C-Reactive Protein and Coronary Disease
Is There a Causal Link?
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Inflammation has been proposed as a contributor to different stages in the pathogenesis of coronary heart disease (CHD), including the lifelong process of atherogenesis, the acute atherothrombotic event that causes ischemic necrosis in acute myocardial infarction, and the myocardial damage after ischemia. C-reactive protein (CRP), an acute-phase plasma protein synthesized by the liver, is the most extensively studied systemic marker of inflammation. Since the original report of an association between modest increases in baseline circulating CRP and subsequent cardiovascular outcomes in patients with unstable angina at the initial examination, CRP has been the focus of intense investigation. Measurement of CRP has been advocated as a means of improving cardiovascular risk prediction. Because CRP binds to low-density lipoprotein (LDL) and is present in atherosclerotic plaques, there is also considerable interest in whether CRP may play a direct causal role in CHD (and, by implication, could be an important therapeutic target for disease prevention). As discussed below, the evidence relating to this hypothesis has been derived from several different routes of enquiry, including observational epidemiology, human genetic studies, experimental and animal models, and randomized clinical trials of statins. Collectively, several recent findings, such as those in this issue of Circulation from Koike et al on human CRP transgenic rabbits, have reduced the likelihood that CRP itself is a major causal mediator in CHD. In this editorial, we briefly summarize the strengths and limitations of the available data, with an emphasis on the recent major findings.

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In regard to observational epidemiology, the first population-based prospective study of CRP concentration and CHD risk appeared in 1996. By 2009, >50 additional such studies had been reported. The rapid appearance of epidemiological studies on CRP has been facilitated greatly by the relative stability of the protein during long-term frozen storage and the availability of standardized assay methods. Furthermore, despite the spikes in CRP concentration during acute-phase reactions, its year-to-year consistency within individuals is similar to those for total cholesterol and systolic blood pressure. A literature-based meta-analysis of 22 of these prospective studies (that were published before 2004) reported an odds ratio for CHD, adjusted for several conventional risk factors, of 1.6 (95% confidence interval, 1.5 to 1.7) in a comparison of people with baseline CRP levels in the top third with those in the bottom third of the population distribution (corresponding to values of ≈2.4 versus 1 mg/L). This odds ratio is similar in magnitude to those reported for some established causative risk factors (eg, LDL cholesterol or systolic blood pressure), as well as to those for some other nonspecific circulating markers of inflammation (eg, fibrinogen, interleukin-6) and other risk markers (eg, triglycerides). An updated and more detailed review of the prospective observational evidence has involved a collaborative analysis of individual data from >160 000 participants in 54 prospective studies. It has found continuous associations of CRP concentration with a variety of different conditions, each of which are broadly similar in magnitude, including CHD, ischemic stroke, and deaths due to several cancers, chronic lung disease, and even external causes (such as injury). The relevance of CRP to such a variety of conditions is unclear. Other inflammatory markers (eg, fibrinogen, leukocyte count, albumin) have been shown previously to have associations with a range of both vascular and nonvascular conditions. Furthermore, in the recent collaborative analysis the strength of association of CRP concentration with ischemic vascular disease reduced considerably after adjustment for several conventional risk factors and other markers of inflammation (such as fibrinogen). The interpretation of this attenuation is not straightforward. If conventional risk factors and/or fibrinogen mediate the associations between CRP concentration and CHD risk, then correction of the association between CRP and CHD risk for these factors would be an overadjustment that could obscure a causal relationship. Alternatively, if these risk factors do not mediate the association, then the attenuation seen in the CRP-CHD associations after adjustment for these risk factors reduces the likelihood of a causal role for CRP.

Studies that employ CRP-related genotypes as proxies for life-long CRP concentration (ie, "mendelian randomization" analyses) should avoid such difficulties in interpretation, provided that the genotypes are not correlated with other risk factors. Mendelian randomization analyses attempt to minimize confounding and avoid reverse association bias by measurement of common polymorphisms or haplotypes in regulatory regions of the CRP gene that have been reliably associated with differences in circulating CRP concentration.
(but not with any known change in CRP function). According to Mendel’s second law, the inheritance of genetic variants should be subject to the random assortment of maternal and paternal alleles at the time of gamete formation. Therefore, if CRP concentration actually increases the risk of CHD, then carriage of alleles (or haplotypes) that expose individuals to a long-term elevation of CRP should confer an increased risk of CHD outcomes in proportion to the difference in CRP levels attributable to the allele. Because of the randomized allocation of alleles from parents to offspring, potential confounders should be distributed evenly among the genotypic classes, and any bias due to reverse causation should be avoided because genotypes are determined at conception and are not prone to modification by the onset of disease. Previous mendelian randomization analyses have yielded findings consistent with causal roles in CHD for LDL cholesterol and lipoprotein(a), and they have reduced the likelihood of causality for fibrinogen concentration.

Recent genetic analyses, including a meta-analysis of studies involving 28,000 CHD patients and 100,000 controls, have reported essentially null associations between CRP-related genotypes and fibrinogen levels, several conventional risk factors, and CHD risk. These findings reduce the likelihood of a major causal role for CRP in CHD, but there are limitations to the data. First, because the CRP-related genotypes studied have relatively moderate effects on CRP concentration, even larger sample sizes than that in the aforementioned meta-analysis would be needed to exclude a modestly causative effect of CRP concentration on CHD. Second, much of the information in the meta-analysis noted above was based on aggregated (ie, study level) data, including the information used to estimate the strength of the association between CRP concentration and CHD risk. By contrast, access to individual data from a similarly large number of cases and controls, for each of whom CRP-related genotypes and CRP concentration have been recorded, is needed for more robust and detailed analyses. Third, as with all mendelian randomization analyses, there is at least in principle, the scope for residual confounding by unrecognized pleiotropic effects of genotypes and by developmental adaptation (“canalization”), although there is no good evidence that such considerations have had any material impact on the findings described above.

In regard to experimental and animal studies on CRP, the possibility that CRP might have proatherogenic actions was first suggested in 1982 by the discovery of its specific binding to LDL and very-low-density lipoprotein cholesterol and was supported by its detection in atherosclerotic plaque. Even before these observations, it was well established that CRP can activate the classic complement pathway and is therefore potentially proinflammatory. However, compelling evidence for a role of CRP in atherosclerosis has not emerged, despite many reports describing a range of proinflammatory, prothrombotic, vasoactive (and thus potentially proatherogenic and proatherothrombotic) effects of CRP preparations on various cell types in vitro. Almost none of these reports, most of which used commercial preparations of CRP, described any characterization of the integrity or purity of the protein, and few included adequate controls. None of the early studies removed either the toxic sodium azide, present in all commercial preparations as a bacteriostatic, or considered the inevitable presence of bacterial endotoxin (lipopolysaccharide) in CRP produced by recombinant Escherichia coli. Recent studies often claim to have removed endotoxin. Complete removal of abundant lipopolysaccharide is, however, challenging, and, despite apparently “negative” Limulus assays, heavily contaminated commercial CRP preparations are not a rigorous starting point for critical functional studies, especially because almost all of the reported effects attributed to CRP in such preparations are manifested potently by bacterial products. Careful studies with authentic pure CRP isolated from human material and with recombinant CRP produced by mammalian cells, and thus free of bacterial contamination, have not reported wide-ranging direct cellular actions of CRP.

There is now an extensive and controversial literature that extends to in vivo studies involving either injection of CRP in different species or transgenic expression of CRP in mice. Injection of even enormous doses (40 mg/kg) of purified authentic human CRP into mice and rats neither elicited inflammation nor produced any clinical ill effects (including changes in blood pressure). This observation is consistent with the fact that human CRP concentrations can cover a 10,000-fold range (from <5 μg/L to >500 mg/L) in the acute-phase response, and this is unlikely to be compatible with significant effects of CRP on vascular tone, activation of inflammatory cells, triggering of coagulation, or any of the other purported signaling functions lately ascribed to CRP on the basis of in vitro studies with commercial CRP preparations. Although there is 1 report of enhanced atherosclerosis in apolipoprotein E knockout mice expressing transgenic human CRP, other and larger studies show no such effect nor any proinflammatory or prothrombotic action even in aged atherosclerotic animals. Furthermore, in the more humanized model of atherosclerosis in LDL receptor knockout mice expressing apolipoprotein B100, transgenic human CRP was found to be atheroprotective.

In this issue of Circulation, Koike et al extend the range of previous in vivo experimental studies by developing novel transgenic rabbits that express either low or high amounts of human CRP. As noted by the investigators, rabbits and humans share more similar lipoprotein metabolism, cardiovascular pathology, inflammatory responses, and CRP structure and function than do mice and humans. Koike et al compared the development of aortic and coronary atherosclerosis induced by a cholesterol-rich diet in 27 transgenic rabbits (including 13 in the low-CRP group and 14 in the high-CRP group) and 24 nontransgenic rabbits. Despite showing that human CRP in the transgenic rabbits was functional in vivo and that human CRP was present in atherosclerotic lesions, the investigators reported that human CRP did not affect aortic or coronary atherosclerosis lesion formation, regardless of the level of CRP expression. Although these findings further reduce the likelihood that human CRP plays a direct role in the pathogenesis of atherosclerosis, there are limitations to the evidence. First, as acknowledged by the investigators, these experiments do not address other possible mechanisms (eg, thrombosis, plaque...
rupture) by which CRP could mediate CHD. Second, even though rabbits may better approximate humans than do mice, extrapolation to humans from in vivo experimental animal studies of CRP function requires careful qualification. This is because, despite the considerable phylogenetic conservation of CRP structure, there are substantial differences between CRPs of different species with respect to the fine details of ligand recognition, secondary effects of ligand binding including complement activation, normal concentrations, and behavior as acute-phase reactants.37,38

In regard to human intervention studies, randomized trials have shown that statins can lower CRP concentration in healthy participants and in people with stable vascular disease.39 The Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER trial) showed that rosuvastatin reduced the risk of first-ever vascular disease in people who have lower than average LDL cholesterol concentration and higher than average CRP concentration.40 However, because statins potently affect LDL cholesterol concentration (an established causal risk factor), statin trials cannot provide specific causal inferences about CRP. Given the possibility that statins may have relevant anti-inflammatory actions, it has been suggested that the cardioprotective benefits of statins may be proportionally greater in people with higher baseline CRP levels than in those with lower CRP levels.41 The JUPITER trial itself may not be the optimum study in which to test this hypothesis because it was restricted to participants with CRP levels ≥2 mg/L. This proposition could, however, be readily tested by analysis of CRP levels in baseline samples of other existing large statin trials that have recruited patients without regard to baseline CRP levels. Although some statin trials have reported associations between the degree of CRP lowering achieved and risk of cardiovascular disease,42 these analyses may have been liable to bias because they have not been based on the trials’ randomized treatment allocations.

In aggregate, therefore, the available evidence does not support the view that CRP itself is a direct causal mediator in the long-term development of CHD, but the matter is not fully resolved, and further investigation is required. High concentrations of CRP produced during acute-phase reactions (as distinct from modestly increased baseline values) could potentially contribute to acute atherothrombotic events. Increased CRP values at the time of acute myocardial infarction are strongly associated with risk of short-term cardiac complications and death,43 and there is compelling experimental evidence that high CRP concentrations may contribute acutely to the severity and outcome of ischemic lesions.44 Clinical studies with specific CRP inhibitor drugs should be able to determine whether these mechanisms operate and are significant in humans.45 Further research is also required to address other clinically relevant questions about CRP, such as evaluation of the incremental value of CRP measurement beyond established risk factors for cardiovascular risk assessment.6 Finally, irrespective of the relevance of CRP itself to CHD, there is considerable evidence that inflammatory processes in general may contribute to CHD,1,2 and there is a need to identify the specific genetic, biochemical, and environmental determinants responsible for these associations.

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References
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