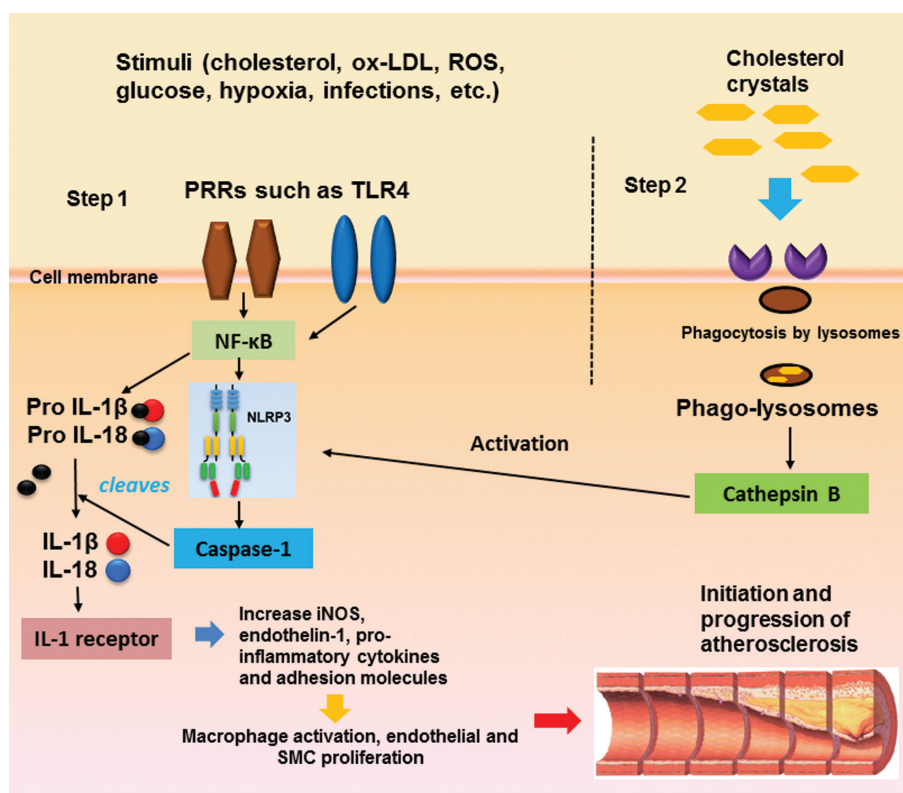
**Figure 1** Basic Structure of Four Well-Characterized Inflammasomes. Reproduced from [8] with permission from Dr. Hiroko Tsutsui.

## NLRP3 Inflammasome and Atherosclerosis

The NLRP3 inflammasome is the most extensively studied inflammasome. It has been implicated in the pathogenesis of several disorders, such as gout, rheumatoid arthritis, type 2 diabetes mellitus, and atherosclerosis [5]. Activation of NLRP3 is a two-step process as illustrated in Figure 2. A priming step consists of activation of nuclear factor  $\kappa$ B by activation of cell membrane PRRs such as Toll-like receptor 4 [8]. This leads to induction of proforms of IL-1 family cytokines (pro-IL-1 $\beta$  and pro-IL-18) as well as NLRP3, and sets the stage for the second step. The second step consists of the phagocytosis of sterile crystalline substances by lysosomes, which transform into phagolysosomes and result in production of cathepsin B; this process initiates the oligomerization of the NLRP3 inflammasome and caspase 1 production [11, 12]. Caspase 1 cleaves the pro-IL-1 forms to active IL-1 $\beta$  and IL-18. IL-1 $\beta$  is often considered the “gatekeeper

of inflammation” [13]. Through activation of IL-1 type I receptor, it mediates a proinflammatory state characterized by increased production of inducible nitric oxide synthase, endothelin 1, and other pro-inflammatory chemokines, cytokines, and adhesion molecules. This results in macrophage activation as well as endothelial and SMC proliferation, leading to progression of atherosclerosis. The role of IL-1 $\beta$  in atherosclerosis has long been established [14, 15]. In apolipoprotein E (ApoE)-deficient mice, lack of IL-1 $\beta$  is the basis for reduction in the severity of atherosclerosis [15]. Carotid ligation induces less neointimal thickening in IL-1 $\beta$ -deficient mice than in controls [14]. IL-18 is widely considered a circulating biomarker of atherosclerosis and enhances atheromatous plaque progression through interferon- $\gamma$  production [16]. However, it took the discovery of inflammasomes to understand how certain cardiac risk factors such as hyperlipidemia result in increased IL production.

Crystalline substances such as urate crystals, silica, and alumina have long been known to initiate



**Figure 2** NLRP3 Activation in Atherosclerosis is a Two-Step Process.

The priming step (step 1) leads to production of nuclear factor  $\kappa$ B and proforms of IL-1 and IL-18. The second step is mediated by phagocytosis of substances such as crystalline cholesterol and leads to production of caspase 1, which cleaves the inactive forms to active forms.

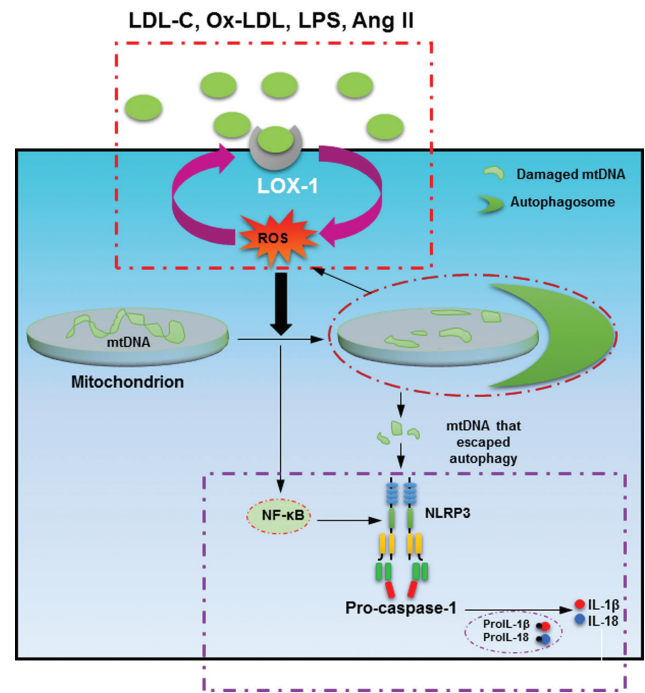
the second step of NLRP3 inflammasome activation. Duewell et al. [11] made the seminal observation that cholesterol crystals lead to activation of inflammasomes. Recent studies from our laboratory suggest that xanthine oxidase, the action of which results in the formation of uric acid, can induce foam cell formation in macrophages and SMCs, and in this process activation of lectin-type ox-LDL receptor 1 (LOX-1), a and C-type lectin receptor, plays a crucial role (unpublished observations).

Both extracellular and intracellular cholesterol crystals can result in NLRP3 activation. Exposure of human macrophages to ox-LDL has long been known to be a potent activator of NLRP3 [17]. This is mediated by endocytosis of ox-LDL by the scavenger receptor CD36 [18]. Ox-LDL then directly primes NLRP3 activation by production of pro-IL forms (priming signal) as well as nucleates to intracellular cholesterol crystals, resulting in activation of NLRP3 (second signal). The process of formation of intracellular cholesterol crystals in human macrophages is now well understood [19]. Cholesterol exists mostly in the arterial wall (as LDL particles) in a esterified form. When LDL is oxidized and assimilated by macrophages, cholesterol ester hydrolase converts esterified cholesterol to free cholesterol, whereas acyl coenzyme A cholesterol acyltransferase 1 converts free cholesterol back to esterified cholesterol, thus maintaining an equilibrium. Disruption in cholesterol homeostasis leads to the formation of crystals of free cholesterol within the macrophage and activates inflammasomes.

Recent studies from our laboratory suggest that NLRP3 mediates the deleterious effects of the activation of LOX-1 [20, 21]. In cultured human macrophages, LOX-1 inhibition with a binding antibody or small interfering RNA resulted in decreased expression of the NLRP3 inflammasome and thus inhibited reactive oxygen species generation, autophagy, and mitochondrial DNA damage [20]. This is confirmed by reduced mitochondrial DNA and reduced NLRP3 activation in LOX-1-knockout mice (Figure 3) [21].

In addition to cholesterol crystals, other lipid and nonlipid signals modulate NLRP3-mediated inflammation, especially in the context of atherosclerosis:

1. Electronegative LDL, a modified LDL fraction, has been shown to promote the release of IL-1 $\beta$



**Figure 3** Lectin-Type Oxidized LDL Receptor 1 (LOX1) Mediates Inflammasome Activation Through Damaged Mitochondrial DNA.

- through both the priming step and the activation step of NLRP3 [22].
2. ATP is secreted by activated or injured endothelial cells, leukocytes, and platelets, and then acts in a paracrine manner to transduce sterile inflammatory signals [23]. This transduction is likely mediated by NLRP3. When exposed to ATP, macrophages from wild-type mice demonstrate NLRP3 activation along with increased lipid deposition in lysosomes and enhanced migration ability [24].
3. Damaged mitochondrial DNA is a common by-product of autophagy and can induce inflammation via NLRP3 activation [20, 21].
4.  $\beta$ -Hydroxybutyrate, a ketone that occurs during starvation, has been shown to reduce inflammasome activation by reducing potassium efflux [25]. Although its effects on atherosclerosis are unknown, it may explain the anti-inflammatory basis of the ketogenic diet.
5. NLRP3 expression may also mediate the atherogenic effects of cellular hypoxia within the atheromatous plaque [26]. Hypoxia is commonly present because of reduced diffusion as well as increased consumption within the plaque.



Cellular hypoxia within the plaque promotes foam cell and necrotic core formation as well as catabolism of extracellular matrix by inducing matrix metalloproteinases. In activated human macrophages, Folco et al. [26] showed that moderate hypoxia also increases the expression of NLRP3, caspase 1 and IL-1 $\beta$ .

Finally, the role of the NLRP3 inflammasome may extend beyond its proinflammatory effects. NLRP3 inflammasomes may also alter macrophage function and alter lipid deposition, which may increase their susceptibility to form foam cells. ASC gene (*PYCARD*) deletion markedly abolished NLRP3 inflammasome activation, attenuated lysosomal lipid deposition, and decreased macrophage migration ability [24].

### Evidence Linking the NLRP3 Pathway to Atherosclerosis and Associated Disease States

In the landmark study by Duewell et al. [11], bone marrow was transplanted from either wild-type or NLRP3-knockout mice into LDL receptor-deficient mice. Despite similar cholesterol levels, the components of NLRP3 inflammasomes, the levels of the cytokines IL-1 and IL-18, and the extent of early atherosclerosis were markedly decreased in mice that received the NLRP3-deficient bone marrow. Silencing of NLRP3 also suppresses atherosclerosis and stabilizes plaques in ApoE-deficient mice [27].

There is growing evidence from human studies that supports this mechanism of development of atherosclerosis. NLRP3 inflammasomes are highly expressed in aorta of patients with atherosclerosis undergoing coronary artery bypass grafting and correlate significantly with traditional risk factors such as LDL as well as the severity of coronary artery disease [28]. In a cohort of 123 patients with acute coronary syndrome, peripheral blood monocyte NLRP3 levels were shown to correlate with the severity of coronary atherosclerosis and were a predictor of major adverse cardiac events in a Cox regression model ( $P=0.043$ ) [29]. Components of the NLRP3 pathway are highly expressed in carotid plaque of patients undergoing carotid endarterectomy, with significantly higher

levels in unstable plaque compared with stable plaque [30]. Single nucleotide polymorphisms in NLRP3 have been associated with the presence of abdominal aortic aneurysms, another manifestation of atherosclerosis [31].

### Inflammasomes as Therapeutic Targets

The discovery of inflammasomes thus provides insight into the mechanism that links hyperlipidemia to inflammation, especially the IL-1 $\beta$  pathway. The finding that cholesterol crystals initiate inflammation supports the two major therapeutic strategies already used to combat atherosclerosis: diet and 3-hydroxy-3-methylglutaryl coenzyme A receptor antagonists. Although lipid deposition in the plaque has long been considered a consequence of inflammation and cell death, activation of inflammasomes by cholesterol crystals provides evidence that hyperlipidemia actually promotes inflammation. Intuitively, a healthy diet and lifestyle could help attenuate the effects of inflammasome activation. The effects of dietary interventions on NLRP3 have been studied in adipose tissue but not in human atherosclerosis [32]. Since adipose tissue NLRP3 levels correlate with severity of atherosclerosis [33], this can be considered indirect evidence that dietary modification can be helpful. Statins have long been known to attenuate inflammation and reduce the levels of markers such as high-sensitivity C-reactive protein [34]. This was regarded a pleiotropic effect and not necessarily related to their lipid-lowering abilities. Conceptually, lipid lowering with statins could also reduce inflammation by reducing inflammasome activation. Preliminary human data suggest that statins reduce NLRP3 activation [35, 36]. Treatment with ezetimibe, a nonstatin drug that works by inhibiting absorption of cholesterol in the intestine, has also been shown to reduce cholesterol crystal formation in rabbits and reduce serum cholesterol levels [37].

The discovery of NLRP3 has provided insight into the anti-inflammatory and antiatherosclerotic mechanism of action of other agents as well. Resveratrol is a flavanoid that has been shown to reduce inflammation; this effect may be mediated by attenuation of the NLRP3 pathway [38]. NLRP3

has also been implicated in the salutary effects of moderate alcohol intake on coronary artery disease [39]. The antiatherosclerotic effect of dipeptidyl dipeptidase 4 inhibitors (antidiabetic drugs) may also be through suppression of NLRP3 via glucagon-like peptide 1 receptor [40].

At least three major components of the inflammasome pathway – NLRP3, caspase 1, and IL-1 $\beta$ , are potential therapeutic targets.

## IL-1 $\beta$ Antagonists

IL-1 $\beta$  antagonists are already in clinical use in the treatment of diseases such as gout and rare genetic syndromes such as Muckle-Wells syndrome (an autosomal dominant disorder characterized by mutations in the *NLRP3* gene resulting in abnormal inflammatory response). The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) is designed to study the efficacy of canakinumab, an antibody that selectively inhibits IL-1 $\beta$  to reduce plaque inflammation and the risk of cardiovascular events [41]. The study will provide indirect evidence of the benefits of attenuating inflammasome formation. In addition, since canakinumab does not affect lipid parameters, the study will provide proof of the benefits of targeting inflammation in coronary artery disease [42].

## Caspase 1 Inhibition

The antiatherosclerotic effects of caspase 1 inhibition in animal models is less clear. Menu et al. [43] fed *ApoE<sup>-/-</sup>/Nlrp3<sup>-/-</sup>*, *ApoE<sup>-/-</sup>/Pycard<sup>-/-</sup>*, and *ApoE<sup>-/-</sup>/Casp1<sup>-/-</sup>* double-deficient mice a high-fat diet for 11 weeks and subsequently assessed atherosclerosis progression and plaque phenotype in comparison with *ApoE<sup>-/-</sup>* mice. No differences in atherosclerosis progression, infiltration of plaques by macrophages, or plaque stability was found [43]. However, Usui et al. [44] demonstrated reduced vascular inflammation and atherosclerosis in ApoE/caspase 1 double-deficient mice. Similarly Gage et al. [45] found a reduction in atherosclerosis despite similar lipid levels in caspase 1/ApoE double-deficient mice compared with ApoE-deficient mice. The difference in these studies has been attributed to the difference in the composition of the diet [46].

Although caspase 1 is primarily responsible for cleavage of pro-IL-1 $\beta$  intracellularly, other proteases such as neutrophil elastase can extracellularly process the pro-IL forms into active cytokines [47, 48]. Caspase 1-deficient mice have low, but comparable, amounts of circulating active IL-1 $\beta$  compared with wild-type controls after carotid ligation and do not exhibit a statistically significant reduction in neointima formation [14]. They also mount a full inflammatory response to subcutaneous turpentine, another IL-1 $\beta$ -dependent process [49]. Thus the effect of caspase 1 inhibition on atherosclerosis is uncertain.

Although several caspase 1 inhibitor molecules are under preliminary investigation in various diseases, none are in clinical trials in patients with atherosclerosis.

## NLRP3 Inhibition

Preliminary animal data suggest that inhibiting NLRP3 itself may be potentially useful. Silencing of NLRP3 suppresses atherosclerosis and stabilizes plaques in ApoE-deficient mice [27]. Arglabin, an anti-inflammasome inhibitor, has been shown to reduce inflammation and atherosclerosis in ApoE-deficient mice [46]. A novel small molecule NLRP3 inhibitor, MCC950, has been developed but has not been studied yet in atherosclerosis [50]. Various acrylamide derivatives are being developed for their anti-inflammasome activity via inhibition of NLRP3 ATPase [51].

## Conclusion

Inflammasomes play an important role in innate immunity, and the NLRP3 inflammasome plays a major role in mediating atherosclerosis. Its discovery has improved our understanding of how disorders of cholesterol metabolism contribute to inflammation. Whether targeting inflammasomes directly would be a viable therapeutic target in atherosclerosis remains to be seen.

## Conflict of Interest

The authors declare no conflict of interest.

## REFERENCES

- Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105(9):1135–43.
- Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- $\beta$ . *Mol Cell* 2002;10(2):417–26.
- Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin Chem* 2008;54(1):24–38.
- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352(16):1685–95.
- Stutz A, Golenbock DT, Latz E. Inflammasomes: too big to miss. *J Clin Invest* 2009;119(12):3502–11.
- Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* 2015;21(7):677–87.
- Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. *Nat Rev Immunol* 2013;13(6):397–411.
- Tsutsui H, Imamura M, Fujimoto J, Nakanishi K. The TLR4/TRIF-mediated activation of NLRP3 inflammasome underlies endotoxin-induced liver injury in mice. *Gastroenterol Res Pract* 2010;2010:641865.
- Di Virgilio F. The therapeutic potential of modifying inflammasomes and NOD-like receptors. *Pharmacol Rev* 2013;65(3):872–905.
- Pétrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ* 2007;14:1583–9.
- Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010;464:1357–61.
- Rajama K, Lappalainen J, Oorni K, Valimäki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* 2010;5:e11765.
- Dinarello CA. A clinical perspective of IL-1 $\beta$  as the gatekeeper of inflammation. *Eur J Immunol* 2011;41(5):1203–17.
- Chamberlain J, Evans D, King A, Dewberry R, Dower S, Crossman D, et al. Interleukin-1 $\beta$  and signaling of interleukin-1 in vascular wall and circulating cells modulates the extent of neointima formation in mice. *Am J Pathol* 2006;168:1396–403.
- Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, et al. Lack of interleukin-1 $\beta$  decreases the severity of atherosclerosis in apoE-deficient mice. *Arterioscler Thromb Vasc Biol* 2003;23:656–60.
- Badimon L. Interleukin-18: a potent pro-inflammatory cytokine in atherosclerosis. *Cardiovasc Res* 2012;96(2):172–5.
- Jiang Y, Wang M, Huang K, Zhang Z, Shao N, Zhang Y, et al. Oxidized low-density lipoprotein induces secretion of interleukin-1 $\beta$  by macrophages via reactive oxygen species-dependent NLRP3 inflammasome activation. *Biochem Biophys Res Commun* 2012;425(2):121–6.
- Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhalawon B, Carpenter SB, et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nat Immunol* 2013;14(8):812–20.
- Janoudi A, Shamoun FE, Kalavakunta JK, Abela GS. Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque. *Eur Heart J* 2016;37(25):1959–67.
- Ding Z, Liu S, Wang X, Dai Y, Khaidakov M, Deng X, et al. LOX-1, mtDNA damage, and NLRP3 inflammasome activation in macrophages: implications in atherogenesis. *Cardiovasc Res* 2014;103(4):619–28.
- Ding Z, Liu S, Wang X, Theus S, Fan Y, Deng X, et al. LOX-1 – dependent mitochondrial DNA damage and NLRP3 activation during systemic inflammation in mice. *Biochem Biophys Res Commun* 2014;451(4):637–43.
- Estruch M, Rajamäki K, Sanchez-Quesada JL, Kovanen PT, Öörni K, Benitez S, et al. Electronegative LDL induces priming and inflammasome activation leading to IL-1 $\beta$  release in human monocytes and macrophages. *Biochim Biophys Acta* 2015;1851(11):1442–9.
- Oury C. CD36: linking lipids to the NLRP3 inflammasome, atherogenesis and atherothrombosis. *Cell Mol Immunol* 2014;11(1):8–10.
- Li X, Zhang Y, Xia M, Gulbins E, Boini KM, Li P-L. Activation of NLRP3 inflammasomes enhances macrophage lipid-deposition and migration: implication of a novel role of inflammasome in atherogenesis. *PLoS One* 2014;9(1):e87552.
- Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite  $\beta$ -hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med* 2015;21(3):263–9.
- Folco EJ, Sukhova GK, Quillard T, Libby P. Moderate hypoxia potentiates interleukin-1 $\beta$  production in activated human macrophages. *Circ Res* 2014;115(10):875–83.
- Zheng F, Xing S, Gong Z, Mu W, Xing Q. Silence of NLRP3 suppresses atherosclerosis and stabilizes plaques in apolipoprotein E-deficient mice. *Mediat Inflamm* 2014;2014:507208.
- Zheng F, Xing S, Gong Z, Xing Q. NLRP3 inflammasomes show high expression in aorta of patients with

- atherosclerosis. *Heart Lung Circ* 2013;22:746–50.
29. Afrasyab A, Qu P, Zhao Y, Peng K, Wang H, Lou D, et al. Correlation of NLRP3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart Vessels* 2016;31(8):1218–29.
  30. Shi X, Xie WL, Kong WW, Chen D, Qu P. Expression of the NLRP3 inflammasome in carotid atherosclerosis. *J Stroke Cerebrovasc Dis* 2015;24(11):2455–66.
  31. Roberts RL, Van Rij AM, Phillips LV, Young S, McCormick SP, Merriman TR, et al. Interaction of the inflammasome genes CARD8 and NLRP3 in abdominal aortic aneurysms. *Atherosclerosis* 2011;218(1):123–6.
  32. Finucane OM, Lyons CL, Murphy AM, Reynolds CM, Klinger R, Healy NP, et al. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1 $\beta$  secretion and insulin resistance despite obesity. *Diabetes* 2015;64:2116–28.
  33. Bando S, Fukuda D, Soeki T, Nishimoto S, Uematsu E, Matsuura T, et al. Expression of NLRP3 in subcutaneous adipose tissue is associated with coronary atherosclerosis. *Atherosclerosis* 2015;242:407–14.
  34. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet* 2009;373(9670):1175–82.
  35. Altaf A, Qu P, Zhao Y, Wang H, Lou D, Niu N. NLRP3 inflammasome in peripheral blood monocytes of acute coronary syndrome patients and its relationship with statins. *Coron Artery Dis* 2015;26(5):409–21.
  36. Satoh M, Tabuchi T, Itoh T, Nakamura M. NLRP3 inflammasome activation in coronary artery disease: results from prospective and randomized study of treatment with atorvastatin or rosuvastatin. *Clin Sci (Lond)* 2014;126(3):233–41.
  37. Patel R, Janoudi A, Vedre A, Aziz K, Tamhane U, Rubinstein J, et al. Plaque rupture and thrombosis is reduced by lowering cholesterol levels and crystallization with ezetimibe and is correlated with FDG-PET. *Arterioscler Thromb Vasc Biol* 2011;31:2007–14.
  38. Deng ZY, Hu MM, Xin YF, Gang C. Resveratrol alleviates vascular inflammatory injury by inhibiting inflammasome activation in rats with hypercholesterolemia and vitamin D2 treatment. *Inflamm Res* 2015;64(5):321–32.
  39. Nurmi K, Virkanen J, Rajamäki K, Niemi K, Kovanen PT, Eklund KK. Ethanol inhibits activation of NLRP3 and AIM2 inflammasomes in human macrophages—a novel anti-inflammatory action of alcohol. *PLoS One* 2013;8:e78537.
  40. Dai Y, Dai D, Wang X, Ding Z, Mehta JL. DPP-4 inhibitors repress NLRP3 inflammasome and interleukin-1 $\beta$  via GLP-1 receptor in macrophages through protein kinase C pathway. *Cardiovasc Drugs Ther* 2014;28:425–32.
  41. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 $\beta$  inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J* 2011;162(4):597–605.
  42. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, et al. Effects of interleukin-1 $\beta$  inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. *Circulation* 2012;126(23):2739–48.
  43. Menu P, Pellegrin M, Aubert JF, Bouzourene K, Tardivel A, Mazzolai L, et al. Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. *Cell Death Dis* 2011;2:e137.
  44. Usui F, Shirasuna K, Kimura H, Tatsumi K, Kawashima A, Karasawa T, et al. Critical role of caspase-1 in vascular inflammation and development of atherosclerosis in Western diet-fed apolipoprotein E-deficient mice. *Biochem Biophys Res Commun* 2012;425(2):162–8.
  45. Gage J, Hasu M, Thabet M, Whitman SC. Caspase-1 deficiency decreases atherosclerosis in apolipoprotein E-null mice. *Can J Cardiol* 2012;28:222–9.
  46. Abderrazak A, Couchie D, Mahmood DF, Elhage R, Vindis C, Laffargue M, et al. Anti-inflammatory and antiatherogenic effects of the NLRP3 inflammasome inhibitor arglabin in ApoE<sub>2</sub>/Ki mice fed a high-fat diet. *Circulation* 2015;131:1061–70.
  47. Hazuda DJ, Strickler J, Kueppers F, Simon PL, Young PR. Processing of precursor interleukin 1 beta and inflammatory disease. *J Biol Chem* 1990;265:6318–22.
  48. Alfaidi M, Wilson H, Daigneault M, Burnett A, Ridger V, Chamberlain J, et al. Neutrophil elastase promotes interleukin-1 $\beta$  secretion from human coronary endothelium. *J Biol Chem* 2015;290(40):24067–78.
  49. Fantuzzi G, Ku G, Harding MW, Livingston DJ, Sipe JD, Kuida K, et al. Response to local inflammation of IL-1 beta-converting enzyme-deficient mice. *J Immunol* 1997;158:1818–24.
  50. Coll RC, Robertson AA, Chae JJ, Higgins SC, Muñoz-Planillo R, Inserra MC, et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nat Med* 2015;21:248–55.
  51. Cocco M, Miglio G, Giorgis M, Garella D, Marini E, Costale A, et al. Design, synthesis, and evaluation of acrylamide derivatives as direct NLRP3 inflammasome inhibitors. *Chem Med Chem* 2016;11(16):1790–813.