

Atherosclerosis: cell biology and lipoproteins

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Abbreviations

CRP	C-reactive protein
MCP-1	monocyte chemoattractant protein-1
NF-κB	nuclear factor κ B

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Recruitment of leukocytes into atherosclerotic lesions occurs throughout the initiation and maturation phases of atherosclerosis. The emigration of leukocytes from blood into arterial tissue involves a complex array of mediators to facilitate the attraction, adhesion, and diapedesis of cells into the subendothelial space. Once resident in the artery, these cells are regulated in this local environment to perform a myriad of disparate activities. These functions include modification and metabolism of lipids and lipoproteins, deposition and destruction of extracellular matrix, regulation of smooth muscle cell growth and migration, and modulation of thrombosis [1].

There has been a recent focus on angiotensin II as a prominent inflammatory stimulant in atherogenesis [2,3]. The ability of angiotensin II to promote arterial accumulation of macrophages is partially attributable to the stimulation of monocyte chemoattractant protein-1 (MCP-1) acting on CCR2 [4]. The source of angiotensin II-induced MCP-1 has not been defined, but could be either endothelial or smooth muscle cells. Sahar *et al.* [5^{*}] demonstrated recently that angiotensin II-induced inflammatory responses enhanced expression of the α subunit of interleukin-18 receptors in both cultured vascular smooth muscle cells and mouse aortas in organ culture. This led to functional changes of enhanced responsiveness to interleukin-18. This was manifested during co-incubation of angiotensin II and interleukin-18 in which was there a synergy in activating nuclear factor κ B (NF- κ B), resulting in enhanced secretion of the inflammatory cytokines interleukin-6 and interleukin-8. This observation provides a mechanism for a mode of enhancing the atherogenic potential of both angiotensin II and interleukin-18.

Although angiotensin II garners the most attention in the renin–angiotensin system, it is but one of a number of bioactive molecules that are derived from angiotensinogen. Included in this family are cleavage products, termed angiotensin III and IV, generated by specific aminopeptidases. Angiotensin IV has been implicated in the atherogenic process via its role in the growth of vascular cells and promotion of thrombotic mechanisms. Angiotensin IV binds to a specific receptor, designated AT4, that has been identified as insulin-regulated aminopeptidase [6]. Esteban *et al.* [7] demonstrated that angiotensin IV induced activation of NF- κ B in cultured vascular smooth muscle cells by a process independent of AT1 or AT2 receptors. Consistent with a role of AT4 receptors, activation of NF- κ B was decreased in a concentration-dependent manner by the antagonist divalinal. Stimulation of AT4 receptors on vascular smooth muscle cells with angiotensin IV increased the expression of a number of inflammatory molecules that promote atherosclerosis, including MCP-1, interleukin-6, intracellular cell adhesion molecule 1 (ICAM-1), and plasminogen-activator inhibitor-1 (PAI-1).

Together, these findings provide further twists on mechanisms by which the renin–angiotensin system promotes atherosclerosis. It also provides a rationale for a more in-depth investigation of the role of specific angiotensin receptors in cells present in atherosclerotic lesions. Thus while there is strong evidence for the atherogenic function of the renin–angiotensin system via promotion of inflammatory processes, there are many mechanistic facets that need to be defined.

With increasing focus on the inflammatory aspect of atherosclerosis, there are extensive efforts to quantify this component. In this regard, C-reactive protein (CRP) has attracted intense interest in the atherosclerosis community, primarily in the debate of its utility as a biomarker for defining lesion severity. In addition to the biomarker function of CRP, a recent flurry of papers have investigated whether this acute-phase protein is an active contributor to this disease. Consistent with its role as an atherogenic molecule, exogenous CRP has been shown to exhibit a wide spectrum of properties including enhanced expression of E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and MCP-1; adverse effects on progenitor cell survival; and upregulation of AT1 receptors [8]. However, there have been concerns that the preparations of CRP used in these studies may have structural

perturbations and contaminants that do not mimic the native protein [9].

One common approach to define atherogenicity of a protein *in vivo* is to use genetically targeted mice to delete the gene of interest. However, unlike humans, CRP in mice does not function as an acute-phase protein. Therefore, an alternative strategy is to increase plasma concentrations of CRP by the generation of transgenic mice. An initial study by Paul *et al.* [10] demonstrated that transgenic expression of human CRP in male apolipoprotein E^{-/-} mice had no effect on aortic root lesions at 15 weeks of age, but increased size at 29 weeks of age. No increase was observed in lesion size using en-face analysis. In contrast, Hirschfield *et al.* [11**] used the same transgenic mice expressing human CRP in an apolipoprotein E^{-/-} background and failed to demonstrate an increase in aortic root lesion size at any interval examined (12, 20, and 56 weeks of age). In both of these studies, transgenic female mice expressed only low levels of CRP and did not influence the development of atherosclerosis [10,11**]. These reports also presented conflicting data of lesion characteristics with respect to immunodetectable VCAM-1 and complement C3.

Two other recent studies have demonstrated that transgenic expression of either human or rabbit CRP failed to increase atherosclerotic lesion size in apolipoprotein E^{-/-} and apolipoprotein E3 Leiden transgenic mice, respectively [12,13]. In addition to genetic manipulations, the function of CRP has also been investigated by injection of human proteins [14]. This study performed weekly injections of either monomeric or pentameric forms of human CRP. These two configurations of the protein provided different results. The monomeric form increased lesion size, while conversely the injection of the pentameric form led to a decrease. In all these studies, the expression of human or rabbit CRP may be a confounding factor in interpretation. Overall, this

recent batch of publications has not provided an unequivocal answer to the question of whether CRP is an active component of the atherogenic process.

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