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# Endothelial Dysfunction: The Role of SREBP-Induced NLRP3 Inflammasome in Atherosclerosis

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## Abstract

**Purpose of review—**Great effort has been devoted to elucidate the molecular mechanisms by which inflammasome in macrophages contributes to atherosclerosis. Inflammasome in vascular endothelial cells (ECs) and its causal relationship with endothelial dysfunction in atherosclerosis are less understood. Here we review recent studies of inflammasome and its activation in ECs, and highlight such endothelial inflammatory response in atherosclerosis.

**Recent findings—**Inflammasomes are critical effectors in innate immunity, and their activation in macrophages and the arterial wall contributes to atherogenesis. Sterol regulatory element-binding protein 2 (SREBP2), a master regulator in cholesterol biosynthesis, can be activated in a non-canonical manner, which leads to activation of the inflammasome NOD-like receptor family pyrin domain-containing protein (NLRP) in macrophages and ECs. Results from *in vitro* and *in vivo* models suggest that SREBP2 is a key molecule in aggravating pro-inflammatory responses in ECs, and promoting atherosclerosis.

**Summary—**The SREBP-induced NLRP inflammasome and its instigation of innate immunity is an important contributor to atherosclerosis. Elucidating the underlying mechanisms will expand our understanding of endothelial dysfunction and its dynamic interaction with vascular inflammation. Furthermore, targeting SREBP-inflammasome pathways can be a therapeutic strategy for attenuating atherosclerosis.

### Keywords

SREBP; inflammasome; endothelial dysfunction; innate immunity; atherosclerosis

## Introduction

The “response-to-injury” hypothesis proposed more than 3 decades ago stated that atherosclerotic lesions result from some form of "injury" to the arterial endothelium [1].

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Relentless research efforts have investigated the contextual basis of endothelial injury leading to atherosclerosis. Because of the chronic nature of atherogenesis, insults from various forms of cardiovascular risk factors, even acute, disturb the homeostasis in vascular endothelial cells (ECs). This vascular impairment leads to a dysfunctional endothelium, marked by the decreased expression and/or activity of endothelial nitric oxide synthase (eNOS) and increased inflammatory response.

Innate immunity is the non-specific first response in phagocytic cells to infectious or sterile injury via the recognition of molecules with characteristic pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) [2, 3]. Lining the luminal surface of blood vessels, ECs are directly exposed to circulating immune and inflammatory mediators and therefore can be considered atypical immunogenic cells in the vascular wall. Like monocytic phagocytes, ECs express a class of pattern-recognition receptors such as Toll-like receptors (TLRs), CD36 scavenger receptor, and the receptor for advanced glycation end products (RAGEs). These receptors induce a coordinated signaling network that ultimately activates nuclear factor-kappa B (NF-κB) and the ensuing production of pro-inflammatory cytokines, chemoattractants, and adhesion molecules (as reviewed in [4]).

Innate immunity in atherosclerosis often refers to the recruitment, infiltration, and differentiation of monocytes/macrophages in the arterial wall with subsequent foam cell formation. Gene targeting in conjunction with bone marrow transplantation are prevalently used to test the role of innate immune response in monocytes/macrophages involved in atherosclerosis. Recent advances have highlighted the induction of the NOD-like receptor (NLR) family pyrin domain-containing protein 3 (NLRP3) inflammasome and subsequent activation of interleukin-1β (IL-1β) family proteins, which underscore the innate immune response in phagocytes, particularly in monocytes/macrophages [5]. The presence of the NLRP3 inflammasome in foam cells of murine models reinforces the concept that the enhanced innate immune response of monocytes/macrophages contributes to atherosclerosis [6]. What is less understood is whether inflammasome is an integral component of endothelial innate immune response, and if so, its causal relationship with endothelial dysfunction leading to atherosclerosis.

Unlike monocytes/macrophages, ECs show little accumulation of cholesterol and lipids in culture, the arterial wall, or atherosclerotic lesions. Sterol regulatory element-binding protein 1 and 2 (SREBP1 and SREBP2) play a canonical role in cholesterol and fatty acid homeostasis via their transcriptional regulation of genes involved in the biosynthesis of cholesterol, triacylglycerides, and phospholipids. Intriguingly, SREBP can be induced in ECs by atherogenic factors such as disturbed flow and oxidized palmitoyl-arachidonyl-phosphatidyl choline (ox-PAPC) [7, 8], which suggests a non-canonical role for SREBP in endothelial biology. This review will assess recent studies suggesting that the NLRP3 inflammasome is indeed present in ECs and that SREBP is crucial for the induction of inflammasome. Moreover, such innate immune responses coincide with dysregulated EC functions rather than lipid accumulation and phagocytosis seen in monocytic cells.

## Endothelial dysfunction

Before discussing the molecular basis underlying inflammasome activity in ECs, it is worthwhile to review the current understanding of endothelial dysfunction that has been greatly advanced by revealing the underpinnings of molecules and pathways related to endothelial biology. At the tissue level, endothelial dysfunction is characterized by impaired vasodilation, increased redox state, as well as augmented pro-inflammatory and pro-thrombotic properties. eNOS-derived NO bioavailability has been a hallmark of a functional endothelium. Besides regulating vascular tone, NO has several beneficial effects against atherosclerosis, including anti-inflammation, anti-oxidation, and anti-proliferation of smooth muscle cells as well as inhibiting the adhesion and infiltration of leukocytes (as reviewed in [9]). In ECs, eNOS expression and activity are regulated by multiple mechanisms, summarized in several excellent reviews [10–12]. Of note, regulators positively modulating eNOS, such as AMP-activated protein kinase (AMPK), Sirtuin 1 (SIRT1), Krüppel-like factor 2 (KLF2), and KLF4, are activated or induced by stimuli (e.g., atheroprotective flow and resveratrol) that enhance eNOS activity. In contrast, vascular insults such as disturbed flow, angiotensin II (Ang II), and oxidized-LDL (ox-LDL), impair the NO regulation network and decrease the activity and/or expression of AMPK, SIRT1, KLF2, and KLF4. In addition, microRNAs (miRs) regulate eNOS. For example, disturbed flow or tumor necrosis factor α (TNFα) activation upregulates miR-92a, which putatively targets SIRT1, KLF2, and KLF4, thus resulting in deficient NO bioavailability and hence endothelial dysfunction [13, 14].

Intriguingly, molecular and cellular events involved in mitochondrial biogenesis, autophagy, inflammasome, and the endothelial-mesenchymal transition (EndoMT) intersect with pathways regulating eNOS. Although ECs do not have high energy demands and the ATP supply is largely depends on glycolysis rather than mitochondrial oxidative phosphorylation [15], the endothelial mitochondria act as critical signaling organelles to modulate the intracellular dynamics of NO, reactive oxygen species (ROS), and $Ca^{2+}$, which in turn control endothelial function (as reviewed in [16, 17•]). Therefore, damage to mitochondrial function or impaired mitochondrial biogenesis may contribute to endothelial dysfunction, thus leading to a pathophysiological state. This observation is supported in part by the increased mitochondrial DNA damage (a marker of impaired mitochondrial biogenesis) seen in ApoE-null mice and in humans with atherosclerosis [18]. Mitophagy, a catabolic process that removes dysfunctional mitochondria, coordinates with mitochondrial biogenesis to maintain functional mitochondria and integrity of the anti-oxidative defense system [19]. In conjunction with mitophagy, the highly regulated auto-digesting events collectively termed autophagy maintain endothelial energy and redox balance by efficient clearance and recycling of damaged cytoplasmic contents [20]. If left uncleared, cellular damage and pathogens elicit an inflammatory response through the activation of inflammasome pathways, initially characterized as innate immune response in phagocytic cells. Recent studies suggest that the activation of inflammasome in ECs under pathophysiological conditions aggravates endothelial dysfunction, which will be further discussed below.

Importantly, the induction or suppression of these novel markers of endothelial function or dysfunction appear to be correlated with that of oxidative stress, the inflammatory state, and

eNOS-derived NO bioavailability. For example, resveratrol and atheroprotective flow have a positive effect on mitochondrial biogenesis and autophagy while inhibiting inflammasome activation [21–23••]. Therefore, risk factors of atherosclerosis, such as disturbed flow, hyperglycemia, and hyperlipidemia, may elevate oxidative stress and inflammasome activity while suppressing mitochondrial biogenesis and autophagy, thereby exacerbating endothelial dysfunction. In addition to the aforementioned processes that can be acutely altered by detrimental factors, gradual loss of endothelial lineage via EndoMT is an emerging marker of dysfunctional endothelium involved in atherosclerosis [24]. Although the molecular mechanisms underlying EndoMT remain elusive, transforming growth factor β (TGFβ)-related signaling appears to be a principal inducer [25, 26]. This scenario recapitulates the intimate cross-talk among multiple pathways implicated in endothelial dysfunction (summarized in Table 1) and emphasizes the importance of uncovering new mechanistic links among these biological processes.

## NLRP inflammasome in atherosclerosis

Inflammasome is a multi-protein complex consisting of three key molecules, an NLRP, apoptosis-associated speck-like protein containing a CARD (ASC), and pro-caspase-1 [79••]. Upon NLRP's recognition of PAMPs (e.g., lipopolysaccharide and double-stranded DNA), or endogenous DAMPs (e.g., ATP, $Ca^{2+}$, urate, or cholesterol crystals), the protein components of inflammasome oligomerize, thus resulting in the self-cleavage and activation of caspase-1 [5]. The activation of inflammasome requires a priming signal (Signal 1) to transcriptionally induce inflammasome components and an activating signal (Signal 2) to cause caspase-1 cleavage. The activated caspase-1 in turn cleaves pro-IL-1β and pro-IL-18 to generate the mature forms of IL-1β and IL-18 [80–83]. These activated IL-1β and IL-18 are then secreted from the cell to promote further inflammatory processes in the immediate surroundings (for more detailed reviews on inflammasomes, see [84–86]). The NLRP family includes NLRP1, NLRP3, NLRP6, NLRP7, NLRP12, and NLR family CARD domain-containing protein 4 (NLRC4). On recognition of specific PAMPs/DAMPs, the respective NLRP activates caspase-1 to increase the secretion of IL-1β and IL-18. The distinct inflammasomes with their cognate PAMP/DAMP recognition patterns are outlined in more extensive reviews [79••, 87]. Here we focus on NLRP3 because of its high prevalence in atherosclerosis.

Saturated fatty acids, high glucose, and cholesterol crystals can all activate NLRP3 inflammasome in myeloid cells [6, 69••, 88, 89]. Pertinent to atherogenesis, inflammasome activation is linked to the formation of foam cells and the development of atherosclerotic lesions [90•]. In support of this notion, hypercholesterolemic LDL receptor (LDLR)-deficient mice transplanted with bone marrow from mice deficient in inflammasome components (i.e., NLRP3, ASC, or IL-1β) showed less atherosclerosis than their littermates receiving wild-type bone marrow [6]. Results from studies with ApoE-null mice are less consistent. Menu et al. reported little change in atherosclerosis among $NLRP3^{-/-}/ApoE^{-/-}$, $ASC^{-/-}/ApoE^{-/-}$, or $capsase\text{-}1^{-/-}/ApoE^{-/-}$ mice as compared with ApoE-null littermates [91]. However, more recent studies showed that genetic ablation of caspase-1 in an ApoE-null background (i.e., $capsase\text{-}1^{-/-}/ApoE^{-/-}$) conferred an anti-atherosclerotic effect [92, 93]. Nonetheless, the causative effects of inflammasome activation in atherosclerosis are

consistent with earlier findings that genetic ablation of IL-1β or IL-1 receptor (IL-1R) attenuates atherosclerosis and that of IL-1R antagonist promotes atherogenesis in hypercholesterolemic mice [94–96]. Additionally, IL-18 intraperitoneally injected into hypercholesterolemic mice caused a two-fold increase in atherosclerotic lesion size, whereas IL-18-deficient mice showed a significant decrease in atherosclerosis [97, 98]. With respect to translational applications, the recently launched Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) [99,100] will clarify the efficacy of using IL-1β antibody to prevent cardiovascular events in patients with atherosclerosis.

Another functional outcome resulting from inflammasome activation is pyroptosis, a pro-inflammatory programmed cell death leading to self-destruction of the infected host cell [101]. Apoptosis, which is non-inflammatory, depends on caspase-3 and cytochrome-c, whereas pyroptosis depends on caspase-1 activation [102]. Therefore, pyroptosis stimulates the pathological ion efflux and release of inflammatory substances, thus aggravating local inflammation [103]. Given that ox-LDL and ROS are well documented in stimulating caspase-1 and inducing cell death [104, 105] and that inflammasome activation is evident in atherosclerotic lesions [6, 92], caspase-1-dependent pyroptosis may very well be involved in atherosclerosis.

## Endothelial innate immune response in atherosclerosis

The reciprocity among oxidative stress, innate immune response, and endothelial dysfunction prelude many cardiovascular diseases. Various atherogenic factors such as hyperlipidemia, hypertension, hyperglycemia, and disturbed flow increase the expression of adhesion molecules and chemoattractants, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), and E-selectin in ECs. Consequently, monocytes are recruited to the foci of the injured endothelium. Despite scarce information, endothelial innate immune response has been suggested to be related to these early events of atherosclerosis. For example, TLR2 expression was confined to the lesser curvature of the aortic arch of LDLR-deficient mice and was further increased by hyperlipidemia [106]. Genetic deficiency of TLR4 or its downstream myeloid differentiation factor 88 (MyD88) in $ApoE^{-/-}$ background reduced atherosclerosis in the atheroprone areas [107]. Atheroprotective flow, mimicked by laminar shear stress in flow channels, can decrease TLR2 expression in ECs [108]. Despite these published results, direct evidence is still needed to support whether endothelial innate immunity is necessary and/or sufficient for atherogenesis. Such shortage of information has been due in part to the scarcity of models of EC-specific genetic ablation or overexpression of molecules crucial in innate immune pathways.

Although the pathological activation of inflammasome in ECs involved in atherosclerosis remains largely unknown, the study by Wang *et al.* showed that macrophage-derived microparticles (MPs) could increase the expression of cell adhesion molecules in ECs via the activation of NLRP3 inflammasome [109]. Knockdown of NLRP3 in macrophages reduced the activity of the MPs, and blockade of the IL-1R in ECs decreased MP-dependent EC activation, which suggests cross-talk between macrophage inflammasomes and EC activation. Furthermore, a study by Xiang *et al.* demonstrated that hemorrhagic shock could

activate TLR2, TLR4, and RAGE in ECs leading to NLRP3 inflammasome activation [110]. Our recently published work showed that disturbed flow activates an innate immune response via induction of the NLRP3 inflammasome in ECs [23••]. Oscillatory shear stress *in vitro*, mimicking disturbed flow, increased the levels of biologically active caspase-1 and IL-1β in ECs. The activation of NLRP3 inflammasome *in vivo* was evident in the lesser curvature of the aortic arch of C57BL/6 mice, as demonstrated by increased levels of caspase-1 and IL-1β. Thus, inflammasome-mediated endothelial innate immune response may flare when ECs are in an atherogenic milieu. Because caspase-1 activation can also lead to pyroptosis, such inflammatory cell death may be an additional functional consequence of activated endothelial innate immunity and further implicated in the development of atherosclerosis.

## SREBP2 activation of NLRP3 inflammasome

SREBP1 and SREBP2 were originally identified by their regulation of cellular levels of lipids or cholesterol [111]. With abundant lipids or cholesterol in the cell, the precursor SREBP is anchored in the ER by binding to SREBP cleavage activating protein (SCAP) and insulin-induced gene 1 or 2 (INSIG-1 or -2). When lipid or cholesterol is depleted, the SCAP-SREBP complex translocates to the Golgi, where SREBP is further processed in a two-step N-terminal cleavage by site-1 and site-2 protease (S1P and S2P). Once cleaved, the N-terminal portion of SREBP [SREBP2(N)] translocates into the nucleus to serve as a transcription factor for genes involved in *de novo* synthesis and uptake of lipids and cholesterol, such as 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and LDLR [112]. Furthermore, the intronic miR-33 is co-transcribed with SREBP2, which targets ATP-binding cassette A1 (ABCA1) and ABCG1, thus inhibiting cholesterol efflux [113–115]. Synergistically, transactivation of SREBP2-miR-33a maintains cellular cholesterol homeostasis.

Im *et al.* reported that SREBP1a activates genes required for lipogenesis as well as those encoding the inflammasome component, namely NLRP1a, in macrophages [116]. More than a decade ago, we found that SREBP was activated by disturbed flow as well as ox-PAPC in ECs [7, 8]. From these earlier studies, we recently found that disturbed flow-activated SREBP2 can induce NLRP3 inflammasome via transactivation of NLRP3 and NADPH oxidase 2 (NOX2) in ECs [23••]. Although both NOX2 and NOX4 are present in ECs and disturbed flow can increase the expression of both [117, 118] SREBP2 induces NOX2, but not NOX4, in ECs [23••]. Because NOX4 appears to be more abundant than NOX2 in ECs [119] it is likely that NOX4 may also play a role in inflammasome activation but under regulation of other transcription factors, which requires further study. The SREBP2 transcriptional activation of NLRP3 and NOX2 suggests that SREBP2 is involved in both Signals 1 and 2 required for inflammasome induction. Interestingly, NF-κB can also transactivate NLRP, pro-IL-1β, and SREBP in Signal 1 [120, 121•]. In terms of endothelial dysfunction, transgenic mice with EC-specific overexpression of N-terminal SREBP2 (i.e., the active form of SREBP2) [EC-SREBP2(N)-Tg] showed decreased EC-dependent vessel dilation (Chen and Shyy, unpublished results). In terms of atherosclerosis, EC-SREBP2(N)-Tg mice in an ApoE-null background showed increased atherosclerosis in both

atheroprotective and atheroprone regions [23••]. Collectively, these results suggest that SREBP2 links endothelial dysfunction and atherosclerosis.

The activity of SREBP can be regulated by mechanisms involving post-translational modifications (PTMs). AMPK, cyclin-dependent kinase 8 (CDK8), and SIRT1 decrease whereas Akt increases SREBP activity [122–125]. Indeed, the reduced AMPK phosphorylation and SIRT1 deacetylation of SREBP1 was associated with increased SREBP activity in the aorta of diabetic atherosclerotic pigs [126•]. Importantly, PTMs of SREBP are translationally implicated in the clinical setting. For example, hyperglycemia potentiates Akt signaling, whereas HMG-CoA reductase inhibitors (i.e., statin) and the anti-diabetic drug metformin both activate AMPK and SIRT1 in ECs [127, 128]. Future development of drugs intervening in atherosclerosis should consider the role of these molecules in attenuating the SREBP-regulated innate immunity in ECs.

## Conclusions and perspectives

Because little evidence supports infectious agents as the primary source of an atherogenic innate immune response [129••], SREBP-dependent endothelial dysfunction could be the culprit of sterile inflammatory responses elicited by various atherogenic risk factors. The canonical SREBP activation often results in lipid and cholesterol accumulation in macrophages and tissues involved in energy metabolism (e.g., hepatocytes). In contrast, ECs are resistant to lipid deposition *in vitro* and *in vivo*. Thus, SREBP activation in ECs responding to various atherogenic stimuli should regulate inflammatory response rather than synthesize lipid/cholesterol. However, cholesterol accumulation in the endothelium in atheroprone areas has been found in some accelerated atherosclerosis models such as $LDLR^{-/-}$ mice [106]. Whether this aberrant level of lipids/cholesterol is due to increased *de novo* synthesis (i.e., HMG-CoA reductase), uptake (i.e., LDLR), and/or retention (i.e., miR-33 and its suppressed ABCA1/ABCG1) warrants further investigation.

Given the current knowledge of SREBP-dependent activation of inflammasome, there appears to be a paradox between the canonical and non-canonical pathways activating SREBP. Under hyperlipidemic conditions, SREBP activation would conceptually be repressed in lipid-laden foam cells and endothelium. However, SREBPs are activated during atherogenesis. Thus, upstream pathways inducing SREBP2 involved in the innate immune response would differ from those of sterol depletion. Another paradox is that the widely prescribed statins, which inhibit HMG-CoA reductase, activate SREBP2 in the liver and macrophages. Yet, statins have pleotropic anti-inflammatory and vascular protective effects. An explanation may be that in ECs, statins activate AMPK [127], which in turn inhibits SREBP cleavage and activation [122].

Atherosclerosis depends on the contributory role of dysfunctional endothelium and the retention of atherogenic lipoproteins in the subendothelial space as well as macrophage-derived foam cells. As discussed, accumulating evidence points to excessively activated inflammasomes in macrophages retained in the subendothelial space. However, the topographical distribution of atherosclerotic lesions suggests that local flow pattern-regulated endothelial innate immune response is an indispensable factor of atherogenesis.

Rather than passively subjected to hemodynamic or biochemical injuries, ECs actively respond to atheroprone flow and/or elevated oxidative stress by activating SREBP2 and thereby provoking the inflammasome cascade. The resulting IL-1β secretion in turn promotes the endothelial inflammatory status by inducing chemoattractant and adhesion molecules such as MCP-1, ICAM, VCAM, and E-selectin. Such sterile inflammation in the endothelium constitutes an early step in atherogenesis, recruiting monocytes to the endothelium and migrate into the subendothelial space, thus aggravating the atherogenic process. The characterization of endothelial inflammasome further expands and refines the "response-to-injury" hypothesis centered on disrupted EC function. More importantly, the reciprocally enhanced SREBP-NLRP3 inflammasome pathways between ECs and monocytes/macrophages link both the "response-to-injury" [1] and "response-to-retention" [130] hypotheses. The SREBP-mediated NLRP inflammasome activation and cross-talk between ECs and macrophages in atherogenesis are summarized and illustrated in Figure 1.

The molecular mechanisms by which SREBP and/or other upstream molecules regulate the inflammasome and innate immunity in ECs (e.g., by activating Signals 1 and 2) remain to be elucidated. Given the multiple molecular and cellular pathways involving the inflammasome and other endothelial functions mentioned in this review (i.e., mitochondrial biogenesis, autophagy, and EndoMT), delineating the cross-talk and interplay among these processes is worth investigating. From a translational point of view, the NLRP3 inflammasome is a promising drug target for atherosclerosis because both endothelial and monocytic innate immunity are crucial for atherogenesis. In addition to the IL-1β antibody investigated in the CANTOS trial, targeting other key molecules of NLRP3 inflammasome may be a promising therapeutic approach. Because atherosclerosis is significantly reduced in caspase-$1^{-/-}$/$ApoE^{-/-}$ mice [92] and that a caspase-1 inhibitor could induce acute repression of apoptosis in atherosclerotic lesions [131], clinically using caspase-1 inhibitors for cardiovascular disease is worthwhile to investigate.

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**Key points**

- Inflammasomes constitute an important component of innate immunity in macrophages and in vascular endothelial cells. Such inflammasome-mediated innate immune responses play an essential role in atherogenesis.
- SREBP is a master regulator of inflammasome, and its activation by cardiovascular risk factors (e.g., disturbed flow and oxidized lipids) contributes to atherosclerosis.
- Endothelial innate immunity mediated by SREBP-induced inflammasome is a link between endothelial activation and monocyte recruitment, which forms a pro-inflammatory milieu disrupting vascular homeostasis, thus leading to atherosclerosis.
- The understanding of SREBP-inflammasome-endothelial innate immune response provides novel insights integrating the “response-to-injury” and “response-to-retention” hypotheses.

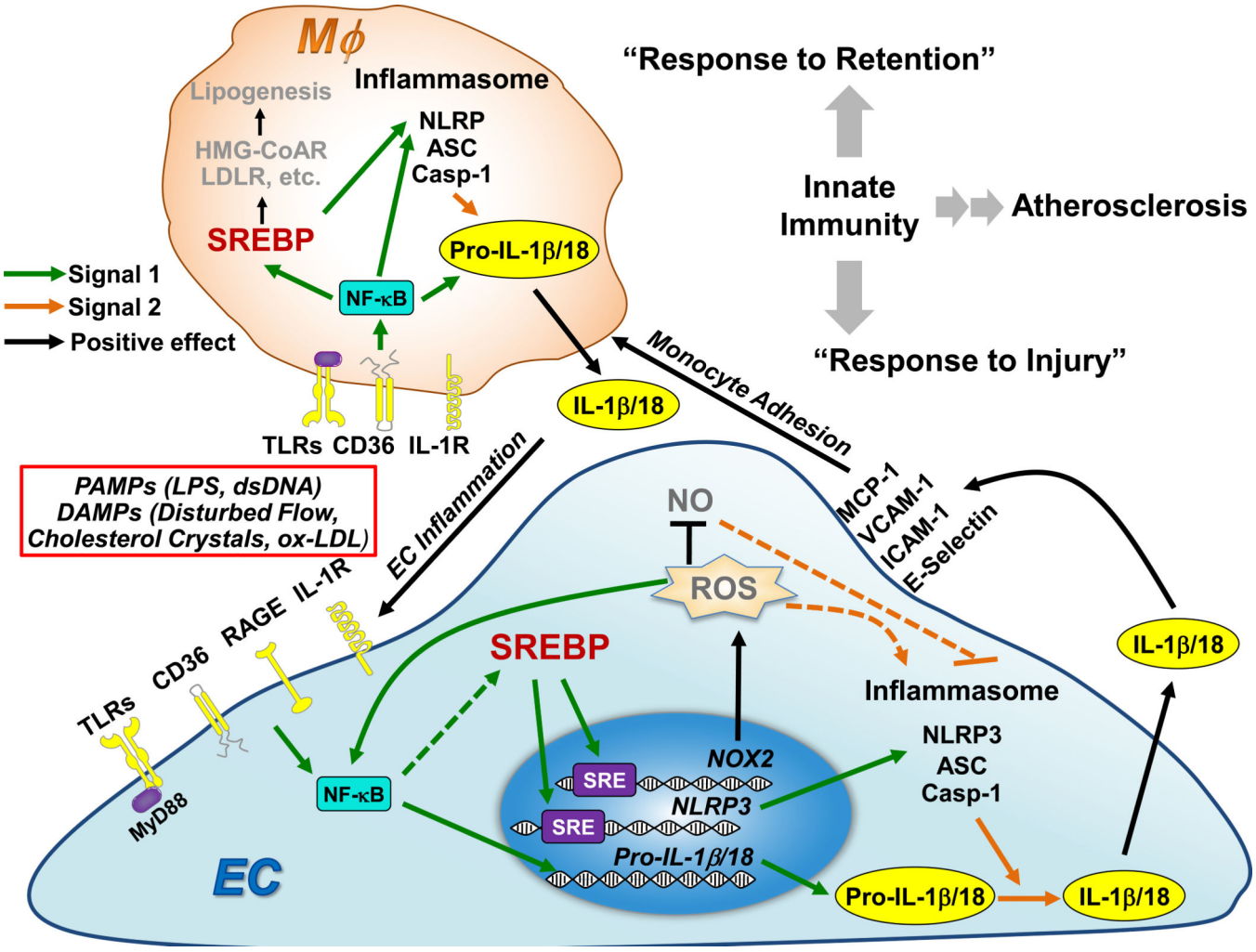


**Figure 1.**

Inflammasome activation and cross-talk between endothelial cells (ECs) and macrophages (MØ) in atherogenesis. In monocytes/macrophages, the activation of NLRP inflammasome is initiated through the recognition of PAMPs and DAMPs by TLRs, CD36, or IL-1R, which activates NF-κB. NF-κB transactivates genes such as NLRP, pro-IL-1β, and SREBP in Signal 1 (i.e., the priming step of the inflammasome). Signal 2 (the activating step) stimulates the oligomerization of inflammasome components (e.g., NLRP, ASC, and pro-caspase-1), thus resulting in the auto-cleavage and activation of caspase-1. Active caspase-1 in turn cleaves pro-IL-1β/pro-IL-18 to the biologically active form of IL-1β/IL-18. Activation of SREBP in monocytes/macrophages also induces genes involved in lipogenesis such as HMG-CoA reductase (HMG-CoAR) and LDLR, which may contribute to the formation of foam cell. In ECs, NLRP3 inflammasome activation is also initiated through the recognition of PAMPs/DAMPs by TLRs-MyD88, CD36, RAGE, and IL-1R signaling pathways. The activated NF-κB pathway can increase the transcription of pro-IL-1β and likely induces SREBP, which in turn stimulates Signal 1 through transactivating NLRP3 and NOX2 genes, the latter of which generates a high level of ROS. ROS production can activate NF-κB to escalate Signal 1 and also induce the oligomerization of the NLRP3

inflammasome by initiating Signal 2. The resulting secretion of IL-1β, and possibly IL-18 in ECs, promotes the expression of chemoattractant and adhesion molecules (e.g., MCP-1, VCAM-1, ICAM-1, and E-selectin). The increased ROS resulting from SREBP2-NOX2 activation inhibits eNOS-derived NO, which can be an inflammasome inhibitor. Collectively, the activation of SREBP in ECs mediates the activation of innate immune response as well as the impaired NO bioavailability, thereby constituting endothelial dysfunction. These detrimental effects in ECs promote the recruitment and transmigration of monocytes. Reciprocally, the cytokines released by macrophages (e.g., IL-1β/18) aggravate the EC inflammasome. The depicted innate immune responses in macrophages and ECs provide a dynamic link between the “response-to-retention” and “response-to-injury” hypotheses. Dash lines are pathways remain to be experimentally validated.

**Table 1**

Endothelial functions, molecular markers, regulators, and pathophysiological or pharmacological stimuli relevant to atherosclerosis

| *EC function* | *Molecular markers* | *Positive regulators* | *Negative regulators* | *Positive stimuli* | *Negative stimuli* |
|---|---|---|---|---|---|
| eNOS-derived NO | eNOS↑ (p-eNOS, Ac-eNOS, expression) NO↑ | KLF2[27], KLF4[28], AMPK[29] SIRT1[30], Akt[31], PKA[32] | miR-92a[13], miR-21[33] | Laminar shear stress[34] Statins[35], Metformin[36] Resveratrol[37], VEGF[38] Bradykinin[39], Estrogen[40] | ADMA[41], ROS[42] Ang II[43], AGEs[44] |
| Mitochondrial biogenesis | PGC1α↑, Trx↑, NRFs↑ TFAM↑, MtDNA↑ | AMPK[45], SIRT1[46] NO/cGMP[47], Akt3[48] | | Pulsatile shear stress[49] Caloric restriction[50], Resveratrol[51], VEGF[48] | Hyperglycemia[52] Ox-LDL[53] |
| Autophagy | Beclin-1↑, LC3-II↑, ULK1↑ ATG5↑, ATG7↑, p62↓ | AMPK[54] | mTOR[55], Akt[55] BIRC2[56] | Ox-LDL[57], Hypoxia [58] Starvation[59], Ischemia[60] LPS[56], Rapamycin[61] | Hyperglycemia[62] Long-term high-fat diet[63] |
| Inflammasome | NLRP3↑ ASC↑, Caspase-1↑ IL-1β↑, IL-18↑ | SREBP2[23••] NOXs[23••] NF-κB[64] TXNIP[64] | AMPK[65] SIRT1[66] NO[67, 68] | Oscillatory shear stress[23••] Ox-PAPC[8], Ox-LDL[69••] Long-chain fatty acid[65] Hyperglycemia[70] Visfatin[71] | Resveratrol[72] Metformin[73•] |
| EndoMT | vWF↓, CD31↓, VE-cad↓ KLF4↓, KLF2↓, eNOS↓ α-SMA↑, N-cad↑, FSP1↑ Vimentin↑ | Smads[74], PKCδ[74] c-Abl [74], GSK3[74], Snail1/2[74] Wnt signaling[75] NOTCH1[75], ET-1[75] | Primary cilia[24] Caveolin-1[76] | TGFβ[74], Ang II[77], TNFα[78] High glucose[74], AGEs[74] Hypoxia[74] | |

The number in superscript is the cited reference

↑: up-regulation; ↓: down-regulation;

eNOS, endothelial nitric oxide synthase; KLF, kruppel-like factor; AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; Akt, protein kinase B; PKA, protein kinase A; VEGF, vascular endothelial growth factor; ADMA, asymmetric dimethylarginine; ROS, reactive oxygen species; Ang II, angiotensin II; AGE, advanced glycation end-product; PGC1α, peroxisome proliferator-activated receptor-gamma coactivator; Trx, thioredoxin; NRF, nuclear respiration factor; TFAM, mitochondrial transcription factor A; MtDNA, mitochondrial DNA; cGMP, cyclic GMP; LC3, microtubule-associated protein 1A/1B-light chain 3; ULK, UNC-51-like kinases; ATG, autophagy protein; p62, Nucleoporin p62; mTOR, mammalian target of Rapamycin; BIRC, baculoviral IAP repeat-containing protein; LPS, lipopolysaccharide; SREBP, sterol regulatory element binding protein; NLRP, NOD-like receptor family, pyrin domain containing; NOX, NADPH oxidase; ASC, apoptosis-associated speck-like protein containing a CARD; IL, interleukin; NF-κB, nuclear factor-kappa B; TXNIP, thioredoxin interacting protein; PAPC, palmitoyl-arachidonyl-phosphatidyl choline; vWF, von Willebrand factor; VE-cad, vascular endothelial cadherin; SMA, smooth muscle actin, N-cad, neuronal cadherin; FSP, fibroblast-specific protein; PKCδ, protein-kinase C-delta; c-Abl, Abelson murine leukemia viral oncogene homolog 1; GSK, glycogen synthase kinase; ET, endothelin; TGF, transforming growth factor; TNF, tumor necrosis factor.