Vascular Inflammation and the Renin-Angiotensin System

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Abstract—It is now well established that vascular inflammation is an independent risk factor for the development of atherosclerosis. In otherwise healthy patients, chronic elevations of circulating interleukin-6 or its biomarkers are predictors for increased risk in the development and progression of ischemic heart disease. Although multifactorial in etiology, vascular inflammation produces atherosclerosis by the continuous recruitment of circulating monocytes into the vessel wall and by contributing to an oxidant-rich inflammatory milieu that induces phenotypic changes in resident (noninflammatory) cells. In addition, the renin-angiotensin system (RAS) has important modulatory activities in the atherogenic process. Recent work has shown that angiotensin II (Ang II) has significant proinflammatory actions in the vascular wall, inducing the production of reactive oxygen species, inflammatory cytokines, and adhesion molecules. These latter effects on gene expression are mediated, at least in part, through the cytoplasmic nuclear factor-κB transcription factor. Through these actions, Ang II augments vascular inflammation, induces endothelial dysfunction, and, in so doing, enhances the atherogenic process. Our recent studies have defined a molecular mechanism for a biological positive-feedback loop that explains how vascular inflammation can be self-sustaining through upregulation of the vessel wall Ang II tone. Ang II produced locally by the inflamed vessel induces the synthesis and secretion of interleukin-6, a cytokine that induces synthesis of angiotensinogen in the liver through a janus kinase (JAK)/signal transducer and activator of transcription (STAT)-3 pathway. Enhanced angiotensinogen production, in turn, supplies more substrate to the activated vascular RAS, where locally produced Ang II synergizes with oxidized lipid to perpetuate atherosclerotic vascular inflammation. These observations suggest that one mechanism by which RAS antagonists prevent atherosclerosis is by reducing vascular inflammation. Moreover, antagonizing the vascular nuclear factor-κB and/or hepatic JAK/STAT pathways may modulate the atherosclerotic process. (Arterioscler Thromb Vasc Biol. 2002; 22:1257-1266.)

Key Words: atherosclerosis ■ interleukin-6 ■ angiotensin II ■ angiotensinogen ■ hepatic acute-phase response

It is now widely accepted that atherosclerosis involves a series of coordinated cellular and molecular events characteristic of inflammation. The fatty streak, the first identifiable precursor of the atherosclerotic lesion, is an inflammatory reaction composed of lipid, monocytes, and T lymphocytes formed in response to vascular injury. Not normally resident in the vessel wall, these monocytes and lymphocytes are recruited into the subendothelial space through the combined actions of locally produced chemotactic cytokines and adhesion molecules expressed on the injured endothelial surface. Continuous recruitment of additional circulating mononuclear cells into the injured vessel wall, LDL oxidation, and reactive smooth muscle proliferation give rise to the complex atherosclerotic lesion. Later, in this established lesion, extracellular secretion of metalloproteinases and cathepsins by resident macrophages destabilizes the fibrous cap, resulting in plaque rupture, vascular occlusion, and the development of acute cardiac or cerebral ischemia. Understanding the mechanisms for early lesion formation and the factors controlling mononuclear recruit-
ment into the vessel wall will identify important therapeutic targets for the prevention of atherosclerosis.

Although classically the renin-angiotensin system (RAS) has been regarded as an endocrine system responsible for correcting acute hypotension through changes in peripheral vascular resistance and electrolyte homeostasis, the RAS is now recognized as being important in the long-term control of blood pressure, progression of diabetic nephropathy, and development of hypertensive cardiomyopathy and, more recently, as being a modulating factor in control of the development (or progression) of atherosclerosis. In the present review, we will focus on the evidence and mechanisms supporting the proatherogenic role of the RAS, focusing on its role in cellular recruitment through cytokine-like actions enhancing vascular inflammation. On the basis of our molecular studies, we present a hypothesis for how vascular inflammation may produce sustained angiotensin II (Ang II) action in the atherosclerotic vessel.

**Linkage of RAS With Atherosclerosis**

A relationship between RAS activity and the risk of myocardial infarction (MI) was suggested over a decade ago, when it was shown that the renin profile was independently associated with subsequent risk of MI in a prospective study of treated moderately hypertensive patients. More convincing support has been provided by the use of ACE inhibitors in recent clinical trials in which profound effects of ACE inhibitors on reducing ischemic vascular events were seen. For example, in the Studies of Left Ventricular Dysfunction (SOLVD) trial, a prospective double-blind trial examining the development of MI or unstable angina in ~6800 patients with low ejection fraction, a 23% reduction in the incidence of recurrent MI was observed in enalapril-treated patients. A similar 25% reduction in recurrent MI was seen in the Survival and Ventricular Enlargement (SAVE) trial in patients with left ventricular (LV) dysfunction after MI. Although these patients were a select group with reduced LV function, the recently completed Heart Outcomes Prevention Evaluation (HOPE) study, involving 9297 high-risk patients with vascular disease and normal LV function, has provided additional compelling data regarding the beneficial effects of ACE inhibition. That study was terminated early because a statistically significant 22% reduction in MI, stroke, or death from cardiovascular causes was seen in the group given the ACE inhibitor ramipril, an effect that was significant after only 2 years of treatment. Because of the only modest effect of ramipril on reducing blood pressure (2 mm Hg diastolic), this effect could not account completely for the risk reduction observed. These data suggest that inhibition of endogenous RAS activity improves the clinical outcome in patients with atherosclerotic burden and have led some to propose expanding the indication for ACE inhibitor therapy to all patients with established atherosclerotic vascular disease without other contraindications.

In experimental models of atherosclerosis, a strong body of evidence supports a modulatory role for the RAS in atherogenesis. The vasoprotective actions of ACE inhibitors on reducing atherogenesis have been extensively documented in a number of experimental animals. For example, in atherosclerosis-prone apoE-deficient (apoE−/−) mice, the ACE inhibitor fosinopril reduced atherosclerotic lesion size by 70% over a 12-week treatment. Similar impressive findings have been reported in Watanabe hypercholesterolemic rabbits, minipigs, and other models (see review). The mechanism by which ACE inhibitors reduce lesion size has been somewhat controversial because ACE is an enzyme critical for the destruction of the vasodilator bradykinin and formation of the vasoconstrictor Ang II. Endothelial cell dysfunction, an impaired vasodilatory response to infused acetylcholine, is one of the earliest physiological hallmarks of atherosclerosis. Presently, it is understood that kinins appear to play a primary role in modulating endothelial function because they inhibit platelet aggregation, stimulate tissue plasminogen activator production, block the deleterious effects of oxidized LDL, reduce oxidative stress on the endothelium, and improve insulin sensitivity. Although ACE inhibitors increase endothelial NO production through the bradykinin pathway, thereby influencing vascular tone, there is no strong evidence currently that ACE inhibitors exert their antiatherogenic effect primarily through indirect effects on kinin metabolism. For example, administration of selective angiotensin type 1 receptor blockers (ARBs), agents that do not influence circulating kinin peptide concentrations, also reduces or reverses endothelial dysfunction. Losartan reduces the medial width of resistance arteries and promotes acetylcholine-induced endothelium-dependent relaxation after chronic treatment. Also, it augments acetylcholine-mediated femoral artery vasodilation and improved flow-mediated brachial artery vasodilation in atherosclerosis. These studies strongly argue for an important direct modulatory role of Ang II early in the atherosclerotic process.

With atherosclerotic plaque as a primary experimental end point, the ARB losartan (Dup 753) reduces lesion size in the cholesterol-fed cynomolgus monkey. Similarly, irbesartan (another type 1–specific ARB) blocked lesion formation in apoE−/− mice. Valsartan reduced intimal lesion and increased lumen area in hypercholesterolemic rabbits. Although more studies need to be performed to determine their effects in humans, these data suggest that specific blockade of endogenously produced Ang II is antiatherogenic and serves to reduce plaque size. Interestingly, in the few human studies in which plaque size was measured in response to ACE inhibitors, the majority have failed to observe significant regression in spite of improvements in ischemic end points (see review). This failure may be due to drug dosage effects, insensitivity of the clinical measurements of atherosclerotic lesion size, or species differences in atherogenesis.

On the other side of the coin, pharmacological administration of Ang II to hypercholesterolemic animals is potently proatherogenic. In transgenic mice coexpressing human renin and angiotensinogen genes, extensive aortic root lesions develop in response to challenge with a high-fat diet. In separate experiments, 2 groups evaluated the effect of a chronic (4- to 8-week) subcutaneous Ang II infusion in apoE−/− mice. This treatment dramatically accelerated the generation of atherosclerotic aortic lesions (from 4% surface area involvement in control mice to 70% in Ang II–treated
apoE−/− mice, enhanced the formation of aortic aneurysms, and induced macrophage recruitment to the underlying adventitia. In control experiments, Ang II infusion into apoE−/− mice did not noticeably induce aneurysms or atherosclerosis, indicating that Ang II, by itself, is not strongly atherogenic. The combined effects of the Ang II infusion with hyperlipidemia appear to result from synergistic effects on the generation of reactive oxygen species (ROS) in the vessel wall. Overall, these data strongly suggest that endogenous and pharmacological administration of Ang II potentiates the development of atherosclerosis in experimental models of hyperlipidemia.

Proatherogenic Actions of Ang II

Ang II has potent and diverse actions throughout the cardiovascular system, including induction of vasoconstriction, aldosterone production, cardiac hypertrophy, vascular smooth muscle cell (VSMC) proliferation, and ROS. Ang II primarily mediates its vascular action through the Ang II type 1 (AT1) receptor subtype, a 7-transmembrane G-protein-coupled receptor expressed on endothelial cells, monocytes, and VSMCs. AT1 activation induces a cascade of cellular responses, including rapid activation of phospholipase C and intracellular calcium, and, later, more sustained effects mediated by changes in gene transcription (see reviews). These proatherogenic genetic responses include the following: inducing VSMC proliferation, an effect mediated through insulin-like growth factor and platelet-derived growth factor (PDGF); altering the structural integrity of the vessel by influencing extracellular matrix production and degradation; and enhancing lipid oxidation. Because of space limitations, we will focus on 3 early effects of Ang II on vessel biology that are probably the most important in its proatherogenic activities.

ROS Production: Role in Paracrine (Endothelial Dysfunction) and Intracellular Signal Cascades

Vascular superoxide appears to function in 2 distinct ways: (1) extracellularly (paracrine), to modulate endothelium-dependent changes in vasomotor tone, and (2) intracellularly, as a second messenger to produce long-term phenotypic alterations of resident cells. In the atherosclerotic vessel, the ability of the endothelium to function as a nonadhesive surface and control vasomotor tone through the release of NO and prostaglandins is disturbed, representing one of the earliest physiological responses to atherosclerosis. Ang II and lipid (oxidized) LDL appear to disrupt normal endothelial function by inducing increases in ROS tone. Ang II–induced production of ROS results in the chemical inactivation of NO, blunting its ability to vasodilate. In rats, chronic Ang II infusion doubles superoxide production in aortic segments through an NAD(P)H-dependent mechanism (described below). Moreover, inhibition of vascular ROS production through the administration of superoxide dismutase increases acetylcholine-induced relaxation, suggesting that ROS species themselves are responsible for the endothelial dysfunction. Similarly, in humans, it has been shown that treatment with ARB reverses endothelial dysfunction in large arteries. Another paracrine effect of ROS is regulation of the activity of the collagen-degrading metalloproteinases pro-MMP-9 and pro-MMP-2. This latter effect may play an important role in plaque instability and rupture.

Although the cell types responsible for Ang II infusion–enhanced vascular superoxide production in the vessel have not yet been unambiguously identified, VSMCs are likely candidates. In these cells, subnanomolar concentrations of Ang II induce NAD(P)H oxidase activity, in which the ROS produced serve as intracellular second messengers, mediating the mitogenic response. Also, ROS activate mitogen-activated kinases, Akt, and the janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, and they induce immediate-early transcription factors (such as activator protein-1), key intracellular signaling molecules responsible for the acquisition of the secretory phenotype. Thus, induction of vascular ROS tone is probably a major mechanism by which Ang II enhances LDL-induced atherosclerosis by inducing endothelial dysfunction and VSMC proliferation.

Because of these important activities of ROS, the mechanisms by which they are generated have been extensively investigated. Among the variety of ROS generators in VSMCs are the mitochondrion and cellular enzymes, such as xanthine oxidase, cyclooxygenase, lipoygenase, NO synthase, heme oxygenases, and peroxidases; the membrane-associated NAD(P)H oxidases have been shown to be of foremost physiological importance. Changes in ROS production are tightly regulated by second-messenger systems modulated by Ang II. Activation of the NAD(P)H oxidase complex has been most thoroughly described in the neutrophil, a cell type that produces bursts of ROS that are orders of magnitude stronger than those in vascular cells. It is known that stimulus-induced NAD(P)H activation involves the assembly of a complex of protein kinase C–phosphorylated cytosolic phagocytic oxidase (p40phox, p47phox, and gp91phox) and Rac-1 (or Rac-2) proteins with the membrane-bound heterodimeric flavocytochrome b558, consisting of gp91phox and p22phox, to generate superoxide anion. The vascular NAD(P)H oxidases use NADH as their preferred substrate and are structurally similar to the neutrophil and (depending on the cell type) contain 2 to 4 of the phox subunits. The critical component directly mediating electron transport, gp91phox, has been identified in endothelial cells and adventitial fibroblasts but has not been unambiguously identified in VSMCs. However, gene homologues of gp91phox have been cloned, and 1 of these, mitogen oxidase [nox1, now termed nox1 for NAD(P)H oxidase], has been shown to be transcribed in VSMCs, in which it contributes to superoxide generation and serum-dependent growth. More recently, another nox family member, nox4, has been cloned, and it has been demonstrated that the expression of nox1 and nox4 greatly exceeds that of the barely detectable gp91phox in VSMCs. Thrombin and Ang II induce consumption of NAD(P)H and translocation of p47phox and Rac2 to the cell membrane, indicating an activation mechanism similar to that used by the neutrophil oxidase. Furthermore, in these cells, nox1 is highly upregulated, and nox4 is downregulated, in response to serum, PDGF, or Ang II. Antisense RNA-mediated depletion of nox1 has indicated its critical
role in Ang II–stimulated superoxide production. In support of the central role of ROS in atherogenesis, compared with apoE−/− mice, apoE−/− mice that are also made genetically deficient in p47phox exhibit decreased progression to atherosclerosis. From these studies, it is clear that the individual components of the vascular NAD(P)H oxidases are highly cell-type dependent and that vascular superoxide has important paracrine and signaling actions that impact atherogenesis.

**LDL Oxidation by Resident Macrophages**

Ang II has important modulatory effects on LDL oxidation in the vessel wall. This is particularly important because the proatherogenic activity of Ang II is primarily seen in the setting of hyperlipidemia, as shown by its infusion in the apoE−/− mouse. An important role of Ang II in LDL oxidation and atherogenesis has been suggested by studies in which the administration of angiotensin receptor blockers (or ACE inhibitors) inhibited both in atherogenic-prone apoE−/− mice. Although the underlying mechanism(s) will require further study, Ang II has been recently reported to increase macrophage uptake of oxidized LDL in the apoE−/− mouse. Macrophage uptake of LDL appears to be mediated through enhanced expression of the scavenger receptor CD36, the predominant receptor found in foam cells. These studies suggest that the effect of Ang II on LDL uptake may be a secondary effect mediated through the local actions of Ang II–induced interleukin-6 (IL-6) expression, a cytokine whose actions are further discussed below. An additional effect of Ang II on vascular lipid metabolism is upregulation of the expression of the lectin-like oxidized LDL receptor-1 in endothelial cells, an effect that may further increase oxidized LDL entry into the lesion. Together, these studies suggest that Ang II modulates LDL oxidation, endothelial cell LDL uptake, and foam cell activity.

**Mononuclear Cell Recruitment**

Ang II influences the expression of proinflammatory molecules in the vessel wall that influence multiple steps in monocyte recruitment into the injured vessel (see Figure 1). In endothelial cells, Ang II upregulates vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule, and E-selectin expression through a ROS-dependent pathway. These are important adhesion molecules that bind very late antigen-4 on the surface of circulating leukocytes, initiating their recruitment into the vessel wall. In VSMCs, Ang II stimulates the production of VCAM-1, chemokine monocyte chemotactic protein-1 (MCP-1), and the cytokine IL-6. MCP-1 is a small (8- to 10-kDa) β (CC-type) chemokine that specifically attracts monocytes and memory T lymphocytes expressing the CCR2 receptor, cell types that are present at all stages of the atherosclerotic lesion. MCP-1 is thought to function locally in the vessel wall by establishing a chemical gradient to attract adherent monocytes and T lymphocytes to the site of injury in the vessel media. MCP-1–CCR2 interactions are important in atherosclerosis because hyperlipidemic-atherosclerotic–prone mice, made genetically deficient in MCP-1 or CCR2, have reduced numbers of vascular macrophages and develop fewer atherosclerotic lesions than do the control mice. Finally, IL-6 is a 26-kDa glycoprotein abundantly secreted by activated monocytes and VSMCs. Initially described as a plasma cell growth factor, IL-6 has local (paracrine) actions to promote smooth muscle cell proliferation involving the local production of PDGF. In addition, as discussed below, IL-6 produces systemic effects by stimulating gene expression in distant organs (primarily the liver). The ability of Ang II to induce coordinate expression of adhesion molecules and chemokines then ensures the recruitment of mononuclear leukocytes into the vessel wall.

Recent studies have shown that endogenous Ang II levels control vascular inflammation. For example, in apoE−/− mice, treatment with the ARB irbesartan reduces mRNA and immunostaining of MCP-1 and expression of macrophage inflammatory protein-1α in atherosclerotic lesions. In addition, losartan attenuated aortic intimal proliferation and caused a marked decrease in the expression of P-selectin and MCP-1 in hyperlipidemic rabbits. Furthermore, recent human studies have shown that treatment with irbesartan significantly reduced serum levels of VCAM-1, tumor necrosis factor (TNF)-α, and superoxide. The complete spectrum of
Ang II–inducible proteins in the vessel wall has yet to be systematically investigated; it is not known, for instance, whether the ARB effect on macrophage inflammatory protein–1α expression is direct or indirect. Nevertheless, these intriguing studies strongly suggest that the proinflammatory properties of endogenously produced Ang II play an important role in atherogenesis and may explain how ACE inhibitors and ARBs influence clinical outcomes in humans without significant changes in lesion size.

The mechanisms by which Ang II induces expression of inflammatory genes have been investigated extensively; our own work has shown that Ang II–induced signal transduction mechanisms overlap those typical of the proinflammatory cytokines, such as TNF. Specifically, Ang II activates the potent cytoplasmic transcription factor, nuclear factor (NF)-κB, a protein that controls networks of chemokine-modulating, growth factor–modulating, translational control, and cellular survival genes. NF-κB is a family of highly inducible DNA-binding proteins that is regulated by protein processing and by association with a family of cytoplasmic inhibitors, the IκBs. Interestingly, Ang II has pleiotropic actions at multiple points in the NF-κB activation pathway in VSMCs. Ang II not only induces the translocation of the sequestered cytoplasmic NF-κB complex through targeted proteolysis of the IκB inhibitors but also induces the processing of the DNA binding form, NF-κB1, and proteolysis of the proto-oncogene, c-Rel. Moreover, NF-κB activation appears to be downstream from the NAD(P)H oxidases, inasmuch as antioxidant treatment interferes with its activation by Ang II. Relevance of the NF-κB signaling pathway to inflammatory molecule expression has been indicated by independent studies showing that inhibition of NF-κB activation blocks Ang II–inducible IL-6, VCAM-1, and MCP-1 expression. A body of work has further shown that NF-κB is inducible by the effects of lipid oxidation, macrophage-derived cytokines interleukin-1 (IL-1) and TNF, and infectious agents. These observations have led to the current model demonstrating that NF-κB plays a key signaling role in multiple proatherogenic insults, thus inducing the inflammatory vascular cytokine cascade, inducing cellular recruitment, and mediating atherosclerotic progression. Importantly, the relevance of these in vitro studies on NF-κB activation to human atherosclerosis was suggested by the earlier identification of activated NF-κB in VSMCs, macrophages, and endothelial cells residing in human atherosclerotic plaques in situ. Because of this central coordinating activity in the inflammatory response, inhibition of vessel wall NF-κB is an attractive therapeutic target for preventing inflammation and, ultimately, atherogenesis.

Systemic Responses to Vascular Inflammation

In otherwise healthy patients, cytokines produced by the atherosclerotic vessels do not produce the obvious systemic manifestations normally associated with inflammation; however, systemic effects are detectable biochemically. For example, in patients with unstable angina, increased IL-6 levels are predictive of a higher incidence of cardiac events, indicating that circulating IL-6 may be a marker for plaque instability. Conversely, in otherwise healthy populations, plasma levels of IL-6 in the upper quartile of the normal range are independently predictive of an increased risk of all-cause as well as cardiovascular mortality and future MI. Furthermore, C-reactive protein (CRP), a biomarker for IL-6, has been shown to be an independent predictor of the risk of future MI, stroke, peripheral vascular disease, and vascular mortality among individuals with no known cardiovascular disease. A series of prospective epidemiological studies from both the United States and Europe demonstrate that elevated circulating CRP levels are a strong predictor of future cardiovascular morbidity and mortality even among individuals with no clinical evidence of vascular disease. For example, in a cohort of 22,000 healthy men, there was a 3-fold increase in the risk of MI and a 2-fold increase in the risk of stroke or peripheral vascular disease among those who had baseline CRP values in the highest quartiles. These effects were independent of coexisting cardiovascular risk factors and were present in smokers as well as nonsmokers. High CRP levels predicted a higher risk of MI for men that were at low risk as well as high risk for coronary heart disease on the basis of lipid values, and in fact, risk stratification that includes both CRP and lipid parameters is superior to that based solely on lipids. These studies suggest that vascular cytokines, notably IL-6, are secreted in sufficient abundance to generate genetic responses of the liver (eg, the hepatic acute-phase reaction).

The liver is the major source of circulating plasma proteins and represents a primary target for the action and metabolism of IL-6 secreted peripherally. The acute-phase response involves a “transcriptional switch,” by which proteins are synthesized de novo by the liver in response to circulating hormones elaborated at the site of injury. Although a spectrum of cytokines induces hepatic acute-phase synthesis, including IL-1, TNF, leukemia inhibitory factor, oncostatin M, interleukin-11, transforming growth factor-β, γ-interferon, and others, the cytokine IL-6 appears to be the primary mediator in humans. For example, in combination with cortisol, IL-6 synergistically stimulates angiotensinogen and fibrinogen synthesis. IL-6 in the presence of IL-1 synergistically upregulates CRP and serum amyloid A (SAA) synthesis. This complex interplay between circulating cytokines allows for heterogeneity in plasma protein synthesis in response to different inflammatory stimuli.

Because of its central role in the hepatic acute-phase reaction and the immune response, IL-6 signaling has been extensively studied. IL-6 induces acute-phase reactant synthesis by 2 distinct signaling pathways initiated through the IL-6 receptor. Type I IL-6 signaling is mediated through a tyrosine kinase mechanism involving the JAK/STAT pathway, whereas type II IL-6 signaling is mediated through a Ras/mitogen-activated protein kinase/NF-IL-6 pathway. Gene transfer studies and genetic knockout experiments have led to the conclusion that the type I (JAK/STAT) pathway is the major pathway responsible for acute-phase reactant synthesis. IL-6 activates the JAK/STAT pathway in hepatocytes by binding to a high-affinity membrane protein receptor on its target cells, termed the IL-6Rα chain, a protein lacking intrinsic kinase activity (schematically diagrammed in Figure 2). Oligomerization of the liganded IL-6–IL-6Rα chain...
complex with a separate transmembrane protein, transducin (gp130), forms the active signaling complex on the plasma membrane. This assembly recruits the gp130-associated tyrosine kinases (JAKs 1 and 2 and Tyk 2 kinases) to molecular distances, allowing them to tyrosine-phosphorylate gp130. Tyrosine-phosphorylated gp130, in turn, recruits the cytoplasmic transcription factors (STAT 1 and STAT 3) to bind gp130 via their src homology-2 (SH2) domains. As substrates of the JAK/Tyk kinases, STATs are also tyrosine-phosphorylated, allowing them to associate (dimerize) and translocate into the nucleus. Once nuclear, STATs bind to target acute-phase response genes, including CRP, SAA, fibrinogen, and angiotensinogen.92 On binding to their cognate response elements, STATs recruit bridging coactivators, such as p300/CBP, which serve as molecular bridges to communicate with RNA polymerase, and through histone acetylase activity, p300/CBP modifies the chromatin structure of its target genes.87,93,94 In this manner, the IL-6/JAK/STAT pathway accounts for the induction of circulating biomarkers of vascular inflammation.

Although the liver represents a major site for the production of circulating proteins, it is important to note that other organs may also contribute to vascular pools. In particular, the pioneering work of Campbell and Habener95,96 has suggested that angiotensinogen is widely expressed in various tissues95 and is particularly highly expressed in brown adipose tissue and fibroblastic cells of the periaortic connective tissue.96 Recent studies in angiotensinogen-deficient mice have shown that transgenic angiotensinogen overexpression selectively in the adipocyte increases fat mass and restores circulating angiotensinogen levels.97 These studies suggest that adipocytes may also contribute to circulating angiotensinogen pools. Whether the adipocyte participates in the acute-phase response and its exact contribution to circulating plasma proteins under normal conditions remain to be determined.

**Relationship of Hepatic Acute-Phase Reactants to Atherogenesis**

Although circulating CRP and other acute-phase reactants may be a marker only for the severity of vascular inflammation, many acute-phase reactants have significant cardiovascular actions. For example, the complement factors (C3, C4, C9, and factor B), coagulant/fibrinolytic factors (fibrinogen, plasminogen, and tissue plasminogen activator), and antiproteases (α1-antitrypsin) are established acute-phase reactants with obvious circulatory and immune activities.59,98 Angiotensinogen, the only known precursor of the angiotensin peptides, circulates at concentrations that control RAS activity (discussed below). CRP, initially identified as a nonspecific opsonin binding to phosphocholine substrates on bacterial and fungal cell walls, has been recently shown to bind apoB-containing lipoproteins, including LDL and VLDL.99,100 CRP is enriched in atherosclerotic lesions, suggesting that it may have a role in foam cell formation.99 CRP has also been shown to induce MCP-1 and adhesion molecule expression in endothelial cells, suggesting a local proinflammatory function.101-102 SAA, a precursor of amyloid protein A deposits, is an apolipoprotein associated with apoA-I and apoA-II in HDL subfractions in plasma.103 SAA enhances cholesterol uptake by smooth muscle...
Inflammation, Angiotensinogen Synthesis, and RAS Activity

The activity of the RAS is controlled by sequential proteolytic processing of the angiotensinogen prohormone, ultimately into the major effector, octapeptide Ang II. In the plasma, angiotensinogen circulates at concentrations less than the Michaelis-Menten constant of renin (1 μmol/L), and its concentration is rate-limiting for the maximal velocity of angiotensin I (Ang I) formation. Physiological observations that support this critical relationship between angiotensinogen synthesis and RAS tone include the following: (1) there is a positive correlation between angiotensinogen concentrations and blood pressure in ambulatory patients, (2) pharmacological manipulations (such as steroid administration) increase angiotensinogen and blood pressure in humans, (3) in mice, gene dosage studies show a linear 8 mm Hg increase in blood pressure with angiotensinogen copy number, (4) transgenic mice overexpressing human angiotensinogen and renin genes are severely hypertensive and (5) human genetic linkage studies have associated polymorphisms of certain angiotensinogen alleles with hypertensive disease states, including essential hypertension and preeclampsia. Together, these studies suggest that changes in the circulating concentration of angiotensinogen have a significant effect on the long-term activity of RAS. Of relevance here, angiotensinogen expression is highly regulated by inflammation. In experimental animals, a single administration of bacterial endotoxin upregulates steady-state angiotensinogen mRNA levels by 5-fold reflected in an increase in circulating angiotensinogen concentration of 3-fold 8 hours after stimulation. Similarly, in humans, 70% of patients with severe infection were found to have increases in circulating angiotensinogen of 2-fold. This 2-fold magnitude of change is sufficient to induce alterations in RAS tone.

Inflammation and the Local Vascular RAS

The observation that angiotensinogen expression is widely distributed has led to the discovery of "self-contained" tissue (or local) Ang II-generating systems in the central nervous system, adipose tissue, heart, and the vessel wall, which operate partly or wholly independently of the circulating RAS. Many of the enzymatic components required for Ang II production are present in the atherosclerotic vessel. Specifically, renin-like enzymes that release Ang I from angiotensinogen, such as cathepsin D, tonins, chymases, and aspartyl proteases, have been identified in the vessel wall. Cathepsin D is an enzyme expressed by VSMCs and macrophages which possesses renin-like activity, being able to liberate Ang I from angiotensinogen. Similarly, chymase, a component of mast cells, has renin-like properties. Moreover, atherosclerotic plaques are rich in classic ACE. In these plaques, ACE is expressed by fat-laden macrophages and T lymphocytes and enriched in the inflamed shoulder of the atherosclerotic plaque. In addition, cathepsin G, expressed in monocytes, has ACE activity. Not surprisingly then, atherosclerotic plaques are positive for Ang II by immunohistochemical staining. These findings strongly suggest that the atherosclerotic plaque has local tissue Ang II-generating mechanisms that process the circulating angiotensinogen substrate locally.

The process of atherosclerosis also influences tissue sensitivity to the angiotensin peptides. Ang II exerts its vasoactive and inflammatory effects primarily through the AT1 receptor. In cultured VSMCs in vitro, LDL cholesterol induces the upregulation of AT1 gene expression and enhances the vasoconstrictive response to exogenous Ang II. This upregulation in VSMCs in vitro is also seen in hypercholesterolemic rabbits. These intriguing studies suggest that the atherosclerotic vessel may be highly sensitive to locally produced Ang II.

A Biological Feedback Loop Between Vascular Inflammation: The RAS

Presently, we know that the actions of Ang II are proinflammatory in the vessel wall, being able to induce the production of IL-6, a systemically acting cytokine that induces the expression of circulating acute-phase reactants, such as CRP and angiotensinogen, through the JAK/STAT/p300 pathway. A schematic model is shown in Figure 2. Although the acute-phase reactants are thought to restore homeostasis by promoting clearance of infectious agents or by facilitating wound repair, they also have significant lipid-metabolic and proinflammatory activities that may exaggerate and sustain the process of atherogenesis. Importantly, enhanced substrate (angiotensinogen) delivery from the combination of hepatic, periadventitial, or adipocyte sources to the Ang II-processing enzymes present locally in the vessel wall further perpetuates the inflammatory cycle. More studies are needed to identify the spectrum of hepatic acute-phase reactants, to determine those that play significant roles in atherogenesis, and to assess how their activities may be targeted for drug intervention.

Summary

The early establishment and development of the atherosclerotic lesion are driven by a coordinated multicellular response to the presence of infectious agents, oxidized LDL, or other nonspecific injury to the vascular wall. The specific recruitment of leukocyte subpopulations (T cells and monocytes/macrophages) is required for foam cell development and lesion progression. Monocyte recruitment into the vessel involves receptor binding to adhesion molecules expressed by activated endothelial cells, followed by transmigration and diapedesis toward chemical gradients of chemotactic cytokines. A body of evidence supports the idea that the RAS, through the actions of Ang II, enhances vascular oxidant tone to produce endothelial dysfunction and vascular LDL oxidation, increases adhesion molecule expression, and upregulates cytokine expression by endothelial cells and VSMCs. Through these processes, Ang II is proatherogenic, being especially potent in the presence of hyperlipidemia. More-
over, present evidence suggests that vascular inflammation induces local and systemic effects (particularly involving liver gene expression) that may be important in further fueling this process. Proteins synthesized by the STAT3/p300 pathway initiated by IL-6 signaling, a cytokine produced at the site of vascular injury, have important biological activities that may further modulate the atherogenic process.

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