CRP—Marker or Maker of Cardiovascular Disease?

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C-reactive protein (CRP) has emerged as an interesting novel and potentially clinically useful marker for increased cardiovascular risk,1,2 This is an attractive concept because atherosclerosis is a disease characterized by chronic arterial inflammation,3,4 and suggests the possibility that subclinical states of atherosclerosis can be identified by an increase in circulating markers of inflammation before acute events occur. Based on data obtained primarily from in vitro studies it has also been proposed that CRP in itself is actively contributing to disease progression and that it should be considered as true risk factor and consequently as a target for intervention. However, in the present issue of Arteriosclerosis, Thrombosis, and Vascular Biology, two independent reports demonstrate that transgenic overexpression of CRP does not affect the development of atherosclerosis in mice, suggesting that this is not the case.

The association between moderately elevated CRP levels and an increased risk for development of cardiovascular disease is well established. Although the increase in risk may have been overestimated in initial studies, recent meta-analysis comprising >7000 patients with coronary events shows that subjects with CRP in the upper tertile have a 50% increased risk for development of acute cardiovascular events.2 There are several possible explanations for this association (Figure). (1) The inflammatory process in arterial tissue affected by atherosclerosis results in release of cytokines into the circulation that subsequently activates expression of CRP in the liver. Plasma CRP levels would then reflect the severity of atherosclerosis and, as a consequence, the risk for development of clinical events. If CRP in addition has atherogenic properties in itself, this would result in activation of a vicious negative cycle. The lack of good support for any strong association between atherosclerosis severity as assessed by different imaging techniques and CRP levels argue against this alternative. The poor association between the major risk factor for atherosclerosis, the LDL cholesterol level, and CRP represents another argument against this hypothesis. (2) CRP is a marker for metabolic disturbances associated with an increased risk for cardiovascular disease. It has been well established that several of the metabolic changes that characterize insulin resistance and type 2 diabetes, such as a high body mass index, hypertension, hypertriglyceridemia, and low HDL cholesterol are associated with increased CRP levels.5 The biological explanation for this association remains to be fully elucidated but may involve an increased release of cytokines from adipose tissue. If this alternative is correct, CRP is only a marker of metabolic disturbances increasing the risk for development of cardiovascular disease but has in itself no association with the actual cardiovascular disease process. (3) CRP is actively contributing to the progression of atherosclerosis. Genetic, metabolic, and other factors stimulate the expression of CRP in the liver and possibly also in the arterial wall. CRP will bind to lipoproteins and damaged cells in atherosclerotic plaques, induce complement activation thus promoting inflammation and disease progression.

What sort of protein, then, is CRP, and what are the evidence supporting that it could play an active role in cardiovascular disease? CRP is a 115-kDa pentamer expressed almost exclusively by hepatocytes as part of a nonspecific acute phase response to tissue damage, infection, inflammation, and malignant neoplasia.6 The fact that it has been highly conserved in evolution and that no human known CRP deficiencies exist suggest that it must provide an important survival value. Yet its role in human physiology and disease remains to be fully understood. It binds with high affinity to phospholipids, primarily phosphocholine, on apoptotic cells, damaged cell membranes, oxidized lipoproteins, and related structures on invading microorganisms, a process that in humans leads to activation of complement as well as to opsonization mediating macrophage removal of the ligand. If this occurs in an atherosclerotic plaque it is conceivable that it may result in increased inflammation and disease progression. However, it cannot be excluded that the facilitation of removal of potentially harmful ligands instead has an antiinflammatory effect. It is interesting to note that CRP in this respect shares many properties with the natural autoantibodies against oxidized LDL phospholipids,7 and that both may be part of a highly conserved, fast-acting, but relatively unspecific defense against invading microorganisms and potentially harmful endogenous neoantigens.

It has also been reported that CRP may have proinflammatory effects unrelated to activation of complement that would further enhance its atherogenic properties. These include increasing the endothelial expression of adhesion molecules, cytokines, decay accelerating factor, as well as induction of apoptosis.8 However, recent studies have shown that most of these effects may be attributable to contaminations with azide and endotoxin in the commercial CRP preparations used.9

It is of considerable importance to clarify whether CRP has a functional role in atherosclerosis because it could represent an interesting novel target for intervention. One obvious
Three possible explanations for the association between CRP and cardiovascular disease. (1) Cytokines released from atherosclerotic arteries induce CRP expression in the liver. (2) Metabolic disturbances cause both atherosclerotic plaque development in arteries and CRP from the liver but there is no direct biological association between CRP and the arterial disease. (3) Metabolic disturbances lead to an increased release of CRP from the liver and CRP subsequently promotes plaque development in arteries.

The approach would be to study the effect of CRP gene knockout in mouse models of atherosclerosis. However, the usefulness of this approach is limited by the fact that CRP is expressed only at extremely low levels and does not function as an acute phase response protein in mice. Another alternative is to study the effect of transgenic non-mouse CRP expression. Two such studies are reported in the present issue of the journal. Trion and coworkers (10) studied the effect of expressing human CRP in apolipoprotein E*3 Leiden mice that have elevated plasma cholesterol and triglyceride levels resembling familial dysbetalipoproteinemia in humans. Analysis of atherosclerosis at the aortic root in 40- to 45-week-old animals demonstrated no difference in lesion size or severity between CRP transgenics and controls. There was also no increase in serum amyloid A (SAA) levels in CRP transgenic mice suggesting that CRP in itself is not sufficient to induce an inflammatory response. Reifenberg and coworkers (11) introduced the rabbit CRP gene into apoE-knockout mice. Atherosclerosis was assessed by en face lipid staining of the aorta of 1-year-old animals, and these investigators also found no difference between CRP transgenic and control mice. These 2 studies do not support the hypothesis that CRP has a proatherogenic role in vivo. This conclusion is also supported by a very recent study by Hirschfield et al (12) demonstrating that expression of human CRP in apoE-knockout mice does not influence the development of atherosclerosis despite deposition of both human CRP and mouse complement in the plaques. Taken together these studies strongly argue against any major role for CRP in the development of atherosclerosis in mice. In contrast, Paul et al (13) have previously reported that expression of human CRP in apoE-knockout mice is associated with a 30% to 50% increase in aortic atherosclerosis. The reason responsible for this discrepancy remains to be identified, but one contributing factor could be the extremely high CRP values observed in the studies by Paul et al (13) (~100 mg/L).

Has the time come to remove CRP from the list of suspected villains in atherogenesis? For mice this is probably the case, but for humans it may still be premature. Activation of complement is one and perhaps the most likely mechanism through which CRP may promote the development of atherosclerosis. As shown by Reifenberg et al, (11) it is unclear whether CRP and CRP/LDL complexes have the same capacity to activate the complement cascade in mice as it has in humans. However, keeping these limitations in mind, the present 2 studies clearly do not support a role for CRP in the development of atherosclerosis. CRP may just be a marker after all.

References

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