C-reactive protein and atherothrombosis—Beyond a biomarker: an actual partaker of lesion formation

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Atherosclerosis and its complications represent the most common cause of death in Western societies. Over the past few years we have witnessed a paradigm shift in our understanding of the underlying principles of atherosclerosis. This new view supports the concept that inflammation is the central orchestrator of atherosclerotic lesion formation, progression, and eventual rupture (5, 6). Chronic inflammation results in endothelial dysfunction and facilitates the interactions between modified lipoproteins, monocyte-derived macrophages, T cells, and normal cellular elements of the arterial wall, inciting early and late atherosclerotic processes. This paradigm has fueled exponential interest in evaluating inflammatory markers of atherosclerosis, of which high sensitivity C-reactive protein (CRP) has emerged as one of the most important. As such, the inflammatory marker CRP is one of the most powerful independent predictors of myocardial infarction, stroke, and vascular death in a variety of settings, with prognostic value extending across various ethnic groups and in men and women in different age groups (1, 10–13). More recently, elegant work by Ridker and colleagues (11) demonstrated that CRP may be a better predictor of future cardiovascular events than low-density lipoprotein (LDL) cholesterol and that baseline CRP evaluation adds prognostic value to the conventional Framingham risk assessment.

The link between CRP and atherosclerosis was initially suggested to be that of a “surrogate biomarker” vs. a mediator of atherosclerosis. This view has been recently revisited, with observations suggesting that CRP has a direct effect to promote atherosclerotic processes and endothelial cell inflammation. In this “Point-Counterpoint” discussion, we summarize the available evidence, which suggests that CRP functions as a powerful proatherogenic factor in addition to being a risk marker of atherosclerotic and metabolic events.

Effects of CRP on endothelial cell activation and angiogenesis. The endothelium functions as a protective biocompatible barrier between all tissues and the circulating blood in addition to being an important secretory organ, releasing a number of endothelium-derived contracting and relaxing factors (16). Endothelial cell dysfunction, an early and central event in lesion formation, results in vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, oxidation, thrombosis, impaired coagulation, vascular inflammation, and eventually atherosclerosis. A growing body of evidence implicates CRP as a direct mediator of endothelial dysfunction. First, CRP, at concentrations known to predict vascular events, directly upregulates endothelial cell adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin (8, 9, 19, 24–26). These adhesion molecules play a key role in facilitating the leukocyte-endothelial interaction, an early step in atherosclerosis. Once the leukocytes adhere to the dysfunctional endothelium, CRP promotes the release of MCP-1, a key chemotactant chemokine, which facilitates leukocyte transmigration through the endothelium (8, 19). Third, CRP directly promotes the release of potent endothelium-derived contracting factors, such as endothelin-1 (ET-1), from endothelial cells (19). ET-1 is not only one of the most potent vasoconstrictors currently known, but it also appears to be a mediator of CRP-induced upregulation of adhesion molecules and MCP-1 (19). More important are observations demonstrating the ability of CRP to directly quench the production of nitric oxide (NO) from the endothelium (15, 21). NO is the key endothelium-derived relaxing factor, which plays a pivotal role in the maintenance of vascular tone and reactivity. In addition to being the main determinant of basal vascular smooth muscle (VSM) tone, NO acts to negate the actions of potent endothelium-derived contracting factors such as ANG II and ET-1 and serves to inhibit platelet and leukocyte activation and maintain the VSM in a nonproliferative state. Human recombinant CRP, when incubated with human endothelial cells at concentrations demonstrated to predict vascular events, potently inhibits both basal and stimulated NO release, in part via destabilizing endothelial nitric oxide synthase (eNOS) transcript (21). In addition, CRP inhibits the eNOS protein expression and the downstream effector of NO, cGMP (21). By virtue of inhibiting eNOS expression and NO release, CRP blocks NO-dependent processes, such as angiogenesis (21). Endothelial cell apoptosis is an important contributor in lesion formation, propagation, and eventual rupture. Through inhibiting NO production, CRP facilitates endothelial cell apoptosis, uncovering yet another proatherogenic and proinflammatory phenotype (21). Recent evidence also implicates CRP as a direct promoter of CD14-induced endothelial cell activation (7). In addition to the aforesmen-

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tioned effects of CRP on endothelial cell adhesion molecules and vasoactive hormone release, CRP functions to upregulate the transcription factor NF-κB (22). NF-κB has been implicated as a key mediator of atherosclerosis. The majority of proinflammatory genes expressed in endothelial cells during the initial phase of lesion formation and in response to inflammatory mediators is dependent on NF-κB activation. Recent evidence suggests that CRP directly increases the degradation of IκB-α and subsequently activates the NF-κB pathway in endothelial cells (22). The proatherogenic effects of CRP on endothelial activation are exaggerated in the hyperglycemic milieu (20), suggesting an important mechanistic link between hyperglycemia, endothelial dysfunction, and cardiovascular disease. Last, patients with elevated levels of CRP have been shown to elicit impaired endothelium-dependent vasodilatation, suggesting that CRP may be a useful clinical tool for endothelial vasomotion (3).

Effects of CRP on macrophage LDL uptake. Uptake of LDL by macrophages is an important process contributing to plaque progression. Recent evidence suggests that CRP directly promotes native LDL uptake into macrophages, a process that is ET-1 dependent and inhibited during coincubation with the ET<sub>AB</sub> receptor blocker bosentan (19, 27).

Effects of CRP on fibrinolytic parameters. Endothelial cells are the major source of plasminogen activator inhibitor-1 (PAI-1), and PAI-1 serves to inhibit endogenous fibrinolysis, promoting atherothrombosis and progression of acute coronary syndromes. Recent evidence suggests that incubation of human coronary artery endothelial cells with CRP results in a time- and dose-dependent increase in secreted PAI-1 antigen, PAI-1 activity, intracellular PAI-1 protein, and PAI-1 mRNA (2).

Effects of CRP on VSM cells and angiotensin receptor regulation. ANG II is one of the most important proinflammatory molecules, capable of promoting diverse proatherosclerotic processes at the level of the endothelium and VSM. The angiotensin type 1 receptor (AT<sub>1</sub>-R) is a key atherosclerotic switch facilitating...
ANG II-induced reactive oxygen species (ROS) production, VSM cell migration, proliferation, and vascular remodeling. Given the central importance of AT1-R in the development and clinical course of atherosclerosis, we recently evaluated the effects of CRP on AT1-R and associated pathophysiologic processes (23). CRP potently upregulates AT1-R mRNA and protein and increases the number of AT1-R binding sites in VSM cells (23). This effect is not related to a change in AT1-R mRNA stability, because the half-life of AT1-R transcript was similar after incubation with actinomycin-D. Additionally, in VSM cells in vitro, CRP markedly stimulated cell migration and proliferation, with an effect approaching 75% of that noted with the prototypical stimulant PDGF (23). The effects of CRP on VSM cells appear to be closely related to the expression of AT1-R, because they were inhibited by losartan, an angiotensin receptor blocker. CRP also augmented ANG II-induced VSM cell migration and proliferation, further supporting a functional relationship between CRP and ANG II in mediating VSM cell pathology. In VSM cells, CRP increased basal ROS production and potentiated the effects of ANG II on ROS formation. These effects were also inhibited by losartan, indicating that increased CRP-mediated ROS formation in VSM cells was related, in part, to increased AT1-R expression. Last, in an in vivo model of carotid balloon angioplasty, CRP exposure facilitated AT1-R expression and differentiation of bone-marrow derived endothelial progenitor cells. Postnatal neovascularization is a process that is vital to the compensatory physiological response in chronic ischemia. Myocardial ischemia provides a potent stimulus to angiogenesis and the subsequent development of collateral vasculature that maintains and/or revitalizes cardiac tissue. The mobilization and differentiation of bone-marrow derived endothelial progenitor cells (EPCs) has recently been shown to be important in this process of neovascularization (14). Recently, the number and migratory activity of circulating EPCs has also been shown to inversely correlate with risk factors for coronary artery disease (4). In this vein, recent work suggests that EPCs incubated with human recombinant CRP, at concentrations known to predict adverse vascular outcomes, exhibited decreased survival and increased apoptosis (18). This reduction in EPC cell number was dose dependent, and at a CRP concentration of 20 μg/ml, there was an ~80% reduction in cell number at 7 days. Additionally, EPCs incubated with human recombinant CRP exhibited decreased expression of endothelial cell-specific markers Tie-2 and endothelial cell-specific lectin, indicating an effect of CRP to inhibit EPC differentiation (18). CRP also caused a significant decrease in EPC eNOS mRNA expression after 24 h of incubation. These observations extend the proatherogenic effects of CRP, beyond the endothelium and VSM to the bone marrow and the systemic response in chronic ischemia.

Conclusions. A growing body of evidence implicates CRP as a powerful risk marker for diverse cardiovascular and metabolic diseases. Initially, this association was suggested to be a surrogate one, wherein CRP functioned to highlight increased levels of vascular inflammation and, in this fashion, identify patients at heightened risk of atherothrombosis. This dogma has been recently revisited, with observations from our group and others suggesting that CRP functions as a direct partaker in lesion formation and directly uncovers a proatherosclerotic and proinflammatory phenotype (Fig. 1). Thus CRP may not just be a marker of atherosclerosis and coronary events, but also a mediator of this disease because it contributes to the substrate underlying lesion formation, plaque rupture, and coronary thrombosis. The CRP-atherosclerosis story is nothing short of a self-fulfilling prophecy.

REFERENCES
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