Brief Reviews

C-Reactive Protein: Risk Marker or Mediator in Atherothrombosis?

Ishwarlal Jialal, Sridevi Devaraj, Senthil K. Venugopal

Abstract—Inflammation appears to be pivotal in all phases of atherosclerosis from the fatty streak lesion to acute coronary syndromes. An important downstream marker of inflammation is C-reactive protein (CRP). Numerous studies have shown that CRP levels predict cardiovascular disease in apparently healthy individuals. This has resulted in a position statement recommending cutoff levels of CRP <1.0, 1.0 to 3.0, and >3.0 mg/L equating to low, average, and high risk for subsequent cardiovascular disease. More interestingly, much in vitro data have now emerged in support of a role for CRP in atherogenesis. To date, studies largely in endothelial cells, but also in monocyte-macrophages and vascular smooth muscle cells, support a role for CRP in atherogenesis. The proinflammatory, proatherogenic effects of CRP that have been documented in endothelial cells include the following: decreased nitric oxide and prostacyclin and increased endothelin-1, cell adhesion molecules, monocyte chemoattractant protein-1 and interleukin-8, and increased plasminogen activator inhibitor-1. In monocyte-macrophages, CRP induces tissue factor secretion, increases reactive oxygen species and proinflammatory cytokine release, promotes monocyte chemotaxis and adhesion, and increases oxidized low-density lipoprotein uptake. Also, CRP has been shown in vascular smooth muscle cells to increase inducible nitric oxide production, increase NFκB and mitogen-activated protein kinase activities, and, most importantly, upregulate angiotensin type-1 receptor resulting in increased reactive oxygen species and vascular smooth muscle cell proliferation. Future studies should be directed at delineating the molecular mechanisms for these important in vitro observations. Also, studies should be directed at confirming these findings in animal models and other systems as proof of concept. In conclusion, CRP is a risk marker for cardiovascular disease and, based on future studies, could emerge as a mediator in atherogenesis. *(Hypertension. 2004;44:6-11.)*

Key Words: endothelium ■ macrophages ■ atherosclerosis

Much evidence supports a pivotal role for inflammation in all phases of atherosclerosis from the initiation of the fatty streak to the culmination in acute coronary syndromes (plaque rupture). The earliest event in atherogenesis appears to be endothelial cell (EC) dysfunction. Various noxious insults including hypertension, diabetes, smoking, dyslipidemia, hyperhomocystinemia, etc, can result in EC dysfunction that manifests primarily as deficiency of nitric oxide (NO) and prostacyclin and an increase in endothelin-1 (ET-1), angiotensin II (Ang II), and plasminogen activator inhibitor-1 (PAI-1), among other aberrations. After EC dysfunction, mononuclear cells such as monocytes and T lymphocytes attach to the endothelium initially loosely and thereafter adhere firmly to the endothelium and then diapedese into the subendothelial space. The rolling and tethering of leukocytes on the endothelium is orchestrated by adhesion molecules such as selectins (E-selectin, P-selectin), cell adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]), and integrins. Chemotaxis and entry of monocytes into the subendothelial space is promoted by monocyte chemoattractant protein-1, interleukin-8 (IL-8), and a newly reported chemokine, fractalkine. Thereafter, macrophage colony-stimulating factor promotes the differentiation of monocytes into macrophages. Macrophages incorporate lipids from oxidized low-density lipoprotein via the scavenger receptor pathway (CD36, scavenger receptor-A), becoming foam cells, the hallmark of the early fatty streak lesion. After the fatty streak lesion, smooth muscle cells migrate into the intima, proliferate, and form the fibrous cap. It is currently believed that lipid-laden macrophages, during the process of necrosis and apoptosis, release matrix metalloproteinases, which cause a rent in the endothelium. Because the lipid-laden macrophage is enriched in tissue factor, this is released from the macrophage and comes in contact with the circulating platelets, resulting in thrombus formation and acute coronary syndromes (unstable angina and myocardial infarction). Various knockouts and transgenic experiments have...
underscored the importance of the various cytokines, chemokines, and adhesion molecules in atherogenesis, emphasizing the importance of the inflammatory component.\textsuperscript{1,2}

There are numerous inflammatory markers that have been shown in various studies to predict cardiovascular events. These include cell adhesion molecules, cytokines, chemokines, acute phase reactants such as fibrinogen, serum amyloid A, and C-reactive protein (CRP). In this brief review, we focus on the inflammatory marker, CRP, because the largest amount of published data support a role for CRP as a risk marker for cardiovascular disease (CVD). Furthermore, data are reviewed to suggest that in addition to being a risk marker, CRP may indeed be a participant and culprit in atherogenesis.

C-Reactive Protein

Biochemistry and Biology

CRP is a member of the pentraxin family. It comprises 5 noncovalently associated protomers arranged symmetrically around a central pore and has a molecular weight of 118 000 Da.\textsuperscript{3} It is a nonglycosylated protein in humans and the gene has been mapped to chromosome 1. Twin studies have shown a highly heritable component to baseline levels of CRP. With regard to its production, the general consensus is that the production is predominantly under the control of IL-6. However, IL-1 and tumor necrosis factor may also contribute to hepatic synthesis and secretion of CRP. CRP has a half-life of \textasciitilde19 hours and this appears to be constant in health and disease. Thus, the sole determinant of CRP levels is the synthetic rate. Its ligand-binding site contains 2 ligated calcium ions and it binds phosphocholine and small ribonucleoproteins. To date in phagocytes, it has been shown to bind Fc-γ receptors I and II and its function appears to clear apoptotic and necrotic cells.

Much recent data challenge the dogma that CRP is exclusively produced by the liver. Indeed, cogent data suggest that it is produced in the atherosclerotic lesion (especially by smooth muscle cells and macrophages), the kidney, neutrons, and alveolar macrophages.\textsuperscript{4–9} Also, there is evidence to suggest that the stimulus for the production of CRP might be lipid peroxidation and infection such as cytomegalovirus that triggers a proinflammatory cytokine cascade resulting in CRP release. In this regard, it is interesting that adipose tissue, previously thought to be an inert triglyceride depot, has been shown to produce cytokines such as tumor necrosis factor-α and IL-6, which also could contribute to production of CRP.\textsuperscript{10}

Hs-CRP and Cardiovascular Risk

Numerous studies from various parts of the world have clearly established that CRP predicts future risk for CVD in apparently healthy persons, independent of established risk factors in the majority of studies. In the studies to date, CRP has been shown to predict myocardial infarction, coronary artery disease (CAD), death, stroke, peripheral arterial disease, sudden death, etc.\textsuperscript{11} In the Women’s Health Study, Ridker et al have shown that CRP is additive to low-density lipoprotein (LDL) cholesterol and the Framingham 10-year risk score in predicting future CVD in healthy American women.\textsuperscript{12} Thus, based on these data, the American Heart Association and Centers for Disease Control and Prevention have issued a statement recommending that CRP be used as a risk marker for CVD in individuals with a Framingham risk score between 10\% and 20\%.\textsuperscript{13} In their recommendations, CRP levels \(<1\) mg/L were considered low-risk, \(1\) to 3 mg/L as average risk, and \(>3\) mg/L as high-risk for CVD (Table). With regard to risk assessment, if the value on 2 occasions 1 month apart is in the same category, ie, \(<1\), \(1\) to 3, and 3 to 10 mg/L, this can be taken as reliable evidence with regard to low, average, and high risk for subsequent CVD. However, if the CRP level is \(>10\) mg/L, then CRP cannot be used to assess cardiovascular risk and other active inflammatory processes (eg, trauma, infection, etc) should be excluded. Thus, when using CRP to assess cardiovascular risk in primary prevention, one needs to adopt the high sensitive (hs) CRP assay, and the patient should be free from any kind of acute inflammation such as infection, trauma, etc, for at least 2 weeks.

Conditions that have been associated with increased levels of CRP include adiposity, chronic inflammation, metabolic syndrome, type 2 diabetes, hypertension, and sleep apnea.\textsuperscript{14,15} It is also important to note that in patients with metabolic syndrome who already have an increased risk of CVD,\textsuperscript{14} CRP levels \(>3\) mg/L predict a greater risk for CVD than in patients with metabolic syndrome with CRP levels \(<3\) mg/L.\textsuperscript{16} To date, modalities that have been shown to lower Hs-CRP levels include weight loss in obese individuals and certain medications such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), peroxisome proliferator-activated receptor-α agonists (fibrates), peroxisome proliferator-activated receptor-γ agonists (glitazones), aspirin in patients with CAD, and high doses of RRR-tocopherol.\textsuperscript{14,17} There is no doubt that this list of therapeutic modalities that modulate CRP will increase as new data are generated.

Thus, it is clearly accepted that CRP is a risk marker for CVD. The more important question that arises, because CRP has now been demonstrated in the vessel wall by numerous investigators, is whether it is an innocent bystander or culprit in atherogenesis.

CRP: A Mediator in Atherogenesis?

In this regard, it should be emphasized that several investigators have now clearly demonstrated the presence of CRP mRNA and protein in human atherosclerotic lesion and vascular cells.\textsuperscript{4–9} Data that support the contention that CRP contributes to atherogenesis derive largely from in vitro observations.

CRP and ECs

The initial data from Yeh’s group\textsuperscript{18,19} showed that incubation of human umbilical vein ECs and human coronary artery ECs...
with CRP induces increased expression of ICAM, VCAM, and E-selectin and the chemokine, monocyte chemoattractant protein-1. They also showed that this increase in adhesion molecule and chemokine expression translated into a biological effect, ie, increased adhesion of U937 cells to human umbilical vein ECs.18,19

Also, at least 3 groups have shown that CRP levels correlate inversely with endothelial vasoreactivity.20–22 This prompted our group to examine the effect of CRP on a critical enzyme in ECs, endothelial nitric oxide synthase (eNOS). In human aortic endothelial cells (HAEC), we showed that CRP resulted in significant reduction in mRNA and protein for eNOS.23 Furthermore, we showed that eNOS activity (ie, conversion of L-arginine to L-citrulline) and bioactivity (secretion of cyclic guanosine 5′-monophosphate [cGMP]) was decreased in aortic ECs. This effect of CRP appeared to be via decreasing the stability of eNOS mRNA. These findings were also confirmed by another group in venous endothelium.24 By virtue of inhibiting eNOS expression and NO release, CRP blocks NO-dependent processes such as angiogenesis. Through inhibiting NO production, CRP facilitates EC apoptosis, uncovering yet another proatherogenic and proinflammatory phenotype. Future studies should now focus on the mechanisms for the reduction in eNOS mRNA stability, the effect of CRP on eNOS phosphorylation, and interaction with heat shock protein-90 and caveolin-1.

Another important product of ECs is prostacyclin, a potent vasodilator, inhibitor of platelet aggregation, and inhibitor of smooth muscle cell proliferation. In our studies, we showed that CRP in doses as low as 10 μg/mL resulted in a decrease in the release of the stable metabolite of prostacyclin, prostaglandin F-1α (PGF-1α), in both HAEC and human coronary artery ECs.25 We also showed that CRP stimulated superoxide anion (O2−) release and because it inhibits eNOS, we investigated another mechanism by which CRP could decrease prostacyclin synthase (PGIS) activity. Ullrich’s group previously showed that PGIS has a great susceptibility to nitration.26 Because an increase in inducible nitric oxide synthase (iNOS) could result in increased formation of peroxynitrite, we examined the effects of CRP on iNOS. We showed that CRP increased iNOS activity, resulting in increased nitration of PGIS. This inhibition of PGIS activity was reversed by the peroxynitrite scavengers, urate, and ascorbate. The schema depicting this mechanistic pathway is shown in Figure 1. For proof of concept, studies need to be undertaken in vitro in aortic rings and with CRP administration in animal models such as rats and rabbits to show that CRP induces impairment in vasoreactivity in vivo. In accordance with the Yeh group, we also showed in aortic ECs that CRP augments both ICAM and VCAM expression and monocyte adhesion to endothelium; however, we were not able to confirm an increase in expression of E-selectin in HAEC.23

PAI-1 is a member of the serine protease inhibitors. It appears that PAI-1 is synthesized in the liver, adipose tissue, EC, vascular smooth muscle cells (VSMCs), and macrophages. PAI-1 is clearly a marker of impaired fibrinolysis and atherothrombosis and is increased in CAD patients. Increased PAI-1 gene expression is present in human atherosclerotic arteries and correlates with the degree of atherosclerosis, and PAI-1 deficiency protects against atherosclerotic progression in the mouse carotid artery. Transgenic mice that express a stable form of the human PAI-1 gene develop coronary artery thrombosis.27 We have shown that CRP induces PAI-1 mRNA, antigen, and activity in HAEC.28 These findings suggest that CRP may be an atherothrombotic agent. This was recently confirmed in the CRP transgenic mice by Danenberg et al, who showed that compared with wild-type mice in which CRP levels are undetectable, in the CRP transgenic mice, CRP levels increased to 18.6 mg/L. After injury to the femoral artery, there was complete thrombotic occlusion in the femoral artery in 75% of the human CRP transgenic mice when compared with 17% of the wild-type mice at 28 days (P<0.05).29 Furthermore, arterial photochemical injury to the carotids shortened clot formation time in the human CRP transgenic mice compared with the wild-type. Thus, this in vivo finding strongly supports the notion that CRP may function as a procoagulant, based on its effects documented in vitro, ie, reduction in eNOS, prostacyclin, increased PAI-1, and tissue factor.

CRP has been shown in venous endothelium to promote the release of the potent endothelial-derived contracting factor, ET-1.30 ET-1 not only is a potent vasoconstrictor but also appears to be a mediator of CRP-induced upregulation of adhesion molecules and monocyte chemoattractant protein-1 in venous EC.

An important chemokine is IL-8, which is a powerful trigger of adhesion of monocytes to endothelium. The mouse homolog of IL-8 triggers arrest of monocytes in carotid arteries of ApoE−/− mice. IL-8 is also an angiogenic factor and inhibits tissue inhibitor of metalloproteinase. Knockout of the homolog of IL-8 receptor, CXCR2, decreases intimal accumulation of macrophages and decreases progression of
atherosclerotic lesions. We have recently shown that CRP induces IL-8 expression in HAEC and human coronary artery ECs. This was evidenced by both secreted IL-8 and also intracellular IL-8 by flow cytometry. The increase in IL-8 was caused by increased mRNA for IL-8. Also, transcription of IL-8 was increased. Thus, we examined the effect of CRP on NFκB activity and showed that CRP increases NFκB activity, as evidenced by increase in nuclear p65 and cytosolic IkB kinase. Inhibitors of NFκB including SN50, parthenolide, and Bay-11 reduced intracellular and secreted IL-8 from HAEC. The increased adhesion of monocytes to endothelium in the presence of CRP was reduced by 30% by pre-incubating the cells with IL-8 antibodies, suggesting that other factors such as ICAM and VCAM may be more important with regard to CRP’s augmentation of monocyte EC adhesion. In all these experiments conducted in our laboratory, we were careful in purifying CRP to an endotoxin level that is not sufficient to induce inflammatory molecules, ie, <12.5 pg/mL. We also showed that addition of polymixin B did not abrogate the effects of CRP, and trypsinizing or boiling CRP abolishes its effects on EC.

CRP and Monocyte-Macrophages

The initial data derive from Cermak et al, who showed that CRP induced monocyte tissue factor secretion. In this study, they showed that CRP induced tissue factor antigen and procoagulant activity. However, no studies were undertaken to elucidate the mechanism.

In monocyte macrophages, CRP, after internalization and degradation, has been shown to induce production of hydrogen peroxide at concentrations >10 μg/mL. Ballou et al conducted a study in which they incubated human monocytes with CRP at different doses for 16 hours and were able to demonstrate significantly increased levels of IL-1β, tumor necrosis factor-α, and IL-6 at concentrations of CRP >5 μg/mL. This induction of cytokine release was unaffected by polymixin B but was completely abrogated by boiling of CRP, confirming that this effect of CRP was not caused by lipopolysaccharide contamination. A single report has shown increased CD11b expression on monocytes incubated with CRP, and this resulted in increased adhesion of these monocytes to lipopolysaccharide-activated human umbilical vein ECs. CRP has been shown to activate complements and stimulate human monocyte chemotaxis. There has been a report that CRP promotes uptake of native LDL. However, this has been brought into question by the Witztum group, who showed recently in an elegant study that CRP promotes the uptake of oxidized but not native LDL because of certain unexposed phosphocholine epitopes on oxidized low-density lipoprotein. A more recent article that further supports the role of CRP in later stages of atherosclerosis is by Williams et al, who showed that CRP stimulated matrix metalloproteinase-1 mRNA, protein, and collagenase activity in human monocyte/macrophages. This appeared to be orchestrated via Fc-γ receptor II, and the signal pathway appeared to be via extracellular signal regulating kinase. CRP had no effect on tissue inhibitor of metalloproteinase-1. Thus, it is clear that CRP is proatherogenic in monocyte-macrophages because it increases tissue factor expression, promotes monocyte chemotaxis and adhesion to EC, reactive oxygen species release, and matrix metalloproteinase-1, and promotes oxidized low-density lipoprotein uptake, thus leading to increased foam cell formation. Furthermore, CRP is present in foam cells in the atherosclerotic lesion and activates complement.

Figure 2. Potential atherothrombotic effects of CRP on vascular cells.
smooth muscle cellular pathology. In an in vivo model of carotid balloon angioplasty, CRP exposure facilitated AT1R expression, with resultant increases in neointimal formation, VSMC migration, and proliferation, and promoted collagen and elastin production, which are key matrix proteins in the vessel wall. These effects were attenuated by angiotensin receptor blockade. Therefore, CRP exerts direct proatherosclerotic effects at the level of VSMC. Also, in VSMC, CRP has been previously shown to upregulate iNOS, certain cell signal transduction pathways including mitogen-activated protein kinase pathway and NFκB.32

Conclusions

In summary, CRP is clearly a risk marker for CVD and is recommended for use in primary prevention for this purpose. In addition, CRP appears to also contribute to atherogenesis (Figure 2). However, much further research is needed, especially in appropriate animal models, to confirm that CRP is a mediator of the proinflammatory, prothrombotic phenotype and does contribute to atherothrombosis. In this regard, it is important to note in Wistar rats after coronary ligation that human CRP enhanced infarct size by 40%.43 Because CRP levels can be modulated by weight loss and certain pharmacological interventions including statin therapy, this further underscores the importance of establishing the role of CRP in atherothrombosis. Thus, in addition to being an adjunct to lipid screening in individuals at high risk for CAD, it is clearly a better method to target therapies in the setting of primary prevention. Potential prognostic value in acute coronary syndrome needs to be confirmed in future studies. It is clear that inflammation is now a new target for both prevention and treatment of CVD. Future research efforts should be directed at investigating the effect of CRP on other atherogenic mediators and elucidating the molecular mechanisms of the procoagulant/proatherogenic effects documented to date.

Acknowledgments

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