Based on a multitude of clinical studies, C-reactive protein (CRP) has emerged as a risk marker for the development of cardiovascular disease, leading to recently published recommendations for screening the general population for plasma CRP level as a predictor for future cardiovascular events. However, uncertainties exist in how to apply these recommendations to populations with very high serum CRP levels and a high prevalence of cardiovascular disease, such as patients with end-stage renal disease. Furthermore, in vitro results are conflicting concerning the role of CRP in the vessel wall. Although many data are in favor of a proinflammatory effect of CRP, evidence is accumulating that CRP also exerts anti-inflammatory actions, mainly in neutrophils and platelets. Many of the apparently contradictory actions of CRP may be attributed to method issues, but, of interest, also may be explained by the existence of 2 distinct conformations of CRP, the native pentamer (nCRP) and modified CRP (mCRP) forms. nCRP is the classical acute-phase reactant detected in serum, whereas mCRP represents a predominantly tissue-bound form. It is detected immunohistochemically, mainly in and around endothelial and vascular smooth muscle cells. Although mCRP activates endothelial cells and neutrophils, induces neutrophil adhesion to the endothelium, and delays apoptosis of neutrophils in vitro, these effects were absent using nCRP. Clearly defined CRP conformers thus may provide a tool for how to reconcile the reported proinflammatory and anti-inflammatory properties of CRP. There is good evidence to believe that CRP is more than just a “bad guy,” and further experiments are needed to determine how these 2 configurations contribute to atherogenesis, development of cardiovascular disease, and acute coronary events.


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INDEX WORDS: Native C-reactive protein; modified C-reactive protein; atherosclerosis; inflammation; cardiovascular disease.

CONFORMATIONS AND BIOLOGICAL ROLE(S)

OF C-REACTIVE PROTEIN

C-reactive protein (CRP) is a normal plasma protein that belongs to the evolutionary ancient and highly conserved pentraxin family. Its plasma level increases 100- to 1,000-fold within 24 to 72 hours in a cytokine-mediated response to most forms of tissue injury, infection, and inflammation. CRP occurs in at least 2 different conformationally distinct forms, native CRP (nCRP) and modified CRP (mCRP).

Native CRP

nCRP is a cyclic disc composed of 5 identical nonglycosylated subunits. It is a highly soluble serum protein that shows calcium-dependent affinity for phosphate monoesters, in particular, phosphocholine. Other intrinsic ligands include native and modified plasma lipoproteins, damaged cell membranes, small ribonucleoprotein particles, apoptotic cells, and fibronectin. Data suggest that binding sites for fibronectin and phosphocholine are distinct. Among extrinsic ligands are components of bacteria, fungi, and parasites, as well as plant products. When bound to these ligands, CRP is recognized by C1q, leading to activation of the classical complement pathway. In addition, bound CRP may bind factor H and thereby regulates alternative-pathway amplification and C5 convertases. In serum, CRP levels increase rapidly after a single stimulus. The half-life of CRP is approximately 19 hours and appears to be similar under physiological and pathological conditions. The human CRP
gene is localized to chromosome 1 and encodes the subunit. The CRP gene in hepatocytes is predominantly under transcriptional control by the cytokine interleukin 6 (IL-6) and, to a lesser degree, IL-1β and tumor necrosis factor α. Native CRP is used in daily clinical practice and represents the classical acute-phase reactant. Median CRP level in middle-aged Americans is approximately 1.5 mg/L.

Modified CRP

nCRP can undergo subunit dissociation into individual monomeric units, eg, when associating with a cell membrane. Such conformational rearrangement significantly modifies CRP structure, solubility, and antigenicity. This conformationally distinct form of CRP is referred to as “modified, monomeric,” or mCRP. Like nCRP, mCRP also is a naturally occurring stable protein, although not detectable in serum. mCRP is characterized by decreased solubility and a tendency to self-aggregate, thus representing the tissue-bound form of CRP. mCRP epitopes can be expressed from nCRP by treatment with urea chelation, acid, or heat or by direct immobilization onto polystyrene plates. Antigens that cross-react with an anti-mCRP antibody have been described in human monocyte/macrophages, epithelial cells of the respiratory tract, and fibrous tissues of normal blood vessel intima. Whether mCRP is produced locally or represents dissociated serum nCRP needs to be clarified.

NATIVE CRP

Immunohistochemistry

In most published studies, commercial CRP preparations or anti-CRP antibodies have been used with no further distinction between the 2 CRP isoforms. Despite this limitation, the effects observed generally have been attributed to the nCRP form; therefore, these are summarized in this section. Immunohistochemical studies provided the first evidence that CRP might not only be a marker in vascular disease, but also is involved causally in the inflammatory process of atherogenesis. Thus, CRP was shown to be deposited together with oxidized or enzymatically modified low-density lipoprotein (LDL), as well as the terminal complement complex CSb-9, within the arterial wall. Other groups reported frequent colocalization of CRP and classical complement components to smooth muscle–like cells and macrophages in both normal arterial and plaque tissue.

However, it should be noted that most CRP polyclonal antisera used for immunohistochimical staining recognize both nCRP and mCRP antigens. In addition, the monoclonal anti-CRP antibody clone 8, widely used for CRP detection in the vessel wall, clearly was shown to predominantly detect the mCRP protein, and not the nCRP protein. Hence, immunohistologic studies evaluating tissue-associated CRP might have reported more accurately on the expression of tissue-associated mCRP, rather than nCRP.

In Vitro Experiments

The effects of CRP on monocyte/macrophages and endothelial and smooth muscle cells in vitro mostly have been interpreted as proinflammatory and atherothrombotic. The potential mechanisms of action on various pathways, reviewed recently, are shown in Fig 1. One important aspect of endothelial injury and early development of atherosclerosis involving CRP may be linked to the enhanced generation of oxygen radicals. Thus, CRP expression in coronary arteries frequently was colocalized with p22phox, an essential component of nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase, an important source of reactive oxygen species in the vasculature. In addition, CRP upregulated angiotensin 1 receptor messenger RNA and protein expression and increased angiotensin 1 receptor number on vascular smooth muscle cells, through which NAD(P)H oxidase may be stimulated. Most recently, it was reported that CRP inhibited endothelium-dependent nitric oxide (NO)–mediated dilation by activating p38 mitogen-activated protein kinase (MAPK) and NAD(P)H oxidase.

However, CRP also elicits effects that can be interpreted as vasculoprotective. These include endothelium–independent vasorelaxation and hypo-reactivity to phenylephrine mediated by increased NO production in human vessels in vitro, upregulation of complement-inhibitory proteins in endothelial cells, and protection against the assembly of the terminal complement attack complex, thus protecting from complement-mediated cell
injury. The dual effects of CRP on both the cellular and humoral (complement) innate immune response may be explained in part by method shortcomings. First, many groups did not control for contaminants in commercial CRP preparations, such as lipopolysaccharide or sodium azide, usually added as a bacterial preservative. Recent studies reported that proinflammatory, proapoptotic, antiproliferative, antimigratory, and antiangiogenic effects of commercial CRP preparations can be attributed to the presence of sodium azide, lipopolysaccharide, or both. Also, the previously described CRP-induced in vitro vasorelaxation now is thought to be an artifact caused by the presence of sodium azide. Second, prolonged storage of purified CRP in the absence of calcium or the presence of chelating agents will result in spontaneous conversion of nCRP to mCRP. Several studies showed that nCRP and mCRP elicited different bioactivities in vitro. nCRP devoid of lipopolysaccharide, sodium azide, or mCRP contamination effectively attenuated the inflammatory response by inhibiting neutrophil activation, adherence, and trafficking. nCRP also inhibited aggregation of human platelets to a variety of stimuli. Conversely, heat-aggregated or minimally oxidized nCRP promoted platelet aggregation. Culture of human coronary artery endothelial cells with nCRP for a short period did not enhance cytokine release or adhesion molecule expression. Conversely, a recent work by Devaraj et al showed that nCRP upregulated IL-8 and plasminogen activator inhibitor 1 production in human aortic endothelial cells, whereas it decreased endothelial NO synthase and prostacyclin release.

Animal Models
Exposure of experimental animals to human nCRP resulted in both protective and deleterious effects. For example, mice expressing the human CRP gene (CRPtg) were resistant to endotoxemia and fatal infection with Streptococcus pneumoniae or Salmonella enterica. Expression of the human CRP gene was accompanied by a significant reduction in bacteremia compared with infected wild-type animals. Conversely, parenteral injection of human CRP in rats enhanced tissue damage in myocardial infarction through a complement-dependent mecha-
nism. Male, but not female, CRPtg mice backcrossed to apolipoprotein E (ApoE)−/− mice showed accelerated aortic atherosclerosis, although the overall effect on the extent of atherosclerosis was relatively modest. It is possible that additional inflammatory stimuli might have accounted for the CRP effects. In these latter 2 studies, human CRP was detected in tissue by means of immunohistochemistry. However, because the primary structure of mouse or rat CRP is 70% homologous to human CRP, significant cross-reactivity between species cannot be excluded in these models. Several recent studies addressed the impact of CRP on atherogenesis by overexpressing human or rabbit CRP in ApoE−/− mice. None of these studies found evidence that CRP was proatherogenic. It was suggested that CRP may promote thrombotic events or revascularization after ischemia because human CRPtg mice showed an increased thrombotic response after vascular injury. However, one should be cautious extrapolating these observations to human disease because transgenically expressed human (or rabbit) CRP does not activate murine complement, a crucial component in plaque development.

**MODIFIED CRP**

**Immunohistochemistry**

mCRP is a natural constituent of normal blood vessel intima with probably specific regulatory functions in vessel homeostasis. Recently, we described mCRP expression in endothelial cells, cytoplasm of smooth muscle cells, and extracellular matrix in vicinity to plaque lesions in ApoE−/− mice. The interaction between the matrix component fibronectin and CRP is controlled by pH. Under physiological conditions, binding of Ca²⁺ to CRP prevented interaction with fibronectin at the extracellular matrix. However, at sites with low pH, such as in atherosclerotic plaques or tumors, Ca²⁺-bound CRP gained the capacity to interact with fibronectin. Whether these different binding capacities involve conformational CRP changes needs to be investigated. In plaques of ApoE−/− mice, we detected frequent colocalization of mCRP with macrophages and apo B, suggesting a role for mCRP in foam cell development. This is in line with the finding that binding of CRP to native LDL, oxidized LDL, or enzymatically modified LDL occurred primarily when CRP underwent conformational changes, yielding mCRP (Wu Y and Potempa LA, unpublished observations).

A growing body of evidence indicates extrahepatic synthesis of CRP. CRP messenger RNA and/or protein were detected in monocyte-depleted peripheral-blood lymphocytes, peripheral-blood mononuclear cells, rat Kupffer cells, macrophages, islet cells of the pancreas, neurons, adipose tissue, endothelial and smooth muscle cells, kidneys, and epithelial cells of the respiratory tract. Most of the described CRP proteins synthesized extrahepatically express poor solubility (ie, the protein is not secreted, is either expressed intracellularly or bound to the cell membrane) and express mCRP (“neo-CRP”) antigenicity. Hence, unless definitively proven, one cannot assume that “CRP” synthesized by any particular cell is the native soluble CRP pentamer. There is a distinct possibility that extrahepatically synthesized CRP subunits are not processed into cyclic pentamer discs, but remain as poorly soluble, self-aggregating, fibrous-like proteins that may deposit within and in the localized areas surrounding the synthesizing cell.

**In Vitro Experiments**

In vitro, loss of pentameric symmetry was associated predominantly with the occurrence of a proinflammatory phenotype (Fig 2). For example, mCRP suppressed spontaneous apoptosis in human neutrophils and promoted neutrophil adhesion to cultured human coronary artery endothelial cells through a CD18-dependent mechanism. In addition, mCRP released IL-8 from neutrophils through peroxynitrite-mediated activation of nuclear factor κB and activator protein 1. Other studies found mCRP inhibition of neutrophil chemotaxis similar to that of nCRP. Therefore, it is possible that neutrophils are recruited to inflammatory sites, halt movement, and convert an activated inflammatory response into a robust one, as exemplified by mCRP potentiation of respiratory burst. Furthermore, mCRP, but not nCRP, rapidly activated endothelial cells through a p38 MAPK-dependent mechanism. Conversely, prolonged (>24 hours) culture with nCRP was needed to detect endothelial cell activation. Using real-time flow cytometry, Khreiss et al reported that whereas nCRP inhibi-
mCRP accelerated shear-induced platelet capture of neutrophils in human whole blood, a critical event in triggering acute coronary events. These actions were mediated through opposing regulation of platelet P-selectin and neutrophil CD11b/CD18 expression by nCRP and mCRP. nCRP actions on neutrophils, endothelial cells, and platelets were markedly attenuated, although never completely inhibited, by an anti-FcγRII (CD32) antibody, whereas mCRP effects were attenuated by only an anti-FcγRIII (CD16) antibody, suggesting involvement of different receptors.

We hypothesize (Fig 3) that endothelial injury might expose the naturally occurring mCRP present in the intima and media of blood vessels. Local mCRP expression might lead to the attraction, attachment, migration, and activation of neutrophils to the injured area. Neutrophils then would be activated to address the threat to homeostasis; hence, a robust acute-phase response would be initiated. Alternatively, mCRP also may be formed from serum nCRP at sites of injury or infection by some as yet unknown mechanism(s). Selective deposition of nCRP has been observed at sites of vascular injury. Endothelial injury may enhance this process through local cytokine production, with subsequent increase of CRP production in the liver or perhaps other cells. The contribution of extrahepatic CRP production sites to systemic serum CRP levels is unclear. Whether mCRP effects on endothelial cells and neutrophils (or other cell types) ultimately would lead to progression or resolution of the atherosclerosis process also needs further investigation.

**Animal Models**

To date, little is known about the in vivo effects of mCRP in disease. In mice bearing a mouse mammary adenocarcinoma cell line, treatment with mCRP effectively slowed or stopped the progression of tumor growth. Most recently, we described a model of early atherogenesis in ApoE−/− mice with low subcutaneous CRP dosing over a long period. We found that human nCRP accelerated atherosclerosis, whereas mCRP partly prevented plaque formation. Enhanced atherogenesis by nCRP was associated with upregulation of CD154, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1. Conversely, mCRP protected against atherogenesis through induction of the anti-inflammatory cytokine IL-10. These in vivo effects of mCRP (Fig 2) are not necessarily in contradiction to the aforementioned studies that addressed immediate responses (over minutes to hours) using isolated cells. The long-term
effect of a stimulatory protein on the overall immune response in a live animal cannot be predicted precisely from isolated systems. We believe that small amounts of mCRP could heighten normal immune surveillance in the mouse, slowing the process of atherosclerotic plaque formation. Our results most likely do not reflect systemic CRP levels or the direct effect of circulating CRP on atherogenic plaque formation. Of note, in an animal model of systemic lupus erythematoses, a single injection of 200 μg of CRP, also administered subcutaneously, prevented or reversed lupus nephritis, a protection that also required IL-10. However, an enhanced IL-10 response may not necessarily confer protection, as shown in animal models of sepsis. Additional studies are needed to elucidate the signaling cascade of mCRP and its relevance to the processes of atherosclerosis in humans.

CRP IN CLINICAL STUDIES: THE GENERAL POPULATION

The introduction of high-sensitivity assays for serum CRP has permitted routine measurements of baseline CRP levels and initiated numerous studies investigating CRP as a risk factor for atherosclerotic diseases. Pioneering work was published in 1982 by de Beer et al., who measured serum CRP and creatine kinase muscle brain levels in patients with cardiovascular disease (CVD) and those without cardiac chest pain. All individuals...
with acute myocardial infarction showed elevated CRP levels. There was a significant correlation between peak CRP and creatine kinase muscle brain values. Subsequently, Ridker et al. reported that baseline serum CRP concentrations predicted risk for future myocardial infarction and stroke in apparently healthy men and women. Since then, numerous studies identified CRP as a risk factor for peripheral and coronary artery disease, myocardial infarction, stroke, and sudden death (for review, see). In the Women’s Health Study, CRP was additive to LDL cholesterol and the Framingham 10-year risk score in predicting future CVD in healthy American women. Based on these studies, it was supposed, but never proven, that CRP is causally involved in atherosclerosis and represents more than a marker of ongoing vascular damage. However, a recent work by Danesh et al. called into question the clinical value of measuring CRP as a predictor of risk for CVD. In the Reykjavik study, a single measurement of CRP at baseline was studied in relation to the 20-year incidence of coronary heart disease. In this large study with a long follow-up, the investigators found that the predictive value of CRP measurements added relatively little in comparison to traditional risk factors. The CRP odds ratio was between those for blood pressure and red blood cell sedimentation rate, but considerably less than that of cholesterol level. The heterogeneity of the results might be related to the duration of follow-up, study design, patient selection, study size, or storage temperature of CRP specimens.

Although sex or food intake do not influence serum CRP levels, adiposity, chronic inflammation, metabolic syndrome, type 2 diabetes, hypertension, and sleep apnea clearly have been associated with increased CRP levels. Hormone replacement therapy increased CRP levels in postmenopausal women. However, weight loss 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, and RRR-α-tocopherol, were associated with decreases in serum CRP levels. Nissen et al. recently reported that statin treatment induced decreases in CRP levels independently and significantly correlated with rate of disease progression in patients with angiographically documented coronary artery disease. Also, in patients with acute coronary syndromes, low CRP levels after statin therapy were associated with better clinical outcomes than higher CRP levels regardless of resultant LDL cholesterol levels. However, randomized clinical trials testing whether reductions in serum CRP levels without affecting traditional risk factors also would lead to a reduction in CVD and cardiovascular mortality are still missing.

CRP IN CLINICAL STUDIES: POPULATIONS AT HIGH RISK

In populations with a high risk for CVD, as encountered in patients with end-stage renal disease, serum CRP level emerged as one of the most powerful predictors of all-cause and cardiovascular death. Much recent interest has focused on this finding because CRP levels are not only relatively high in this population (8 to 10 mg/L), but traditional risk factors do not explain the extent and severity of cardiovascular complications. Therefore, inflammation, represented by elevated CRP levels, is thought to be the “culprit” in these patients. Without doubt, we deal here with a highly atherogenic milieu in which inflammation and oxidative stress interfere with each other. Renal insufficiency itself increases plasma CRP levels, which are enhanced further by the dialysis procedure (dialysis membranes and water) and chronic persistent infections caused by grafts and catheters. Chronic elevations in serum concentrations of proinflammatory cytokines caused by reduced renal clearance or increased heart production in a constant state of fluid overload also might have a role. However, the pathophysiological process probably is too complex to clearly distinguish different pathways. Therefore, one needs to simplify studies in well-defined systems.

CLINICAL APPLICATIONS OF CRP MEASUREMENTS

The clinical relevance of serum CRP measurements in the prediction of risk for CVD in the healthy population appears to be established. The American Heart Association and Centers for Disease Control and Prevention recommended the use of CRP as a risk marker for CVD in individu-
als with a Framingham risk score between 10% and 20%. CRP levels less than 1 mg/L are considered low risk; 1 to 3 mg/L, average risk; and greater than 3 mg/L, high risk for CVD. If plasma CRP values on 2 occasions 1 month apart are in the same category, these may be taken as reliable evidence with regard to low, average, and high risk for subsequent CVD. However, if CRP level is greater than 10 mg/L, CRP may not be used to assess cardiovascular risk, and other active inflammatory processes (trauma, infection, and so on) need to be excluded. Thus, when using CRP level to assess cardiovascular risk in primary prevention, the high-sensitivity CRP assay should be adopted and the patient should be free from any kind of detectable confounding acute inflammation for at least 2 weeks. Of note, approximately 40% of patients with stable CVD showed unexpectedly high variability in CRP levels, even in the absence of clinically detectable active inflammatory processes. Intriguingly, patients in the high-risk category subsequently were found to be at low risk, and vice versa. Reasons for such variability are unknown at present.

What should the recommendations be for patients with high CRP levels, such as patients with end-stage renal disease? In a longitudinal study (16 weeks), Tsirpanlis et al found that in the presence of a greater than normal microinflammatory background (mean CRP level, 3.7 mg/L) that varies with time, the inflammation pattern of 37 hemodialysis patients was characterized by waves of “true” inflammation caused by clinical events (CRP level >9.5 mg/L). They suggested that this waveform pattern might be a risk factor for atherosclerosis in this population. However, in the absence of larger studies, clinical recommendations can only be based on opinion at present. Hopefully, additional analysis of the inflammation pattern in hemodialysis patients with type 2 diabetes mellitus administered the statin atorvastatin will provide new answers. Cutoff values for risk stratification probably should be set somewhat higher than those recommended by the American Heart Association for CVD in the general population. We propose that in patients with end-stage renal disease, CRP levels up to 10 mg/L may represent a state of “microinflammation” and suggest to distinguish further for levels greater than 10 mg/L. CRP values between 10 and 50 mg/L may be interpreted as suspicious of a superimposed inflammatory event. CRP values greater than 50 mg/L may indicate the presence of acute infection.

CONCLUSION

The multitude of compelling clinical studies has led to valuable recommendations for clinical practice. However, we believe that describing CRP only as the “bad guy” is a too simplified view of its complex role in the inflammatory process. Data supporting the notion that CRP contributes directly to atherogenesis derive largely from in vitro observations with no clear-cut picture emerging from murine models of atherosclerosis. In addition, the absence of any known CRP deficiency in humans (or CRP-deficient mice) suggests that this protein has a survival value. The accumulating data on opposing functions of different CRP configurations may shed new light on the role of the innate immune system in atherogenesis.

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