The role of C-reactive protein as a cardiovascular risk predictor

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Abstract

A wealth of evidence supports the concept of inflammation playing a pivotal role in the development and progression of atherosclerosis. C-reactive protein (CRP) is a sensitive acute--phase inflammatory marker for the development of cardiovascular events such as coronary artery disease, myocardial infarction and stroke. Numerous studies indicate that CRP is not only a biomarker but also an active mediator of inflammation and atherogenesis through its direct effects on leukocytes and endothelial cells. We review the evidence and suggest mechanisms by which CRP can affect arterial endothelial cell activation, macrophage recruitment and foam cell generation and the development of atherosclerotic lesions. We emphasize the direct role of CRP in sustaining a proinflammatory and procoagulant milieu within the arterial neointima. This review also proposes that CRP can directly injure arterial endothelium and is a molecule involved in the maintenance of a systemic proatherosclerotic environment. In addition, we review therapies that target CRP for both the prevention and treatment of atherosclerotic diseases.

Key words: C-reactive protein, atherosclerosis, cardiac, vascular, inflammation, coronary artery disease, atherosclerosis

Introduction

The importance of acute-phase molecules as risk factors for human cardiovascular disease has been emphasized in a number of reports [1, 2]. The principal acute-phase protein, C-reactive protein (CRP), so named for its ability to precipitate the C-polysaccharide of Streptococcus pneumoniae, has been linked to atherosclerosis and its complications. CRP is highly stable, and its serum concentration, normally less than 1 mg/L, can increase 10000-fold during the acute phase response [3-5]. Over the past decade, multiple clinical studies have associated CRP with an increased risk of developing myocardial infarction, stroke, peripheral arterial disease, cardiovascular and sudden death [1, 6]. In addition, elevated levels of CRP are one of the strongest predictors of progressive vascular disease [7] and future cardiovascular events in apparently healthy men and women [6, 8, 9].

The relationship between CRP levels and the acute phase response to infection, inflammatory disease, surgery, trauma and cancer is well known [3]. However, its specific roles in the acute phase response remain unclear. In contrast to acute inflammation, CRP levels are minimally elevated (<10 mg/L) in patients at risk for atherosclerotic disease and remain elevated for many months to years. The biological effects of minimal elevations of CRP and the subsequent development of cardiovascular disease remains unexplained [1].

Biochemical and immunological characteristics of CRP

The possible involvement of CRP in atherosclerotic cardiovascular disease has attracted an increasing attention in recent years and gave the impetus to intense molecular and physiological investigations of the protein. CRP, a pentamer composed of five identical non-glycosylated 23 kDa subunits, is a member of a highly conserved family of proteins known as pentraxins. Along with more than 40 other molecules, the pentraxins are recognized as acute-phase response proteins, and are involved in innate immunity and protection against a variety of pathogens through opsonization and activation of the complement cascade. CRP is rapidly synthesized (mainly, but not exclusively, by hepatocytes) in response to an acute inflammatory stimulus, and is internalized through specific receptors on a variety of cells. The circulating protein is a pentamer, but

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may be dissociated into a (denatured) monomeric form that exhibits distinct immunological and biochemical properties. The pentameric CRP binds to phosphocholine on membranes of damaged and apoptotic cells, to C1q component of the complement cascade and to the immunoglobulin Fc gamma receptors I and II (CD64 and CD32, respectively). In contrast, the monomer displays unique antigenic epitopes and binds preferentially to Fc gamma receptor III (CD16). Recent results from our laboratory indicate that the denatured CRP – but not the pentamer – has a promiscuous ability to bind to IgG molecules regardless of their source and antigenic specificity. This property appears restricted to CRP because other pentraxins, (serum amyloid component P and long pentraxin PTX3) do not interact with antibodies other than those specifically directed against them (Fig. 1).

Clinical and epidemiological relevance of CRP blood levels: CRP as a risk marker

During the past decade, numerous primary prevention studies associated elevated CRP blood levels with increased numbers of cardiovascular events. However, traditional assays for CRP lacked the ability to accurately measure CRP levels that were below 8 mg/L, levels associated with vascular disease prediction. The newer high-sensitivity assays are able to determine CRP levels within the 0.3-10 mg/L ranges [10]. CRP levels of <1.0, 1.0 to 3.0, and >3.0 mg/L are associated with low-, moderate-, and high-risk for future cardiovascular events. Parenthetically, we suggest that the term „high sensitivity" be used in reference to the assay methodology, and not to give an impression that one is detecting a novel form of CRP.

Nested case-control studies, as well as a significant number of large prospective studies and meta analyses, established the prognostic power of the high-sensitivity CRP measurement for prediction of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even among individuals without a previous history of cardiovascular disease [11]. The relationship between levels of CRP and future vascular risk has been consistent in studies performed in both the United States and Europe. The predictive power of CRP measurements for cardiovascular events holds for men as well as women, middle age as well as older individuals, non-smokers and smokers, as well as diabetic and non-diabetic, hyperlipidemic and normolipidemic, and hypertensive and normotensive individuals.

CRP levels also predict the risk of both recurrent ischemia and death among individuals with known atherosclerotic disease such as stable and unstable angina, patients undergoing percutaneous angioplasty, and those individuals presenting to the emergency room with acute coronary syndromes. CRP levels consistently predict the likelihood of new coronary events in patients with unstable angina and acute myocardial infarction [12-21]. However, in acute coronary syndromes, CRP predicts recurrent myocardial infarction independent of troponin levels (an indicator of myocardial cell damage), suggesting that CRP is not a marker associated with the presence of myocardial damage [19-21]. Elevated CRP levels also seem to predict the prognosis of recurrent events in patients with stroke [22, 23], and may be a marker for risk of restenosis following percutaneous coronary interventions [24, 25]; this issue remains controversial [26].

In many studies, CRP was a stronger predictor of cardiovascular events than LDL and HDL cholesterol. In addition, CRP levels contribute to the cardiovascular risk associated with other risk factors such as hypertension, diabetes mellitus, smoking, and obesity. The additive value of CRP to lipid screening in terms of coronary risk prediction has been demonstrated in several settings [11, 27-30].

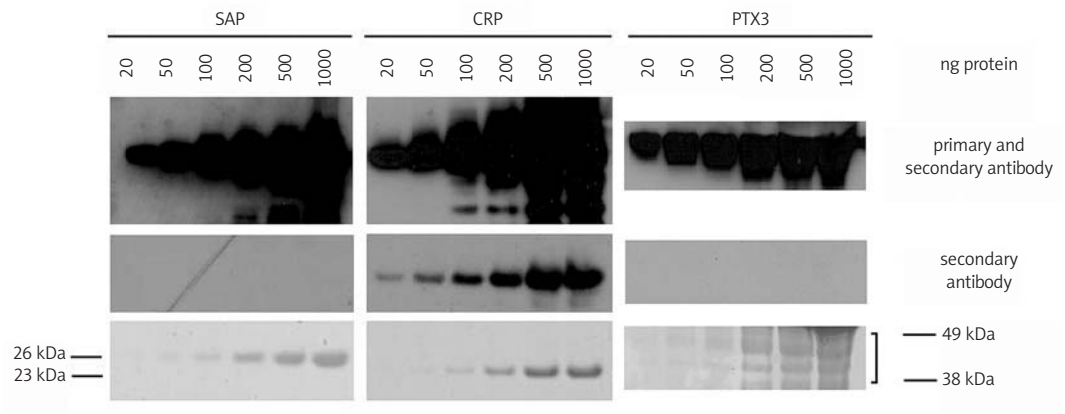

Fig. 1. CRP is unique among pentraxins in reacting with non-specific antibodies. Three human pentraxins, SAP, CRP and PTX3 were probed, respectively, with the cognate rabbit polyclonal antibody, mouse monoclonal antibody and rat monoclonal antibody, followed by detection with a secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG, sheep anti-mouse IgG and goat anti-rat IgG, as appropriate). Only CRP reacted with the secondary antibody alone. Blue panels show protein staining. SAP and CRP migrated as 26 kDa and 23 kDa bands, respectively, under denaturing conditions. PTX3 has the calculated molecular mass of 42 kDa, but because of glycosylation, the protein migrates as a series of bands between 40-45 kDa

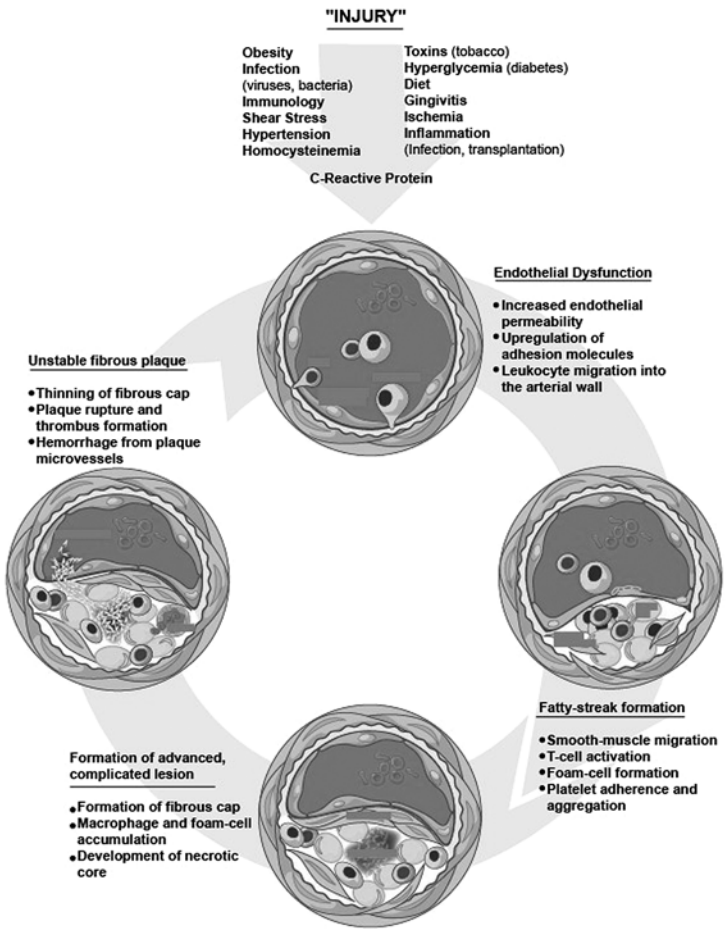

Fig. 2. C-reactive protein and response to injury. C-reactive protein may be one of the factors causing injury to arterial endothelial cells leading to the generation of a dysfunctional endothelium. Endothelial dysfunction, characterized by upregulation of endothelial adhesion molecules, allows leukocyte migration into the arterial wall. Migration of leukocytes and smooth muscle cells into the arterial intima and foam cell formation leads to generation of the fatty-streak. Progression of the atherosclerotic plaque is characterized by formation of an advanced, complicated lesion with the generation of a necrotic core and development of the fibrous cap. An unstable plaque characterized by thinning of the fibrous cap can lead to plaque rupture and thrombus formation as well as hemorrhage from plaque microvessels

Vascular inflammation: a role for CRP

Accumulating evidence suggests that atherosclerosis is an inflammatory vascular disease and that inflammation plays a central role in the development and progression of atherosclerosis and its complications [31, 32]. Among potential risk factors associated with inflammatory processes, the following are of particular note: CRP, serum amyloid A, selectins and adhesion molecules ICAM-1 and VCAM-1 (intercellular and vascular adhesion molecules, respectively). An intriguing question is whether some of these inflammatory molecules represent merely reporters of systemic inflammation or whether they actively contribute to the formation of atherosclerotic lesions and the associated complications.

Atherosclerosis begins with the formation of fibrofatty and fibrous lesions and ends with the generation of more advanced lesions characterized by fibrous plaques (Fig. 2), as a response to insults to the endothelium and smooth muscle cells of the arterial wall. These insults include diet (generation of oxidized or modified LDL-cholesterol, high concentrations of omega-6 polyunsaturated fatty acids), mechanical shear, hypertension, infection (viruses, bacteria), homocysteine, hyperglycemia, obesity, immunologic damage, toxins (tobacco smoke), and other agents. Acute-phase proteins such as CRP can also directly contribute to the generation of a dysfunctional endothelium (Fig. 3) and the recruitment of macrophages and leukocytes into the arterial intima.

This recruitment process occurs in a series of steps tightly regulated by cytokines and adhesion molecules [33] (Fig. 3). Significantly, CRP activates endothelium and induces adhesion molecule expression on human aortic endothelial cells to a level comparable to that induced by cytokines [34, 35]. Furthermore, CRP induces the expression of monocyte chemoattractant protein-1 [35, 36], and interleukin-8 [37] which facilitate monocyte chemotaxis and entry of monocytes into the sub-endothelial space and recruitment into the arterial intima. Interestingly, CRP-mediated monocyte chemotaxis is abolished by a specific monoclonal antibody to CRP [38]. Recent studies demonstrate that CRP can increase chemokine CC receptor 2 expression on monocytes [39], which – together with interleukin-8 – may facilitate adherence of the monocytes to the arterial endothelium and subsequent migration into the intima [37, 40]. Macrophage-derived foam cells in the intimal space are active participants in the development of the vascular lesions of atherosclerosis [31, 32, 41]. The development of macrophage foam cells containing large amounts of cholesterol esters is a hallmark of atherosclerosis and emphasizes the importance of low-density lipoprotein (LDL)-cholesterol in the development of these lesions. Macrophage foam cells sustain a proinflammatory milieu within the arterial intima through increased synthesis and secretion of cytokines and CRP (Fig. 3). Recruitment and transmigration of phagocytes, especially neutrophils, across the endothelium into inflammatory sites within advanced, complicated or unstable fibrous plaques could be facilitated by interleukin-8, a member of the CXC family of chemokines. Recent findings have demonstrated that CRP induces interleukin-8 expression at mRNA and protein levels through nuclear factor κB (NFκB) activation in human aortic and coronary artery endothelial cells [37].

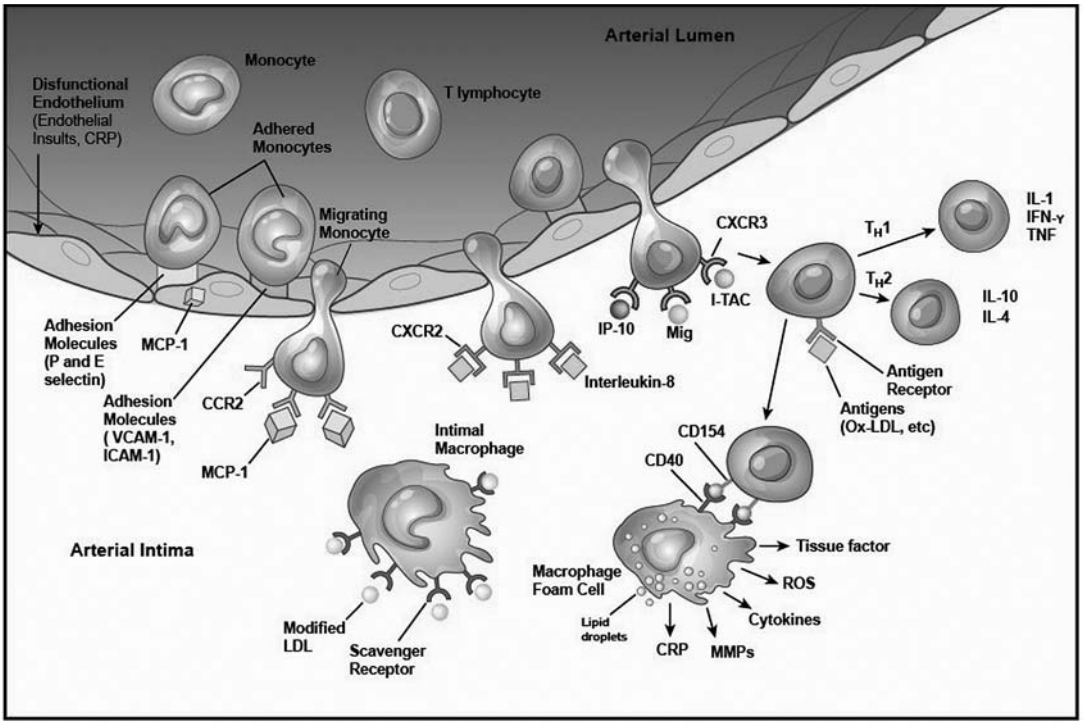

Fig. 3. Mononuclear cell recruitment into the arterial intima: A role for C-reactive protein (CRP). This figure schematizes steps (left to right) in the recruitment of mononuclear cells into the arterial intima and some of the functions of these cells in the atheromatous lesion. Endothelial activation (endothelial insults, CRP) increases expression of P and E-selectins, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Mononuclear cell migration into the arterial intima is facilitated by a variety of chemokines such as monocyte chemoattractant protein-1, interleukin-8 and interferon-?; once into the intima, monocytes become macrophages. In the atheroma, macrophages express scavenger receptors that bind to modified LDL particles (for example, oxidized or glycated LDL) and generating foam cells, a hallmark of the atherosclerotic lesion. Foam cells secrete pro-inflammatory cytokines, C-reactive protein, reactive oxygen species, as well as matrix metalloproteinases and tissue factor, promoting further inflammation and thrombotic complications. T-lymphocytes within the arterial intima release cytokines and engage in crosstalk with foam cells though CD154-CD40 interactions. This crosstalk allows the pro-inflammatory and procoagulant milieu in the atheroma to be sustained over time

CRP and atherosclerosis

A. CRP and endothelial activation

Activation of arterial endothelium is associated with the development of the native form as well as the transplant--associated form of atherosclerosis [32, 41, 42]. An aspect of activation is an increase in ICAM-1 production, and CRP is capable of directly inducing expression of ICAM-1 on human aortic endothelial cells (Fig. 4) [34]. Importantly, induction of adhesion molecule expression is achieved with concentrations of CRP found in patients at risk of developing cardiovascular disease. A positive relationship between elevated CRP levels and ICAM-1 expression on endothelium and in blood was recently found in heart transplant patients who subsequently developed coronary artery disease [42]. Additionally, elevated plasma levels of ICAM-1 have been found in apparently healthy men who subsequently developed myocardial infarction, suggesting adhesion molecules play a significant role in the etiology of cardiovascular disease [43].

CRP can facilitate the release of cytokines such as interleukin 1β, IL-6 and tumor necrosis factor-α and increase the release of soluble IL-6 receptor by macrophages and foam cells in the neointima (Fig. 4) [35, 44, 45]. The production of IL-6 in arteries is likely due to the presence of macrophages within the vascular wall, since IL-6 is produced by venous but not arterial endothelium [46]. In addition, CRP can evoke the production of the potent endothelium-derived vasoactive factor, endothelin-1 [35]. Endothelin-1 and IL-6 are two mediators of CRP-induced expression of adhesion molecules, monocyte chemoattractant protein-1 secretion and LDL uptake by macrophages. The release of IL-6 and soluble IL-6 receptor by endothelial cells and inflammatory cells within the areas of atherogenesis can lead to the appearance of complexes of IL-6 and IL-6 receptors in circulation. These complexes bind to arterial endothelium through glycoprotein 130, which is constitutively expressed on endothelial cells (Fig. 4) [47], sustaining a proinflammatory milieu in these arteries. Such a milieu could favor further synthesis and release of CRP by human arterial endothelial cells. This possibility is strengthened by the recent demonstration of intracellular CRP mRNA and protein in, as well as increased CRP secretion by, human aortic endothelial cells following incubation with interleukins-1β and -6 [48]. Increased synthesis of both intracellular and secreted CRP was also achieved following incubation

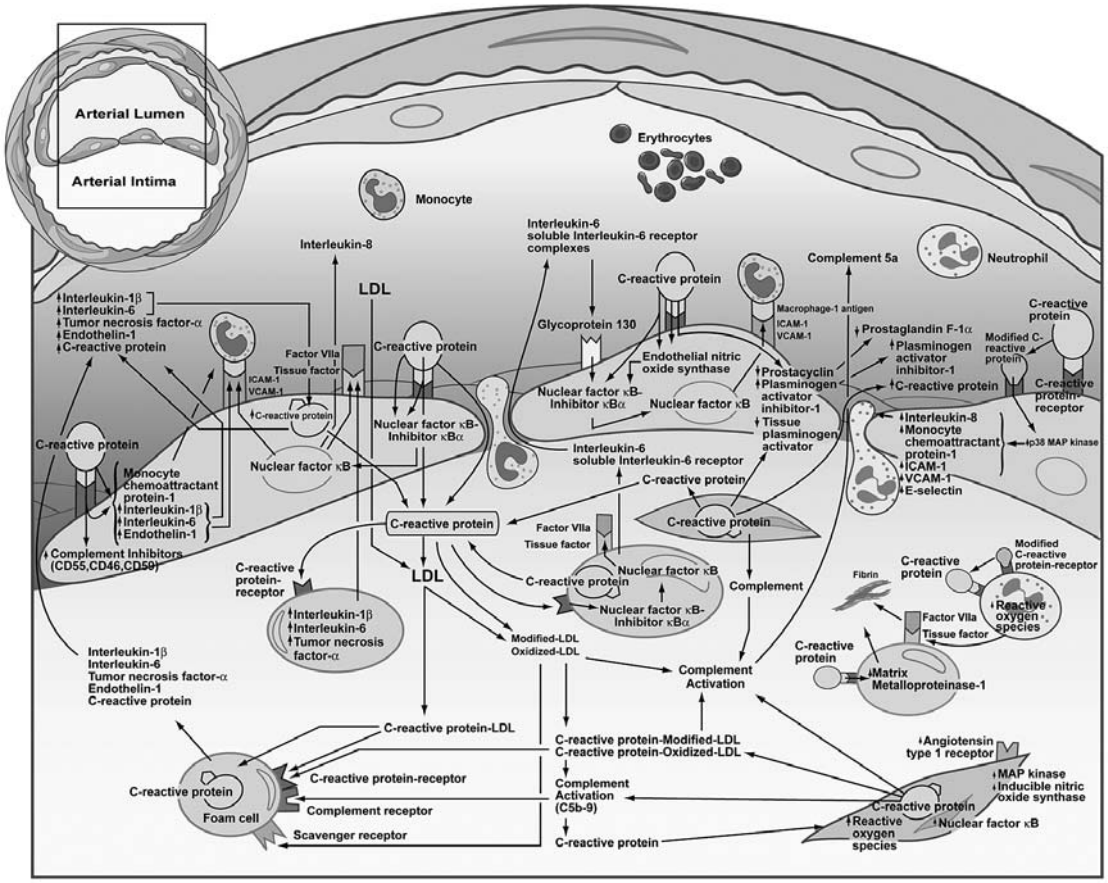

Fig. 4. C-reactive protein, arterial endothelial activation and foam cell formation in atherosclerosis. Once C-reactive protein enters endothelial cells, it activates nuclear factorκB, inducing the expression of intercellular adhesion molecule-1 (ICAM-1) and tissue factor. C-reactive protein inhibits endothelial nitric oxide synthase production and induces synthesis of cytokines and chemokines, promoting further endothelial activation and monocyte recruitment into the arterial intima. Within the intima, C-reactive protein facilitates the uptake of LDL (native or modified/oxidized) by macrophages, and this promotes foam cell formation and induces the macrophage/foam cell tissue factor expression. In addition, C-reactive protein induces synthesis of cytokines and activates complement sustaining a proinflammatory milieu within the atheromatous lesion

with macrophage conditioned media, and these effects were reversed by neutralizing antibodies directed against interleukins-1β and -6 [48]. These data support the idea that macrophages within the atherosclerotic plaques could facilitate cytokine-mediated CRP synthesis and release by arterial endothelial cells. Moreover, a proinflammatory microenvironment within the arterial intima can favor CRP production, since it has been demonstrated that interleukins-1 and -6 induce CRP mRNA and protein in human coronary artery smooth muscle cells [49].

CRP can activate endothelium through modulation of the endothelial nitric oxide synthase-nitric oxide pathway (Fig. 4). Elevated levels of CRP are associated with a significant decrease in nitric oxide production that is the consequence of CRP acting to lower the endothelial nitric oxide synthase mRNA stability and protein levels [50, 51]. Increased CRP levels also result in an increase in monocyte adhesion to the aortic endothelium. These responses are associated with an increased expression of endothelial ICAM-1 and VCAM-1. The effect of decreased nitric oxide production on endothelial adhesion molecule expression is partially mediated via activation of NFκB (Fig. 4) [52]. Such activation represents one mechanism for CRP-associated proinflammatory activity within the arterial vasculature. This idea is strengthened by the recent demonstration that circulating monocytes from unstable angina patients with persistently elevated levels of CRP exhibited activation of NFκB [53]. In addition, CRP activated NFκB in human saphenous vein endothelial cells [54] and in human aortic endothelial cells [37, 55]. CRP increased interleukin-8 mRNA and protein in human aortic endothelial cells, and NFκB inhibitors diminished this upregulation. Since activation of NFκB is associated with a concomitant increase of IL-6 levels, it is possible that proinflammatory effects of CRP are mediated through NFκB [56].

On the basis of its documented effects *in vitro*, CRP may function as a procoagulant, being able to reduce the endothelial nitric oxide synthase and prostacyclin, and to increase plasminogen activator inhibitor-1 and, indirectly, tissue factor [57], a protein expressed on monocytes/macrophages and endothelial cells following activation [58]. Tissue factor represents a linkage between inflammation and

coagulation. The presence of this molecule in atherosclerotic lesions contributes to thrombosis in coronary arteries following plaque rupture and it could explain the prothrombogenic status found in patients that develop the transplant-associated form of the disease [59]. The participation of CRP in atherothrombosis is further supported by the recent demonstration that CRP increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells [46]; moreover, it decreases tissue plasminogen activator antigen expression and activity in human aortic endothelial cells through generation of proinflammatory cytokines interleukin-1β and tumor necrosis factor-α [60]. Additionally, a proatherogenic and prothrombotic role for CRP is suggested by the experiments in which CRP decreased the release of the stable metabolite of prostacyclin, prostaglandin F-1α, in both human aortic and human coronary artery endothelial cells [55, 61]. The effects of CRP upon arterial endothelium seem to be mediated via Fcγ receptors II and I, since it was recently demonstrated that the increase in interleukin-8, ICAM-1 and VCAM-1, as well as the decrease in endothelial nitric oxide synthase and prostacyclin induced by CRP, was abrogated with antibodies to CD32 and CD64 [62].

A direct role for CRP in the development of vascular disease is supported by recent studies performed in CRP transgenic mice [63-65]. Compared with normal mice, the CRP transgenic mice have a significantly increased and faster rate of arterial thrombosis following vascular injury [65]. In addition, CRP accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice [66]. CRP transgenic/apolipoprotein E-deficient mice demonstrated increased arterial deposition of CRP and complement C3, and enhanced expression of angiotensin type 1 receptor, VCAM-1, and collagen, an observation that supports a proatherogenic role for CRP *in vivo*.

B. CRP and LDL uptake, foam cell and plaque formation

Native LDL is not taken up by macrophages rapidly enough to generate foam cells, leading to the proposition that LDL needs to be modified in the vessel wall before being transported inside the macrophage (Fig. 4) [67, 68]. Foam cells in atherosclerotic lesions are generated through the activity of scavenger receptors, which allow macrophages to take up such oxidized or modified LDL particles. Because CRP influences the production of reactive oxygen species by macrophages and smooth muscle cells [69, 70] it is likely that CRP can also facilitate LDL oxidation in atherosclerotic lesions.

CRP deposited within the arterial intima can bind oxidized LDL through recognition of phosphorylcholine residues on oxidized phospholipids [67, 71]. Chang et al. [72] demonstrated recently the binding of CRP to oxidized LDL via phosphorylcholine and the recognition of these complexes by scavenger receptors. CRP displays calcium-dependent binding to native LDL and to very low-density LDL [73], facilitating LDL uptake into macrophages and contributing to the formation of foam cells [74] (Fig. 4). The binding of LDL to extracellular matrix proteins in the arterial wall can expose phosphorylcholine residues on native LDL allowing the binding of CRP [72]. The fact that foam cells in early atherosclerotic lesions show the presence of CRP receptors as well as of CRP [38] is consistent with the hypothesis that CRP participates in foam cell formation through opsonization of lipid particles.

The colocalization of CRP with enzymatically modified LDL was recently demonstrated in early atherosclerotic lesions [67]. In addition to the evidence that foam cell formation can occur through the uptake of modified LDL by scavenger receptors, the uptake could be further facilitated by the binding of CRP to such modified LDL and subsequent incorporation via macrophage-associated CRP receptors (Fig. 4). The major CRP receptor in human macrophages seems to be Fcγ receptor II (CD 32). This receptor could play a significant role in the generation of foam cells [75]. Other CRP-specific receptors are thought to exist in monocytes/macrophages [76], but the relevance of these receptors for subsequent foam cell generation remains to be established.

Complement can facilitate the opsonization of CRP-bound LDL by macrophages, and it has been demonstrated that the CD32 CRP receptor can cluster with other receptors such as complement receptors [75]. This is particularly relevant when one considers that CRP can directly activate complement [77]. The interactions between complement and CRP can be facilitated by the synthesis and release of CRP and complement components by macrophages and smooth muscle cells within the plaques (Fig. 4) [78, 79]. However, CRP also appears to upregulate complement-inhibitory proteins and protects endothelial cells from complement-mediated cell injury [80], suggesting that a balance of proatherogenic and antiatherogenic effects of CRP on the vessel wall may be important in the development of atherosclerosis.

Elevated circulating levels of CRP can increase levels of CRP within vascular lesions by way of diffusion or receptor-mediated uptake by endothelial cells [46]. CRP within the plaques can be also increased by additional synthesis and release by intimal macrophages and smooth muscle cells [38, 75, 77, 78] (Fig. 4). CRP can directly contribute to plaque generation and progression through smooth muscle cell migration and proliferation via angiotensin 1 receptor upregulation [70]. CRP also increases smooth muscle cell basal reactive oxygen species production and potentiates the effects of angiotensin II on reactive oxygen species formation. CRP that accumulates in atherosclerotic plaques might enhance vascular oxidative stress via $p22^{phox}$-based NADH/NADPH oxidase [81] CRP could also inhibit adrenomedullin in arterial endothelial and smooth muscle cells, thereby facilitating oxidative stress [82]. It has been recently demonstrated that CRP stimulates matrix metalloproteinase-1 expression by human macrophages through FcγRII and extracellular signal-regulated kinase pathway [83]. This activity of CRP may promote matrix degradation and thus contribute to plaque vulnerability.

It has been proposed that there are distinct forms of CRP that are formed during inflammation. Conformationally rearranged CRP molecules (monomeric species) express epitopes not seen in native CRP, and these molecules are referred to as modified CRP (mCRP). Patients with more

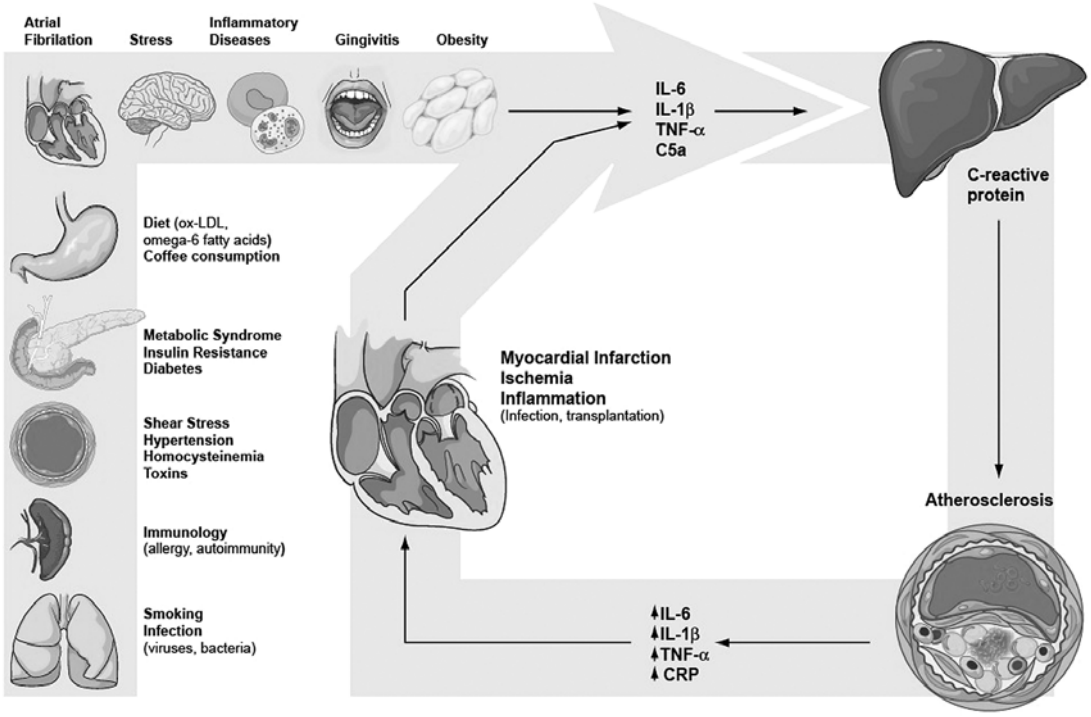

Fig. 5. Cytokine release, C-reactive protein and atherosclerosis. Numerous injurious stimuli such as inflammatory diseases, immunological reactions, endocrine disorders, unhealthy diet, smoking, shear stress, hypertension, homocysteine or other toxins can lead to cytokine release and subsequent increased synthesis of C-reactive protein by the liver. C-reactive protein can promote the generation and progression of atherosclerotic disease through increased synthesis and release of cytokines and more of C-reactive protein. The presence of ischemia secondary to atherosclerosis, and special situations such as myocardial infarction, infection or transplantation in organs like the heart, can further contribute to the sustenance of a proinflammatory and proatherosclerotic environment

monomeric CRP might exhibit a greater proinflammatory phenotype and hence be at a greater risk for adverse cardiovascular events [84]. Indeed, the loss of pentameric symmetry in CRP, resulting in formation of mCRP, promotes a proinflammatory phenotype in human aortic endothelial cells characterized by the expression of ICAM-1, E-selectin and VCAM-1 protein and mRNA (Fig. 4). Incubation of human aortic endothelial cells with mCRP also results in concentration-dependent increases in interleukin-8 and monocyte chemoattractant protein-1 secretion and mRNA levels. These effects are mediated by a p38 MAP kinase-dependent mechanism (Fig. 4) [84]. The presence of cytokines such as interleukin-8 in the arterial intima could facilitate the recruitment of polymorphonuclear leukocytes, and these cells may play an important role in the pathogenesis of thrombosis and atherosclerosis by exerting concentration--dependent regulatory effects on the tissue factor production by mononuclear cells via the release of reactive oxygen species [85]. In contrast to native CRP that predominantly binds the low-affinity IgG FcγRIIa (CD32) receptor [86], mCRP utilizes the low-affinity immune-complex FcγRIII (CD16) on leukocytes [87]. This seems to apply also to arterial endothelial cells since an anti-FcγRIII (CD16) but not an anti- FcγRIIa (CD32) antibody significantly reduced the mCRP effects [84]. The role of mCRP in atherosclerosis is still controversial since calcium is always present in the lesions and mCRP has not yet been demonstrated in atherosclerotic plaques [88].

The role of CRP promoting a proinflammatory status leading to the development and progression of atherosclerosis is depicted in Fig. 5. Briefly, several inflammatory states generate cytokines that in turn induce synthesis and release of CRP by the liver. Elevated CRP can act as an injurious agent upon arterial endothelium, promoting generation and progression of atherosclerosis. Atherosclerotic lesions can be the source of further cytokine and CRP synthesis and release. In this „self-perpetuating" manner atherosclerosis-mediated ischemia and inflammation can contribute to the creation and maintenance of a proinflammatory and proatherosclerotic environment.

C. CRP and atherosclerosis: therapeutic approaches

Numerous clinical studies indicate that CRP levels are associated with the development and progression of atherosclerotic disease. These studies also indicate that CRP is a direct mediator of biochemical processes associated with atherosclerosis such as inflammation and activation of the coagulation cascade (Tab. I). In support of a role for CRP in inflammation and coagulation activation, infusion of recombinant human CRP into healthy volunteers significantly

increased serum levels of interleukins-6 and -8, serum amyloid A, serum phospholipase A_2, prothrombin 1 and 2, D-dimer and plasminogen activator inhibitor 1 [89]. Furthermore, although to-date there is no proof that reduction of CRP levels alone moderates the progression of atherosclerotic disease, treatments that reduce CRP levels have been associated with a diminished cardiovascular risk. Thus, CRP may be a target for the prevention and modulation of atherosclerotic lesions. Definitive proof of independent benefits of reducing CRP levels in humans awaits the development of specific inhibitors of CRP synthesis or action.

Dietary supplements

Diet-induced weight loss is a primary means of reducing CRP levels and the cardiovascular risk in patients who are overweight. It is probably not accidental that obesity is an independent risk factor for the development of cardiovascular disease and that adipocytes (fat cells) synthesize IL-6, the principal CRP activator. There is a positive association of weight and body mass index with CRP and IL-6 levels. Insulin resistance, which is common in obese patients, is also associated with elevated CRP levels. Diet-induced (ie. low calorie, low fat) or surgery-related (gastric surgery) weight loss decreases CRP and IL-6 levels [90-92]. Reduction of insulin resistance through weight loss or with insulin-sensitizing agents also decreases CRP levels.

Some dietary supplements have known anti-inflammatory properties. Omega-3 long chain polyunsaturated fatty acids exhibit anti-inflammatory activities and have been associated with a decreased incidence of atherosclerotic cardiovascular disease and mortality following myocardial infarction. The effect of omega-3 fatty acids on CRP levels remains unclear [93] with some studies suggesting that they reduce CRP [94, 95] while other studies reporting no effects [96, 97]. Moderate alcohol intake is associated with decreased CRP levels in both cross-sectional population-based studies [98] and in a prospective randomized trial [99]. Multivitamins have been shown to reduce CRP levels [100]. Similarly, diets high in plant sterols, alpha-tocopherol and arginine have been reported to lower CRP levels [101]. Also effective are diets rich in soy protein, viscous fiber and almonds, and foods with low glycemic indexes.

Aspirin and statins

Aspirin use modifies the cardiovascular risk associated with elevated CRP levels in both primary prevention trials and unstable angina [6]. However, the direct effects of aspirin on CRP levels are still controversial. Some studies report a decrease in CRP levels with aspirin [102] while other studies report no effects [103].

No such uncertainty exists in the case of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors). These compounds reduce cardiovascular events and CRP levels, independent of their reduction in LDL levels [104-106]. For example, Albert et al. [105] studied 1702 individuals with no prior history of cardiovascular disease (primary pre-

Tab. I. Mechanisms for CRP-induced atherogenesis

1. Induction of adhesion molecules (ICAM-1, VCAM-1, E-selectin) on arterial endothelium
2. Activation of nuclear factor κB in arterial endothelium and intimal foam cells
3. Stimulation of proinflammatory cytokines (interleukin-1β, interleukin-6, tumor necrosis factor-α) in arterial endothelium and intimal foam cells
4. Induction of monocyte chemoattractant protein-1 on arterial endothelial cells
5. Induction of endothelin-1 on arterial endothelium
6. Soluble interleukin-6 receptor release by intimal foam cells and subsequent increased formation of interleukin-6-soluble interleukin-6 receptor complexes in arterial blood
7. Inhibition of endothelial nitric oxide synthase and decreased nitric oxide action on arterial endothelial cells
8. Increased monocyte/macrophage adherence on arterial endothelium
9. Induction of tissue factor on arterial endothelium and intimal foam cells
10. Increased plasminogen activator inhibitor-1 and decreased tissue plasminogen activator in arterial endothelial cells
11. Increased uptake of LDL or oxidized/modified LDL by intimal macrophages with formation of foam cells
12. Complement activation and facilitation of LDL uptake by intimal macrophages
13. Increased generation of reactive oxygen species by foam cells and smooth muscle cells in the arterial intimal space
14. Increased smooth muscle cell migration and proliferation linked to angiotensin type 1 receptor upregulation
15. Increased synthesis and release of interleukin-8 by arterial endothelial cells
16. Decreased prostacyclin release by arterial endothelial cells

vention trial) and 1182 patients with cardiovascular disease (secondary prevention trial). Patients were randomized with respect to pravastatin or placebo for 24 weeks. In the primary prevention trial, median CRP levels were reduced by 17% at 24 weeks in the pravastatin group (no change in the placebo group). In the secondary prevention trial, median CRP levels were reduced 13% in the pravastatin group. The benefits of statins therapy are the greatest among the patients with high levels of CRP, regardless of the level of cholesterol or LDL.

In a 5 year study of the use of statins for primary prevention of coronary events, lovastatin reduced CRP levels by 15% [28]. The drug was effective in preventing coronary events in patients with elevated CRP levels regardless of the ratio of cholesterol to high-density lipoprotein. In another study, the effects of simvastatin, pravastatin, and atorvastatin upon CRP levels were evaluated in a randomized double-blind crossover trial in patients with combined hyperlipidemia [106]. After 6 weeks of treatment, all three statins decreased CRP levels to a similar extent. Statins may also be useful for reducing the development of transplant-associated coronary artery disease and stroke, both diseases associated with elevated CRP levels. These beneficial actions of statins may be related in part to the cholesterol-independent effect of statins on inflammatory processes [107], perhaps mediated through a reduction in CRP levels. Similarly, for coronary artery disease patients undergoing intensive (as contrasted with moderate) statin therapy, the diminished rate of progression of atherosclerosis was significantly associated with reductions in the levels of both atherogenic lipoproteins and CRP [108].

Hypertension, hyperlipidemia and CRP

Studies on a large population of cardiovascular disease patients treated with either angiotensin-converting enzyme inhibitors or angiotensin type 1 receptor antagonists demonstrated some additive effects of these drugs on the cardiovascular endpoints [109-111]. These effects were unrelated to the degree of blood pressure lowering. The additivity of actions could be related partly to the blockade of the renin-angiotensin system. Interestingly, recent investigations have shown that the blockade of the renin-angiotensin system is associated with reduction of CRP levels in patients with coronary artery disease, acute myocardial infarction and stroke [112-114], suggesting a potential use for angiotensin-converting enzyme inhibitors or angiotensin type 1 receptor antagonists in atherosclerosis and its complications. This possibility is supported by the observation that the combination of renin-angiotensin system blockers and aspirin reduces nuclear factor κB activation and CRP expression within atherosclerotic plaques [115].

Both peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ agonists (fibrates and thiazolidinediones, respectively) currently used for the management of hyperlipidemic disorders or diabetes, have been shown to possess anti-inflammatory and antiatherosclerotic properties [36, 116-118]. Treatment of non-diabetic hypertensive patients with rosiglitazone (PPAR-γ agonist) was characterized by a significant decline in CRP levels, together with blood pressure reduction and improvement in insulin sensitivity [119]. Fibric acid derivatives reduced CRP levels in patients with combined hyperlipidemia [120]. Bezafibrate was also effective in reducing CRP levels in patients with severe hypertriglyceridemia and insulin resistance [121]. Furthermore, fenofibrate significantly lowered plasma concentrations of CRP in patients with coronary artery disease [122].

CRP levels can be reduced using heparin-induced extra-corporeal low-density lipoprotein precipitation (HELP)-apheresis [123]. HELP-apheresis has been effective in the treatment of the native as well as transplant-associated forms of atherosclerosis. The use of a combination of HELP-apheresis and statins significantly prevented the development of transplant associated coronary artery disease when compared to patients receiving only statin therapy [124]. Preliminary studies indicate that HELP-apheresis results in a rapid and long-term reduction of CRP levels in patients with familial hypercholesterolemia and advanced coronary artery disease [123].

D. Clinical usefulness of measuring CRP levels

Numerous reports to date support the clinical use of high-sensitivity CRP measurements as independent predictors of an increased cardiovascular risk such as ischemic coronary artery disease (angina, myocardial infarction, sudden death) and stroke. CRP levels are independently associated with risk in patients with known atherosclerotic disease, healthy individuals, and in patients with other risk factors. The levels of high-sensitivity CRP do not correlate with the degree of angiographically defined atherosclerotic disease [18] and therefore are of little value in the selection of patients for coronary artery or peripheral artery procedures. The predictive value of CRP for other cardiovascular events such as renal failure and occlusive peripheral vascular disease requires an additional study.

By evaluating genetic variations in the CRP gene, at-risk genotypes may be identified, providing additional information for overall risk assessment [125]. Baseline levels of CRP show a clear heritability of approximately 40% in family studies [126]. To-date, three polymorphisms that are associated with changes in CRP levels have been identified in the CRP gene [127-129]. Genotype-specific risk categories may identify individuals who have relatively low serum CRP levels yet display an enhanced proinflammatory phenotype. Such identification could lead to more targeted therapies directed to reduce the proinflammatory load in the individuals at risk.

There is no direct evidence to support independent treatment of CRP levels. However, measurement of high-sensitivity CRP levels is reasonable to consider in patients who develop cardiovascular complications in the absence of known cardiovascular risk factors. Although not proven, such patients may benefit from treatments that lower CRP levels. However, proof for such a therapeutic strategy requires a clinical study. An interesting but not yet tested use for high-sensitivity CRP measurement is as a means for motivating individuals with moderate to high cardiovascular risk to improve their lifesty-

les (i.e., smoking cessation, dietary modification, exercise, weight loss) or to comply with drug therapies. However, it is unclear whether CRP would add anything over that of monitoring other risk factors. Furthermore, the use of CRP as a predictor for cardiovascular disease and its complications still needs to be proved in prospective studies since a recent study showed CRP concentration was a relatively moderate predictor of risk of coronary artery disease and added only marginally to the predictive value of already established risk factors for coronary heart disease [130].

If utilized to predict the cardiovascular risk, patients should be free of known inflammatory processes and CRP levels should be measured at least twice over a period of several weeks [131, 132]. Repeated measurement provides an assurance that a subtle undiagnosed inflammatory state was not present. Levels can be measured in the fasting or non-fasting state, since they are minimally affected by food intake and demonstrate no circadian variation. Relative risk categories (low, moderate, high) based upon population studies correspond to the following CRP values: <1.0 mg/L, 1.0 to 3.0 mg/L, and >3.0 mg/L. The finding of a high relative risk level (CRP >3.0 mg/L) should lead to a complete reassessment of therapeutic strategies and drug therapies to maximally lower all cardiovascular risk factors [131-134].

Once the risk is established, there is little evidence upon which to base repeated assessment of CRP levels. Future trials are needed to determine the benefits of repeated testing of CRP levels upon cardiovascular events and other outcomes.

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