

## Review

# C-reactive protein and atherosclerosis. Is there a causal link?

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**Summary.** C-reactive protein (CRP) is a powerful cardiovascular risk marker. Evidence suggests that this may be due to its direct proatherogenic properties. Because of different biological functions of CRP in different species, an appropriate animal model for the study of its role in atherogenesis is difficult to set up. Binding to low density lipoprotein (LDL), activation of the complement system and interaction with monocyte/macrophages are rigorously defined pathogenic properties of CRP which might contribute to an active role of the molecule in human atherogenesis. Furthermore, direct effects on arterial wall cells, i.e. endothelial cells and smooth muscle cells, have been reported. The molecular basis of CRP interaction with these cells, however, remains unclear. Should CRP indeed be actively involved in human atherogenesis, the molecule may become a target for therapy. Pharmaceutical companies develop CRP-inhibitors.

**Key words:** C-reactive protein, complement, atherosclerosis

### Introduction

C-reactive protein (CRP) is the prototype acute phase protein in humans and plays an important role in innate immunity (Mortensen, 2001; Volanakis, 2001). In acute phase response, plasma CRP concentrations may rise up to 1000-fold compared to normal. CRP possesses diverse biological functions like opsonizing pathogens (Kindmark, 1971; Volanakis and Kaplan, 1971; Mortensen et al., 1976; Kindmark, 1971; Mold, 2001) or damaged cells (Martin et al., 1995; Gershov et al., 2000) and regulating inflammatory processes (Mold et al., 2002). One of the major and most rigorously defined biological functions of CRP in humans is activation of the complement system via the classical pathway

(Kaplan and Volanakis, 1974; Volanakis and Kaplan, 1974; Claus et al., 1977; Volanakis, 1982).

### CRP - a cardiovascular risk marker

CRP was established as a sensitive marker of inflammation a long time ago (Tillett and Francis, 1930). Accumulating evidence from various epidemiological prospective studies over the recent years indicates that CRP is an important marker of future cardiovascular risk (Danesh et al., 1998; 2000; Koenig et al., 1999; Speidl et al., 2002; Haidari et al., 2001; Folsom et al., 2002). If subjects are both in the top quintile of CRP and low density lipoprotein (LDL) cholesterol plasma levels their risk of a first cardiovascular event is increased up to eight- to nine-fold (Rifai and Ridker, 2001). Recently, data from a large population study (Women's Health Study) demonstrated worse survival rates for women belonging to the upper quintile of CRP plasma levels as compared to those from the upper quintile of LDL cholesterol plasma levels (Ridker et al., 2002). These data suggest that CRP plasma levels are a stronger predictor of cardiovascular events than LDL cholesterol plasma levels. The question arises whether CRP is just a cardiovascular risk marker or whether it is a cardiovascular risk factor intimately involved in the pathogenesis of atherosclerosis.

### CRP - a cardiovascular risk factor?

Data from a variety of experimental studies indicate an active role of CRP in atherogenesis. However, some of these studies are seemingly preliminary and the described effects need to be investigated in detail before the impact of such studies can be assessed properly. Furthermore, it is not yet proven in an animal model that CRP is causal in atherogenesis. It is difficult to set up an appropriate animal model as the biological functions of CRP differ from species to species.

The following paragraph summarizes the evidence for an involvement of CRP in atherogenesis and comments on the plausibility of results.

1. CRP deposition in human atherosclerotic lesions

is now well established (Torzewski et al., 1998; Zhang et al., 1999; Yashojima et al., 2001). The molecule is already detectable in the arterial intima in the earliest stages of atherogenesis and accumulates with lesion progression.

2. CRP binds to apolipoprotein-containing lipoproteins like LDL and very low density lipoprotein (VLDL). This is also well established and may be of considerable importance in the pathogenesis of atherosclerosis. Initial reports have already demonstrated binding to the native molecules (de Beer et al., 1982; Cabana et al., 1982; Pepys et al., 1985). Recently, a further investigation in the matter revealed that CRP preferentially binds to modified forms of LDL like enzymatically modified LDL (E-LDL) (Bhakdi et al., 1999) and oxidized LDL (oxLDL) (Chang et al., 2002).

3. CRP interacts with vascular cells. The proposed interactions need to be investigated in detail. This seems especially important because the molecular basis of CRP interaction with vascular cells remains largely undefined. The following effects of CRP on vascular cells have been reported:

(a) CRP interaction with monocytes/macrophages. CRP has been demonstrated to induce Tissue Factor expression and release in monocytes/macrophages (Cermak et al., 1993). Furthermore, CRP acts as a chemottractant for monocytes (Torzewski et al., 2000). This observation, in combination with the fact that CRP deposition in the arterial wall precedes the appearance of monocytes in atherogenesis, suggests that CRP may be a major monocyte chemoattractant. Importantly, soluble CRP (Zwaka et al., 2001) and immobilized CRP (Fu and Borenstajn, 2002) have been demonstrated to mediate the uptake of native LDL into macrophages. Intracellular vesicle formation and accumulation of cholesterol suggest a contribution of CRP to foam cell formation. As CRP is known to opsonize biological particles (see above), these data indicate a mechanism for foam cell formation independent from biochemical modification of LDL.

(b) CRP interaction with endothelial cells. Some reports have described effects of CRP on endothelial cells that were supposed to be atherogenic (Pasceri et al., 2000, 2001; Verma et al., 2002a,b; Devaraj et al., 2003). Dose-dependent significant expression of ICAM-1, VCAM-1 and E-selectin was detected in human umbilical vein endothelial cells (HUVEC) following CRP stimulation (Pasceri et al., 2001). Furthermore, CRP was demonstrated to induce expression of monocyte chemoattractant protein-1 (MCP-1) in HUVECs (Pasceri et al., 2001). An involvement of endothelin-1 (ET-1) and interleukin-6 (IL-6) in these CRP-mediated effects (see above) was demonstrated in saphenous vein endothelial cells (Verma et al., 2002a). CRP was also shown to inhibit nitric oxide (NO) production and stimulation of NO release via downregulation of endothelial NO synthase (eNOS) (Verma et al., 2002b). Lastly, CRP was demonstrated to increase plasminogen activator inhibitor-1 (PAI-1) in human aortic endothelial cells

(Devaraj et al., 2003). It is important to note that to date no CRP-receptors have been demonstrated to be expressed on endothelial cells. Thus, the mechanisms of CRP-interaction with endothelium remain unclear.

(c) CRP interaction with vascular smooth muscle cells (VSMCs). It has been proposed that CRP induces relaxation of human vessels independent from endothelium (Sternik et al., 2002). Vasorelaxation was attenuated using potassium channel inhibitors suggesting that CRP may exert a relaxing effect on VSMCs involving K channels. Again, it has to be noted that to date no CRP-receptors have been demonstrated to be present on VSMCs.

### **Mechanisms of CRP action in atherosclerosis**

#### *CRP activates the complement system*

Ligand-bound CRP activates the complement system via the classical pathway (Kaplan and Volanakis, 1974; Volanakis and Kaplan, 1974; Claus et al., 1977; Volanakis, 1982). It has been known for many years now that complement plays a role in atherosclerosis (Torzewski et al., 1997a,b). Animals lacking components of the complement system are protected against diet-induced atherogenesis (Geertinger and Soerensen, 1977; Schmiedt et al., 1998; Buono et al., 2002). Various complement proteins, including the terminal complement complex C5b-9, have been shown to deposit in human and animal atherosclerotic lesions (Niculescu et al., 1985; Vlaicu et al., 1985; Seifert et al., 1989; Torzewski et al., 1997a,b), but not in healthy arterial tissue. Potential complement-activating molecules have been detected in atherosclerotic lesions in recent years (Seifert et al., 1989, 1990). Given the role of CRP as a robust cardiovascular risk marker and given the fact that CRP colocalizes with activated complement fragments in atherosclerotic lesions (Torzewski et al., 1998; Yashojima et al., 2001), CRP-mediated complement activation in the arterial wall may be considered as an important pathogenic feature in human atherogenesis. It is tempting to speculate that CRP, bound to LDL or modified LDL in the arterial wall, might play a pivotal role in atherogenesis by local perpetuation of the humoral immune response via activation of the complement system.

#### *CRP interacts with phospholipids on cell membranes*

CRP-binding to cells has long been investigated. It was initially demonstrated that CRP binds to necrotic cells or damaged cell membranes only (Kushner and Kaplan, 1961), but not to normal cells. CRP was shown to bind to phosphorylcholine in the membranes of damaged cells and this process requires partial hydrolysis of the membrane phospholipids (Volanakis and Wirtz, 1979). Also, CRP was reported to bind to apoptotic cells (Gershov et al., 2000). In the light of the aforementioned reports of CRP-mediated activation of

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vascular cells (cells that are not known to express CRP-receptors), it has to be considered that CRP-induced effects in these cells are not mediated via specific receptors but may be due to an interaction of CRP with phospholipids of damaged cells in cell culture.

### *CRP interacts with cellular CRP receptors*

CRP-binding to cellular receptors has long been investigated. CRP-Rs were demonstrated to be present on various types of leukocytes: polymorphonuclear neutrophils (PMNs) (Müller and Fehr, 1986; Buchta et al., 1987); blood monocytes (Zeller et al., 1986); and lymphocytes (Mortensen et al., 1975; James et al., 1981). Whereas some reports provided evidence for specific CRP-receptors on leukocytes (Tebo and Mortensen, 1990; Zen et al., 1997) other experiments demonstrated interaction with Fc-receptors (Marnell et al., 1995; Bharadwaj et al., 1999). The high affinity IgG-receptor Fc $\gamma$ RI was identified as a low affinity CRP-receptor (Marnell et al., 1995), whereas, recently, the low affinity IgG-receptor Fc $\gamma$ RIIa was described as the major receptor for CRP (Bharadwaj et al., 1999). There are two codominantly expressed allelic variants of Fc $\gamma$ RIIa resulting in a change at amino acid position 131 displaying different affinity to IgG<sub>1</sub> and IgG<sub>2</sub>. Fc $\gamma$ RIIa-131R/R has been defined as the "high responder" form of the receptor and Fc $\gamma$ RIIa-131H/H as the "low responder" form for IgG binding. It has been reported that these two allelic variants confer distinct CRP-binding to leukocytes (Stein et al., 2000). In monocytes, CRP-binding to cells expressing Fc $\gamma$ RIIa-131H/H was hardly detectable whereas significant binding has been demonstrated for Fc $\gamma$ RIIa-131R/H and Fc $\gamma$ RIIa-131R/R. However, as the initial reports (Bharadwaj et al., 1999; Stein et al., 2000) used anti-CRP antibodies in order to demonstrate CRP-binding to Fc $\gamma$ RIIa it was suggested that detection of CRP-binding to Fc $\gamma$ RIIa results from interaction of the Fc-part of the anti-CRP antibodies with Fc $\gamma$ RIIa itself (Saeland et al., 2001). In experiments using F(ab')<sub>2</sub> fragments of anti-CRP antibodies (Clone CRP 8, Sigma), no CRP-binding to Fc $\gamma$ RIIa-R131 was found on polymorphonuclear leukocytes and Fc $\gamma$ RIIa-transfected IIA.6 cells. Other authors proposed that the observation of CRP-binding to Fc $\gamma$ RIIa might be due to IgG-contamination of the CRP reagent used in the binding studies (Hundt et al., 2001). Finally, Ca-dependent high affinity CRP binding to the extracellular portion of Fc $\gamma$ RI has recently been demonstrated using surface plasmon resonance (BIAcore<sup>®</sup> system) (Bodman-Smith et al., 2002). This observation again contradicts previous results of low affinity CRP-binding to Fc $\gamma$ RI expressed on COS-7 cells (Marnell et al., 1995). Thus, the data surrounding the area of CRP binding to cellular receptors are confusing and the question of the major cellular CRP-receptor has not yet been resolved. In the light of increasing evidence for an involvement of CRP in the pathogenesis of atherosclerosis the identification of the major CRP

receptor and its consecutive blockage might be of therapeutic relevance.

In summary, a causal role for CRP in atherogenesis is most likely but not yet proven. CRP may be a target for therapy. Potential therapeutic strategies involve lowering CRP plasma levels by direct inhibition of CRP synthesis in hepatocytes, interfering with CRP-mediated complement activation or blockage of cellular CRP-receptors. Pharmaceutical companies develop CRP-inhibitors.

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