REVIEW

Crucial role of renin-angiotensin system in the pathogenesis of atherosclerosis

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Abstract: The renin-angiotensin system (RAS) has been demonstrated to play a critical role in the initiation and progression of atherosclerosis, thereby contributing to development of cardiovascular diseases. Angiotensin II (Ang II), a major substrate in RAS, stimulates atherosclerosis through various deleterious effects such as endothelial dysfunction, cellular proliferation and inflammation. Recently, local RAS in vasculature is reported to play an important role. Many of these atherogenic actions of Ang II are mediated by reactive oxygen species (ROS). Investigation of the role of ROS and inflammation induced by RAS may provide a clue to understanding the pathophysiology of atherosclerotic diseases, and may lead to a new therapeutic strategy. J. Med. Invest. 57: 12-25, February, 2010

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INTRODUCTION

The renin-angiotensin system (RAS) has been considered as a circulating hormonal system that regulates blood pressure, blood flow, fluid volume and electrolyte balance (1, 2). Angiotensinogen produced in the liver is cleaved to angiotensin (Ang) I in circulation by renin that is secreted from the kidney. Ang I is cleaved to Ang II by angiotensin converting enzyme (ACE) that is mainly distributed in pulmonary circulation. Ang II plays a main role in the RAS by interacting with its specific receptor, Ang II type 1 receptor (AT1R). Ang II-AT1R interaction causes vasoconstriction and aldosterone release from the adrenal gland. This classical view of the RAS has been expanded by recent findings that RAS is activated locally, particularly in the heart (3, 4), the vessel wall (5-7), the kidney (8, 9) and the brain (10-12). There are RAS components in these tissues, allowing local synthesis of Ang peptides. Recent reports also identified other receptors (13-15) and angiotensin-related peptides such as Ang (1-7) (16). Ang II was also reported to be generated by other enzymes such as chymase (17). These findings indicate that RAS could be activated locally and regulated by the complicated crosstalk of the RAS components in each organ.

AT1R blockers (ARBs) specifically block Ang II binding to AT1R. Eventually, Ang II is directed to stimulate AT2R. On the other hand, ACE inhibitors (ACEIs) suppress angiotensin II production. ACEIs also inhibit break down of bradykinin, leading to increase in nitric oxide production. It has been reported that ARBs or ACEIs exert various favorable effects on endothelial function (18, 19), cardiac function (20, 21), cerebral vascular function (22, 23) and renal function (24, 25) other than blood pressure lowering. These findings suggest that blockade of
RAS is an effective strategy for organ protection. In fact, many clinical studies demonstrated that AT1R blockers (ARBs) or ACE inhibitors are effective for patients with cardiovascular, cerebrovascular and renal diseases (20, 24, 26, 27).

Atherosclerosis occurs in whole arteries and results in various organ damages, including myocardial infarction, cerebral infarction, and peripheral arterial diseases, the main cause of death in Western countries (28). Atherosclerosis is considered to be one of the chronic inflammatory diseases (29, 30). Ang II has significant pro-inflammatory actions on the vessel wall, leading to progression and destabilization of atherosclerotic lesions (29, 31). Although multifactorial in etiology, continuous recruitment of circulating leukocytes into the vessel wall plays crucial roles in the pathogenesis of atherosclerosis. Inflammatory cells detected in atherosclerotic lesions are derived from bone marrow. A locally activated RAS has been suggested to contribute to differentiation and proliferation of bone marrow-derived cells (32-34). Recently, we proposed a hypothesis that the local RAS in bone marrow plays crucial roles in atherosclerosis (35, 36). We demonstrated that Ang II-AT1R pathway in bone marrow contributes to atherosclerotic development in the hypercholesterolemic mice. In this review, we briefly summarize recent evidence on the roles of RAS in the pathogenesis of atherosclerosis and in the differentiation of bone marrow cells. We describe our findings on potential participation of bone marrow RAS in progression and destabilization of atherosclerotic plaques.

LOCAL EFFECTS OF AN ACTIVATED RAS IN VASCULATURE

A growing body of evidence suggests that atherosclerosis is a chronic inflammatory disease (29, 30). Recent advances in immunology have dissected several molecular pathways that induce and promote inflammatory responses in atherosclerotic lesions. The RAS serves as a key player in the pathogenesis of atherosclerosis by stimulating a series of coordinated cellular and molecular events observed in the lesions (37-41). It is now well established that Ang II has significant pro-inflammatory actions on the vessel wall, leading to progression of atherosclerosis (29). There are two different types of Ang II receptors, AT1R and AT2R, in mammals (13). Both AT1R and AT2R have been identified in the vessel wall, although AT1R is believed to mediate most of the atherogenic actions of Ang II (42, 43). The greatest AT1R density has been found on vascular smooth muscle cells and endothelial cells. In the vascular wall, ACE is readily detectable on endothelial cells and smooth muscle cells (44-46). Thus, most of the components of RAS could be detected in vasculature (47, 48). RAS is activated locally in the atherosclerotic lesions (49) and in the damaged vessels (50). Thus, these results suggest that not only systemic but also local Ang II-AT1R pathway could contribute to initiation and progression of atherosclerosis.

EFFECTS OF RAS ON VASCULAR CELLS

Ang II up-regulates expression of adhesion molecules (37, 51), chemokines (39, 52) and cytokines (53, 54). These molecules induce endothelial cell dysfunction (55), oxidation and uptake of LDL (56, 57), and proliferation of smooth muscle cells (58). In advanced atherosclerotic lesions, Ang II stimulates expression of matrix metalloproteinases (MMPs) (59-61) and plasminogen activator inhibitor-1 (62), leading to destabilization of atherosclerotic plaque and alteration of fibrinolytic balance. Ang II also up-regulates expression of VEGF that promotes adventitial angiogenesis (63-65) (Fig. 1).

Conversely, previous reports demonstrated that...
inhibition of the Ang II-AT1R pathway reduces atherosclerosis (36, 66-68). It is generally assumed that the beneficial effects obtained by Ang II-AT1R blocking are mediated by reduction of oxidative stress, inhibition of inflammation and improvement of endothelial cell function (66, 67, 69, 70). We generated ApoE-/-AT1aR-/- double knockout mice by cross-breeding ApoE-/-AT1aR+/+ mice and ApoE+/+ AT1aR-/- mice (35). We also administered ARB, olmesartan, into ApoE-/- AT1aR+/+ mice. Both genetic ablation and pharmacological blockade of AT1R effectively suppressed atherosclerotic lesion formation in ApoE deficient mice. Moreover, genetic disruption or pharmacological blockade of AT1R resulted in reduced lipid deposition and increased collagen contents in the atheroma. These results demonstrated that blockade of Ang II-AT1R pathway not only reduces atherosclerotic lesions but also stabilizes the plaque (35).

It should be noted that the production of Ang II could be increased and may act on the AT2R, when AT1R is genetically disrupted or pharmacologically blocked (71). Previous reports suggested an anti-atherogenic effect of AT2R, although its function and distribution are still under debate (72-74). Wu et al. demonstrated that organ-protective actions of valsartan, an ARB, were attenuated in AT2R-deficient mice, suggesting that beneficial effects of AT1R blockers are at least partly due to AT2R-deficient mice, suggesting that beneficial effects of AT1R blockers are at least partly due to AT2R receptor stimulation (75, 76). AT2R stimulation interacts with AT1R stimulation at intracellular signaling molecules, such as through activation of phosphatase (77). In fact, Iwai et al. demonstrated that AT2R stimulation attenuates atherosclerosis through inhibition of oxidative stress and that the anti-atherosclerotic effect of an ARB could be at least partly due to AT2R stimulation by analyzing AT2R/ApoE-double-knockout mice (73).

ROLES OF REACTIVE OXYGEN SPECIES IN ATEROGENESIS

Accumulating evidence indicates that vascular reactive oxygen species (ROS) play a crucial role in atherogenesis. Among many ROS generator, nicotinamide dinucleotide phosphate (NAD(P)H) oxidase-dependent pathway is important in vascular system (78). Barry-Lane et al. demonstrated that NAD(P)H oxidase is important in the pathogenesis of atherosclerosis by analyzing the genetically modified mice that are deficient for both apolipoprotein E (ApoE) and p47phox, one subunits of NAD(P)H oxidase (79). In this study, the double knockout mice showed significant reduction in atherosclerotic lesion compared with that of ApoE-deficient mice. ROS acts not only as a modulator of vascular tonus but also as a second messenger to alter the vascular cell phenotypes. ROS activates mitogen-activated protein kinase (80), Akt (81), and JAK (janus kinase)/STAT (signal transducers and activators of transcription) (82) pathways. These signals play a crucial role in cell proliferation, apoptosis and phenotypic modification that are observed in atherosclerotic lesions.

Association between RAS and ROS has been investigated extensively (5). Ang II induces production of ROS, one of the most important mediators of the atherogenic actions of RAS (70). Although Ang II up-regulates expression of cytokines such as interleukin-6 and tumor necrosis factor-α, pharmacological blockade of AT1R with ARBs would not be so effective to inhibit cytokine production completely. It was demonstrated that cytokines such as TNF-α, IL-1β and IFN-γ increase mitochondrial- and NADPH oxidase-generated ROS (83). Thus, the in vivo inhibition of intracellular ROS production by blocking vascular AT1R may play an adjunct rather than a major role to prevent or reduce atherosclerosis. The above suggestion could be also compatible with the accumulating findings that AT1R blocker could only have a modest effect on atherosclerosis diseases in patients (84).

ROLES OF INFLAMMATORY CELLS IN ATEROGENESIS

In initiation and progression of atherosclerotic lesions, RAS is activated locally and stimulates expression of vascular cellular adhesion molecule-1, intra-cellular adhesion molecule-1 and monocyte chemotactic protein-1 (MCP-1) (37, 39, 51, 52). These molecules accelerate recruitment of inflammatory cells into the vessel walls. It is generally believed that the vascular endothelium serves as an inflammatory barrier by providing a nonadherent surface to leukocytes. However, upon Ang II stimulation, endothelium turns to promote infiltration of inflammatory cells by expressing adhesion molecules and chemokines. After migrating into the vessel wall, monocytes transform into macrophages and contribute to lipid deposition in the plaque (57, 85). Monocytes/macrophages secret chemokines (86) and
MMPs (60), leading to acceleration of atherosclerotic lesion development. Moreover, recruited leukocytes themselves have NAD(P)H oxidase subunits and serve as a source of ROS (87, 88). Thus, activated RAS promotes interaction between circulating leukocytes and vascular cells, an important step in the pathogenesis of atherosclerosis (40, 41). High levels of ACE expression and Ang II have been shown in experimental and human atherosclerotic lesions (89-91). In human atherosclerotic lesions, ACE, Ang II, and its receptor are co-localized at the areas of inflammation (5). Taken together, these results suggest that local effects of an activated RAS in vessel walls promote infiltration of inflammatory cells into the vessel walls, a key feature of atherosclerosis.

LOCAL EFFECTS OF AN ACTIVATED RAS IN BONE MARROW

Bone marrow is a highly organized organ. All blood cells derive from hematopoietic stem cells through complex steps of division and maturation. Previous reports elucidated the surface receptors, cytokines, and growth factors that potentially regulate hematopoiesis (92-94). However, the precise mechanism by which the proliferation and differentiation of hematopoietic stem cells are regulated is not fully understood.

Randomized clinical trials have proved beneficial effects of ACE inhibitors or ARBs in the treatment of cardiovascular diseases (21, 23). However, it was reported that ACE inhibitors or ARBs may have suppressive effects on hematological processes. It is reported that ACE inhibitors induced anemia and leukocytopenia (95-97). ACE inhibitors and ARBs have been shown to effectively reduce hematocrit values in patient with renal transplantation (98, 99). Haznedaroglu et al. proposed the existence of a locally activated RAS in bone marrow that contributes to hematopoiesis (100). Others also demonstrated the presence of RAS components in bone marrow and circulating blood cells. Rodgers et al. showed the presence of AT1R in CD34+CD38- cells, CD34+CD38- cells and lymphocytes (101). The authors demonstrated that Ang II accelerated colony formation of hematopoietic progenitor cells from murine lineage negative bone marrow cells in a dose dependent manner. Ang II also stimulated differentiation of human CD34+ hematopoietic progenitors from cord blood. The effects of Ang II on hematopoietic progenitors were clearly inhibited by an ARB, losartan. It was also reported that Ang II and Ang (1-7) accelerated recovery of circulating leukocytes and the myeloid lineage cells in bone marrow after chemotherapy and irradiation (102, 103). Similarly, other reports demonstrated that RAS components in bone marrow contribute to hematopoiesis (104-106). On the other hand, several papers reported that a local RAS in bone marrow plays a role in the pathological hematopoiesis (107, 108). bone marrow stromal cells also express AT1R, whose activation possibly causes secretion of growth factors or cytokines that increase hematopoietic progenitor cells (109). Thus, it is likely that angiotensin peptides are potential stimulators of proliferation and differentiation of multiple hematopoietic lineages under physiological and pathological conditions.

ANG II STIMULATES CONTRIBUTION OF BONE MARROW-DERIVED CELLS TO THE PATHOGENESIS OF ATHEROSCLEROSIS

Recently, we proposed that bone marrow-derived cells significantly contribute to pathogenesis of atherosclerosis (35, 36, 110-117). This phenomenon was confirmed not only in various animal models of vascular diseases, but also in human samples (118, 119). Ang II is supposed to promote contribution of bone marrow-derived cells to atherosclerosis by enhancing their mobilization, recruitment, differentiation, and proliferation (35, 36). To confirm this notion, we performed bone marrow transplantation from GFP (Green Fluorescent Proteins)+/+ApoE-/- mice to GFP-/-ApoE-/- mice. Administration of Ang II to these bone marrow chimeric mice promoted atherosclerosis lesion formation, which was associated with increased infiltration of bone marrow-derived GFP-positive cells to the lesion (35, 36) (Fig. 2A). We also observed that Ang II infusion increased the number of smooth muscle progenitor cells, which are peripheral blood cells that turn to α-smooth muscle actin-positive cells after culture in the presence of PDGF-BB (116) (Fig. 2B). These smooth muscle-like cells expressed abundant matrix metalloproteinase-9 (MMP-9), which substantially contribute to destabilization of atherosclerotic plaques.
ROLE OF BONE MARROW RAS IN THE PATHOGENESIS OF ATHEROSCLEROSIS

Although interaction between leukocytes and vascular cells plays a crucial role in the pathogenesis of atherosclerosis, it remained to be elucidated whether a local RAS, especially the Ang II-AT1R pathway, in bone marrow contributes to vascular diseases. To evaluate the potential participation of AT1aR in bone marrow in the pathogenesis of atherosclerosis, we generated several combinations of bone marrow chimeric mice in a murine model of hyperlipidemia and atherosclerosis (120). In rodents, two AT1R subtypes, AT1aR and AT1bR, have been identified. In the vasculature, AT1aR is predominant and mediates most of the physiological and pathophysiological responses to Ang II in mice (121, 122). We also revealed that AT1aR was abundantly expressed in bone marrow, whereas other receptors were hardly detected in bone marrow cells by RT-PCR.

At first, we performed bone marrow transplantation (BMT) from the ApoE-/-AT1aR-/- mice to the ApoE-/-AT1aR-/- mice (BMT ApoE-/-AT1aR-/- > ApoE-/-AT1aR-/- mice). These bone marrow chimeric mice had no AT1aR in their body. We also performed BMT from the ApoE-/-AT1aR+/+ mice to the ApoE-/-AT1aR-/- mice (BMT ApoE-/-AT1aR+/+ > ApoE-/-AT1aR-/- mice). These bone marrow chimeric mice had AT1aR in bone marrow, but not in their innate vascular cells. We infused Ang II (5 mg/kg/day) into these bone marrow chimeric mice for 8 weeks using an osmotic mini-pump. There was no significant difference in systolic blood pressure or plasma cholesterol level between these BMT mice. After 8 weeks of infusion, en face Sudan IV staining of the aortic arch revealed that atherosclerotic lesions in the BMT ApoE-/-AT1aR+/+ > ApoE-/-AT1aR-/- mice were significantly larger than those in the BMT ApoE-/-AT1aR-/- > ApoE-/-AT1aR-/- mice. Histological analysis of atherosclerotic lesions

![Ang II promotes accumulation of macrophages in atherosclerotic plaque](image_url)

**A.** Ang II infusion into the bone marrow-chimeric mice promoted atherosclerotic lesion formation as determined by en face Sudan IV staining. Bone marrow-derived GFP-positive cells accumulated at the sites of atherosclerosis.

**B.** α-smooth muscle actin-positive cells could be obtained from the culture of human peripheral mononuclear cells. Those smooth muscle-like cells expressed MMP-9.
in the aortic root revealed that lipid deposition detected by oil red O staining was significantly accelerated in the BMT ApoE-/AT1aR+/+ -> ApoE-/AT1aR+/+ mice compared with those in the BMT ApoE-/AT1aR+/+ -> ApoE-/AT1aR+/+ mice. Collagen content was significantly decreased in the BMT ApoE-/AT1aR+/+ -> ApoE-/AT1aR+/+ mice compared with that in the BMT ApoE-/AT1aR+/+ -> ApoE-/AT1aR+/+ mice as determined by Sirius red staining. Taken together, these results suggest that bone marrow transplantation from the ApoE-/AT1aR+/- animals to the ApoE-/AT1aR+/- mice could restore Ang II-induced acceleration of atherosclerosis and plaque destabilization, even when the recipient vascular cells did not express AT1aR (35).

Next, to investigate the role of bone marrow AT1aR and to keep track of bone marrow-derived cells in the process of atherosclerotic lesion progression, we replaced the bone marrow of the ApoE-/-AT1aR+/- mice with that of the ApoE-/-AT1aR+/-GFP+/- mice (BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice) or the ApoE-/-AT1aR+/-GFP+/- mice (BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice). The former bone marrow chimeric mice lacked AT1aR only in bone marrow, and the latter chimeric mice had AT1aR in both bone marrow and vasculature. In these bone marrow chimeric mice, we compared the effects of continuous Ang II infusion on atherosclerotic lesion formation. Ang II (5 mg/kg/day) was infused after BMT for 8 weeks. Atherosclerotic lesion formation was significantly attenuated in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice compared with that in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice as determined by en face Sudan IV staining of the aortic arch. In these two types of bone marrow chimeric mice, there was no significant difference in blood pressure or total cholesterol level. In atherosclerotic plaques in the aortic root, the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice showed significantly reduced lipid deposition and increased collagen content compared with those in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice. These results suggest that AT1aR in bone marrow-derived cells may play a role in the pathogenesis of accelerated atherosclerosis induced by Ang II. Infiltration of macrophage into the lesions was significantly reduced in BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice compared with that in BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice as determined by immunostaining against MOMA-2. Lack of AT1aR in bone marrow cells decreased atherosclerotic lesion progression and stabilized plaques, despite the existence of AT1aR in vascular cells (35).

We examined gene expression in the plaques by means of a laser microdissection system and quantitative RT-PCR after 4 weeks infusion of Ang II. Expression of MMP-9 and MCP-1 in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice was significantly suppressed compared with those in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice. On the other hand, there was no significant difference in VCAM-1 expression between the two bone marrow transplantation mice. Immunohistochemical analysis revealed that accumulation of bone marrow-derived GFP-positive cells was significantly attenuated in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice compared with that in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice. Most of the bone marrow-derived cells in the lesions were positive for macrophage marker. Furthermore, the percentage of bone marrow-derived GFP-positive cells among MMP-9-positive cells or MCP-1-positive cells was greater in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice than in the BMT ApoE-/-AT1aR+/- -> ApoE-/-AT1aR+/+ mice, suggesting that AT1aR in bone marrow could influence the instability of the atherosclerotic lesions (35). Our findings indicate that AT1aR expressed not only on vascular cells but also on bone marrow cells plays a role in the pathogenesis of atherosclerosis, at least in part. Consistent with our results, contribution of AT1aR in bone marrow cells to the pathogenesis of atherosclerosis was demonstrated in LDL-receptor-deficient mice (123).

It is a generally accepted view that atherosclerotic lesions are initiated by endothelial cell damage, followed by monocyte/macrophage adhesion and invasion as well as smooth muscle cell migration and proliferation (30, 124). Although there are a number of cellular and molecular differences, restenosis after angioplasty shares an important pathophysiological process with atherosclerosis, where injuries to the endothelium are followed by impaired re-endothelialization (125, 126). It has been believed that re-endothelialization is caused only by migration and proliferation of adjacent endothelial cells in the vessel wall (127). However, accumulating evidence indicates that bone marrow derived endothelial progenitor cells (EPCs) also participate in this process (128-131). EPC-dependent neovascularization has been implicated in collateral development in occlusive vascular diseases (129, 132-135). Bone marrow cells including stem cells express AT1R. Thus, it is possible that a local RAS in bone marrow has a role in EPC biology leading to neovascularization. Actually, it was demonstrated that activation of RAS stimulates EPC proliferation and neovascularization (136). These studies suggest that...
ROS may be involved in the balance between self-renewal and differentiation of progenitors and that anti-oxidant may play a role in preservation of stemness of progenitors (137-139). Murohara and his colleagues showed that the Ang II-AT1R pathway plays an important role in ischemia-induced angiogenesis by supporting inflammatory cell infiltration and angiogenic cytokine expression (140). On the other hand, it was reported that blockade of RAS increase the number of EPC and neovascularization in animals models of metabolic diseases (141-143). These studies suggested that Ang II accelerates the onset of EPC senescence by a gp91phox-mediated increase of oxidative stress leading to impairment of EPC proliferation. Under pathological conditions, RAS may be over-activated and the excess production of Ang II might accelerate EPC senescence, resulting in the impairment of EPC function. Future study is required to confirm that RAS is essential for EPC proliferation and neovascularization but excessive activation of RAS may turn to enhance senescence and dysfunction of EPCs (117).

CONCLUSIONS

Our findings demonstrate that AT1aR expressed not only on vascular cells but also on bone marrow-derived cells plays a role in the pathogenesis of atherosclerosis, at least in part, by accelerating infiltration of bone marrow-derived inflammatory cells in the vessel wall (35, 113, 144). Therefore, blockade of AT1R not only in vascular cells but also in bone marrow could be an important strategy to prevent progression and destabilization of atherosclerotic plaques.

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