C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases

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Received 3 May 2003; accepted 9 December 2003

Summary  Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and mediate many of the stages of atheroma development from initial leukocyte recruitment to eventual rupture of the unstable atherosclerotic plaque. C-reactive protein (CRP), an acute phase reactant that reflects different degree of inflammation, has been indicated an independent risk factor in a variety of cardiovascular disease (CVD), especially in unstable coronary syndrome. Our data have showed that increased level of CRP in patients with unstable angina was associated with short-term clinical outcomes, response for conventional therapy, and activation of nuclear factor-kappa B (NF-\textkappa B), but it is not correlated to coronary artery stenosis as well as lipid profile.

Traditionally, CRP has been thought of as a bystander marker of vascular inflammation, without playing a direct role in the CVD. More recently, accumulating evidence suggest that CRP may have direct proinflammatory effects, which is associated with all stages of atherosclerosis. In our recent study, the results demonstrate that monocytes exhibit an enhanced production of interleukin-6 (IL-6) in response to CRP, and this response is significantly inhibited by simvastatin in a dose-dependent manner. This may be of important interest in the connection between CVD and CRP.

Based on those evidence, we hypothesis that CRP is not only an inflammatory marker but also a direct cause of CVD, and treatments that reduce CRP should be benefit for primary and secondary prevention of CVD. Administration of several agents, especially statin has been showed to modify CRP concentrations with a concurrent fall in cardiovascular events. Our clinical investigation suggested that treatment with a single high-dose or a short-term common dose of simvastatin could rapidly reduce CRP level. Those data indicated that the benefit to the vascular endothelium might occur quickly in patients with CVD, which is critical issue for high-risk subgroup. Other interventions, such as lifestyle changes, weight loss, and stop smoking are also warrant attention.

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Introduction

Cardiovascular disease (CVD) is the major cause of death in the developed world. Atherosclerosis, the underlying cause of most CVD, is an inflammatory process that starts early in life and progresses...
slowly and silent for decades. An accumulating
data suggested that such inflammatory process
have been recognized to play a central role in the
pathogenesis of atherosclerosis and its complica-
tions [1]. Inflammatory mechanism plays a central
role in mediating all phases of atherosclerosis,
from initial recruitment of circulating leucocytes
to the arterial wall to eventual rupture of unstable
plaque. Thus attention has focused on whether
circulating plasma levels of markers of vascular
inflammation may help identify those at high risk
for future cardiovascular events [2,3]. These
markers include P-selectin, interleukin-6 (IL-6),
IL-1, tumor necrosis factor-α (TNF-α), soluble
intercellular adhesion molecule-1 (ICAM-1), and
C-reactive protein (CRP). Among of those inflam-
matory markers, produced in the liver in response
to IL-6, CRP has emerged as the most powerful
inflammatory marker of future cardiovascular risk,
and has been the most intensively investigated,
especially in clinical trials [4]. Initially considered
as innocent bystander in the atherosclerotic pro-
cess, recent evidence suggested that CRP may have
direct proinflammatory effects, and mediated in
the initiation, and progression of the atheroscle-
rotic lesion, as well as contributing decisively to
the ultimate development of acute ischemic syn-
dromes. CRP may help predict short- and long-term
cardiovascular outcomes, and the additional CRP
screening to traditional lipid testing has the po-
tential to identify individuals at high risk for future
cardiocascular events who may benefit from target
preventive interventions [5,6].

Characteristics of C-reactive protein

C-reactive protein was first detected in 1930 by
Tillet and Frances, who identified a substance in
the sera of patients acutely infected with pneu-
ococcal pneumonia that formed a precipitate
when combined with polysaccharide C of Strepto-
coccus pneumonia [7]. Subsequently, it was found
that this reaction was not unique to pneumococcal
pneumonia but could be found with variety of other
cutaneous infections. This was early evidence of the
body's chemical response to inflammatory states
and led to the characterization of other so-called
"acute phase proteins" [8]. CRP is synthesized by
hepatocytes with an overall molecular weight of
approximately 11,800 Da [9]. In response to infec-
tion or tissue inflammation, CRP production is
stimulated by several cytokines, particularly, IL-6,
IL-1, and TNF-α. Like many acute phase protein,
CRP is normally present in trace levels in serum but
increases rapidly and dramatically in response to a
variety of infectious or inflammatory conditions
[10].

C-reactive protein has a normal range of less
than 2 mg/L in healthy individuals; with illness such
as rheumatoid arthritis or sepsis, this level in-
creases in the first 6–8 h and can reach peak levels
approaching 300 mg/L after approximately 48 h
[11]. Upon resolution of inflammation or tissue
destruction, CRP level rapidly decline with an
elimination half-life estimated at 4–9 h [12]. It is
found that mean CRP level in serum was increasing
slightly with age [8]. There is no difference in mean
concentrations between men and women although
higher levels are found late in pregnancy [12].

Several studies have shown that CRP concen-
trations are actually relatively constant in an in-
dividual, both with regards to time of day, and over
days to months, even over months to years [5]. A
report indicate that CRP level in healthy subjects
over 24 h measured hourly was no significant indi-
vidual diurnal variation, which makes it possible
to measure CRP at any time of the day [13]. Ock-
eke et al. [14] found that 63% of CRP values in
healthy individuals were in the same quartile as
when re-measured at three months. The relatively
stable level of CRP is probably due to a half-life
that is determined only by synthesis and not by
catabolism.

The exact function of CRP is largely unknown.
But two functional properties of CRP including the
ability to activate complement through the classic
complement pathway, and the ability to modulate
the function of phagocytic cells have been dem-
onstrated. In addition, the principle source of CRP
production is the liver. However, recent data show
that arterial tissue can produce CRP as well as
complement proteins [3].

Measurement of C-reactive protein

When CRP is used primarily for measuring states
of extremely active inflammation, such as sepsis or
arthritis, values of 50–100 mg/L are most relevant.
Until the late 1970s, CRP was measured using
qualitative or semi-quantitative laboratory tech-
nique, most commonly latex agglutination, which
precluded its use as differential diagnostic test
because any degree of inflammation produced po-
sitive results [15]. Presently, accurate and rapid
quantitative measures of CRP are obtained using
laser nephelometry, rate immunonephelometry or
turbidimetry, and enzyme immunoassay [9]. Early
research used an ELISA based assay, which was
shown to correlate well with a commercially available lax method in the physicians’ Health Study (PHS) data set. The development of high sensitivity methods with lower detection limits of 0.2 mg/L allowed differentiation of low-level states of inflammation that are important in coronary heart disease risk. Accurate results can be obtained in 30 min with an analytic sensitivity of 0.04 mg/L [16].

**CRP: evidence as a marker of cardiovascular disease**

The relevance of elevated levels of inflammatory marker in CVD is gaining increasing recognition. Of all the plasma markers of vascular inflammation, CRP has been the most intensively investigated in clinical studies. Levels of CRP have been found to predict future risk among patients with stable and unstable angina, in the chronic phase of myocardial infarction, and among patients undergoing revascularization procedures [3—7].

Several studies have demonstrated that CRP, measured at either presentation or discharge, may have prognostic value in patients with acute coronary syndromes. One of earliest studies from 1982 showed peak CRP concentrations correlated with CK-MB ($r = 0.441$, $p < 0.001$) and those with a complicated myocardial infarction course had a prolonged increase in CRP [17]. Similar conclusion from a small interventional trial more firmly established the relationship between CRP and myocardial infarction. During their first myocardial infarction, patients who were treated with thrombolytic therapy had CRP concentrations 4 h after the onset of pain that correlated with infarct size, as assessed by CK-MB and radioisotope scan. This data from the patients with acute myocardial infarction showed that patients successfully treated with streptokinase had a rise in CRP of only 20% of the levels seen in those not treated, when matched for infarct size [18].

In addition, some reports have also examined the risk stratification of patients by CRP alone or in combination with cardiac troponins. Liuzzo et al. [19] showed that in 31 patients with severe unstable angina and no evidence of myocardial necrosis, as demonstrated by the absence increased cardiac troponin T, CRP concentration more than 3 mg/L at admission were associated with an increased incidence of recurrent angina, coronary revascularization, myocardial infarction, and cardiovascular death. Our recent study has showed that increased level of CRP in patients with unstable angina was associated with worse short-term clinical outcomes, relatively bad response to conventional therapy [20], and activation of nuclear factor-kappa B (NF-κB) [21]. Data from the Thrombolysis in Myocardial Infarction 11A (TIMI 11A), a study of unstable angina and non-Q-wave myocardial infarction, showed that significantly increased CRP levels at presentation in 437 patients was a good predictor of 14-day mortality in that population [22]. A recent report by de Winter et al. [23] demonstrated that CRP concentrations more than 5 mg/L at admission in 150 patients with non-ST-elevation acute coronary syndromes were associated with an increased incidence of major cardiac events within six months, regardless of cardiac troponin I values. However, patients with coronary vasospasm have persistently normal CRP levels, despite frequent episodes of ST-segment elevation [24], suggesting that the rise in CRP may be secondary to an underlying proinflammatory state, rather than due to myocardial necrosis.

Furthermore, baseline levels of CRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral vascular disease, and vascular death among healthy individuals including man and women without known vascular disease by several investigators [25,26]. Data in support of a role for CRP for cardiovascular risk prediction among apparently healthy individuals are robust and remarkably consistent across several European and US cohort. To date, 10 prospective studies, six in the US and four in Europe, have consistently shown that CRP is a powerful predictor of future first coronary event in apparently healthy men and women. Findings from Multiple Risk Factors Interventional Trail (MRFIT) demonstrated a direct positive association between CRP and coronary heart disease mortality in men followed over a 17-year period (RR = 2.8; 95% CI, 1.4—5.4) [27]. Similar positive association between CRP and future coronary events in apparently healthy men was also demonstrated by PHS data set [28]. A recent analysis indicated that CRP was the single most powerful predictor of cardiovascular risk in all of the inflammatory and lipid markers. In this multivariate analysis, matched for age and smoking and adjusted for other cardiovascular risk factors, found that only CRP and total cholesterol/high-density cholesterol ratio were independent predictors of future cardiovascular risk. Furthermore, available studies suggest that the concentration of CRP testing with traditional lipid screening may significantly improve cardiovascular risk prediction, particular when low-density lipoprotein cholesterol (LDL-C)
What meaning CRP as a risk marker is involved in CVD is still hard to elucidate. Several explanations have posited by some investigators. Firstly, CRP may reflect inflammation of arterial walls by pathogenic agents such as cytomegalovirus, Chlamydia pneumoniae, or Helicobacter pylori infection, which was supported by measurable antibodies in serum as well as detectable those organisms in atherosclerotic lesions. In addition, although it is controversial whether increased CRP level was associated with coronary artery stenosis evaluated by angiography, CRP may reflect inflammation related to the extent and severity of atherosclerosis; Concerning myocardial injury, CRP may reflect inflammation related to the extent of myocardial ischemia or to the extent of myocardial necrosis; Finally, CRP may reflect the amount and activity of circulating proinflammatory cytokines, for example, TNF-α, IL-1, and IL-6.

However, we hypothesize that CRP is not only an inflammatory marker but also a direct cause of CVD, and treatment that reduce CRP should be benefit for primary and secondary prevention of CVD.

**CRP: evidence as a cause of cardiovascular disease**

Few effects have been undertaken to elucidate the role of CRP in atherosclerotic lesion formation. Until 1985, CRP was extracted from and quantified in human aortic atherosclerotic intima, thus providing the first evidence for its presence in atherosclerotic lesions. However, in an attempt by Rowe et al. [29] to localize CRP in atherosclerotic lesions, no CRP could be detected. In contrast, Reynold and Vance [30] and Hatanaka et al. were able to demonstrate CRP in atherosclerotic lesions of human aortas, and both reported that CRP was localized around foam cells and the deep fibroelastic layer and in the fibromuscular layer adjacent to the media. However, in the latter study, only one fatty streak lesion and only atheromatous plaque lesion were examined, and thus, the data cannot be regarded as sufficiently conclusive. CRP, therefore, has been traditionally thought of as a bystander marker of vascular inflammation, without playing a direct role in the CVD.

Over the last several years, increasing evidence suggests CRP may contribute directly to the proinflammatory state, indicating that CRP may play a direct role in vascular injury process. Several reports demonstrated that CRP has been localized directly within atheromatous plaque where it precedes and mediates monocyte recruitment. CRP is an activator of complement, and it has been shown to colocalize with the membrane attack complex in early atherosclerotic lesions [31]. In recent study, the localization of CRP and the terminal membrane attack complex, C5b-9, was investigated by immunohistochemistry in 15 early atherosclerotic lesions of human coronary arteries collected from autopsies [32]. In this study, CRP was found to be widely distributed in early human atherosclerotic lesions, with two predominant manifestations. First, the majority of foam cells below the endothelium showed positive staining for CRP. This staining was clearly cell associated, mainly along the cell surface. Second, CRP was deposited diffusely rather than focally in the deep fibroelastic layer and the fibromuscular layer of the intima adjacent to the media. No C5b-9 deposition was seen in close apposition to foam cells. In contrast, serial sections and double immunohistochemistry with antibodies to CRP and C5b-9 showed, at sites of early atherosclerotic lesions, frequent colocalization of both antigens in the fibromuscular layer of the intima, which contains predominantly smooth muscle cells. Thus, CRP, C5b-9, and smooth muscle cells can be found in close apposition to each other in the deep intima of the early coronary lesions.

Moreover, CRP can stimulate tissue factor production and activate complement when aggregated. Tissue factor may be the main stimulus for initiating coagulation, which may account for its important role in CHD [33]. Similar conclusions were reported that CRP stimulates monocyte release of inflammatory cytokines such as IL-1β, IL-6, and TNF-α and may also directly act as a proinflammatory stimulus to phagocytic cells by binding to the FcγRII receptor [34]. In vitro studies have shown that aggregated CRP binds to low and very low density lipoprotein, which in turn activates complement, stimulates tissues factor production by macrophages, and thus starts coagulation [5]. In our recent study, the data showed that monocytes from healthy subjects exhibit an enhanced production of IL-6 in response to CRP [35]. Preliminary time-course studies have shown that IL-6 production increases very rapidly, 4 h stimulated by CRP and therefore continues to rise at a slower rate, reaching a peak at 24 h. This may provide an important information in the connection between CVD and CRP.

In addition, it has been recently demonstrated that CRP causes expression of ICAM-1 and vascular adhesion molecule-1 (VCAM-1) by endothelial cells and mediate monocyte chemotactic protein-1 (MCP-1) induction in endothelial cells [36,37]. Re-
cent data suggest CRP in the presence of serum mediates the uptake of LDL into the macrophages, which then become foam cells. CRP opsonization of low-density lipoprotein (LDL) also mediate LDL uptake by macrophages [38]. Another explanation for the association between CRP and CHD is as a marker that increases in inflammatory states associated with CHD, for example, cytomegalovirus, Chlamydia pneumoniae, or Helicobacter pylori infection. Several studies have shown that markers of an individual disease do not correlate well with CRP concentration. A new theory sees CRP as a culprit in atherogenesis; perhaps it is pro-coagulant, it increases opsonization [39]. In another concept, CRP is a marker of vascular inflammation that it is released from atherosclerotic sites, a theory supported by the finding of decreased forearm vascular responsiveness in patients with increased CRP, and the inflammatory history of unstable coronary plaques.

In brief, CRP is an important cardiovascular risk factor and deposits in the arterial wall during atherogenesis, colocalizing with the terminal complement complex and foam cells. CRP upregulates the expression of adhesion molecules, and mediates proinflammatory factor induction in several kinds of cells in artery wall as well as circulating monocytes [40]. It increases opsonization of LDL and mediates the uptake of LDL into the macrophages, which then become foam cells. In our recent study, the results demonstrate that monocytes exhibit an enhanced production of interleukin-6 (IL-6) in response to CRP, and this response is significantly inhibited by simvastatin in a dose-dependent manner. This may be of important interest in the connection between CVD and CRP [35]. Those data suggest that CRP may indeed a direct proinflammatory factor involving in the initiation, evolution and progression of atherosclerosis.

Clinical implications and interventions for CRP

The findings described above raise the question of whether the CVD risk associated with increased CRP is modifiable. Although no specific therapies have been developed to decrease CRP and there is no direct evidence that risk of future cardiovascular events is diminished by reducing CRP, studies have been showed that aspirin and statin are effective in decreasing the incidence of future coronary events in those with increased CRP concentration. These studies suggest that the two examined drugs possess anti-inflammatory characteristics. Recently, several other drugs administration have also shown the decreased effects on serum CRP level including angiotensin II converting enzyme inhibitor (ACEI), angiotensin II receptor blocker (ARB) [41], β-blocker [42] as well as antiplatelet drugs, for example ezetimibe [43] and clopidogrel [44] in small sample size clinical trials. The other interventions, such as lifestyle changes, weight loss, stop smoking have also been demonstrated a benefit effects on those inflammatory markers, including CRP [45,46].

Aspirin has been known to decrease risk of future cardiovascular events due to anti-platelet. Among apparently healthy men in the PHS with in CRP (>2.1 mg/L), aspirin use (325 mg of aspirin every other day) decreased the risk of future myocardial infarction by almost 60% [26]. In contrast, aspirin use was associated with a much smaller, although statistically significant, 14% decrease in future myocardial infarction among men with low CRP (<0.55 mg/L). A randomized double blind crossover trial found patients with stable angina and normal cardiac function who took 300 mg aspirin daily for 3 weeks had lower CRP level by 27% [47]. Although the magnitude of reduction in future risk of myocardial infarction dependent on the concentration of CRP, it is important to note that all subjects benefited from aspirin use. These findings suggest that aspirin was acting not only as an antiplatelet agent but also as an anti-inflammatory drug. In addition, it is possible that the small dose of aspirin, for example, 100 mg per day, may be as effective in lowering CRP, and in decreasing risk of cardiovascular event, this question is warrant attention.

It is demonstrated that statin can decrease the risk of myocardial infarction. Similar findings like aspirin were also noted with pravastatin use in the CARE study, which is a prospective study of men and women with average lipid concentrations who have suffered a myocardial infarction [48]. Participants were randomized between 40 mg of pravastatin per day and placebo and followed for 5 years. Study participants with high CRP (>9.9 mg/L or 90th percentile) at baseline experienced a reduction of 54% in the incidence of recurrent coronary events compared with a reduction of 25% in those with low CRP (<9.9 mg/L or 90th percentile), although baseline lipid values were almost identical in the two groups. Moreover, during the 5-year follow-up, pravastatin lowered mean CRP by almost 40%. This represented a 22% difference at 5 years in median CRP between the pravastatin and placebo groups. Furthermore, the magnitude of changes in CRP appeared to be unrelated to that of...
LDL-C in both the pravastatin and placebo groups. In the Air Force-Texas Coronary Atherosclerotic Prevention Study (AFCAPS/TexCAPS), treatment with lovastatin 20–40 mg per day resulted in a 37% reduction in the incidence of first acute major coronary events [49]. Patients who subsequently developed a major coronary event had median baseline CRP levels higher than those who did not and treatment with lovastatin significantly reduce CRP by 14.8% in those who remained free of coronary events during the follow-up period.

Simvastatin and atorvastatin were as effective as pravastatin and lovastatin in lowering CRP in a small crossover trial, and once again, the degree of decrease was not related to the reduction in LDL-C. Our recent data showed that both doses of simvastatin (20 and 40 mg per day) induced significant reductions in median CRP levels and in mean CRP levels following a 14-day period treatment (22.3% and 23.1%) without a dose-dependent manner, regardless of lipid profile status [50], and that single high-dose of simvastatin could significantly reduce CRP levels in patients with unstable angina within 48 h [51]. This is of an important interest, especially in acute coronary syndromes, because it may signal early onset of vascular endothelial benefit after short-term simvastatin therapy. Cerivastatin was also effective in lowering median CRP by ~13%. The change in CRP was not dose dependent similar as our study, was seen in just 8 weeks of treatment [52]. Briefly, Those findings suggest that pravastatin may have anti-inflammatory characteristics that are independent from its lipid-lowering property. Clinical trials are currently ongoing to future explore the interaction between pravastatin, aspirin, and the inflammatory response in primary and secondary prevention settings.

Elevated CRP levels have been cross-sectionally associated with proxy indicators of elevated body fitness (Body weight and body mass index [BMI]) and with CVD risk factor and insulin resistance. Recent study indicates that weight loss reduces CRP levels in obese postmenopausal women [53]. The physiological rational underlying this mechanism is that obesity has been positively associated with plasma CRP, and adipose tissue has been proposed as a factor directly modulating CRP levels. The contribution of adipose tissue in IL-6 secretion has been proposed to be the link between plasma CRP and adiposity.

In addition, effect of Hormone replacement therapy (HRT) on cardiovascular risk has been controversial for a long period. The recent heart and Estrogen/Progestin Replacement Study (HERS) results indicate that hormone replacement may actually increase risk for cardiovascular events. The underlying causes for this adverse effect is largely unclear. However, two large trials have demonstrated that increased CRP effect of HRT may help to explain the mechanism for the negative effect of HRT on cardiovascular risk [54,55].

Conclusions

Inflammatory processes play a pivotal role in a variety of clinical settings of atherogenesis [56]. Emerging evidence suggests that plasma markers of chronic low-grade vascular wall inflammation may help predict individuals at risk for plaque rupture. Among of those markers, CRP has been the most intensively investigated, especially in clinical trials. CRP, a hepatic acute phase reactant produced in response to IL-6, appears to be the strongest predictor of future cardiovascular risk. Accumulating studies have been showed that CRP may be a direct cause of CVD, and treatments that reduce CRP should be benefit for primary and secondary prevention of CVD. Several studies demonstrated that aspirin and statin are effective in decreasing the incidence of future coronary events in those with increased CRP concentration. However, large-scale randomized trials are needed to directly test this effect. The attentions for other interventions, such as lifestyle changes, weight loss, and stop smoking have also paid by investigators.

Acknowledgements

The study was supported in part by grant from Hubei Education Bureau (1999B010) and Scientific Research Foundation for the Returned Overseas Chinese Scholars from State Education Ministry, People’s Republic of China (1998679) to Dr Li.

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