CRP or not CRP? That Is the Question

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C-reactive protein (CRP) and cardiovascular disease is a very hot topic at present. Excitement and interest have spilled over dramatically from the scientific literature into the media and popular press leading to much speculative comment. However, rigorously controlled and reproducible studies are now laying the basis for a more realistic consensus. The article in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* from Carmen van den Berg’s laboratory makes a notable contribution to this process.

Shortly after its discovery in 1929 as “the acute phase protein,” increased production of CRP was recognized as a characteristic feature of the response to acute myocardial infarction, and until the 1960’s detection of CRP was widely used for routine monitoring of acute rheumatic fever. In 1961 Irving Kushner, in one of the first applications of the then new technique of immunofluorescence, demonstrated the deposition of rabbit CRP in experimental myocardial infarction lesions. The association between CRP and cardiovascular disease thus has a very long history. In 1979 Kushner reported the kinetics of the acute phase CRP response to human acute myocardial infarction, and soon afterward we first investigated critically the behavior of CRP in clinical coronary artery disease and myocardial infarction. We observed that persistently elevated circulating CRP concentrations after an infarct were associated with a poor prognosis, although at that time we were focusing mainly on CRP as a sensitive nonspecific marker of all the various complications of coronary occlusion and their treatment.

The current phase of interest in CRP and cardiovascular disease started in the early 1990’s with observations of increased CRP concentrations in some patients with “active coronary syndromes” and some individuals with acute myocardial infarction tested very soon after onset of pain, before the acute phase response to infarction could have started. These findings were consistent with the growing recognition at that time of the importance of inflammation in atherogenesis and the inflammatory nature of unstable atherosclerotic lesions. At the same time the large European prospective study (ECAT) of coagulation factors as possible prognostic markers in outpatients with stable and unstable angina unexpectedly revealed that the baseline value of CRP, which had only been included as a control for the acute phase behavior of some clotting proteins, significantly predicted future coronary events. The assay used was insufficiently sensitive to detect CRP in many of the samples in the ECAT study, and we therefore developed the first automated high-sensitivity method to re-assay their 3000 samples. Separately we also used this method to study, in collaboration with the Maseri group, carefully characterized patients with severe unstable angina who had not yet experienced any myocardial necrosis. We selected a cut point of 3 mg/L as the upper limit of normal, based on our original 1981 study of 468 healthy volunteer blood donors in whom this was the 90th centile of the CRP distribution. In both the ECAT and the unstable angina studies, increased baseline CRP values were associated with significantly increased risk of future coronary events.

Subsequently several groups showed independently that baseline CRP measurements are associated with future coronary events in general populations, without known preexisting coronary artery disease. In 2000 we reported with Danesh and colleagues the results from the British Regional Heart Study comprising 506 coronary events, >2× as many as in any other such study up to that time, together with a meta-analysis of all previous studies. This showed that the relative risk of having a coronary event among individuals with a baseline CRP value in the upper compared with the lower tertile of the CRP distribution was ≈2.0. In 2004 the Reykjavik Study, comprising 2459 coronary events, showed this relative risk to be 1.45 (95% CI, 1.25 to 168), and meta-analysis of all previously published general populations studies, comprising 7068 patients with coronary events, gave a similar result.12 The results from this now quite rigorously large experience indicate that CRP value is a relatively modest predictor of coronary events. Higher odds ratios have been reported only in studies containing relatively small numbers of actual events despite large numbers of individuals at entry.

These observations in general populations identify an association between low grade inflammation, or at least a metabolic state characterized by low level upregulation of inflammatory markers, and future atherothrombotic events. However, the exquisitely sensitive CRP response is an entirely nonspecific systemic marker of tissue damage, infection, and/or inflammation. The association of very modestly increased baseline CRP values with future cardiovascular disease does not of itself implicate CRP in the pathogenesis of cardiovascular disease. It also does not identify the source of the stimuli triggering the increased CRP production, and does not distinguish between stimuli arising in the arteries and/or elsewhere in the body or reflecting individual varia-
tions in acute phase responsiveness to noncardiovascular events that may be associated with propensity to atherothrombosis. It is also extremely important to note that many other nonspecific systemic markers of inflammation show similar associations with coronary heart disease, including fibrinogen and other acute phase coagulation proteins, serum amyloid A protein (SAA), erythrocyte sedimentation rate and plasma viscosity, albumin as a negative acute phase reactant, and total white blood cell count. The spotlight on CRP largely reflects the fact that it is a sensitive marker with readily available, inexpensive, routine assays standardized on the World Health Organization International Reference Standard for CRP Immunoassay (85/506) that we produced more than 20 years ago.

It remains controversial whether routine measurement of CRP, or other inflammatory markers, adds to classical assessment of the risk of cardiovascular disease in individual patients based on the known causative and modifiable factors, including LDL and HDL cholesterol, smoking, blood pressure, body mass index and adiposity, and exercise. Despite the recent recommendations of the Center for Disease Control and the American Heart Association, critical review of the accumulating evidence now suggests that CRP is a relatively poor predictor that does not contribute usefully in this regard.

In parallel with the growing enthusiasm for CRP as a risk marker, a number of reports have appeared implicating CRP as a proinflammatory and pathogenic factor in atherogenesis and atherothrombosis. The genesis of this idea is also not new. It is more than 30 years since the discovery that aggregated or ligand-complexed human CRP can potently activate the classical complement pathway and thereby generate all the opsonic and proinflammatory effector functions of the complement system. Furthermore, CRP has long been known to bind avidly to many autologous ligands which, under physiological conditions, are likely to exist in vivo, including lysophospholipids, the plasma membranes of damaged cells, small nuclear ribonucleoprotein particles, and apoptotic cells. Finally it is more than 20 years since we first showed that CRP can bind selectively to LDL and VLDL, and speculated that CRP might therefore be involved in atherosclerosis. Subsequently it was shown that CRP binds avidly to modified LDL of the type that accumulates in atherosclerotic plaques and both activates and modulates complement activation by such ligands. In fact, CRP is present in almost all atherosclerotic plaques, often colocalized with activated complement components. However, presence at the scene of a crime is not in itself necessarily compelling evidence of guilt. The party in question, CRP, could be an innocent bystander, a victim, or even have an atheroprotective function. Importantly, evidence from recent Mendelian randomization epidemiological studies does not support the suggestion that CRP plays a major causative role in coronary events.

The recent concept that CRP contributes significantly to pathogenesis of atherosclerosis and atherothrombosis comes from studies in which preparations containing CRP have been found to have proinflammatory and prothrombotic effects in vitro. If these effects are counterparts of phenomena that occur in vivo, they must be compatible with the known in vivo behavior of human CRP. In particular, CRP has a remarkable, rapidly responsive dynamic range of up to 10 000 fold, from 0.05 to 500 mg/L, which would be extraordinary for a potent inflammatory mediator. Although healthy subjects maintain rather steady individually characteristic baseline CRP values over many years for most of the time, everybody mounts acute phase responses of varying intensity after trauma of all types, infections, and other illnesses, and patients with chronic diseases may run markedly elevated CRP concentrations for years. If CRP were an important proinflammatory molecule in vivo, substantial consequences would be expected during the acute phase response that are not seen in clinical practice. Furthermore, injection of even enormous doses of purified human CRP into mice and rats neither elicits inflammation nor produces any clinical ill effects. What then is responsible for the in vitro effects attributed to CRP?

Most of the published studies have used commercially purchased CRP and few report any controls to establish that results obtained are in fact attributable to CRP itself. There is little or no information about the source or the structural or functional integrity of the CRP used nor, remarkably, about other potentially proinflammatory contaminants that may be present. Many authors deal with the question of possible contamination by bacterial lipopolysaccharide (LPS; endotoxin), but the total exclusion of LPS is known to be exceptionally problematic. In the case of human CRP produced in E coli by recombinant technology, which is the most widely used reagent in the studies in question, there must also be concern about the possible presence of other highly potent bacterial products, such as lipopeptides and peptidoglycans. Surprisingly, although all such commercial reagents contain sodium azide as a bacteriostatic preservative, very few reports mention whether this was removed before addition to cell cultures. We have raised these issues before, and reports of experiments addressing them have lately begun to appear, culminating in the rigorous study published by van den Berg and colleagues in this issue. They compared the effects on cells in vitro of commercial bacterial recombinant human CRP, natural human CRP isolated from human effusion fluids, and recombinant human CRP produced in mammalian CHO cells rather than bacteria. They also examined the effects of the bacterial recombinant CRP after dialysis to remove azide and other dialysable small molecules. Only the undialysed bacterial product had proinflammatory effects, all of which were replicated by addition to the cultures of either bacterial LPS or azide. Because neither natural human CRP nor human CRP made recombinantly in mammalian cells had any proinflammatory action, it seems unlikely that CRP itself actually has such properties.

Transgenic expression of human CRP in apoE−/− mice has been reported to exacerbate their spontaneous atherosclerosis and to be associated with upregulation of some cellular activation markers. However, we have not detected any proatherogenic, proinflammatory, or indeed any atheroprotective effect of human CRP in this same model. Mice expressing transgenic human CRP show no systemic sign of inflammation and maintain normal values of their most sensitive autologous acute phase proteins, serum amyloid P
component (SAP) and SAA, throughout their lives. These observations do not support the idea that human CRP is a proinflammatory molecule in healthy individuals. Confirming this, we have lately shown that a single intravenous injection of 4 mg/kg of pure natural human CRP into normal C57BL/6 mice has no effect on their circulating SAP and SAA concentrations, whereas the same dose of commercial bacterial recombinant CRP, even after removal of azide by dialysis, caused a 3-fold increase in plasma SAP and a 40-fold increase in SAA at 24 hours. The recent finding by Bisoendial et al.29 that injection into human subjects of this same commercial bacterial recombinant CRP, even after its partial purification by gel filtration chromatography, induced a similar dramatic acute phase response, is thus not surprising. How confidently can one conclude that this amazing proinflammatory effect was caused by CRP rather than by contaminating bacterial products?

This skepticism does not imply that human CRP may not be a proinflammatory molecule under certain circumstances, and there are longstanding observations to suggest that this may indeed be the case (reviewed in Refs. 30 and 31). More recently we have shown that after induction of ischemic tissue necrosis in the heart or the brain by arterial ligation in the rat, introduction of human CRP specifically exacerbates tissue damage.32,33 Rats have their own CRP but it does not activate rat complement, whereas human CRP activates both rat and human complement and can therefore be proinflammatory in both rat and man.34 The pathogenic effect of human CRP in the rat acute myocardial infarction model is completely abrogated by complement depletion, and is thus apparently mediated by human CRP-dependent complement activation.32 However, the pathogenic effect of human CRP is only seen in rats subjected to ischemic injury. Injection of pure natural human CRP into healthy rats has no adverse effects.24,32

The peak and post infarct CRP concentrations in patients with myocardial infarction and with stroke are significantly predictive of outcome, and all human acute myocardial infarction lesions contain CRP codeloposited with activated complement and CRP-containing complexes that activate complement.35 It is therefore possible that CRP exerts in patients the same adverse effects observed in the rat model and that inhibition of these effects is therefore a valid therapeutic strategy. Work is in progress on drugs to specifically target and inhibit CRP effects,36 and these agents may have useful cardioprotective and cerebroprotective effects in patients experiencing heart attack and stroke. Availability of specific CRP blocking drugs should also eventually enable definitive direct determination of whether or not CRP contributes to the pathogenesis of atherosclerosis and atherothrombosis.

References


