NLRP3 Inflammasome and the IL-1 Pathway in Atherosclerosis

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Abstract: **Inflammation is an important driver of atherosclerosis, the underlying pathology of cardiovascular diseases. Therefore, therapeutic targeting of inflammatory pathways is suggested to improve cardiovascular outcomes in patients with cardiovascular diseases. This concept was recently proven by CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), which demonstrated the therapeutic potential of the monoclonal IL (interleukin)-1β–neutralizing antibody canakinumab. IL-1β and other IL-1 family cytokines are important vascular and systemic inflammatory mediators, which contribute to atherogenesis. The NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3) inflammasome, an innate immune signaling complex, is the key mediator of IL-1 family cytokine production in atherosclerosis. NLRP3 is activated by various endogenous danger signals abundantly present in atherosclerotic lesions, such as oxidized low-density lipoprotein and cholesterol crystals. Consequently, NLRP3 inflammasome activation contributes to the vascular inflammatory response driving atherosclerosis development and progression. Here, we review the mechanisms of NLRP3 inflammasome activation and proinflammatory IL-1 family cytokine production in the context of atherosclerosis and discuss treatment possibilities in light of the positive outcomes of the CANTOS trial. (*Circ Res*. 2018;122:1722-1740. DOI: 10.1161/CIRCRESAHA.118.311362.)**

Key Words: atherosclerosis ■ canakinumab ■ cytokines ■ inflammasomes ■ inflammation

Cardiovascular diseases (CVDs), which comprise several disorders of the heart and the vasculature, are a severe global health burden currently representing the leading cause of death worldwide.[1] Cardiovascular events like myocardial and cerebral infarction, which are responsible for the majority of CVD deaths, are most commonly caused by atherothrombotic occlusion of blood vessels. Preceding longtime atherosclerotic changes of the blood vessels are driven by dyslipidemia and vascular inflammation.

Several environmental, behavioral, and genetic risk factors, including hyperlipidemia, hypertension, diabetes mellitus, Western-type diet, lack of exercise, smoking, male gender, and aging, have been associated with CVDs. Among these, elevated blood cholesterol levels, or more precisely LDL-C (low-density lipoprotein cholesterol) levels, are considered a major risk factor and were causally linked to the pathogenesis of atherosclerosis.[2] The current conservative treatment of atherosclerosis is mainly focused on lowering plasma cholesterol levels, but this treatment is insufficient to reduce the risk of future cardiovascular events in all patients. However, atherosclerosis has increasingly been recognized as a chronic inflammatory disease, which paved the way for new therapeutic approaches targeting vascular inflammation. Several clinical studies are currently underway assessing various anti-inflammatory agents for the treatment of atherosclerosis.[3] Recently, the results of the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) were published.[4] They demonstrate that treatment with the monoclonal IL (interleukin)-1β–neutralizing antibody canakinumab reduces the risk of recurrent cardiovascular events in patients with prior heart attack. In light of this positive outcome of the CANTOS trial, we review the origin and the impact of the inflammation hypothesis, with special focus on the NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3) inflammasome and proinflammatory IL-1 family cytokines in atherogenesis.

Inflammation Hypothesis of Atherosclerosis

Already in the 19th century, leading pathologists like Rokitansky and Virchow described the inflammatory character of atherosclerotic plaques. Although Rokitansky considered the cellular inflammatory changes to be a secondary phenomenon, Virchow proposed that inflammatory processes actively contribute to the pathological changes in the arteries and the progression of atherosclerotic plaques.[5] However, this concept was almost buried in oblivion in the early 20th century, when excessive amounts of free and esterified cholesterol were identified in atherosclerotic lesions, and experimental evidence for the causative role of cholesterol-rich diets in atherosclerosis development in rabbits was provided.[6] High concentrations of blood cholesterol, in particular, LDL-C

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Nonstandard Abbreviations and Acronyms

AIM2	absent in melanoma 2
ApoE	apolipoprotein E
ASC	apoptosis-associated speck-like protein containing a CARD
Ca^{2+}	calcium
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcomes Study
CARD	caspase recruitment domain
CC	cholesterol crystal
CD36	cluster of differentiation 36
CIRT	Cardiovascular Inflammation Reduction Trial
CRP	C-reactive protein
CVD	cardiovascular disease
GSDMD	gasdermin D
HDL-C	high-density lipoprotein cholesterol
IFN	interferon
IL	interleukin
IL-18BP	IL-18 binding protein
IL-1R	IL-1 receptor
IL-1Ra	IL-1R antagonist
JUPITER	Justification for the Use of Statin in Prevention: an Intervention Trial Evaluating Rosuvastatin
K^+	potassium
LDL-C	low-density lipoprotein cholesterol
LDLR	LDL receptor
LDM	low-dose methotrexate
LRR	leucine-rich repeat
MCP-1	monocyte chemoattractant protein 1
MI	myocardial infarction
NE	neutrophil elastase
NET	neutrophil extracellular trap
NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells
NLRC	NOD-, LRR- and CARD-containing
NLRP	NOD-, LRR- and PYD-containing
NOD	nucleotide oligomerization domain
oxLDL	oxidized LDL
P2X7R	purinergic 2X7 receptor
PCSK9	proprotein convertase subtilisin/kexin type 9
POP	pyrin-only protein
PR3	proteinase 3
PRR	pattern recognition receptor
PYD	pyrin domain
TLR	Toll-like receptor

were found to be a major risk factor for atherosclerosis.[7,8] In turn, atherosclerosis was mainly considered as a consequence of passive lipid accumulation in the vessel wall and pathological changes were mainly attributed to smooth muscle cell migration and proliferation in response to endothelial injury.[9] Consequently, pharmacological lowering of blood cholesterol levels, in particular, LDL-C with statins, became a standard drug treatment for atherosclerosis. Although statin therapy proved to be very effective in improving clinical outcomes, CVDs still remained the leading cause of mortality in industrialized societies.[10]

In the early 90s, the concept that inflammatory processes are causally involved in atherosclerosis progression was rediscovered and started to propagate as the inflammation hypothesis of atherosclerosis. More and more evidence for the inflammatory nature of atherosclerotic plaques accumulated. Finally, the identification of elevated CRP (C-reactive protein) plasma levels as an independent predictive risk factor for myocardial infarction (MI) and stroke,[11] subverted the paradigm of atherosclerosis being solely a vascular cholesterol storage disorder. Atherosclerosis was increasingly also regarded as a chronic inflammatory disease, and a causative vicious circle of arterial lipid deposition and inflammation was proposed.[12] Since then, epidemiological data emphasizing the correlation between inflammation and the risk of cardiovascular events has accumulated.[13] In parallel, a plethora of experimental in vitro, ex vivo, and in vivo studies revealed the importance of the innate and adaptive immune system in all stages of atherosclerotic disease development and progression.[14,15]

Etiologic Agent(s) of Low-Grade Chronic Vascular Inflammation in Atherosclerosis

Inflammation is the typical response of the host immune system to microbial infection or tissue injury. However, excessive and unresolved inflammation often results in detrimental chronic inflammatory diseases.

Accordingly, propagation of the inflammation hypothesis raised the question of the origin of vascular and low-grade systemic inflammation. Naturally, pathogenic infections were considered as initiators of atherosclerosis-associated inflammation. This so-called infection hypothesis is actually a very old concept dating back to 19th century when Rokitansky and Virchow described the inflammatory component of atherosclerosis.[16] Since then, infections with a variety of different pathogens including bacteria and viruses, such as *C. pneumoniae*, *P. gingivalis*, cytomegalovirus, human immunodeficiency virus, and influenza A virus, have been correlated to increased CVD risk.[17] In addition, epidemiological data suggest that the frequency of cardiovascular events is transiently increased for a few weeks after an acute infection.[18,19] It is suggested that acute infections cause a rapid increase in inflammation in the coronary arteries, either directly by microbes present in the vascular wall or indirectly by systemic inflammation caused by the acute infection.[17] This increased vascular inflammation then contributes to plaque destabilization and triggers the coagulation cascade, together favoring the rupture of vulnerable atherosclerotic plaques and subsequent acute cardiovascular events.[20] Taken together, it is, therefore, likely that either infections with a single pathogen or the entirety of an infectious burden can contribute to atherogenesis in an indirect manner by promoting local vascular as well as systemic inflammation.[21] However, despite profound and constantly increasing evidence for the involvement of microbial infections in the pathogenesis of atherosclerosis, to date an etiologic relationship between a single microbe and atherosclerosis development could not be demonstrated. Yet, a variety of chronic inflammatory and autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, gout, and

systemic lupus erythematosus, were linked to an increased risk of cardiovascular events.[22] This underlines the detrimental role of systemic inflammation, independent of its origin, as CVD risk factor.

Inflammation can also be triggered by host-derived molecules associated with cell death, tissue injury, and metabolic disturbances. A variety of these so-called danger signals have been implicated in eliciting proinflammatory immune responses in atherosclerotic lesions and thus promoting atherosclerosis development. These danger signals comprise, for example, aggregated host molecules such as crystalline cholesterol or calcium precipitates, tissue damage- and cell death-associated molecules such as extracellular matrix components, heat shock proteins, alarmins, and ATP, as well as modified host molecules, such as advanced glycation end products and oxLDL (oxidized LDL).[23]

In particular, the finding that modified or aggregated lipids and lipoproteins, such as oxLDL and crystalline cholesterol, which are abundantly present in atherosclerotic lesions, represent potent inflammatory molecules,[24–27] provided a link between vascular cholesterol deposition and vascular inflammation. Underlining the importance of endogenous inflammatory triggers over microbial infections, a study using germ-free atherosclerosis-prone mice demonstrated that initiation and progression of diet-induced atherosclerotic lesions can occur in the absence of infectious microbes.[28] The authors conclude that increased levels of circulating cholesterol are sufficient to initiate diet-induced atherogenesis, which seems to conflict with the inflammation hypothesis. However, it turned out that cholesterol and inflammation are interconnected, because cellular cholesterol accumulation promotes inflammatory responses, whereas immune cell activation promotes cholesterol accumulation by impairing cellular cholesterol efflux.[29]

Although the mechanisms driving the sustained, nonresolving vascular inflammation in atherosclerosis are still not entirely understood, the current concept proposes that deposited material within the atherosclerotic lesion induces the chronic low-grade inflammation driving atherogenesis.

IL-1 Cytokines in Atherosclerosis

Cytokines are immunomodulatory signaling molecules and thus central mediators of inflammation. The IL-1 family cytokines comprise several proinflammatory cytokines (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ) and 1 anti-inflammatory cytokine (IL-37).[30] They are critically involved in shaping both, the innate and the adaptive immune responses. Most innate immune cells express either IL-1 family cytokines, their receptors, or both, and therefore almost all innate immune cells are affected by IL-1 signaling. Moreover, IL-1 family cytokines also play an important role in the differentiation, polarization, and function of innate and adaptive lymphoid cells.[31]

Signaling of these cytokines is transduced by members of the IL-1R (IL-1 receptor) family, which form mainly 4 heterodimeric signaling complexes, namely the IL-1, IL-18, IL-33, and IL-36 receptors. Moreover, the IL-1 cytokine and receptor families contain various molecules, which interfere with IL-1 cytokine signaling at different levels, thereby providing tight control of IL-1–mediated inflammatory responses. These inhibitory molecules include receptor antagonists (IL-1Ra [IL-1R antagonist], IL-36Ra, and IL-38), receptor chains with modified signaling domains interfering with downstream signaling adapters, therefore acting as negative regulators (TIR8 [Toll/IL-1R8] and IL-1RAcPb [IL-1R accessory protein b]), and 2 decoy receptors (IL-1R2 and IL-18BP [IL-18 binding protein]), which sequester IL-1α/IL-1β and IL-18, respectively.[31]

To date, the best-studied members of the IL-1 family cytokines are IL-1α, IL-1β, IL-18, and the IL-1Ra. Among those, IL-1β has attracted the most attention, because of its crucial involvement in inflammatory diseases.

Both, IL-1α and IL-1β, 2 proteins encoded by different genes, signal via IL-1R and therefore have similar downstream biological characteristics. However, they are differing in terms of their cellular source, maturation requirements and release, which affect their impact on inflammation.

IL-1 cytokines are produced as precursor molecules, which generally require enzymatic cleavage for maturation to the biologically active cytokine. In this regard, IL-1α is unique, as its precursor form is already biologically active.[32] It is mainly present on the surface of cells, particularly on monocytes and macrophages, in a membrane-bound form.[33] Although the plasma membrane protease calpain is capable of cleaving the IL-1α precursor into its mature form, IL-1α is rarely secreted on cell stimulation. Therefore, IL-1α predominantly mediates local inflammatory effects. However, IL-1α is constitutively expressed by various nonimmune cell types including epithelial cells. Tissue damage and necrotic cell death of IL-1α expressing cells result in the release of biologically active IL-1α precursors. Therefore, IL-1α functions as an alarmin, which can rapidly and potently induce proinflammatory immune responses contributing to sterile inflammation.[32]

The activation of IL-1β, which is primarily produced by hematopoietic cells, including blood monocytes, tissue macrophages, and dendritic cells, is controlled at several levels. The IL-1β precursor is not constitutively expressed and thus needs to be induced by signals activating NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells)–mediated transcription. As such, IL-1β transcription is mediated by proinflammatory stimuli that activate TLRs (Toll-like receptors), but also by autocrine signaling of IL-1α and IL-1β via IL-1R, thus providing a positive feedback loop.[34]

In contrast to IL-1α, pro–IL-1β, the IL-1β precursor, is not biologically active, but requires proteolytic processing by caspase-1, resulting in the secretion of the active IL-1β cytokine. Caspase-1, the predominant IL-1 processing protease, is abundantly present in hematopoietic cells as a proenzyme, which requires activation by the inflammasome.

However, mature IL-1β can also be produced independent of caspase-1, especially in the context of local inflammation. In turpentine-induced tissue necrosis, the same amount of mature IL-1β was detected at the injection site in caspase-1–deficient mice as in wild-type animals, whereas IL-1β–deficient mice were completely protected.[35] Similarly, in acute neutrophil-dominated arthritis neutrophil PR3 (proteinase 3) was shown to be the major pro–IL-1β processing enzyme, whereas caspase-1 rather gained significance in a chronic model of arthritis.[36] Because neutrophils die within

hours after extravasation to inflammatory sites, it can be expected that they release pro–IL-1β from intracellular stores. Mature caspase-1 has a very short half-life[37] and is therefore not well suited for the extracellular maturation of pro–IL-1β. In contrast, extracellular IL-1β precursors can be processed by neutrophil PR3.[38] Further proteases such as NE (neutrophil elastase), MMP9 (matrix metalloprotease 9), and granzyme A were also implicated in the extracellular processing of pro–IL-1β.[39] Additionally, it was shown that mast cell chymase, a constituent of secretory granules that are released on mast cell degranulation, can generate active IL-1β.[40]

The local activation of IL-1β is central in mediating the proinflammatory response resulting in activation of secondary inflammatory mediators, including IL-6. In turn, IL-6 acts systemically to elicit the acute phase response with hepatic production of acute phase proteins, such as CRP, fibrinogen, and plasminogen activator inhibitor.[41] Therefore, IL-1β has a crucial role in activating the humoral arm of the innate immune system.

IL-18 is biologically and structurally related to IL-1β. Similar to IL-1β, it is produced as an inactive precursor, which requires cleavage by caspase-1 for maturation to the biologically active cytokine. Extracellular maturation of IL-18 can also be achieved by neutrophil PR3.[42] However, similar to IL-1α, IL-18 is constitutively expressed in various cell types and can also exist as a membrane-bound form in human macrophages.[43] It is suggested that IL-18, because of its similarities to IL-1β, contributes to inflammasome-mediated caspase-1–dependent autoinflammatory diseases, as discussed later.

A plethora of data implicates a role of IL-1 in atherosclerosis and CVDs. In humans, increased levels of IL-1β were observed in atherosclerotic compared with normal human coronary arteries and were positively correlated to disease severity.[44] Furthermore, IL-1 was shown to act on many cells present in the human atheroma. For example, it induces various inflammatory functions in human vascular endothelial cells, including procoagulant activity, increased expression of adhesion molecules, important for leukocyte recruitment, and production of MCP-1 (monocyte chemoattractant protein 1).[45,46] Together these changes enable the increased recruitment of monocytic phagocytes, which were strongly implicated in atherogenesis. IL-1 also acts on human vascular smooth muscle cells and promotes their proliferation.[47] However, human vascular endothelial cells and smooth muscle cells are not only target cells for IL-1, but can also produce IL-1 in response to inflammatory stimuli.[48,49]

The proatherogenic character of IL-1 cytokines was furthermore demonstrated in various animal models.

In pigs, chronic administration of IL-1β resulted in intimal thickening of the arteries.[50] Consistently, IL-1 was implicated in neointima formation after vessel wall injury, which was significantly limited by IL-1 inhibition by application of IL-1Ra.[51] Similarly, in mice injury-induced neointima formation was promoted by IL-1Ra deficiency,[52] and accordingly reduced by IL-1Ra treatment or a deficiency in IL-1R and IL-1β, but not IL-1α,[53] indicating a particular role for IL-1β signaling in neointima formation in response to vessel wall injury.

Mice with constitutively increased IL-1 signaling because of IL-1Ra deficiency develop a transmural arterial inflammation leading to lethal aneurysms later in life.[54] Therapeutic administration of IL-1Ra inhibits fatty-streak formation in *Apoe*$^{-/-}$ mice (apolipoprotein E-deficient), indicating an important role of IL-1 in early atherosclerotic lesion development.[55] Consistently, overexpression of IL-1Ra reduced diet-induced atherosclerotic lesion development in *Ldlr*$^{-/-}$ (LDL receptor-deficient) mice,[56] and IL-1Ra deficiency promoted spontaneous development of atherosclerotic lesions in *Apoe*$^{-/-}$ mice.[52] Consistently, IL-1β deficiency attenuated spontaneous development of atherosclerotic lesions in *Apoe*$^{-/-}$ mice[57] and transplantation of IL-1α/IL-1β–deficient bone marrow into *Ldlr*$^{-/-}$ mice impaired diet-induced atherosclerosis.[25] Moreover, administration of a monoclonal antibody targeting IL-1β reduced the development of diet-induced atherosclerotic lesions in *Apoe*$^{-/-}$ mice.[58]

However, a study investigating the effects of IL-1R deficiency on diet-induced atherosclerosis in *Apoe*$^{-/-}$ mice revealed a reduction in atherosclerotic lesion size. Yet, the size of the vessel lumen was decreased because of impaired outward vessel remodeling, and the atherosclerotic lesions presented signs of plaque instability, including increased intraplaque hemorrhage, reduced vascular smooth muscle cell, and decreased collagen content.[59] These results indicate that because of its profibrotic functions and proliferative effects on smooth muscle cells, IL-1 can contribute to lesion stability, suggesting a protective role for IL-1 signaling in advanced atherosclerotic lesions. Nevertheless, a wealth of data demonstrates overall proatherogenic effects of IL-1 cytokines, in particular, during early atherosclerosis development and progression.

The proatherogenic role of IL-1 was predominantly studied addressing IL-1β or blockage of IL-1α and IL-1β signaling by IL-1Ra. However, IL-1α could also be implicated in atherogenesis. *Apoe*$^{-/-}$ mice transplanted with bone marrow of *Il-1α*$^{-/-}$ mice showed a more pronounced reduction of atherosclerotic plaque size than animals transplanted with IL-1β–deficient bone marrow, indicating a more prominent role of IL-1α in atherosclerosis development.[60] Similar results were obtained by transplantation of *Ldlr*$^{-/-}$ mice with IL-1α– and IL-1β–deficient bone marrow, respectively.[61]

Moreover, IL-18 was associated to atherosclerosis development. Similar to IL-1, IL-18, and IL-18R were found to be expressed in human atheroma cells, including vascular endothelial cells, smooth muscle cells, and macrophages.[62] Furthermore, blocking IL-18 activity in *Apoe*$^{-/-}$ mice in vivo by overexpression of IL-18BP[63] and IL-18 deficiency in *Apoe*$^{-/-}$ mice[64] resulted in impaired atherosclerosis lesion development. IL-18 was originally described as IFN (interferon)-γ–inducing factor and indeed, Whitman et al[65] showed that intraperitoneal injections of IL-18 into *Apoe*$^{-/-}$ mice aggravated atherosclerosis development in an IFN-γ–dependent manner.

More recently, researchers started to evaluate the potential beneficial impact of the anti-inflammatory IL-1 family cytokine IL-37 on atherogenesis. Current results indicate that IL-37 can modulate atherosclerosis progression and therefore represents a promising therapeutic target (recently reviewed by McCurdy et al[66]).

Inflammasomes: Central Producers of IL-1 Family Cytokines

Innate immune cells detect pathogen-derived or sterile danger signals (also known as pathogen-associated molecular

patterns or damage-associated molecular patterns, respectively) via germ line-encoded PRRs (pattern recognition receptors). Inflammasome formation is induced by several intracellular PRRs. On activation, they form large multimolecular signaling platforms, which among others catalyze the maturation of pro–IL-1β and pro–IL-18.[67]

Inflammasome research has become prominent within the field of innate immunity since the first report of inflammasomes in 2002.[68] The number of receptors described to be capable of inducing inflammasome formation has been constantly growing. Today, 5 PRRs are widely accepted as inflammasome receptors, namely NLRP1, NLRP3, NLRC4 (NOD-, LRR-, and CARD [caspase recruitment domain]-containing protein 4]) and AIM2 (absent in melanoma 2), and pyrin.[69] However, the role of other PRRs, such as RIG-I (retinoic acid-inducible gene I), IFI-16 (IFN-γ inducible protein 16), NLRP6, NLRP7, and NLRP12, as inflammasome-inducing receptors is either not well-defined or controversially discussed.[70] Depending on their receptors, inflammasomes become activated by a diverse set of pathogen-associated molecular patterns and damage-associated molecular patterns. For example, NLRP1 detects anthrax lethal toxin in mice, NLRC4 is activated by different NAIPs (NOD-like receptor family apoptosis inhibitory proteins) which recognize flagellin or components of the bacterial type III secretion system, AIM2 binds cytosolic double-stranded DNA and pyrin is activated by toxin-induced modifications of Rho-GTPases.[71] In this regard, NLRP3 is unique, because it is activated by a large variety of diverse pathogen-associated molecular patterns and damage-associated molecular patterns.[72]

Inflammasome assembly is organized in a hierarchical order and in most cases requires a sensor protein, an adapter protein, and an effector protein.[67] On activation, the inflammasome receptor either undergoes conformational changes allowing for self-oligomerization or several receptors bind to a common oligomeric ligand.[73,74] In both cases, receptor oligomerization results in the recruitment of the common inflammasome adaptor ASC (apoptosis-associated speck-like protein containing a CARD). ASC is composed of 2 death domains; an N-terminal PYD and a C-terminal CARD, both of them known to promote oligomeric homotypic interactions.[75] Thus, recruitment of monomeric ASC molecules to the oligomerized receptor signaling domains, promotes the formation of an ASC-PYD filament.[74] The CARDs of ASC molecules face to the outside of the PYD filament. Via homotypic CARD–CARD interactions, they can either crosslink different ASC-PYD filaments or recruit the immature form of the inflammasome effector caspase-1.[76–78] Pro-caspase-1 oligomerization on the ASC filament enables proximity-driven autocatalytic caspase-1 maturation.[68,79,80] Cleaved caspase-1 in turn forms an active heterotetramer which can process pro–IL-1β and pro–IL-18,[81–83] and induces the release of their mature forms, which exert potent proinflammatory effects.[67,84,85] Moreover, it was recently discovered that mature caspase-1 also mediates proteolytic cleavage of GSDMD (gasdermin D),[86,87] which in turn forms oligomeric membrane pores responsible for inflammasome-induced inflammatory cell death termed pyroptosis.[88–90]

In homeostasis, the homotypic oligomerization events during inflammasome assembly are inhibited by a high energy barrier. However, any preformed cluster can act as a seed that lowers the activation threshold and allows for a prion-like polymerization. Once inflammasome formation is initiated, all cellular ASC molecules are recruited into the inflammasome in an irreversible process which is no longer dependent on the initial trigger.[91] Thereby, inflammasome assembly strongly amplifies the initial activation signal.[80]

Inflammasome activation causes a very potent self-amplifying response, which might cause damage to the host if not properly controlled. Therefore, a multitude of checkpoints evolved, including gene expression control, posttranslational modifications, autophagy of inflammasome complexes and various other positive and negative feedback loops.[92] Furthermore, POPs (pyrin-only proteins), COPs (CARD-only proteins), and splice variants of ASC, such as ASC-c, can act as decoy interaction partners for inflammasome components containing the respective domains.[93] In addition, some POPs were shown to interfere with NF-κB signaling which is required for transcriptional induction of some inflammasome sensors and pro–IL-1β. Caspase-1 activity is limited by the short half-life of active caspase-1[37] and, as described above, the IL-1 signaling pathway is also tightly controlled on cytokine and receptor level by various mechanisms, such as transcriptional and translational regulation of cytokine synthesis, requirement for proteolytic cytokine maturation, and presence of receptor antagonists, negative regulators, and decoy receptors.[31] Nevertheless, dysregulation of inflammasome activation can result in detrimental IL-1β–driven chronic autoinflammatory disorders.

Autoinflammatory diseases are primarily driven by the innate immune system and are therefore not to be confused with autoimmune diseases, which are caused by autoreactive antibodies or other dysregulated effectors of the adaptive immune system. Autoinflammatory diseases can be caused by misbalanced regulation of innate inflammatory processes as well as by inherited or newly acquired genetic mutations.[92] For instance, gain-of-function mutations in the genes coding for the inflammasome sensors NLRP3, NLRC4, and pyrin were reported to cause inflammasome-dependent autoinflammatory diseases, which commonly present with systemic inflammation, periodic fever, rashes, neutropenia, myalgia, and fatigue. Among those, familial Mediterranean fever is the most common autoinflammatory disease, which is caused by mutations in the gene coding for pyrin. Mutations in the NLRP3 gene cause a spectrum of different inflammasome-dependent diseases, summarized as cryopyrin-associated periodic syndromes.[92]

Patients with autoinflammatory diseases benefit from therapies interfering with IL-1 signaling, such as anakinra, a recombinant IL-1Ra, and rilonacept, which traps IL-1α and IL-1β. Moreover, successful treatment with monoclonal IL-1β-neutralizing antibodies, such as canakinumab, highlights the important pathological role of IL-1β in these inflammasome-driven autoinflammatory disorders.[39,92]

NLRP3 Inflammasome

Activation of the NLRP3 inflammasome (Figure 1) is regulated at several levels and in macrophages usually requires 2 independent signals: an initial priming signal activating a PRR or cytokine receptor induces the transcriptional upregulation

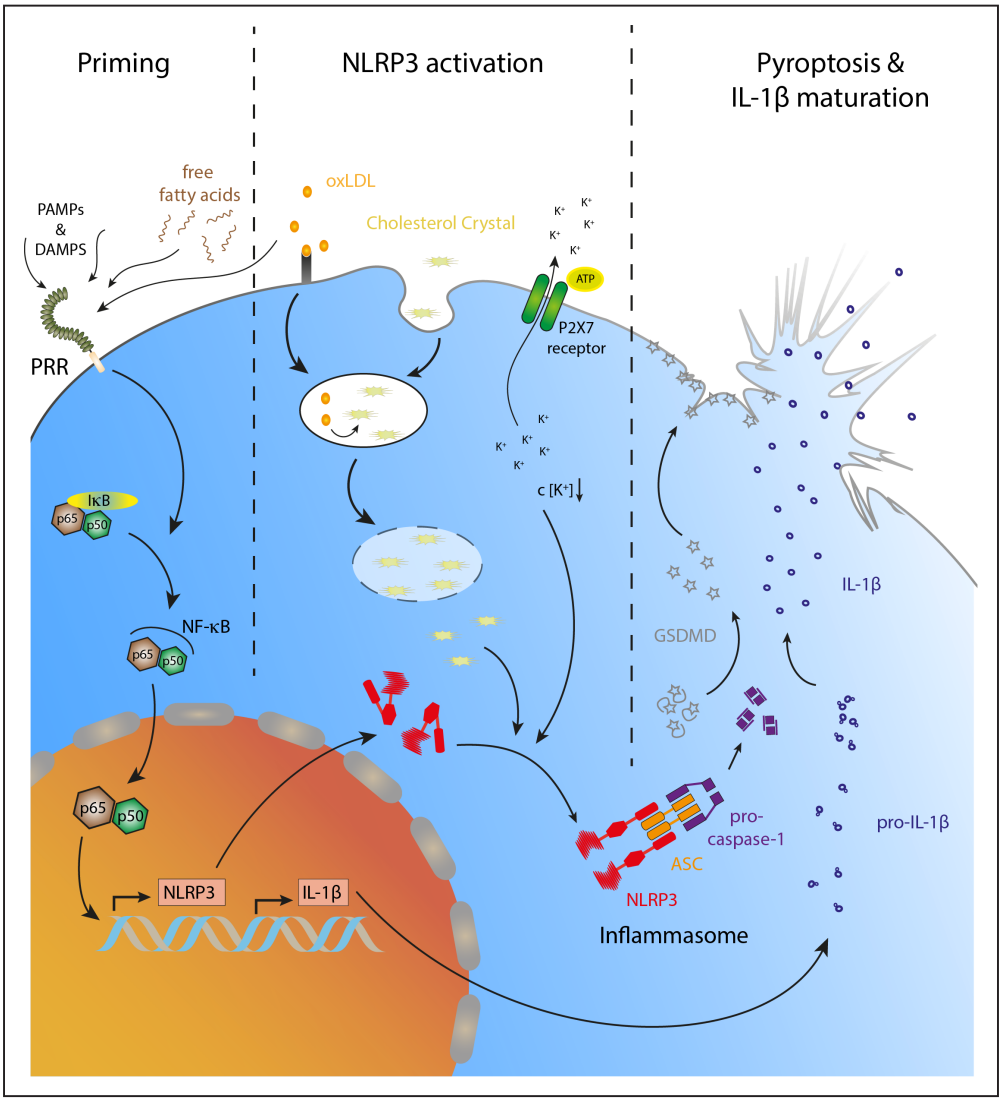

Figure 1. IL (interleukin)-1β secretion in response to NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3) inflammasome activation. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) induce proinflammatory NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) signaling after detection by PRRs (pattern recognition receptors). The transcription factor NF-κB localizes to the nucleus and among others induces the expression of the IL-1β precursor pro–IL-1β and NLRP3. Cholesterol crystals can be phagocytozed or oxLDL (oxidized low-density lipoprotein) can be taken up via receptor-mediated phagocytosis resulting in cholesterol crystal formation within phagolysosomes. The inactive NLRP3 protein can be activated by crystal-induced lysosomal rupture or a decrease in the intracellular potassium concentration caused, for example, by the ATP-gated P2X7R (purinergic 2X7 receptor). On activation, NLRP3 recruits the adapter molecule ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), which links NLRP3 to pro-caspase-1. Caspase-1 is activated by autoproteolysis and formation of the enzymatically active heterotetramer. Active caspase-1 catalyzes the processing of IL-1β and the pyroptosis mediator GSDMD (gasdermin D).

of NLRP3 and pro–IL-1β via NF-κB signaling. A second stimulus is required to activate NLRP3 to induce inflammasome assembly.[94] In addition, fast, nontranscriptional priming processes via posttranslational modifications of presynthesized NLRP3 protein were described.[95–97] However, the effect of posttranslational modifications on NLRP3 activation is rather complex and incompletely understood, but it seems to depend on the exact position and type of the posttranslational modification. For example, phosphorylation at serine 5 within the PYD of NLRP3 blocks its activation, most likely by interfering with a PYD–PYD interaction interface, thereby preventing receptor oligomerization.[98] Consistently, phosphorylation and nitrosylation at other positions were also described to inhibit NLRP3 activation.[99–103]

As mentioned above, the NLRP3 inflammasome can be activated by a large number of chemically and structurally diverse pathogen-associated molecular patterns and damage-associated molecular patterns. Accordingly, it is quite unlikely that all of these stimuli directly bind to NLRP3. Hence, it was suggested that NLRP3 might indirectly sense these stimuli by a common upstream mediator.[104] Although some common events upstream of NLRP3 have been postulated by now, none of them seems to be capable of integrating all different triggers. The best-characterized activators can be summarized

as either inducers of phagolysosomal rupture or as modulators of ion homeostasis. Additionally, mitochondrial damage and release of cardiolipin, mitochondrial DNA, and reactive oxygen species have been discussed as further common upstream mechanisms for NLRP3 activation.[105,105a,106] However, although these events occur simultaneously, it was shown that only potassium (K^+) efflux activates NLRP3.[107] In homeostasis, the cytosolic concentration of K^+ is much higher than in the extracellular space, and the electrochemical gradient is tightly controlled.[72] Some classical NLRP3 activators, such as the bacterial toxins gramicidin, nigericin, and valinomycin, act as ionophores and similar to the ATP-gated P2X7R (purinergic 2X7 receptor) allow for a net K^+ efflux.[72,108–110] Besides K^+ efflux, calcium (Ca^{2+}) fluxes have also been associated with NLRP3 activation. Some reports claim a significant role for Ca^{2+} mobilization, whereas the overall evidence rather suggests that an elevated intracellular Ca^{2+} concentration is not involved in NLRP3 activation.[72]

Although the involvement of phagolysosomal rupture in NLRP3 inflammasome activation by crystals is widely accepted, the exact downstream mechanism that regulates NLRP3 is still not completely understood. NLRP3 was postulated to sense lysosomal damage because of cathepsin B release.[111] Yet, other experiments with cathepsin B–deficient mice challenged this idea and rather suggest a combined effect of several cathepsins, which are all blocked by the originally used inhibitor.[112,113] Indeed, a recent study could confirm that multiple cathepsins seem to have redundant roles and only deficiency in or blocking of several cathepsins prevented crystal-induced cell death.[114] Furthermore, crystal-induced cell death is independent of NLRP3 and caspase-1, although blocking of multiple cathepsins still blocked the crystal-induced IL-1β release.[112,114] Another report places lysosomal damage upstream of the TAK1–JNK pathway which can regulate inflammasome activation by promoting ASC oligomerization.[115] Taken together, there is a large body of evidence linking crystal-induced lysosomal damage and cathepsin release to NLRP3 activation and cell death, however, further investigation is needed to completely understand this relationship.

Recently, 3 independent screens identified, that the interaction of the NEK7 (never in mitosis A-related kinase 7) with NLRP3 is a prerequisite for its activation.[116–118] However, the exact mechanism of this regulation still needs to be identified.

Besides of cellular components, the innate immune system also comprises humoral factors including acute phase proteins and complement factors, which are classically considered as opsonins and lytic agents.[119,120] However, factors of the complement system have also been shown to upregulate IL-1β expression by promoting TLR signaling.[121] Moreover, complement activation resulting in the formation of the complement membrane attack complex at subcytotoxic levels was shown to activate the NLRP3 inflammasome.[122,123] Interestingly, cathepsin L, which was also linked to crystal-induced inflammasome activation, is responsible for the intracellular cleavage of the complement factor C3.[124] Furthermore, at least in T cells, complement signaling was shown to regulate the expression of nutrient channels, the uptake of amino acids and glucose, glycolysis, and oxidative phosphorylation.[124] Cross talk between metabolism and the complement system could be shown for a variety of pathways, and the resulting complement–metabolism–inflammasome axis was reviewed in detail elsewhere.[125]

The standard concept for NLRP3 activation includes 2 different signals for priming and activation, which is commonly referred to as canonical inflammasome activation. However, it is possible that both signals are delivered by one molecule, resulting in noncanonical activation of the NLRP3 inflammasome. In the murine system, it was demonstrated that intracellular LPS can bind caspase-11, which results in a TLR4-independent activation of NLRP3.[126,127] In humans, the functional homologs are caspase-4 and caspase-5.[128,129]

The activation of the NLRP3 inflammasome has been implicated in a multitude of diseases. In contrast to inflammasome-triggered autoinflammatory diseases, they may not be intrinsically caused by NLRP3, but are closely linked to and often aggravated by NLRP3 inflammasome activation, rendering NLRP3 activation a serious health issue.

Macrophages are professional phagocytes, which try to clear various kinds of particulate matter, including aggregated or crystalline materials. However, these aggregates or crystals cause phagolysosomal rupture and reactive oxygen species and consequently activate the NLRP3 inflammasome. As such, NLRP3 is activated by a broad variety of exogenous and endogenous particulate materials, which are widely linked to chronic inflammatory diseases.

For example, inhalation of asbestos, silica, and other crystals, which were shown to activate the NLRP3 inflammasome, lead to the development of progressive pulmonary fibrosis.[111,130,131] Even everyday inhaled particulate matter because of air pollution causes NLRP3 activation[132] and most likely contributes significantly to a large number of chronic inflammatory airway diseases and systemic inflammation, which could contribute to the risk of CVDs.

Moreover, neurodegenerative diseases such as Alzheimer, Parkinson, and even multiple sclerosis are strongly influenced by NLRP3. Aggregates of β-amyloid in case of Alzheimer disease or α-synuclein in case of Parkinson disease are NLRP3 inflammasome activators promoting inflammation and cell death.[85] Mice lacking NLRP3 showed a delay in experimental autoimmune encephalomyelitis development, less inflammation, and weaker cognitive symptoms.[85]

NLRP3 activation was also linked to various metabolic disorders, such as gout and type 2 diabetes mellitus. In gout, monosodium urate crystals deposited within the joints potently activate the NLRP3 inflammasome.[133] Type 2 diabetes mellitus is generally associated with increased levels of inflammatory cytokines, including IL-1β.[134] It was shown that the pancreatic islet amyloid polypeptide, which is co-secreted with insulin, forms aggregates, which induce NLRP3-dependent IL-1β secretion as well as NLRP3-dependent cell death of insulin-producing β-cells.[135,136] Moreover, saturated fatty acids were also shown to activate NLRP3 and thus promote type 2 diabetes mellitus.[137]

Recently, hexokinase, a glycolytic enzyme, has been involved in NLRP3 inflammasome activation.[138] Inhibition of hexokinase by bacterial-derived N-acetylglucosamine, glycolytic inhibitors, or feedback inhibition by glycolytic products results in hexokinase release from the mitochondria, which triggers NLRP3 activation in a K^+ efflux- and

pyroptosis-independent manner. This not only defines a metabolic enzyme as a PRR, but it also supports the concept that NLRP3 can act as general sensor for so-called homeostasis-altering molecular processes[139] and does not rely on a specific pathogen-derived ligand. However, this data are conflicting with older studies, showing that mTORC1 (mechanistic target of rapamycin complex 1)- and PKM2 (pyruvate kinase isozyme M2)-dependent induction of glycolysis via hexokinase is required for NLRP3 activation.[140,141] Yet, other studies have also provided evidence for an intense cross talk between metabolic conditions and NLRP3 activation. For example, β-hydroxybutyrate, a ketone metabolite, produced during starvation or low carbohydrate prevalence, acts as an inhibitor of NLRP3[142] and omega-3 fatty acids were also shown to act as negative regulators of the NLRP3 inflammasome.[143,144] The topic of metabolic regulation of the inflammasome pathways and innate immunity in general has recently been reviewed in detail elsewhere.[125,145–147]

Although chronic NLRP3 activation is generally considered detrimental to the host an evolutionary driving force for its expression and functionality is expected.

Role of the NLRP3 Inflammasome in Atherogenesis

Because the NLRP3 inflammasome, a major producer of cleaved IL-1 family cytokines, links metabolic disturbances and inflammation, its role in the pathogenesis of atherosclerosis was intensely studied.

First experimental evidence for the importance of NLRP3 inflammasome activation in diet-induced atherosclerosis development and progression was provided by bone marrow transplantations of *Ldlr*$^{-/-}$ mice with bone marrow-derived from either wild-type, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$, or *Il-1α*$^{-/-}$/*Il-1β*$^{-/-}$ mice.[25] Bone marrow deficiency of NLRP3, ASC, or IL-1α and IL-1β resulted in significantly reduced development of atherosclerotic lesions compared with *Ldlr*$^{-/-}$ mice transplanted with wild-type bone marrow. However, a study using *Apoe*$^{-/-}$/*Nlrp3*$^{-/-}$, *Apoe*$^{-/-}$/*Asc*$^{-/-}$, and *Apoe*$^{-/-}$/*Caspase-1*$^{-/-}$ double knockout mice did not reveal a reduction in atherosclerosis progression, suggesting that atherogenesis can progress independently of NLRP3 inflammasome activation.[148] These discrepancies might be explained by the different experimental conditions applied, including the mouse model, the gender, the time of high-fat diet feeding as well as the type of atherogenic diet. Indeed, the second study[148] used *Apoe*$^{-/-}$ mice, which show faster and more severe diet-induced atherosclerosis development than *Ldlr*$^{-/-}$ mice, and even develop spontaneous atherosclerosis under prolonged chow diet.[149] Moreover, the mice received a much stronger atherogenic diet with more than 8-fold higher cholesterol content, and were fed for a period of 11 weeks,[148] thus 3 weeks longer than in the other study.[25] Although it is likely that these conditions were used to ensure cholesterol crystals (CC) formation and NLRP3 activation, it is possible that the overabundance of dietary cholesterol in combination with the prolonged feeding period triggers other inflammatory pathways apart from the NLRP3 inflammasome, potentially making NLRP3 inflammasome activation dispensable for further atherosclerosis development and progression at later stages of the disease. This hypothesis is in line with observations on the requirement for IL-1β signaling in atherogenesis. *Ldlr*$^{-/-}$ mice transplanted with bone marrow of *Il-1β*$^{-/-}$ mice develop only insignificantly smaller atherosclerotic lesions than control mice after receiving an atherogenic diet for a prolonged period of 16 weeks.[61] In the same study atherosclerotic lesion development and progression was significantly impaired in *Ldlr*$^{-/-}$ mice transplanted with bone marrow of *Il-1α*$^{-/-}$ mice. This indicates that IL-1α, rather than IL-1β is involved in atherogenesis after prolonged exposure to large amounts of atherogenic triggers. Of note, the alarmins IL-1α and high mobility group box protein 1 (HMGB1), which potently induce proinflammatory immune responses, can be released by macrophages in an inflammasome-independent manner[61,151] and were both shown to promote atherogenesis.[61,152] Therefore, it is possible that other innate immune signaling pathways with redundant roles become activated under these conditions and bypass the proatherogenic effects of the inflammasome-dependent cytokines IL-1β and IL-18.

The NLRP3 inflammasome is controlled by priming and multiple signaling pathways which regulate NLRP3 posttranscriptional modifications. Hence, differences in the local or systemic environment, including differences in the microbiome composition, likely influence the gene dose effect. NLRP3 bone marrow deficiency in *Ldlr*$^{-/-}$ mice was recently shown to impair exaggerated atherogenesis due to other pathways contributing to the local inflammatory response, yet in these studies, NLRP3 deficiency alone showed only small effects.[153,154] It is possible that confounding factors like gender, the microbiome or environmental conditions influenced the contribution of NLRP3 toward atherogenesis. However, despite these discrepancies, several other studies supported the initially proposed importance of the NLRP3 inflammasome in atherosclerosis. For example, two independent studies demonstrated that *Apoe*$^{-/-}$/*Caspase-1*$^{-/-}$ double knockout mice show impaired development of diet-induced atherosclerotic lesions when being fed an atherogenic diet with a lower cholesterol content,[155,156] as well as a reduced spontaneous development of atherosclerotic lesions after chow diet feeding for 26 weeks.[156] A more recent study has confirmed the importance of caspase-1 and caspase-11 activation in murine diet-induced atherogenesis in *Ldlr*$^{-/-}$ mice transplanted with bone marrow of *Caspase-1/11*$^{-/-}$ mice, which develop significantly smaller atherosclerotic plaques than *Ldlr*$^{-/-}$ mice transplanted with control bone marrow.[157] Further evidence for the involvement of NLRP3 inflammasome activation in diet-induced atherogenesis was provided by lentiviral gene silencing of *Nlrp3* in *Apoe*$^{-/-}$ mice, which impaired atherosclerosis progression.[158] A recent study demonstrated that cholesterol accumulation in myeloid cells activates the NLRP3 inflammasome. In turn, a deficiency in NLRP3 or caspase-1/11–reduced atherosclerotic lesion size in *Ldlr*$^{-/-}$ mice lacking myeloid cholesterol efflux transporters ABCA1 (ATP-binding cassette transporter A1) and ABCG1 (ATP-binding cassette transporter G1).[154] In addition, it was recently reported that the NLRP3 inflammasome is triggered by Western diet early during atherogenesis, even before significant plaque burden can be observed. After 8 weeks of Western diet feeding, *Ldlr*$^{-/-}$/*Nlrp3*$^{-/-}$ double knockout mice develop significantly reduced atherosclerotic lesion sizes.[159]

Several epidemiological studies provided indirect evidence for the importance of the NLRP3 inflammasome in humans by correlating aortic NLRP3 expression and CVD prevalence. It was found that patients with coronary atherosclerosis display high aortic expression of NLRP3, which is directly correlating to disease severity and several clinical risk factors for CVD, including smoking, hypertension, diabetes mellitus, LDL-C, and HDL-C (high-density lipoprotein cholesterol) levels.[160] Furthermore, 2 independent studies have shown significantly increased expression of NLRP3, ASC, caspase-1, IL-1β, and IL-18 in human carotid atherosclerotic plaque tissue obtained by carotid endarterectomy in comparison to nonatherosclerotic mesenteric or iliac arteries.[161,162] NLRP3 expression levels were significantly higher in symptomatic compared with asymptomatic patients,[162] and all NLRP3 inflammasome signaling components were higher expressed in unstable compared with stable atherosclerotic plaques.[161] Moreover, NLRP3 protein levels in peripheral blood monocytes were found to be increased in patients with acute coronary syndrome and directly correlated to disease severity.[163] This study even asserted a prognostic value of NLRP3 protein levels in peripheral blood monocytes for the prediction of major adverse events in patients with acute coronary syndrome.

To date, studies on potential associations between NLRP3 polymorphisms with CVDs revealed contradicting results. One study demonstrated a significant association between the *NLRP3 rs10754558* polymorphism and the occurrence and severity of coronary artery disease as well as increases in IL-1β serum concentrations.[164] This finding could be explained by a prior functional analysis of the *NLRP3 rs10754558* polymorphism, which is located in the 3′ untranslated region of the *NLRP3* gene, and was shown to increase *NLRP3* mRNA stability.[165] Conversely, another study testing various NLRP3 polymorphisms could not confirm this result.[166] However, the cohort size in both studies was relatively small, therefore, larger studies will be needed to clearly assess the effect of NLRP3 polymorphisms on CVDs.

Nevertheless, increasing experimental evidence and epidemiological associations are supporting the critical contribution of the NLRP3 inflammasome to atherogenesis and CVDs.

Atherogenic Triggers of NLRP3 Inflammasome Activation

Within human carotid atherosclerotic plaques, components of the NLRP3 inflammasome are mainly expressed in macrophages and foam cells, and only sporadically in smooth muscle cells.[161,162] Consequently, the role of NLRP3 inflammasome activation in the pathogenesis of atherosclerosis is mainly studied in monocytes and macrophages, which are abundantly present in the developing atherosclerotic lesion.

NLRP3 inflammasome activation in vitro requires an initial priming signal via receptors that activate NF-κB–mediated transcription, such as TLRs or IL-1R. Increased expression of various TLRs was observed in murine[167,168] and human[169,170] atherosclerotic plaques. Furthermore, genetic deletion of MyD88 (myeloid differentiation primary response protein 88), the key adaptor protein for TLR and IL-1R signaling, in *Apoe*$^{-/-}$ mice reduced diet-induced atherosclerotic lesion development,[171,172] suggesting proatherogenic roles of TLR or IL-1R signaling. In turn, several studies investigated the role of individual TLRs in murine atherogenesis (reviewed in more detail elsewhere[23,173]). TLR2 and TLR4 are considered to be proatherogenic because genetic deletion of these receptors results in reduced diet-induced atherosclerotic lesion development in mice.[174,175] Consistent with this, in particular, cell surface TLRs are involved in recognition of various endogenous danger signals that were implicated in atherosclerosis development, such as extracellular matrix components, cell death-associated molecules, and oxLDL.[23] All these molecules can, therefore, provide or contribute to the required priming stimulus for NLRP3 inflammasome activation in vivo.

One of the most extensively studied danger signals in atherosclerotic plaques is oxLDL. Macrophage scavenger receptors, such as CD (cluster of differentiation) 36, mediate the uptake of oxLDL into macrophages.[176] Moreover, oxLDL binding to macrophage CD36 induces the assembly of a TLR4/TLR6 heterodimer, resulting in NF-κB signaling and priming of these cells for inflammasome activation.[24] Recently, oxLDL immune complexes, which constitute 90% of circulating oxLDL, were also shown to prime dendritic cells and macrophages for inflammasome activation via Fcγ receptor, CD36, and TLR4, which was even more potent than oxLDL priming via CD36 and the TLR4/TLR6 heterodimer.[177]

Crystalline cholesterol was the first NLRP3 activator associated with atherosclerosis. CCs, a hallmark of advanced atherosclerotic plaques, were implicated in plaque instability and vulnerability.[178,179] Moreover, they are already present in early diet-induced atherosclerotic lesions, corresponding to the first appearance of plaque macrophages.[25] Stimulation of murine and human macrophages in vitro demonstrated the potential of CCs to activate the NLRP3 inflammasome, suggesting an important role for CC-mediated NLRP3 inflammasome activation and subsequent IL-1β secretion in the development and progression of atherosclerosis.[25,26] Comparable to other previously known crystalline NLRP3 activators, CCs were shown to activate the NLRP3 inflammasome via lysosomal damage. Overexpression of the TFEB (transcription factor EB), the master regulator of lysosomal biogenesis, which activates lysosomal and autophagy genes in response to lysosomal stress, rescued oxLDL- and CC-mediated lysosomal dysfunction, and reduced IL-1β secretion in response to these atherogenic triggers.[180] Consistently, autophagy deficiency in macrophages increased CC-mediated inflammasome activation, and *Apoe*$^{-/-}$ mice with macrophage-specific autophagy deficiency developed enlarged atherosclerotic plaques with increased CC content.[181] These results indicated an atheroprotective function of macrophage autophagy by dampening CC-mediated NLRP3 inflammasome activation.

Macrophages can be efficiently primed by oxLDL for CC-mediated NLRP3 inflammasome activation.[25] However, oxLDL uptake by CD36 additionally results in intracellular cholesterol crystallization and can thereby directly activate the NLRP3 inflammasome in macrophages.[27] Of note, CD36 deficiency in *Apoe*$^{-/-}$ mice resulted in impaired atherosclerotic lesion development with significantly reduced CC deposition within the atherosclerotic lesions. This was furthermore associated with decreased serum concentrations of IL-1 family cytokines. These results indicate the important atherogenic role of oxLDL in diet-induced atherosclerosis by demonstrating its dual role as NLRP3 priming and activating stimulus.

Moreover, a recent study demonstrated that CCs act in a feed-forward loop by increasing CD36 expression, thereby promoting further oxLDL uptake into macrophages.[182]

In whole blood assays, CCs were shown to activate the complement system. This pathway is considered highly relevant for the activity of inflammasomes in vivo as the activated complement factors provided the priming signal for monocytes and promoted the phagocytosis of CCs.[183] Furthermore, CCs were shown to trigger the release of neutrophil extracellular traps (NETs), which in turn prime macrophages.[184] Moreover, atherosclerosis development and IL-1β plasma levels were reduced in NET-deficient mice, which lack both PR3 and NE, indicating an important role of NET formation for NLRP3 inflammasome priming and atherogenesis in vivo.[184]

However, although NETs were repeatedly demonstrated to be associated with atherosclerosis[185,186] their exact role in disease pathogenesis remains under debate. Although pharmacological inhibition of NETosis reduced atherogenesis,[187] experiments in NE-deficient mice, which display impaired NET formation, showed no reduction in atherosclerotic lesion size.[188] However, NET-associated extracellular DNA was also shown to activate plasmacytoid dendritic cells, which boosts atherosclerosis via IFN-α.[189] It should be kept in mind that the 2 NETosis-related enzymes PR3 and NE were also reported to directly cleave pro–IL-1β (see above), which complicates the interpretation of the interplay between NETosis, inflammasomes, IL-1β, and atherosclerosis.

Nevertheless, NET formation can also be promoted by IL-1β and IL-18,[190] suggesting that CCs might induce a vicious circle of NLRP3 inflammasome activation in macrophages and macrophage priming by NET-releasing neutrophils.

The above-mentioned studies highlight that both oxLDL and CCs represent important, interrelated danger signals in atherosclerosis, which can provide both, priming and activation signals for NLRP3 inflammasomes. Therefore, the NLRP3 inflammasome links the vascular deposition of lipids and lipoproteins to the inflammatory responses driving the atherosclerotic process. Furthermore, several feed-forward mechanisms have been demonstrated, which indicate that CCs can induce a self-feeding cycle of further CC formation and NLRP3 inflammasome activation. This vicious circle most likely contributes to the nonresolving chronic vascular inflammation that drives atherosclerosis progression (Figure 2).

Although the presence of oxLDL and CCs could be sufficient to activate the NLRP3 inflammasome in atherosclerotic lesions, other proinflammatory mediators, such as TLR triggers, most likely contribute to and intensify NLRP3 inflammasome priming and activation. Indeed, further NLRP3 inflammasome activators were implicated in the pathogenesis of atherosclerosis. For example, calcium phosphate crystals, which are associated with vascular calcification and abundantly present in advanced, calcified atherosclerotic lesions, were shown to elicit IL-1β and IL-1α secretion from macrophages in a caspase-1–dependent manner.[155] Furthermore, extracellular ATP is a well-known NLRP3 inflammasome activator, which is released during cell death. A hallmark of advanced atherosclerotic lesions is the necrotic core region. Recent studies revealed that P2X7R, which mediates ATP-dependent NLRP3 activation, is highly expressed in diet-induced atherosclerotic lesions of *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, as well as in human carotid atherosclerotic plaque tissue.[191,192] Furthermore, P2X7R knockdown in *Apoe*$^{-/-}$ mice,[191] as well as P2X7R deficiency in *Ldlr*$^{-/-}$ mice,[192] were shown to reduce lesional inflammasome activation and to limit atherosclerotic lesion size, indicating that ATP-mediated NLRP3 inflammasome activation via P2X7R promotes atherosclerosis development.

Possible Contribution of Other Inflammasome Pathways in Atherogenesis

Although a large body of evidence connects the NLRP3 inflammasome and atherosclerosis, very little is known about the involvement of other inflammasomes in atherogenesis. Given the presumably large amounts of DNA released by dying cells in the necrotic core of atherosclerotic lesions, a role of the AIM2 inflammasome could be expected. Although AIM2 was so far only described to be activated by cytosolic DNA, the transfer of DNA from dying cells via apoptotic bodies or extracellular vesicles to the cytosol of immune cells might be possible but lacks evidence. However, extracellular DNA can act as priming signal to induce expression of inflammasome components, including AIM2.[193,194] In this regard, it should be considered that results obtained from mouse studies might not be transferable to humans, as it was recently shown, that a cGAS–NLRP3 axis rather than AIM2 is responsible for the DNA-dependent inflammasome activation in human myeloid cells.[195]

Recently, it was demonstrated that the inflammasome can be activated by cholesterol accumulation in macrophages, independent of NLRP3 and CCs, but dependent on AIM2.[196] High intracellular cholesterol content leads to reduced mitochondrial respiratory capacity and release of mtDNA into the cytosol driving AIM2 activation. Although high blood cholesterol levels are a hallmark of atherosclerosis, the intracellular level of cholesterol is normally tightly regulated, and the authors of the above-mentioned study could only show this effect in cholesterol 25-hydroxylase–deficient macrophages, which display reduced cholesterol biosynthesis and intracellular cholesterol content on LPS challenge.

Moreover, activation of the noncanonical NLRP3 inflammasome pathway via caspase-11 was suggested to play a role in atherogenesis in *Ldlr*$^{-/-}$ mice lacking myeloid cholesterol efflux transporters ABCA1 and ABCG1, which also have increased intracellular cholesterol accumulation.[154] It remains to be determined how this activation occurs. The most prominent ligand of caspase-11 is LPS, a component of the outer membrane of Gram-negative bacteria, but oxidized phospholipids, released from dying cells were demonstrated as another ligand of caspase-11.[197] Accordingly, it is conceivable that the noncanonical inflammasome is activated in necrotic areas of atherosclerotic lesions, but direct evidence is still missing. Furthermore, it needs to be determined why in 2 different models of cellular cholesterol accumulation, namely Abca1/g1- and cholesterol-25-hydroxylase-deficiency, which both induce an increase in cellular cholesterol levels, 2 different inflammasome pathways, namely the noncanonical NLRP3 inflammasome and the AIM2 inflammasome, seem to be exclusively activated in each model.

However, activation of the AIM2 or the noncanonical inflammasome in the context of infections[198] might further drive

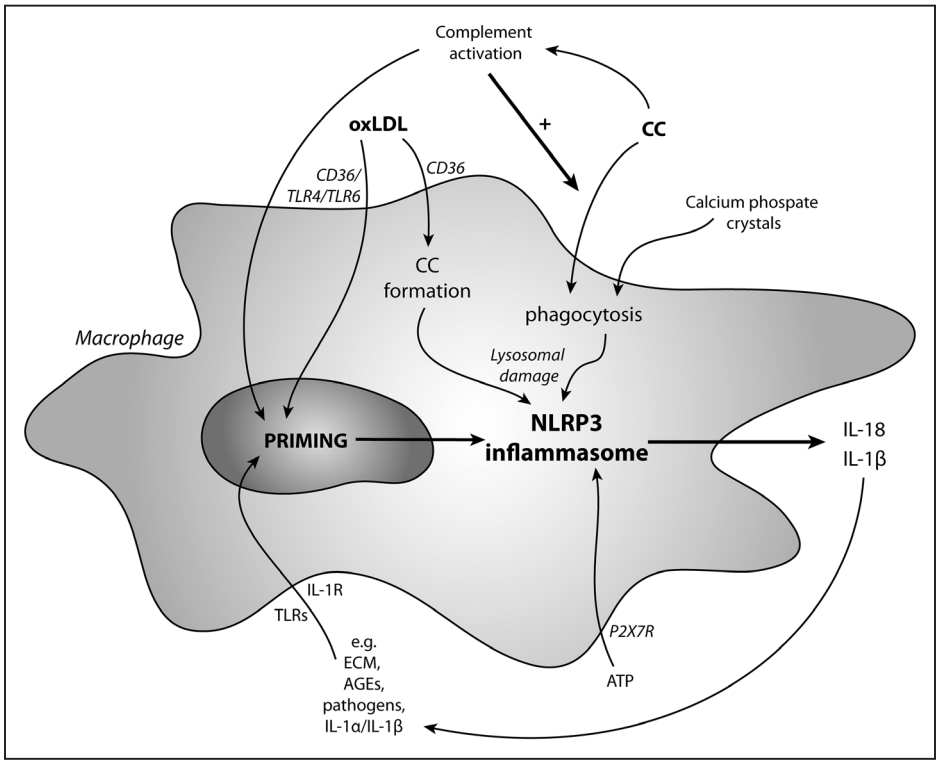

Figure 2. Vicious circle of NLRP3 (NOD [nucleotide oligomerization domain]-, LRR [leucine-rich repeat]-, and PYD [pyrin domain]-containing protein 3) inflammasome activation in atherosclerosis. oxLDL (oxidized low-density lipoprotein) and cholesterol crystals (CCs), which are abundantly present in atherosclerotic lesions, provide a self-feeding cycle of continuous NLRP3 inflammasome priming and NLRP3 inflammasome activation in macrophages. oxLDL binds to CD (cluster of differentiation)36, which mediates a priming signal via TLR (Toll-like receptor)4/TLR6, as well as the phagocytosis of oxLDL, resulting in intracellular CC formation. Phagocytosis of extracellular CCs, as well as intracellular CC formation, causes lysosomal damage, which triggers NLRP3 inflammasome activation. CCs also activate the complement system, which promotes macrophage priming as well as CC phagocytosis and hence, NLRP3 activation. Secreted IL-1β and IL-18 also promote macrophage priming via IL-1R signaling. This vicious circle can be further exacerbated by various endogenous or pathogen-derived danger signals present in the atherosclerotic lesion, providing further TLR-mediated priming signals (eg, extracellular matrix [ECM] components, advanced glycation end products [AGEs]), or additional NLRP3 activation signals (eg, ATP and calcium phosphate crystals).

acute vascular inflammation and contribute to the increased risk of cardiovascular events after infections.

Targeting the IL-1 Pathway in Atherosclerosis—the CANTOS Trial

The CANTOS trial is a large-scale clinical trial designed to test the inflammation hypothesis of atherosclerosis in humans, and to evaluate the protective potential of anti-inflammatory therapies for CVD.[199] This trial is largely based on the findings of previous clinical trials on statin therapy, which demonstrated that statin therapy was most effective in reducing cardiovascular events when reducing not only LDL-C but also CRP plasma levels,[200–202] which serve as inflammatory biomarker for CVDs. In particular, the JUPITER trial (Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin), demonstrated that statin therapy reduces the risk for future cardiovascular events in subjects with high CRP but low LDL-C levels,[203] indicating that individuals with elevated CRP levels benefit from anti-inflammatory treatments.[204] Although these studies strongly supported the hypothesis that decreasing inflammation will reduce CVD risk, statins have intrinsic anti-inflammatory as well as LDL-C lowering properties. Therefore, it remained to be shown that reducing inflammation alone, more precisely without affecting cholesterol levels, also lowers the risk of developing cardiovascular events.

Although CRP as a marker for systemic inflammation represents a useful measure to evaluate the effectiveness of anti-inflammatory treatments, it is not causal for atherosclerosis development, and therefore does not qualify as a target for therapeutic intervention itself.[205,206] However, because IL-1 cytokines and particularly IL-1β are importantly involved in atherogenesis and induce the production of IL-6 and the downstream inflammatory biomarker CRP, the IL-1 signaling pathway displays a valuable treatment target for atherosclerosis. Canakinumab is a human monoclonal antibody binding to IL-1β and thereby blocking the interaction of IL-1β with IL-1R, which prevents subsequent proinflammatory signaling events. Canakinumab is already approved for the treatment of cryopyrin-associated periodic syndrome patients.[207] A clinical trial in patients with diabetes mellitus revealed that administration of canakinumab efficiently reduces plasma markers of inflammation, such as CRP and IL-6, for up to 3 months without affecting LDL-C or HDL-C levels,[208] validating canakinumab as a candidate to evaluate the inflammation hypothesis of atherosclerosis.

Consequently, the CANTOS trial was initiated to test whether reducing inflammation by neutralizing IL-1β with canakinumab in patients with a history of MI and elevated CRP plasma levels (>2 mg/L) lowers their risk of recurrent cardiovascular events on top of standard secondary prevention therapies.[4] Canakinumab treatment dose-dependently decreased baseline levels of IL-6 and CRP compared with placebo, whereas LDL-C and HDL-C levels remained unaffected. Treatment with 150 mg canakinumab every 3 months resulted in a significant 15% reduction of the primary end point events, namely MI, stroke, or cardiovascular death, compared with placebo. Although this number might not seem to be very high, it needs to be considered, that this is an effect on top of standard secondary prevention care, including statin therapy with its anti-inflammatory effects. Therefore, the CANTOS trial revealed an improvement in the cardiovascular outcome of patients with prior MI by canakinumab treatment in combination with current standard secondary prevention therapies.

Two important conclusions can be drawn from the CANTOS trial. First, CANTOS clearly demonstrated that reducing low-grade systemic inflammation in CVD patients without affecting blood cholesterol levels decreases the risk of developing cardiovascular events. Therefore, it is the first large-scale randomized, double-blinded, and placebo-controlled clinical trial providing a proof-of-principle for the inflammation hypothesis of atherosclerosis. Second, it confirms the clinical importance of the proatherogenic characteristics of IL-1β. Thus, it provides evidence that therapeutic interference with IL-1β production or function can improve clinical outcomes of CVDs.

What Can We Learn From the CANTOS Trial?

CANTOS confirmed that MI patients on statin therapy with well-controlled LDL-C levels but residual inflammatory risk, as determined by CRP levels >2 mg/L, have at least the same risk of recurrent cardiovascular events as those with residual cholesterol risk because of insufficient LDL-C lowering. Previous clinical trials have shown that statin-treated patients with residual cholesterol risk (LDL-C levels >70 mg/dL) could benefit from additional treatment with either the cholesterol-absorption inhibitor ezetimibe[209] or PCSK9 (proprotein convertase subtilisin/kexin type 9)-neutralizing antibodies.[210,211] The CANTOS trial results now indicate that statin-treated patients with residual inflammatory risk (elevated CRP levels) benefit from additional anti-inflammatory therapies, such as canakinumab. Importantly, canakinumab treatment of patients at residual inflammatory risk was as efficient in reducing recurrent cardiovascular events as treatment of patients at residual cholesterol risk with PCSK9 inhibitors[4] indicating the equal importance of these 2 different types of residual risk. This emphasizes the need for personalized biomarker-based therapeutic approaches in addition to standard secondary prevention measures and demonstrates that both LDL-C and CRP should be assessed to evaluate an individual's residual risk for recurrent cardiovascular events.

Furthermore, a secondary analysis of the CANTOS trial data confirmed the previously established positive correlation between the magnitude of CRP reduction with cardiovascular event reduction also for canakinumab treatment.[212] These findings even suggest that the extent of CRP reduction after the first canakinumab application could already predict the clinical benefits of canakinumab treatment for individual patients. Therefore, the CANTOS trial opens new paths to personalized therapeutic approaches for secondary prevention of CVDs.

However, in this context, it needs to be emphasized that polymorphisms in the CRP gene are associated with a considerable lifelong increase in CRP plasma levels, which do not increase the risk of CVDs.[206] The CANTOS trial population was only recruited by elevated CRP levels, but not controlled for CRP polymorphisms. Thus, it is possible, that some individuals in the CANTOS trial population were selected based on genetically increased CRP levels, which are most likely not affected by canakinumab treatment, which only blocks IL-1β–induced CRP production. This might have weakened the overall outcome of the CANTOS trial.

Of note, the trial does not allow for any definite conclusions about the relationship of CRP levels to the trial outcome as the study cohort was not randomized for CRP levels.

Overall, the CANTOS trial results emphasize that targeting vascular and systemic inflammation represents a valuable treatment strategy for decreasing CVD risk. However, targeting inflammatory pathways systemically always bears the risk of disturbing immune homeostasis and of detrimentally affecting protective immune responses, for example during infection. Indeed, the CANTOS trial revealed a small, but significant increase in fatal infections and sepsis on canakinumab treatment.[4] However, all-cause mortality was unaffected on canakinumab treatment, which might be explained by a significant reduction in cancer mortality. Patients enrolled in the CANTOS trial have elevated CRP levels and often smoke, which increases their risk of several inflammatory cancers, including lung cancer.[213,214] Furthermore, IL-1β is an important constituent of the inflammatory tumor microenvironment and was attributed an important role in promoting carcinogenesis in certain tumors in which smoldering inflammation is part of the pathogenesis.[31] Interestingly, canakinumab treatment significantly reduced incident lung cancer and lung cancer mortality in the CANTOS trial population.[215] This unexpected finding suggests that an anti-inflammatory therapy targeting IL-1β with canakinumab could also be beneficial for patients with certain cancers, in particular, lung cancer.

Although CANTOS confirmed that blocking IL-1β is a valuable treatment approach for CVDs, it also demonstrated that the clinical use of anti-inflammatory therapies may require careful evaluation of potential risks because of interfering with immune homeostasis. Canakinumab specifically inhibits IL-1β, whereas leaving other IL-1 family cytokines unaffected. Therefore, despite the observed increase in fatal infections, long-term treatment with canakinumab might still be safer compared with treatment with anakinra or rilonacept, which affect both, IL-1α and IL-1β. Beside of CANTOS, several clinical trials are currently underway assessing the effects of other anti-inflammatory therapies for the treatment of CVDs, which have been extensively reviewed.[3,216] Of note, in parallel to the CANTOS trial, Ridker et al[199,217] initiated a second trial to directly test the inflammation hypothesis: the CIRT trial (Cardiovascular Inflammation Reduction Trial).

CIRT has a similar trial design as the CANTOS trial and thus will assess the effects of low-dose methotrexate (LDM) treatment on the risk of recurrent cardiovascular events in stable post-MI patients on standard secondary prevention care. LDM is commonly used as a treatment for patients with rheumatoid arthritis and psoriasis, where it efficiently reduces inflammatory biomarkers such as CRP and IL-6, without affecting plasma lipid levels. Patients with rheumatoid arthritis and psoriasis have an increased risk of developing CVDs, but LDM treatment was associated with reduced cardiovascular mortality.[218] Furthermore, mechanistic studies provide evidence for direct atheroprotective effects of LDM, presumably related to increased cholesterol efflux and reverse cholesterol transport from foam cells in the atherosclerotic lesion.[219] The outcome of CIRT will be of particular interest, because LDM, in contrast to canakinumab, is administered orally once a week. Consequently, it would provide a more cost-effective and more convenient treatment associated with higher patient compliance. Thus, LDM might be more suitable for routine therapy than subcutaneous monoclonal antibody treatment.

Inflammasomes are central in IL-1 cytokine production, and particularly the NLRP3 inflammasome was implicated in atherosclerosis and vascular inflammation. Although the CANTOS trial cannot definitely answer to what extent the NLRP3 inflammasome contributes to atherogenesis and atherosclerosis progression—given that proatherogenic IL-1β can also be produced by other inflammasomes and inflammasome-independent pathways—it might still represent a valuable therapeutic target for treatment of CVDs. Currently, there is great interest in developing small molecule inhibitors of the NLRP3 inflammasome (reviewed in detail elsewhere[220]). Recently, the selective NLRP3 inhibitor CRID3 (also known as CP-456773 or MCC950) was indeed shown to mediate a reduction in size and inflammatory character of atherosclerotic lesions in *Apoe*$^{-/-}$ mice,[221] highlighting the therapeutic potential of small molecule inhibitors of the NLRP3 inflammasome for CVDs. Although concerns of hepatotoxicity were raised for this particular NLRP3 inhibitory compound, which was included in the ToxCast initiative by Pfizer as a discontinued pharmaceutical,[222] the identification of other, nontoxic small molecule inhibitors of the NLRP3 inflammasome would allow for testing of the therapeutic potential of these in clinical trials.

Pharmacological inhibition of NLRP3 inflammasome activation might provide a more specific and less harmful therapeutic approach for CVDs. Compared with canakinumab, anakinra and rilonacept, which all block IL-1β (and in case of anakinra also IL-1α) function, the risk of fatal infections will likely be lower because of specific NLRP3 inflammasome inhibition. Besides NLRP3, other inflammasomes are involved in recognition of microbes. Hence, because of this functional redundancy, IL-1 family cytokines can be produced in response to infectious triggers independent of the NLRP3 inflammasome. Direct inhibition of the NLRP3 inflammasome will not only inhibit IL-1β, but as well IL-18 maturation and secretion, which could be beneficial in terms of CVD outcomes. Moreover, NLRP3 inflammasome inhibition will prevent inflammasome-mediated inflammatory cell death, which consequently could further lower local inflammation.

Another way to target specifically atherosclerosis-driving vascular immune responses is the removal of the atherogenic NLRP3 inflammasome triggers. We have recently shown that treatment of *Apoe*$^{-/-}$ mice with cholesterol solubilizing substances, such as ursodeoxycholic acid[223] or 2-hydroxypropyl-β-cyclodextrin[224] reduces vascular CC deposition and impairs diet-induced atherosclerosis development. Moreover, we found that treatment with 2-hydroxypropyl-β-cyclodextrin is able to mediate atherosclerosis regression. However, we suggested that besides removing CCs, 2-hydroxypropyl-β-cyclodextrin promoted cholesterol metabolism, which resulted in transcriptional reprogramming of macrophages favoring cholesterol efflux and reverse cholesterol transport. These studies indicate that the removal of inflammatory atherogenic triggers represents a valuable treatment approach to locally reduce vascular inflammation and atherosclerosis development.

In conclusion, the CANTOS trial provided a proof-of-principle for the inflammation hypothesis of atherosclerosis. It highlighted the potential of anti-inflammatory therapies to improve the clinical outcomes of CVDs, exemplified by IL-1β inhibition. Furthermore, the CANTOS trial results emphasize the potential of personalized biomarker-based therapeutic approaches in the secondary prevention of CVDs.

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Dr Latz is co-founder and a consultant to IFM Therapeutics.

References

1. Benjamin EJ, Blaha MJ, Chiuve SE, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation*. 2017;135:e146–e603. doi: 10.1161/CIR.0000000000000485.
2. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38:2459–2472. doi: 10.1093/eurheartj/ehx144.
3. Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br J Pharmacol*. 2017;174:3898–3913. doi: 10.1111/bph.13818.
4. Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914.
5. Mayerl C, Lukasser M, Sedivy R, Niederegger H, Seiler R, Wick G. Atherosclerosis research from past to present–on the track of two pathologists with opposing views, Carl von Rokitansky and Rudolf Virchow. *Virchows Arch*. 2006;449:96–103. doi: 10.1007/s00428-006-0176-7.
6. Classics in arteriosclerosis research: On experimental cholesterin steatosis and its significance in the origin of some pathological processes by N. Anitschkow and S. Chalatow, translated by Mary Z. Pelias, 1913. *Arteriosclerosis*. 1983;3:178–182.
7. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann Intern Med*. 1979;90:85–91.
8. Grundy SM. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and

treatment of high blood cholesterol in adults (Adult Treatment Panel II). *JAMA*. 1993;269:3015–3023.
9. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. *Science*. 1973;180:1332–1339.
10. Braunwald E. Cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med*. 1997;337:1360–1369.
11. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979. doi: 10.1056/NEJM199704033361401.
12. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340:115–126. doi: 10.1056/NEJM199901143400207.
13. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109:II2–I10. doi: 10.1161/01.CIR.0000129535.04194.38.
14. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;12:204–212. doi: 10.1038/ni.2001.
15. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity*. 2013;38:1092–1104. doi: 10.1016/j.immuni.2013.06.009.
16. Nieto FJ. Infections and atherosclerosis: new clues from an old hypothesis? *Am J Epidemiol*. 1998;148:937–948.
17. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost*. 2011;106:858–867. doi: 10.1160/TH11-06-0392.
18. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med*. 2004;351:2611–2618. doi: 10.1056/NEJMoa041747.
19. Dalrymple LS, Mohammed SM, Mu Y, Johansen KL, Chertow GM, Grimes B, Kaysen GA, Nguyen DV. Risk of cardiovascular events after infection-related hospitalizations in older patients on dialysis. *Clin J Am Soc Nephrol*. 2011;6:1708–1713. doi: 10.2215/CJN.10151110.
20. Harskamp RE, van Ginkel MW. Acute respiratory tract infections: a potential trigger for the acute coronary syndrome. *Ann Med*. 2008;40:121–128. doi: 10.1080/07853890701753672.
21. Frostegård J. Immunity, atherosclerosis and cardiovascular disease. *BMC Med*. 2013;11:117. doi: 10.1186/1741-7015-11-117.
22. Mason JC, Libby P. Cardiovascular disease in patients with chronic inflammation: mechanisms underlying premature cardiovascular events in rheumatologic conditions. *Eur Heart J*. 2015;36:482–489c. doi: 10.1093/eurheartj/ehu403.
23. Zimmer S, Grebe A, Latz E. Danger signaling in atherosclerosis. *Circ Res*. 2015;116:323–340. doi: 10.1161/CIRCRESAHA.116.301135.
24. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA, Lacy-Hulbert A, El Khoury J, Golenbock DT, Moore KJ. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat Immunol*. 2010;11:155–161. doi: 10.1038/ni.1836.
25. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938.
26. Rajamäki K, Lappalainen J, Oörni K, Välimäki E, Matikainen S, Kovanen PT, Eklund KK. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One*. 2010;5:e11765. doi: 10.1371/journal.pone.0011765.
27. Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B, Carpenter SB, Becker CE, Ediriweera HN, Mullick AE, Golenbock DT, Stuart LM, Latz E, Fitzgerald KA, Moore KJ. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nat Immunol*. 2013;14:812–820. doi: 10.1038/ni.2639.
28. Wright SD, Burton C, Hernandez M, Hassing H, Montenegro J, Mundt S, Patel S, Card DJ, Hermanowski-Vosatka A, Bergstrom JD, Sparrow CP, Detmers PA, Chao YS. Infectious agents are not necessary for murine atherogenesis. *J Exp Med*. 2000;191:1437–1442.
29. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev Immunol*. 2015;15:104–116. doi: 10.1038/nri3793.
30. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281:8–27. doi: 10.1111/imr.12621.
31. Garlanda C, Dinarello CA, Mantovani A. The interleukin-1 family: back to the future. *Immunity*. 2013;39:1003–1018. doi: 10.1016/j.immuni.2013.11.010.
32. Chen CJ, Kono H, Golenbock D, Reed G, Akira S, Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med*. 2007;13:851–856. doi: 10.1038/nm1603.
33. Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER. Identification of a membrane-associated interleukin 1 in macrophages. *Proc Natl Acad Sci USA*. 1985;82:1204–1208.
34. Warner SJ, Auger KR, Libby P. Interleukin 1 induces interleukin 1. II. Recombinant human interleukin 1 induces interleukin 1 production by adult human vascular endothelial cells. *J Immunol*. 1987;139:1911–1917.
35. Fantuzzi G, Ku G, Harding MW, Livingston DJ, Sipe JD, Kuida K, Flavell RA, Dinarello CA. Response to local inflammation of IL-1 beta-converting enzyme- deficient mice. *J Immunol*. 1997;158:1818–1824.
36. Joosten LA, Netea MG, Fantuzzi G, Koenders MI, Helsen MM, Sparrer H, Pham CT, van der Meer JW, Dinarello CA, van den Berg WB. Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. *Arthritis Rheum*. 2009;60:3651–3662. doi: 10.1002/art.25006.
37. Walsh JG, Logue SE, Lüthi AU, Martin SJ. Caspase-1 promiscuity is counterbalanced by rapid inactivation of processed enzyme. *J Biol Chem*. 2011;286:32513–32524. doi: 10.1074/jbc.M111.225862.
38. Coeshott C, Ohnemus C, Pilyavskaya A, Ross S, Wieczorek M, Kroona H, Leimer AH, Cheronis J. Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc Natl Acad Sci USA*. 1999;96:6261–6266.
39. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol*. 2009;27:519–550. doi: 10.1146/annurev.immunol.021908.132612.
40. Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS. Rapid and specific conversion of precursor interleukin 1 beta (IL-1 beta) to an active IL-1 species by human mast cell chymase. *J Exp Med*. 1991;174:821–825.
41. Castell JV, Gómez-Lechón MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology*. 1990;12:1179–1186.
42. Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, Hanzawa K, Kumagai K, Okamura H, Takada H. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. *J Immunol*. 2001;167:6568–6575.
43. Bellora F, Castriconi R, Doni A, Cantoni C, Moretta L, Mantovani A, Moretta A, Bottino C. M-CSF induces the expression of a membrane-bound form of IL-18 in a subset of human monocytes differentiating in vitro toward macrophages. *Eur J Immunol*. 2012;42:1618–1626. doi: 10.1002/eji.201142173.
44. Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE, Holt CM. Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. *Arterioscler Thromb Vasc Biol*. 1996;16:1000–1006.
45. Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA Jr. Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. *J Exp Med*. 1984;160:618–623.
46. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA Jr. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J Clin Invest*. 1985;76:2003–2011. doi: 10.1172/JCI112200.
47. Libby P, Warner SJ, Friedman GB. Interleukin 1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids. *J Clin Invest*. 1988;81:487–498. doi: 10.1172/JCI113346.
48. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA. Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. *Am J Pathol*. 1986;124:179–185.
49. Libby P, Ordovas JM, Birinyi LK, Auger KR, Dinarello CA. Inducible interleukin-1 gene expression in human vascular smooth muscle cells. *J Clin Invest*. 1986;78:1432–1438. doi: 10.1172/JCI112732.
50. Shimokawa H, Ito A, Fukumoto Y, Kadokami T, Nakaike R, Sakata M, Takayanagi T, Egashira K, Takeshita A. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest*. 1996;97:769–776. doi: 10.1172/JCI118476.
51. Morton AC, Arnold ND, Gunn J, Varcoe R, Francis SE, Dower SK, Crossman DC. Interleukin-1 receptor antagonist alters the response to vessel wall injury in a porcine coronary artery model. *Cardiovasc Res*. 2005;68:493–501. doi: 10.1016/j.cardiores.2005.06.026.
52. Isoda K, Sawada S, Ishigami N, Matsuki T, Miyazaki K, Kusuhara M, Iwakura Y, Ohsuzu F. Lack of interleukin-1 receptor antagonist modulates

plaque composition in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2004;24:1068–1073. doi: 10.1161/01.ATV.0000127025.48140.a3.
53. Chamberlain J, Evans D, King A, Dewberry R, Dower S, Crossman D, Francis S. Interleukin-1beta and signaling of interleukin-1 in vascular wall and circulating cells modulates the extent of neointima formation in mice. *Am J Pathol*. 2006;168:1396–1403.
54. Nicklin MJ, Hughes DE, Barton JL, Ure JM, Duff GW. Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *J Exp Med*. 2000;191:303–312.
55. Elhage R, Maret A, Pieraggi MT, Thiers JC, Arnal JF, Bayard F. Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. *Circulation*. 1998;97:242–244.
56. Devlin CM, Kuriakose G, Hirsch E, Tabas I. Genetic alterations of IL-1 receptor antagonist in mice affect plasma cholesterol level and foam cell lesion size. *Proc Natl Acad Sci USA*. 2002;99:6280–6285. doi: 10.1073/pnas.092324399.
57. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2003;23:656–660. doi: 10.1161/01.ATV.0000064374.15232.C3.
58. Bhaskar V, Yin J, Mirza AM, Phan D, Vanegas S, Issafras H, Michelson K, Hunter JJ, Kantak SS. Monoclonal antibodies targeting IL-1 beta reduce biomarkers of atherosclerosis in vitro and inhibit atherosclerotic plaque formation in Apolipoprotein E-deficient mice. *Atherosclerosis*. 2011;216:313–320. doi: 10.1016/j.atherosclerosis.2011.02.026.
59. Alexander MR, Moehle CW, Johnson JL, Yang Z, Lee JK, Jackson CL, Owens GK. Genetic inactivation of IL-1 signaling enhances atherosclerotic plaque instability and reduces outward vessel remodeling in advanced atherosclerosis in mice. *J Clin Invest*. 2012;122:70–79. doi: 10.1172/JCI43713.
60. Kamari Y, Werman-Venkert R, Shaish A, Werman A, Harari A, Gonen A, Voronov E, Grosskopf I, Sharabi Y, Grossman E, Iwakura Y, Dinarello CA, Apte RN, Harats D. Differential role and tissue specificity of interleukin-1alpha gene expression in atherogenesis and lipid metabolism. *Atherosclerosis*. 2007;195:31–38. doi: 10.1016/j.atherosclerosis.2006.11.026.
61. Freigang S, Ampenberger F, Weiss A, Kanneganti TD, Iwakura Y, Hersberger M, Kopf M. Fatty acid-induced mitochondrial uncoupling elicits inflammasome-independent IL-1α and sterile vascular inflammation in atherosclerosis. *Nat Immunol*. 2013;14:1045–1053. doi: 10.1038/ni.2704.
62. Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schönbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *J Exp Med*. 2002;195:245–257.
63. Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, Humbert Y, Chvatchko Y, Tedgui A. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res*. 2001;89:E41–E45.
64. Elhage R, Jawien J, Rudling M, Ljunggren HG, Takeda K, Akira S, Bayard F, Hansson GK. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. *Cardiovasc Res*. 2003;59:234–240.
65. Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma. *Circ Res*. 2002;90:E34–E38.
66. McCurdy S, Liu CA, Yap J, Boisvert WA. Potential role of IL-37 in atherosclerosis. *Cytokine*. 2017:S1043–S4666(17)30282-X. doi: 10.1016/j.cyto.2017.09.025.
67. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. *Nat Rev Immunol*. 2013;13:397–411. doi: 10.1038/nri3452.
68. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*. 2002;10:417–426.
69. de Vasconcelos NM, Van Opdenbosch N, Lamkanfi M. Inflammasomes as polyvalent cell death platforms. *Cell Mol Life Sci*. 2016;73:2335–2347. doi: 10.1007/s00018-016-2204-3.
70. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol*. 2016;16:407–420. doi: 10.1038/nri.2016.58.
71. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev*. 2017;277:61–75. doi: 10.1111/imr.12534.
72. Próchnicki T, Mangan MS, Latz E. Recent insights into the molecular mechanisms of the NLRP3 inflammasome activation. *F1000Res*. 2016;5:1469. doi: 10.12688/f1000research.8614.1.
73. Hu Z, Yan C, Liu P, Huang Z, Ma R, Zhang C, Wang R, Zhang Y, Martinon F, Miao D, Deng H, Wang J, Chang J, Chai J. Crystal structure of NLRC4 reveals its autoinhibition mechanism. *Science*. 2013;341:172–175. doi: 10.1126/science.1236381.
74. Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, Schröder GF, Fitzgerald KA, Wu H, Egelman EH. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell*. 2014;156:1193–1206. doi: 10.1016/j.cell.2014.02.008.
75. Srinivasula SM, Poyet JL, Razmara M, Datta P, Zhang Z, Alnemri ES. The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. *J Biol Chem*. 2002;277:21119–21122. doi: 10.1074/jbc.C200179200.
76. Fernandes-Alnemri T, Wu J, Yu JW, Datta P, Miller B, Jankowski W, Rosenberg S, Zhang J, Alnemri ES. The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ*. 2007;14:1590–1604. doi: 10.1038/sj.cdd.4402194.
77. Sborgi L, Ravotti F, Dandey VP, Dick MS, Mazur A, Reckel S, Chami M, Scherer S, Huber M, Böckmann A, Egelman EH, Stahlberg H, Broz P, Meier BH, Hiller S. Structure and assembly of the mouse ASC inflammasome by combined NMR spectroscopy and cryo-electron microscopy. *Proc Natl Acad Sci USA*. 2015;112:13237–13242. doi: 10.1073/pnas.1507579112.
78. Schmidt FI, Lu A, Chen JW, Ruan J, Tang C, Wu H, Ploegh HL. A single domain antibody fragment that recognizes the adaptor ASC defines the role of ASC domains in inflammasome assembly. *J Exp Med*. 2016;213:771–790. doi: 10.1084/jem.20151790.
79. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature*. 1992;356:768–774. doi: 10.1038/356768a0.
80. Lu A, Wu H. Structural mechanisms of inflammasome assembly. *FEBS J*. 2015;282:435–444. doi: 10.1111/febs.13133.
81. Howard AD, Kostura MJ, Thornberry N, Ding GJ, Limjuco G, Weidner J, Salley JP, Hogquist KA, Chaplin DD, Mumford RA. IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1 beta precursor at two distinct sites and does not cleave 31-kDa IL-1 alpha. *J Immunol*. 1991;147:2964–2969.
82. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, Quintal L, Sekut L, Talanian R, Paskind M, Wong W, Kamen R, Tracey D, Allen H. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature*. 1997;386:619–623. doi: 10.1038/386619a0.
83. Fantuzzi G, Dinarello CA. Interleukin-18 and interleukin-1 beta: two cytokine substrates for ICE (caspase-1). *J Clin Immunol*. 1999;19:1–11.
84. Broderick L, De Nardo D, Franklin BS, Hoffman HM, Latz E. The inflammasomes and autoinflammatory syndromes. *Annu Rev Pathol*. 2015;10:395–424. doi: 10.1146/annurev-pathol-012414-040431.
85. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med*. 2015;21:677–687. doi: 10.1038/nm.3893.
86. Kayagaki N, Stowe IB, Lee BL, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*. 2015;526:666–671. doi: 10.1038/nature15541.
87. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526:660–665. doi: 10.1038/nature15514.
88. Aglietti RA, Estevez A, Gupta A, Ramirez MG, Liu PS, Kayagaki N, Ciferri C, Dixit VM, Dueber EC. GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc Natl Acad Sci USA*. 2016;113:7858–7863. doi: 10.1073/pnas.1607769113.
89. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, Sun H, Wang DC, Shao F. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 2016;535:111–116. doi: 10.1038/nature18590.
90. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, Lieberman J. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. 2016;535:153–158. doi: 10.1038/nature18629.
91. Cai X, Chen J, Xu H, Liu S, Jiang QX, Halfmann R, Chen ZJ. Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell*. 2014;156:1207–1222. doi: 10.1016/j.cell.2014.01.063.

92. Hoffman HM, Broderick L. The role of the inflammasome in patients with autoinflammatory diseases. *J Allergy Clin Immunol*. 2016;138:3–14. doi: 10.1016/j.jaci.2016.05.001.
93. Hoss F, Rodriguez-Alcazar JF, Latz E. Assembly and regulation of ASC specks. *Cell Mol Life Sci*. 2017;74:1211–1229. doi: 10.1007/s00018-016-2396-6.
94. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V, Latz E. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol*. 2009;183:787–791. doi: 10.4049/jimmunol.0901363.
95. Ghonime MG, Shamaa OR, Das S, Eldomany RA, Fernandes-Alnemri T, Alnemri ES, Gavrilin MA, Wewers MD. Inflammasome priming by lipopolysaccharide is dependent upon ERK signaling and proteasome function. *J Immunol*. 2014;192:3881–3888. doi: 10.4049/jimmunol.1301974.
96. Juliana C, Fernandes-Alnemri T, Kang S, Farias A, Qin F, Alnemri ES. Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. *J Biol Chem*. 2012;287:36617–36622. doi: 10.1074/jbc.M112.407130.
97. Py BF, Kim MS, Vakifahmetoglu-Norberg H, Yuan J. Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Mol Cell*. 2013;49:331–338. doi: 10.1016/j.molcel.2012.11.009.
98. Stutz A, Kolbe CC, Stahl R, Horvath GL, Franklin BS, van Ray O, Brinkschulte R, Geyer M, Meissner F, Latz E. NLRP3 inflammasome assembly is regulated by phosphorylation of the pyrin domain. *J Exp Med*. 2017;214:1725–1736. doi: 10.1084/jem.20160933.
99. Hernandez-Cuellar E, Tsuchiya K, Hara H, Fang R, Sakai S, Kawamura I, Akira S, Mitsuyama M. Cutting edge: nitric oxide inhibits the NLRP3 inflammasome. *J Immunol*. 2012;189:5113–5117. doi: 10.4049/jimmunol.1202479.
100. Spalinger MR, Kasper S, Gottier C, et al. NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. *J Clin Invest*. 2016;126:1783–1800. doi: 10.1172/JCI83669.
101. Mao K, Chen S, Chen M, et al. Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. *Cell Res*. 2013;23:201–212. doi: 10.1038/cr.2013.6.
102. Mishra BB, Rathinam VA, Martens GW, Martinot AJ, Kornfeld H, Fitzgerald KA, Sassetti CM. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. *Nat Immunol*. 2013;14:52–60. doi: 10.1038/ni.2474.
103. Mortimer L, Moreau F, MacDonald JA, Chadee K. NLRP3 inflammasome inhibition is disrupted in a group of auto-inflammatory disease CAPS mutations. *Nat Immunol*. 2016;17:1176–1186. doi: 10.1038/ni.3538.
104. Horvath GL, Schrum JE, De Nardo CM, Latz E. Intracellular sensing of microbes and danger signals by the inflammasomes. *Immunol Rev*. 2011;243:119–135. doi: 10.1111/j.1600-065X.2011.01050.x.
105. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011;469:221–225. doi: 10.1038/nature09663.
105a. Shimada K, Crother TR, Karlin J, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity*. 2012;36:401–414. doi: 10.1016/j.immuni.2012.01.009.
106. Iyer SS, He Q, Janczy JR, Elliott EI, Zhong Z, Olivier AK, Sadler JJ, Knepper-Adrian V, Han R, Qiao L, Eisenbarth SC, Nauseef WM, Cassel SL, Sutterwala FS. Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity*. 2013;39:311–323. doi: 10.1016/j.immuni.2013.08.001.
107. Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith BL, Rajendiran TM, Núñez G. K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity*. 2013;38:1142–1153. doi: 10.1016/j.immuni.2013.05.016.
108. Pressman BC. Biological applications of ionophores. *Annu Rev Biochem*. 1976;45:501–530. doi: 10.1146/annurev.bi.45.070176.002441.
109. Perregaux D, Gabel CA. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J Biol Chem*. 1994;269:15195–15203.
110. Kelkar DA, Chattopadhyay A. The gramicidin ion channel: a model membrane protein. *Biochim Biophys Acta*. 2007;1768:2011–2025. doi: 10.1016/j.bbamem.2007.05.011.
111. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol*. 2008;9:847–856. doi: 10.1038/ni.1631.
112. Lima H Jr, Jacobson LS, Goldberg MF, Chandran K, Diaz-Griffero F, Lisanti MP, Brojatsch J. Role of lysosome rupture in controlling Nlrp3 signaling and necrotic cell death. *Cell Cycle*. 2013;12:1868–1878. doi: 10.4161/cc.24903.
113. Dostert C, Guarda G, Romero JF, Menu P, Gross O, Tardivel A, Suva ML, Stehle JC, Kopf M, Stamenkovic I, Corradin G, Tschopp J. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS One*. 2009;4:e6510. doi: 10.1371/journal.pone.0006510.
114. Orlowski GM, Sharma S, Colbert JD, Bogyo M, Robertson SA, Kataoka H, Chan FK, Rock KL. Frontline science: multiple cathepsins promote inflammasome-independent, particle-induced cell death during NLRP3-dependent IL-1β activation. *J Leukoc Biol*. 2017;102:7–17. doi: 10.1189/jlb.3HI0316-152R.
115. Okada M, Matsuzawa A, Yoshimura A, Ichijo H. The lysosome rupture-activated TAK1-JNK pathway regulates NLRP3 inflammasome activation. *J Biol Chem*. 2014;289:32926–32936. doi: 10.1074/jbc.M114.579961.
116. He Y, Zeng MY, Yang D, Motro B, Núñez G. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature*. 2016;530:354–357. doi: 10.1038/nature16959.
117. Schmid-Burgk JL, Chauhan D, Schmidt T, Ebert TS, Reinhardt J, Endl E, Hornung V. A genome-wide CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. *J Biol Chem*. 2016;291:103–109. doi: 10.1074/jbc.C115.700492.
118. Shi H, Wang Y, Li X, et al. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat Immunol*. 2016;17:250–258. doi: 10.1038/ni.3333.
119. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature*. 2007;449:819–826. doi: 10.1038/nature06246.
120. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol*. 2005;131:417–430. doi: 10.1111/j.1365-2141.2005.05753.x.
121. Zhang X, Kimura Y, Fang C, Zhou L, Sfyroera G, Lambris JD, Wetsel RA, Miwa T, Song WC. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. *Blood*. 2007;110:228–236. doi: 10.1182/blood-2006-12-063636.
122. Laudisi F, Spreafico R, Evrard M, Hughes TR, Mandriani B, Kandasamy M, Morgan BP, Sivasankar B, Mortellaro A. Cutting edge: the NLRP3 inflammasome links complement-mediated inflammation and IL-1β release. *J Immunol*. 2013;191:1006–1010. doi: 10.4049/jimmunol.1300489.
123. Triantafilou K, Hughes TR, Triantafilou M, Morgan BP. The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. *J Cell Sci*. 2013;126:2903–2913. doi: 10.1242/jcs.124388.
124. Liszewski MK, Kolev M, Le Friec G, et al. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity*. 2013;39:1143–1157. doi: 10.1016/j.immuni.2013.10.018.
125. Haneklaus M, O'Neill LA. NLRP3 at the interface of metabolism and inflammation. *Immunol Rev*. 2015;265:53–62. doi: 10.1111/imr.12285.
126. Hagar JA, Powell DA, Aachoui Y, Ernst RK, Miao EA. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. *Science*. 2013;341:1250–1253. doi: 10.1126/science.1240988.
127. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, Miyake K, Zhang J, Lee WP, Muszyński A, Forsberg LS, Carlson RW, Dixit VM. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science*. 2013;341:1246–1249. doi: 10.1126/science.1240248.
128. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature*. 2014;514:187–192. doi: 10.1038/nature13683.
129. Casson CN, Yu J, Reyes VM, Taschuk FO, Yadav A, Copenhaver AM, Nguyen HT, Collman RG, Shin S. Human caspase-4 mediates noncanonical inflammasome activation against gram-negative bacterial pathogens. *Proc Natl Acad Sci USA*. 2015;112:6688–6693. doi: 10.1073/pnas.1421699112.
130. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science*. 2008;320:674–677. doi: 10.1126/science.1156995.
131. Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, Carter AB, Rothman PB, Flavell RA, Sutterwala FS. The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci USA*. 2008;105:9035–9040. doi: 10.1073/pnas.0803933105.
132. Hirota JA, Hirota SA, Warner SM, Stefanowicz D, Shaheen F, Beck PL, Macdonald JA, Hackett TL, Sin DD, Van Eeden S, Knight DA. The airway epithelium nucleotide-binding domain and leucine-rich repeat protein 3 inflammasome is activated by urban particulate matter. *J Allergy Clin Immunol*. 2012;129:1116–25.e6. doi: 10.1016/j.jaci.2011.11.033.

133. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;440:237–241. doi: 10.1038/nature04516.
134. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes*. 2003;52:812–817.
135. Lorenzo A, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature*. 1994;368:756–760. doi: 10.1038/368756a0.
136. Masters SL, Dunne A, Subramanian SL, et al. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nat Immunol*. 2010;11:897–904. doi: 10.1038/ni.1935.
137. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol*. 2011;12:408–415. doi: 10.1038/ni.2022.
138. Wolf AJ, Reyes CN, Liang W, Becker C, Shimada K, Wheeler ML, Cho HC, Popescu NI, Coggeshall KM, Arditi M, Underhill DM. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell*. 2016;166:624–636. doi: 10.1016/j.cell.2016.05.076.
139. Liston A, Masters SL. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat Rev Immunol*. 2017;17:208–214. doi: 10.1038/nri.2016.151.
140. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, Nakahira K, Choi AMK. mTORC1-Induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. *Cell Rep*. 2015;12:102–115. doi: 10.1016/j.celrep.2015.05.046.
141. Xie M, Yu Y, Kang R, Zhu S, Yang L, Zeng L, Sun X, Yang M, Billiar TR, Wang H, Cao L, Jiang J, Tang D. PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. *Nat Commun*. 2016;7:13280. doi: 10.1038/ncomms13280.
142. Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med*. 2015;21:263–269. doi: 10.1038/nm.3804.
143. Yan Y, Jiang W, Spinetti T, Tardivel A, Castillo R, Bourquin C, Guarda G, Tian Z, Tschopp J, Zhou R. Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity*. 2013;38:1154–1163. doi: 10.1016/j.immuni.2013.05.015.
144. Williams-Bey Y, Boularan C, Vural A, Huang NN, Hwang IY, Shan-Shi C, Kehrl JH. Omega-3 free fatty acids suppress macrophage inflammasome activation by inhibiting NF-κB activation and enhancing autophagy. *PLoS One*. 2014;9:e97957. doi: 10.1371/journal.pone.0097957.
145. Phan AT, Goldrath AW, Glass CK. Metabolic and epigenetic coordination of T cell and macrophage immunity. *Immunity*. 2017;46:714–729. doi: 10.1016/j.immuni.2017.04.016.
146. Próchnicki T, Latz E. Inflammasomes on the crossroads of innate immune recognition and metabolic control. *Cell Metab*. 2017;26:71–93. doi: 10.1016/j.cmet.2017.06.018.
147. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol*. 2016;16:553–565. doi: 10.1038/nri.2016.70.
148. Menu P, Pellegrin M, Aubert JF, Bouzourene K, Tardivel A, Mazzolai L, Tschopp J. Atherosclerosis in ApoE-deficient mice progresses independently of the NLRP3 inflammasome. *Cell Death Dis*. 2011;2:e137. doi: 10.1038/cddis.2011.18.
149. Getz GS, Reardon CA. Do the Apoe-/- and Ldlr-/- Mice Yield the Same Insight on Atherogenesis? *Arterioscler Thromb Vasc Biol*. 2016;36:1734–1741. doi: 10.1161/ATVBAHA.116.306874.
150. Deleted in proof.
151. Willingham SB, Allen IC, Bergstralh DT, Brickey WJ, Huang MT, Taxman DJ, Duncan JA, Ting JP. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol*. 2009;183:2008–2015. doi: 10.4049/jimmunol.0900138.
152. Li W, Sama AE, Wang H. Role of HMGB1 in cardiovascular diseases. *Curr Opin Pharmacol*. 2006;6:130–135. doi: 10.1016/j.coph.2005.10.010.
153. Tumurkhuu G, Shimada K, Dagvadorj J, Crother TR, Zhang W, Luthringer D, Gottlieb RA, Chen S, Arditi M. Ogg1-dependent DNA repair regulates NLRP3 inflammasome and prevents atherosclerosis. *Circ Res*. 2016;119:e76–e90.
154. Westerterp M, Fotakis P, Ouimet M, et al. Cholesterol efflux pathways suppress inflammasome activation, NETosis and atherogenesis [published online ahead of print March 27, 2018]. *Circulation*. doi: 10.1161/CIRCULATIONAHA.117.032636. http://circ.ahajournals.org/content/early/2018/03/23/CIRCULATIONAHA.117.032636.long.
155. Usui F, Shirasuna K, Kimura H, Tatsumi K, Kawashima A, Karasawa T, Hida S, Sagara J, Taniguchi S, Takahashi M. 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;425:162–168. doi: 10.1016/j.bbrc.2012.07.058.
156. Gage J, Hasu M, Thabet M, Whitman SC. Caspase-1 deficiency decreases atherosclerosis in apolipoprotein E-null mice. *Can J Cardiol*. 2012;28:222–229. doi: 10.1016/j.cjca.2011.10.013.
157. Hendrikx T, Jeurissen ML, van Gorp PJ, Gijbels MJ, Walenbergh SM, Houben T, van Gorp R, Pöttgens CC, Stienstra R, Netea MG, Hofker MH, Donners MM, Shiri-Sverdlov R. Bone marrow-specific caspase-1/11 deficiency inhibits atherosclerosis development in Ldlr(-/-) mice. *FEBS J*. 2015;282:2327–2338. doi: 10.1111/febs.13279.
158. Zheng F, Xing S, Gong Z, Mu W, Xing Q. Silence of NLRP3 suppresses atherosclerosis and stabilizes plaques in apolipoprotein E-deficient mice. *Mediators Inflamm*. 2014;2014:507208. doi: 10.1155/2014/507208.
159. Christ A, Günther P, Lauterbach MAR, et al. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell*. 2018;172:162–175.e14. doi: 10.1016/j.cell.2017.12.013.
160. Zheng F, Xing S, Gong Z, Xing Q. NLRP3 inflammasomes show high expression in aorta of patients with atherosclerosis. *Heart Lung Circ*. 2013;22:746–750. doi: 10.1016/j.hlc.2013.01.012.
161. Shi X, Xie WL, Kong WW, Chen D, Qu P. Expression of the NLRP3 inflammasome in carotid atherosclerosis. *J Stroke Cerebrovasc Dis*. 2015;24:2455–2466. doi: 10.1016/j.jstrokecerebrovasdis.2015.03.024.
162. Paramel Varghese G, Folkersen L, Strawbridge RJ, et al. NLRP3 inflammasome expression and activation in human atherosclerosis. *J Am Heart Assoc*. 2016;5:e003031.
163. Afrasyab A, Qu P, Zhao Y, Peng K, Wang H, Lou D, Niu N, Yuan D. Correlation of NLRP3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart Vessels*. 2016;31:1218–1229. doi: 10.1007/s00380-015-0723-8.
164. Zhou D, Wang X, Chen T, Wen W, Liu Y, Wu Y, Yuan Z. The NLRP3 rs10754558 polymorphism is associated with the occurrence and prognosis of coronary artery disease in the Chinese Han population. *Biomed Res Int*. 2016;2016:3185397. doi: 10.1155/2016/3185397.
165. Hitomi Y, Ebisawa M, Tomikawa M, et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *J Allergy Clin Immunol*. 2009;124:779–785.e6. doi: 10.1016/j.jaci.2009.07.044.
166. Zhao X, Gu C, Yan C, Zhang X, Li Y, Wang L, Ren L, Zhang Y, Peng J, Zhu Z, Han Y. NALP3-inflammasome-related gene polymorphisms in patients with prehypertension and coronary atherosclerosis. *Biomed Res Int*. 2016;2016:7395627. doi: 10.1155/2016/7395627.
167. Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. *Circulation*. 2001;104:3103–3108.
168. Mullick AE, Soldau K, Kiosses WB, Bell TA III, Tobias PS, Curtiss LK. Increased endothelial expression of Toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events. *J Exp Med*. 2008;205:373–383. doi: 10.1084/jem.20071096.
169. Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation*. 2002;105:1158–1161.
170. Otsui K, Inoue N, Kobayashi S, Shiraki R, Honjo T, Takahashi M, Hirata K, Kawashima S, Yokoyama M. Enhanced expression of TLR4 in smooth muscle cells in human atherosclerotic coronary arteries. *Heart Vessels*. 2007;22:416–422. doi: 10.1007/s00380-007-1001-1.
171. Björkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT, Freeman MW. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med*. 2004;10:416–421. doi: 10.1038/nm1008.
172. Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and

alters plaque phenotype in mice deficient in apolipoprotein E. *Proc Natl Acad Sci USA*. 2004;101:10679–10684. doi: 10.1073/pnas.0403249101.
173. Cole JE, Georgiou E, Monaco C. The expression and functions of toll-like receptors in atherosclerosis. *Mediators Inflamm*. 2010;2010:393946. doi: 10.1155/2010/393946.
174. Madan M, Amar S. Toll-like receptor-2 mediates diet and/or pathogen associated atherosclerosis: proteomic findings. *PLoS One*. 2008;3:e3204. doi: 10.1371/journal.pone.0003204.
175. Mullick AE, Tobias PS, Curtiss LK. Modulation of atherosclerosis in mice by Toll-like receptor 2. *J Clin Invest*. 2005;115:3149–3156. doi: 10.1172/JCI25482.
176. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem*. 2002;277:49982–49988. doi: 10.1074/jbc.M209649200.
177. Rhoads JP, Lukens JR, Wilhelm AJ, Moore JL, Mendez-Fernandez Y, Kanneganti TD, Major AS. Oxidized low-density lipoprotein immune complex priming of the Nlrp3 inflammasome involves TLR and FcγR cooperation and is dependent on CARD9. *J Immunol*. 2017;198:2105–2114. doi: 10.4049/jimmunol.1601563.
178. Abela GS, Aziz K, Vedre A, Pathak DR, Talbott JD, Dejong J. Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes. *Am J Cardiol*. 2009;103:959–968. doi: 10.1016/j.amjcard.2008.12.019.
179. Abela GS. Cholesterol crystals piercing the arterial plaque and intima trigger local and systemic inflammation. *J Clin Lipidol*. 2010;4:156–164. doi: 10.1016/j.jacl.2010.03.003.
180. Emanuel R, Sergin I, Bhattacharya S, Turner J, Epelman S, Settembre C, Diwan A, Ballabio A, Razani B. Induction of lysosomal biogenesis in atherosclerotic macrophages can rescue lipid-induced lysosomal dysfunction and downstream sequelae. *Arterioscler Thromb Vasc Biol*. 2014;34:1942–1952. doi: 10.1161/ATVBAHA.114.303342.
181. Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, Ting JP, Virgin HW, Kastan MB, Semenkovich CF. Autophagy links inflammasomes to atherosclerotic progression. *Cell Metab*. 2012;15:534–544. doi: 10.1016/j.cmet.2012.02.011.
182. Kotla S, Singh NK, Rao GN. ROS via BTK-p300-STAT1-PPARγ signaling activation mediates cholesterol crystals-induced CD36 expression and foam cell formation. *Redox Biol*. 2017;11:350–364. doi: 10.1016/j.redox.2016.12.005.
183. Samstad EO, Niyonzima N, Nymo S, Aune MH, Ryan L, Bakke SS, Lappegård KT, Brekke OL, Lambris JD, Damås JK, Latz E, Mollnes TE, Espevik T. Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. *J Immunol*. 2014;192:2837–2845. doi: 10.4049/jimmunol.1302484.
184. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science (80-)*. 2015;349:316–320.
185. Megens RT, Vijayan S, Lievens D, Döring Y, van Zandvoort MA, Grommes J, Weber C, Soehnlein O. Presence of luminal neutrophil extracellular traps in atherosclerosis. *Thromb Haemost*. 2012;107:597–598. doi: 10.1160/TH11-09-0650.
186. Borissoff JI, Joosen IA, Versteylen MO, Brill A, Fuchs TA, Savchenko AS, Gallant M, Martinod K, Ten Cate H, Hofstra L, Crijns HJ, Wagner DD, Kietselaer BLJH. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. *Arterioscler Thromb Vasc Biol*. 2013;33:2032–2040. doi: 10.1161/ATVBAHA.113.301627.
187. Knight JS, Luo W, O'Dell AA, Yalavarthi S, Zhao W, Subramanian V, Guo C, Grenn RC, Thompson PR, Eitzman DT, Kaplan MJ. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. *Circ Res*. 2014;114:947–956. doi: 10.1161/CIRCRESAHA.114.303312.
188. Soehnlein O, Ortega-Gómez A, Döring Y, Weber C. Neutrophil-macrophage interplay in atherosclerosis: protease-mediated cytokine processing versus NET release. *Thromb Haemost*. 2015;114:866–867. doi: 10.1160/TH15-08-0623.
189. Döring Y, Manthey HD, Drechsler M, et al. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. *Circulation*. 2012;125:1673–1683. doi: 10.1161/CIRCULATIONAHA.111.046755.
190. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. *J Immunol*. 2013;190:1217–1226. doi: 10.4049/jimmunol.1202388.
191. Peng K, Liu L, Wei D, Lv Y, Wang G, Xiong W, Wang X, Altaf A, Wang L, He D, Wang H, Qu P. P2X7R is involved in the progression of atherosclerosis by promoting NLRP3 inflammasome activation. *Int J Mol Med*. 2015;35:1179–1188. doi: 10.3892/ijmm.2015.2129.
192. Stachon P, Heidenreich A, Merz J, et al. P2X7 deficiency blocks lesional inflammasome activity and ameliorates atherosclerosis in mice. *Circulation*. 2017;135:2524–2533. doi: 10.1161/CIRCULATIONAHA.117.027400.
193. Hakimi M, Peters A, Becker A, Böckler D, Dihlmann S. Inflammation-related induction of absent in melanoma 2 (AIM2) in vascular cells and atherosclerotic lesions suggests a role in vascular pathogenesis. *J Vasc Surg*. 2014;59:794–803. doi: 10.1016/j.jvs.2013.03.048.
194. Lugrin J, Martinon F. The AIM2 inflammasome: sensor of pathogens and cellular perturbations. *Immunol Rev*. 2018;281:99–114. doi: 10.1111/imr.12618.
195. Gaidt MM, Ebert TS, Chauhan D, Ramshorn K, Pinci F, Zuber S, O'Duill F, Schmid-Burgk JL, Hoss F, Buhmann R, Wittmann G, Latz E, Subklewe M, Hornung V. The DNA inflammasome in human myeloid cells is initiated by a STING-cell death program upstream of NLRP3. *Cell*. 2017;171:1110–1124.e18. doi: 10.1016/j.cell.2017.09.039.
196. Dang EV, McDonald JG, Russell DW, Cyster JG. Oxysterol restraint of cholesterol synthesis prevents AIM2 inflammasome activation. *Cell*. 2017;171:1057–1071.e11. doi: 10.1016/j.cell.2017.09.029.
197. Zanoni I, Tan Y, Di Gioia M, Broggi A, Ruan J, Shi J, Donado CA, Shao F, Wu H, Springstead JR, Kagan JC. An endogenous caspase-11 ligand elicits interleukin-1 release from living dendritic cells. *Science*. 2016;352:1232–1236. doi: 10.1126/science.aaf3036.
198. Man SM, Karki R, Sasai M, Place DE, Kesavardhana S, Temirov J, Frase S, Zhu Q, Malireddi RKS, Kuriakose T, Peters JL, Neale G, Brown SA, Yamamoto M, Kanneganti TD. IRGB10 liberates bacterial ligands for sensing by the AIM2 and Caspase-11-NLRP3 inflammasomes. *Cell*. 2016;167:382–396.e17. doi: 10.1016/j.cell.2016.09.012.
199. Ridker PM. Closing the loop on inflammation and atherothrombosis: why perform the CIRT and CANTOS trials? *Trans Am Clin Climatol Assoc*. 2013;124:174–190.
200. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, Flaker GC, Braunwald E. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation*. 1998;98:839–844.
201. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P; Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med*. 2005;352:29–38. doi: 10.1056/NEJMoa042000.
202. Morrow DA, de Lemos JA, Sabatine MS, Wiviott SD, Blazing MA, Shui A, Rifai N, Califf RM, Braunwald E. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation*. 2006;114:281–288. doi: 10.1161/CIRCULATIONAHA.106.628909.
203. Ridker PM. The JUPITER trial: results, controversies, and implications for prevention. *Circ Cardiovasc Qual Outcomes*. 2009;2:279–285. doi: 10.1161/CIRCOUTCOMES.109.868299.
204. Ridker PM, Lüscher TF. Anti-inflammatory therapies for cardiovascular disease. *Eur Heart J*. 2014;35:1782–1791. doi: 10.1093/eurheartj/ehu203.
205. Teupser D, Weber O, Rao TN, Sass K, Thiery J, Fehling HJ. No reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. *J Biol Chem*. 2011;286:6272–6279. doi: 10.1074/jbc.M110.161414.
206. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med*. 2008;359:1897–1908. doi: 10.1056/NEJMoa0707402.
207. Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN; Canakinumab in CAPS Study Group. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med*. 2009;360:2416–2425. doi: 10.1056/NEJMoa0810787.
208. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, Thuren T; CANTOS Pilot Investigative Group. Effects of interleukin-1β

inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. *Circulation*. 2012;126:2739–2748. doi: 10.1161/CIRCULATIONAHA.112.122556.

209. Cannon CP, Blazing MA, Giugliano RP, et al; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397. doi: 10.1056/NEJMoa1410489.
210. Ridker PM, Revkin J, Amarenco P, et al; SPIRE Cardiovascular Outcome Investigators. Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med*. 2017;376:1527–1539. doi: 10.1056/NEJMoa1701488.
211. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR; FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713–1722. doi: 10.1056/NEJMoa1615664.
212. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ; CANTOS Trial Group. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet*. 2018;391:319–328. doi: 10.1016/S0140-6736(17)32814-3.
213. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *J Clin Oncol*. 2009;27:2217–2224. doi: 10.1200/JCO.2008.19.8440.
214. Chaturvedi AK, Caporaso NE, Katki HA, Wong HL, Chatterjee N, Pine SR, Chanock SJ, Goedert JJ, Engels EA. C-reactive protein and risk of lung cancer. *J Clin Oncol*. 2010;28:2719–2726. doi: 10.1200/JCO.2009.27.0454.
215. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ; CANTOS Trial Group. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet*. 2017;390:1833–1842. doi: 10.1016/S0140-6736(17)32247-X.
216. Bäck M, Hansson GK. Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol*. 2015;12:199–211. doi: 10.1038/nrcardio.2015.5.
217. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, Gupta M, Clearfield M, Libby P, Hasan AA, Glynn RJ, Ridker PM. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. *Am Heart J*. 2013;166:199–207.e15. doi: 10.1016/j.ahj.2013.03.018.
218. Micha R, Imamura F, Wyler von Ballmoos M, Solomon DH, Hernán MA, Ridker PM, Mozaffarian D. Systematic review and meta-analysis of methotrexate use and risk of cardiovascular disease. *Am J Cardiol*. 2011;108:1362–1370. doi: 10.1016/j.amjcard.2011.06.054.
219. Coomes E, Chan ES, Reiss AB. Methotrexate in atherogenesis and cholesterol metabolism. *Cholesterol*. 2011;2011:503028. doi: 10.1155/2011/503028.
220. Baldwin AG, Brough D, Freeman S. Inhibiting the inflammasome: a chemical perspective. *J Med Chem*. 2016;59:1691–1710. doi: 10.1021/acs.jmedchem.5b01091.
221. van der Heijden T, Kritikou E, Venema W, van Duijn J, van Santbrink PJ, Slütter B, Foks AC, Bot I, Kuiper J. NLRP3 inflammasome inhibition by MCC950 reduces atherosclerotic lesion development in apolipoprotein E-deficient mice-brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:1457–1461. doi: 10.1161/ATVBAHA.117.309575.
222. Shah F, Greene N. Analysis of Pfizer compounds in EPA's ToxCast chemicals-assay space. *Chem Res Toxicol*. 2014;27:86–98. doi: 10.1021/tx400343t.
223. Bode N, Grebe A, Kerksiek A, Lütjohann D, Werner N, Nickenig G, Latz E, Zimmer S. Ursodeoxycholic acid impairs atherogenesis and promotes plaque regression by cholesterol crystal dissolution in mice. *Biochem Biophys Res Commun*. 2016;478:356–362. doi: 10.1016/j.bbrc.2016.07.047.
224. Zimmer S, Grebe A, Bakke SS, et al. Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. *Sci Transl Med*. 2016;8:333ra50. doi: 10.1126/scitranslmed.aad6100.