

Invited Mini Review

Role of vascular smooth muscle cell in the inflammation of atherosclerosis

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Atherosclerosis is a pathologic process occurring within the artery, in which many cell types, including T cell, macrophages, endothelial cells, and smooth muscle cells, interact, and cause chronic inflammation, in response to various inner- or outer-cellular stimuli. Atherosclerosis is characterized by a complex interaction of inflammation, lipid deposition, vascular smooth muscle cell proliferation, endothelial dysfunction, and extracellular matrix remodeling, which will result in the formation of an intimal plaque. Although the regulation and function of vascular smooth muscle cells are important in the progression of atherosclerosis, the roles of smooth muscle cells in regulating vascular inflammation are rarely focused upon, compared to those of endothelial cells or inflammatory cells. Therefore, in this review, we will discuss here how smooth muscle cells contribute or regulate the inflammatory reaction in the progression of atherosclerosis, especially in the context of the activation of various membrane receptors, and how they may regulate vascular inflammation. [BMB Reports 2014; 47(1): 1-7]

THE ROLES OF vSMC IN THE PROGRESSION OF ATHEROSCLEROSIS

Atherosclerosis is a complicated chronic inflammatory disease, characterized by the development of atheromatous plaque in the intimal layer. Currently, the progression of atherosclerosis is divided into three stages. The initial step is the initiation of atherosclerosis, due to the activation of endothelial cells by metabolic risk factors, such as hyperlipidemia, hypertension, or pro-inflammatory mediators. The resultant endothelial dysfunction allows for the blood monocytes to permeate the endothe-

lial cell layer, and infiltrate into the intima and subintima, resulting in phagocytosis of the LDL cholesterol, or oxidized phospholipids and foam cell formation. Necrosis of the lipid containing foam cells will result in propagation of inflammation, as well as coalescence of the lipid contents, resulting in necrotic core formation. In the next step, SMCs migrate from the media to the intima, proliferate, and form plaque. Pidkova and Berliner demonstrated that oxidized phospholipids, called POVPC, lead to vSMC proliferation and apoptosis, by inhibiting cellular differentiation at high concentration (1-3). Migrated SMCs in intima can produce extracellular matrix (ECM), to make a fibrous cap. The last step is thrombosis, and rupture of the atherosclerotic plaque. Typically, the thin layer of the fibrous cap easily ruptures, in which few SMCs and abundant macrophages exist, because inflammatory cells cause the death of SMCs, which produce collagen for the fibrous cap as a main source (4). Macrophages are observed to induce apoptosis of SMCs by the TNF- α /NO signaling pathway, and decrease collagen synthesis by the secretion of MMPs, such as MMP-9 and MMP-12 (5-7). Subsequently, these series of reactions cause diseases, such as myocardial infarction, stroke, or congestive heart failure (8).

THE ROLE OF MEMBRANE RECEPTORS OF vSMC IN VASCULAR INFLAMMATION

AT1R (Angiotensin II type 1 receptor)

The role of the Renin-Angiotensin system in the pathophysiology of cardiovascular diseases has been extensively studied. Angiotensin II (Ang II), through their activation of the angiotensin type 1 receptor, has been known to play an important role in the development of atherosclerosis, as well as cardiac dysfunction (9, 10). AT1R was first shown to be expressed in vascular smooth muscle cell in 1973 (11). Extensive researches have been done regarding the role of Ang II in promoting inflammation by increasing oxidative stress, as well as by upregulating various proinflammatory signals, such as RAGE, VCAM-1, ICAM-1, and MCP-1 in vSMCs (12). Moreover, Cai *et al.* showed that Ang II-induced proinflammatory signals allow monocytes to bind to vSMCs, indicating a highly significant association of vSMCs in inflammation (13). Ang II, besides the

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proinflammatory effect, also induces SMCs proliferation/migration and hypertrophic response, which can contribute to the development of atherosclerosis. However, there are conflicting reports on whether the presence of AT1aR activation in the endothelial cells or vSMCs is required for atherosclerosis development. In a recent study, AT1aR in endothelial or smooth muscle cell was not necessary to induce atherosclerosis and abdominal aortic aneurysm in mice having endothelial or smooth muscle cell-specific deletion of AT1aR (14). However, in another study, endothelial cell-specific deficiency of AT1aR showed the attenuation of Ang II-induced ascending aortic aneurysm in LDL receptor KO mice (15). ATRs have been extensively researched, and angiotensin type 1 receptor antagonists are being clinically used for the treatment of cardiovascular disease (16, 17). However, more research needs to be done regarding the role of other receptors in the renin-angiotensin system, such as the MAS receptor and angiotensin II type 2 receptor, in the pathogenesis of atherosclerosis.

RAGE (Receptor for advanced glycation end products)

RAGE is a transmembrane receptor, and recognizes various endogenous ligands, including AGE, HMGB1 and S100 proteins, as a multi-ligand receptor (18-20). RAGE is a pattern recognizing receptor that is important in mediating innate immunity. RAGE is abundantly expressed in various tissues, and are known to be expressed in endothelial cells and vSMCs (21). Since RAGE was first described and demonstrated from cardiovascular cells (22, 23), many studies have focused on the proinflammatory effect of RAGE activation in diabetes and atherosclerosis. As direct evidence, soluble RAGE (sRAGE), a decoy receptor that blocks RAGE activation, inhibited atherosclerotic severity in streptozotocin-induced diabetic ApoE-null mice (24). Our recent study has shown that infusion of Angiotensin II in ApoE knockout mice results in increased atherosclerosis and overexpression of RAGE activation, a process that has been shown to be attenuated by RAGE inhibition (12). Studies have shown that ligands of RAGE, such as S100B, HMGB1 increase inflammation, and promote atherosclerosis. Hayakawa and colleagues showed that the treatment of S100B in RAGE-overexpressed SMC cell line was associated with the expression of proinflammatory genes, such as MCP-1, IL-6, and ICAM-1, and activates mitogen-activated protein kinase and the NF- κ -B signaling pathway (25). HMGB1, a chromatin structural protein that is released extracellularly during tissue necrosis, has been shown to trigger inflammation during atherogenesis, and amplify inflammatory reaction, through increasing IL-1 β , an inflammatory cytokine, in vSMCs (26). Recent studies suggest the involvement of new signaling pathways in HMGB1-induced inflammation, in which caspase-1 activation mediated by active inflammasome, such as NLRP3, results in increased IL-1 β production and the propagation of inflammation. HMGB1 mediated inflammation is mediated through various pattern recognizing receptors, including TLR2, TLR4, and RAGE (27, 28). Although these results were demon-

strated in other cell types, the involvement of the novel signaling pathway in mediating vascular inflammation is highly expected. Since several receptors can interact under inflammatory stimuli, researchers have focused on the main receptors involved in mediating vascular inflammation. Kokkola compared the proinflammatory activity among TLR2, RAGE, and IL-1 receptor type I using KO mouse, and demonstrated that RAGE is the major receptor activated from stimuli in macrophages (29). Although RAGE activation is associated with increased oxidative stress and proinflammatory signaling activation through NF- κ B activation, the intracellular signaling cascade for these processes is not well defined. For RAGE interacting adaptor proteins, few molecular mechanisms have been elucidated. However, a recent study by Schmidt *et al.* revealed that mDia1, a member of the formin family, binds to cytoplasmic domain of RAGE under the activation of S100B, which can induce oxidative stress, migration and vascular remodeling, through c-Src translocation and Rac1 activation, followed by NOX1-PI3K-AKT-GSK3 β signaling pathway in SMCs, of the wire-injured mouse model (30). A new role of RAGE in vascular osteogenesis has recently been demonstrated, which showed that RAGE promotes vascular calcification, through the activation of Wnt/ β -catenin signaling (31). Another article demonstrates that RAGE can play a crucial role in vascular calcification, in peritoneal dialysis patients (32). Although RAGE-ligand induced signaling has been implicated in the inflammation associated with chronic diseases, such as diabetes and atherogenic process, the physiological roles or signaling pathways in vSMCs have still not been fully elucidated.

LOX-1 (lectin-like oxidized LDL receptor)

The importance of LDLR in atherosclerosis has been demonstrated in the middle of the 1970s (33, 34), from the accelerated atherosclerosis found in familial hypercholesterolemia patients lacking LDLR. The lack of LDL receptor is associated with decreased clearance of LDL in the circulation and hypercholesterolemia, resulting in the accelerated development of atherosclerosis. Since then, mouse models of atherosclerosis have been developed, by knocking out the LDL receptor in mice (35), and LDLR-knockout mice have been widely used to study several diseases or symptoms, including atherosclerosis, fibrosis, and nephrotic syndrome. In addition, LDLR is also known to regulate the amount of apolipoprotein E or amyloid β in the brain (36). However, the LDL receptor is not the main pathway for the uptake of cholesterol in the arteries, as evidenced by the accelerated atherosclerosis in familial hypercholesterolemia (34, 37). In macrophage, LDL needs to be modified by oxidation or acetylation for phagocytosis (38). The modified LDLs are bound and internalized through LOX-1. In vSMCs, overexpression of LOX-1 levels in response to ox-LDL results in activation of inflammatory signals, such as NF- κ B and JNK (39). LOX-1, identified as the major ox-LDL receptor in endothelial cells, may play a critical role in the initiation and progression of atherosclerosis, through their uptake

of modified LDLs, and activation of the proinflammatory pathway in vSMCs (40). In vSMCs, proinflammatory cytokines, such as TGF- β , IL-1 α , and IL-1 β , increase LOX-1 expression; whereas, LOX-1 inhibition leads to significant reduction in the formation of the atherosclerotic lesions (41). Since ox-LDL uptake through LOX-1 is critical for foam cell formation, which is the hallmark of the atherosclerotic region, and vSMCs in intimal area highly express LOX-1, some pioneer studies have addressed the role of vSMC related to LOX-1. Although these studies did not present direct evidence, high possibilities are suggested for the transformation from SMCs into foam cells through LOX-1 (42, 43). Statins such as pravastatin showed a new pleiotropic effect, in which pravastatin downregulated LOX-1 expressions in human vSMCs and atherosclerotic lesions, and reduced intimal media thickness and lipid core area (43). In a study using mice overexpressing LOX-1 from ApoE KO background, proatherosclerotic role of LOX-1 was demonstrated. Also, a synergistic role with LDLR was suggested from LOX-1 and LDL double knockout mice, in which the double knockout mice had a significantly reduced atherosclerotic lesion formation, compared to that of LDLR knockout mice (44).

TLRs (Toll-like receptors)

TLRs, one of the well-characterized-pattern-recognition receptors, are important receptors for mediating innate immunity. Around 13 different TLRs have been demonstrated in humans and mammals, and exogenous and endogenous ligands have been identified for 9 different types of TLRs, in monocytes/macrophages, dendritic cells, T and B lymphocytes, mast cells and vascular cells (45). Among the TLRs, increased expression levels of TLR 2 and 4 have been shown in atherosclerotic plaques, and their importance for initiation and progression have been demonstrated from several studies (46, 47).

TLR2 is important for the recognition of bacterial lipoproteins, and known to promote inflammation in vSMCs, which has been demonstrated by previous studies, using apolipoprotein E-knockout (ApoE KO) mouse (48-50). Although researches related to TLR2 have been performed in vSMCs, most studies focus on the proliferation or migration mechanisms of vSMCs, rather than being related to inflammatory response. In the case of vSMCs proliferation, TLR2 and TLR4 signaling pathway are known to be involved in neointimal lesions under HSP60 stimulation (51). According to recent studies, TLR2 can activate p38 MAPK, ERK1/2, JNK1/2 and AKT, in vSMCs from TLR2 KO mouse. Also, cAMP response element-binding protein (CREB)-dependent IL-6 production was highly related to TLR2, not TLR4, in vSMCs migration (52). Interestingly, Lee *et al.* suggested a new role of TLR2 in vSMCs, where MMP2 and proinflammatory cytokines can be produced through TLR2-Nox1-dependent manner, leading to increasing monocyte-endothelial cell adhesion, and transendothelial migration of monocytic cells (53).

TLR4 is mainly stimulated by lipopolysaccharide (LPS) in

vSMCs, and is known to induce inflammatory signals, as well as TLR2. Yang and colleagues showed that TLR4 stimulated by LPS activated phosphorylation of extracellular regulated kinase 1/2 (ERK1/2), induced monocyte-chemoattractant protein-1 (MCP-1) release and interleukin (IL)-6, and increased IL-1 α expression (54). Another research group also examined increased TLR4 expression and nitric oxide (NO) production by LPS playing very important roles to induce inflammation in human aortic vascular SMCs (55). TLR4 can increase MMP-9 expression, which is known to be involved in the pathogenesis of atherosclerotic plaque instability, as well as abnormal arterial remodeling. Li *et al.* demonstrated that TLR4/NF- κ B pathway, stimulated by LPS, was able to increase the expression of MMP-9 mRNA and protein for human aortic vascular SMCs migration, indicating a high possibility for the correlation between TLR4/NF- κ B pathway, and pathogenesis of atherosclerosis (56). Up to date, studies have demonstrated that TLR2 and TLR4 share common pathways, or have synergetic effects in the inflammatory signaling. However, recent study suggested that TLR4 has a protective role in atherosclerosis progression, whereas TLR2 has a proinflammatory response, followed by periodontal pathogen (57). Therefore, further in-depth researches are needed to understand the differing roles of TLR4 in vascular inflammation.

NLRs (Nod-like receptors)

NLRs are another type of cytoplasmic pattern-recognition receptors, which play important roles in innate immunity (58). Among members of the NLRs family, NLRP3 is the receptor studied most extensively for its association with cardiovascular diseases, such as cardiomyopathy and heart failure (59). Microbial molecules or endogenous danger signals can activate NLRP3, and in turn, NLRP3 recruits and makes a complex with apoptosis-associated speck-like protein (ASC), and pro-caspase-1 (60). This complex is called an active NLRP3 inflammasome, which activates caspase-1, IL-1 β , and IL-18. NLRP3 is also involved with autophagy, which is a quality control system by degradation or re-use of intracellular organelles and proteins, and is essential for cell survival (61). NLRP3 inflammasome was reported to co-localize with autophagosome, as autophagy mechanism can regulate inflammasome activation, through the interaction between autophagic adaptor p62 and ASC (62). Another study demonstrated the importance of this relationship, by showing that LPS induces more mortality, through the active NLRP3 inflammasome in autophagy-deficient (LC3B-deficient) mouse model (63). New roles of NLRP3 have been elucidated, with regard to the pathogenesis of cardiovascular diseases. In human coronary atherosclerosis, NLRP3 was reported to have a positive correlation with an increased epicardial adipose tissue volume, which has been shown to be associated with increased cytokine production and proatherogenesis (64). In heart from NLRP3 KO mice, the protective effect of ischemic preconditioning is increased against ischemia-reperfusion injury, through the reduced expression of

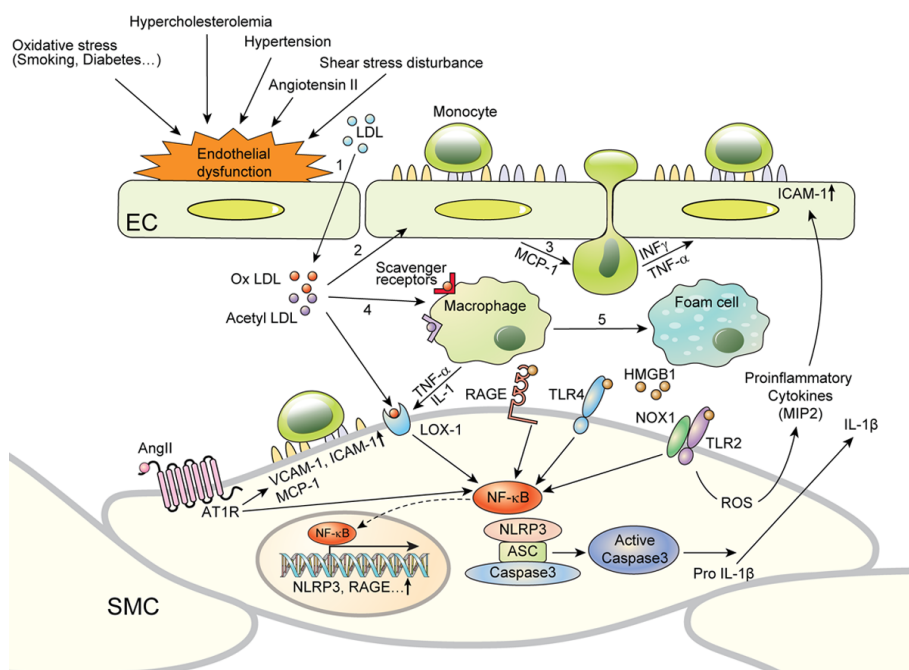


Fig. 1. Involvement of vSMCs in the inflammatory response of atherosclerosis. 1-5 briefly show LDL-induced inflammatory reactions, in which modified LDL particles are accumulated in the artery under various risk factors, resulting in the formation of foam cells. This process is mediated by uptake of ox-LDL particles from the activation of scavenger receptors of macrophages, such as CD36, SRA1/2, LOX-1, and TLRs. In addition, SMCs can produce proinflammatory cytokines, through the activation of LOX-1, RAGE, TLR2, TLR4, AT1R, or NLRP3 by their specific agonists, and by their interaction with endothelial cells and macrophages.

STAT3 (65). There are some debates as to whether NLRP3 inflammasome is involved in the progression of atherosclerosis, or not. In the LDLR KO-diabetic mice model, activation of NLRP3 inflammasome with increased inflammatory responses, as well as IL-1 β production, has been demonstrated in the atherosclerotic lesions (66). On the other hand, NLRP3 did not show any difference from ApoE KO and NLRP3-/ApoE-double KO mice study (68). Nevertheless, recent studies strongly suggest the involvement of NLRP3 inflammasome in atherosclerosis. Qial *et al.* showed that activated NF- κ B due to TLR4 activation can bind to the NLRP3 promoter, resulting in the increase of NLRP3 expression from macrophages (67). Xiao *et al.* also demonstrated that sterol regulatory element binding protein 2 (SREBP2) overexpress NLRP3, which results in endothelial inflammation, and atherosclerosis formation. SREBP2, as an initial activator, was able to be activated in endothelial cells by atheroprone arterial blood flow, leading to NLRP3 inflammasome activated IL-1 β signaling, followed by subsequent activation of NADPH oxidase2 (NOX2), in the area of atherosclerotic lesions (69). In a study regarding the role of NLRP3 within vSMCs in mediating inflammation during atherogenesis, Xiao *et al.* demonstrated that NLRP3 activation due to atheroprone blood flow is important for inflammation and progression of atherosclerosis. As disturbed arterial blood flow has been known to induce phenotypic changes in vSMCs that are highly involved in inflammatory reaction through the overexpression of receptors affecting inflammation and adhesion, we speculate that NLRP3 may have an important role in mediating activation of vSMCs in atherogenesis (70, 71).

CONCLUSIONS

Until recently, vSMCs had been considered to passively transform or differentiate into foam cells, by factors from macrophage stimulation. However, recent studies have focused on the active roles of vSMCs in propagating inflammation, during the development of atherosclerosis. In this review, we summarized the possible role of vSMCs in actively mediating inflammation, through their regulation of cytokine secretion and membrane receptors, during the pathogenesis of atherosclerosis (Fig. 1).

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