Review

C-reactive protein in atherosclerosis: A causal factor?

Elaine Paffen a, Moniek P.M. deMaat b,*

aHemostasis and Thrombosis Research Centre, Dept. of Hematology, Leiden University Medical Centre, Leiden, The Netherlands
bDepartment of Hematology, Room Ee 13.93, Erasmus University Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

Received 3 August 2005; received in revised form 3 March 2006; accepted 6 March 2006
Available online 13 March 2006
Time for primary review 28 days

Abstract

Atherosclerosis is considered a to be multifactorial disease driven by inflammatory reactions. The process of inflammation also contributes to the pathogenesis of acute atherothrombotic events. C-reactive protein (CRP) is an acute phase protein and its concentration in serum reflects the inflammatory condition of the patient. Levels of CRP are consistently associated with cardiovascular disease (CVD) and predict myocardial infarctions and stroke. Since CRP is present in the atherosclerotic lesion, it may actively contribute to the progression and/or instability of the atherosclerotic plaque. The role of CRP in inflammation and its causality in atherosclerosis are the subject of many investigations but are not yet fully elucidated. This review focuses on recently identified mechanisms by which CRP may modulate and evolve the process of atherosclerosis. We discuss the function of CRP and review the most recent evidence for an independent role of CRP in the development of atherosclerosis. Many studies suggest such a role, but a number of the described effects may be the result of contamination of the CRP preparations.

© 2006 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: C-reactive protein; Azide; Atherosclerosis; Inflammation; Thrombosis

1. Introduction

Inflammation plays a key role in the pathogenesis of cardiovascular disease (CVD), acute atherothrombotic events and atherosclerosis [1,2]. Inflammation also regulates the production of the acute phase proteins such as C-reactive protein (CRP), fibrinogen and serum amyloid A [3,4].

The serum concentration of CRP can increase >1000-fold upon inflammation and, with a half life of 19h, CRP is a very stable downstream marker of the inflammatory process [5]. Because CRP is such a sensitive indicator of the inflammatory process, it has been extensively studied whether plasma concentrations of CRP and other circulating inflammatory proteins (e.g. fibrinogen, interleukin-6) have a predictive value in the pathogenesis of CVD. Many clinical and population studies, with cross-sectional and nested case-control designs, proved these inflammatory mediators to be predictors of CVD [6–9].

Several reports describe human CRP to be involved in the induction of ischemic tissue damage in the brain, myocardial infarction and the increase of stroke volume in rats [10].

Most clinical studies report that CRP is an independent predictor of risk of atherosclerosis [11], cardiovascular events [5], atherothrombosis [12], hypertension [13] and myocardial infarction [14], even after considering other cardiovascular risk factors such as age, smoking, obesity, diabetes, hypercholesterolemia and hypertension. However, absence of a relationship between CRP and risk of myocardial infarction has been reported as well, especially after comprehensive adjustment for established risk factors [15].

An early indication that CRP may be more than just a risk marker was the observation that, of several inflamma-
tory markers studied (such as P-selectin, interleukin-6, interleukin-1, tumor necrosis factor-α (TNFα), soluble intercellular adhesion molecule-1 (sICAM-1), fibrinogen), CRP emerged as the most powerful inflammatory predictor of future cardiovascular risk [16,17].

Assuming that, in the physiological condition of human pathology and the atherosclerotic lesion, CRP exerts direct functions, the question is whether it is possible to discriminate between the direct effects of CRP and the parallel presence of other factors that determine the risk of CVD. It was recently observed that several of the biological effects contributed to CRP in vitro are in fact caused by contamination of the CRP preparation by azide or bacterial lipopolysaccharides [18,19]. However, in other studies a contribution of contamination can be excluded, indicating that a causal role of CRP is clearly present. Examples are the recent ex vivo and in vivo studies where CRP was administered to healthy volunteers [20] and the in vitro experiments on the effect of CRP on tissue plasminogen activator activity, interleukin-1β and tumor necrosis factor-α in human aortic endothelial cells [21]. The functional effects of CRP that may be relevant for the development of CVD and will be discussed in this review.

2. Components of atherosclerotic lesions

The first step in the development of an atherosclerotic plaque and the resulting local inflammatory process is endothelial dysfunction. Upon injury, endothelial cells (ECs) express the vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and the endothelial leukocyte adhesion molecule-1 (ELAM) on the cell surface [22]. Leukocytes, especially T-lymphocytes (CD8+ cytotoxic T-cells, CD4+ help Th1/Th2-cells) and monocytes, are then recruited from the blood and cross the endothelial cell barrier via a process called diapedesis. Lipoprotein particles (LDLs and variants such as oxidized LDL) accumulate in the lesion, are taken up by monocyte-derived macrophages, which as a result develop into foam cells, and form a fatty streak. Smooth muscle cells (SMCs) proliferate and extracellular matrix components extend to form a fibrous cap, enclosing and defining the morphology of the atherosclerotic lesion [23].

The cells involved in formation of the atherosclerotic plaque (e.g. ECs, monocytes, T-cells, SMCs) are stimulated to produce many different substances, such as inflammatory mediators (interleukin-6, tumor necrosis factor-α, interleukin-1) [24], complement factors (C1q, C3, C5–C9) [25,26], chemokines (monocyte chemoattractant protein-1, interleukin-8), adhesive molecules (selectins P/E, integrins CD18/CD11) [27], metalloproteinases (MMP-1/9) [28], collagenases, reactive oxygen species (such as nitric oxide (NO)) and CRP [29]. These mediators contribute to the development, instability and eventual rupture of the plaque [30].

3. CRP

CRP is one of the substances present in the atherosclerotic lesion, more specifically in the vascular intima, where it co-localizes with monocytes, monocyte-derived macrophages and lipoproteins [31,32]. This localization makes a direct contribution to the atherosclerotic process possible.

CRP is a phylogenetically highly conserved plasma protein, with homologues in vertebrates and many invertebrates, that is part of the systemic response to inflammation [5]. It is an acute phase protein and a member of the family of pentraxins. CRP was originally observed in 1930 in the plasma of patients with acute infections, where it reacted with the C polysaccharide of pneumococcus [33].

The major part of the CRP present in the plasma comes from the liver, where the synthesis of CRP is mainly regulated by interleukin-6, which in turn is upregulated by other inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α [34]. Small amounts of CRP can also be produced locally [35]. For example, CRP has been detected on the surface of about 4% of normal blood lymphocytes and it has been demonstrated that this CRP is produced by the lymphocytes themselves [36]. CRP also can be produced locally in atherosclerotic lesions by SMCs [29] and monocyctic cells [37].

The structure of CRP is important for its stability and for the execution of its function [38,39]. CRP is composed of five identical, 15,000Da subunits. Upon dissociation of its pentameric structure, CRP subunits undergo a spontaneous and irreversible conformational change. The loss of the pentameric structure of CRP results in modified or monomeric CRP (mCRP), which is a naturally occurring form of CRP and it is a tissue-based rather than a serum-based molecule [40]. mCRP is less soluble than CRP and tends to aggregate, and it has been described to induce mRNA of chemokines and the expression of adhesion molecules in human cultured coronary artery endothelial cells (HCAECs) [41]. Thus, next to circulating native pentameric CRP, mCRP can also promote a pre-inflammatory phenotype and exert atherogenic effects in human endothelial cells, although it may be in less potent manner than native CRP [42]. In ApoE(−/−) mice, mCRP has been described to have opposite effects on atherosclerosis compared to normal CRP [43]. These data may explain in part the conflicting activities previously reported for CRP in models of atherogenesis.

4. Functionality of CRP and purity of CRP preparations

The assumption that CRP is a causal factor in the development of the atherosclerotic lesion is based on its
rapid accessibility to the plaque, localization in the plaque and the results of in vitro studies, in which CRP has been demonstrated to actively contribute to inflammatory processes.

The involvement of CRP in several mechanisms executing and maintaining the inflammatory process has been widely studied with the use of different commercially human CRP preparations. These CRP preparations differ in source (serum, plasma, recombinant) and quality.

Use of commercially CRP preparations introduces two means of interference: 1) by contamination with bacterial lipopolysaccharides (LPS) or 2) by contamination with the preservative sodium azide (NaN₃).

Presence of endotoxin in commercially available CRP preparations has been eloquently described [44] and we ourselves measured concentrations between 3 and 600 pg endotoxin/100 μg CRP in different purified and recombinant CRP preparations (with the Limulus Ameocyte test which is endotoxin-positive > 10 pg = 0.12 EU endotoxin present; unpublished data 2003). The presence of even very low concentrations of endotoxin can interact with the contents of the vessel wall [45], activate gene expression and a cascade of reactions in monocytes [46], activate procoagulant activity in monocytes and macrophages [47,48] through induction of tissue factor [49]. Endotoxins trigger an atherogenic response in SMCs and block the induction of secretion of interleukin-1 and MCP-1 by human endothelial cells [50].

The amount of sodium azide present in different commercially CRP preparations, varies from 0.05% to 1.00% of the CRP present. Recent studies describe an effect of this preservative, when tested in vitro, on several processes involved in atherosclerotic development, which were formerly subscribed to CRP. Addition of sodium azide, in amounts comparable to its concentration as a preservative, to cultures of endothelial cells resulted in a decrease of migration, proliferation and angiogenic properties of these cells [18]. In SMCs, addition of sodium azide (without CRP) did induce vasorelaxation [51] and evoked inducible nitric oxide synthase (iNOS) induction and nitric monoxide (NO) release. These effects were initially ascribed to the CRP. It is not clear yet how many of the reported effects of CRP can be ascribed to contamination and whether the effects can be explained by contamination completely or whether there will simultaneous effects of contaminants and CRP. However, several studies showed direct effects of CRP preparations that were free of contaminants, showing that CRP may indeed have a direct role. Examples are studies comparing the effect of plasma from CRP transgenic mice and wild-type mice [21] or studies in which the effect of CRP containing a sodium azide contamination exceeds the effect of sodium azide alone [52], which deliver strong evidence of CRP having a direct and causal effect on several cell types and the inflammatory processes. Furthermore, the specific interaction of CRP with complement factors, cell receptors, lipids and other inflammatory mediators assures the possibility of CRP being directly involved atherosclerosis. Therefore, in this review, we will discuss the possible direct roles of CRP in atherosclerosis.

5. Effects of CRP on atherosclerosis

Inflammatory mechanisms play a central role in all phases of atherosclerosis, from the initial recruitment of circulating leukocytes to the arterial wall to the rupture of unstable plaques, which results in the clinical manifestations of the disease. CRP may be involved in each of these stages by direct influencing processes like complement activation, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation and thrombosis. Each of these processes offers several mechanisms by which CRP may influence its progress, as will be described in more detail below.

5.1. Complement activation

Activation of the classical pathway of the complement system is a well known and direct biological function of CRP [53]. Via this action, CRP directly amplifies and facilitates innate immunity [31,54], a process that has already been associated with initiation and progression of CVD for a long time. In situ hybridization showed intense mRNA signals for CRP and complement component C4 in SMCs and macrophages present in the thickened intima of the lesion. CRP also co-localizes with C5–C9, the membrane attack complex, of complement [37]. Activation of this membrane attack complex (MAC) is initiated by the direct binding of CRP to C1q, also present in the atherosclerotic lesion [54], and characterized by elevated levels of component C5a [55]. C5a itself exerts potent chemotactic and pro-inflammatory effects and its plasma levels have been associated with increased cardiovascular risk in patients with advanced atherosclerosis [26].

CRP is also involved in the inhibition of complement activation through interaction with factor H (fH), which is also present in injured areas. The CRP–fH complex interferes with the activity of C3b (see Fig. 1) [56], and thus will prevent formation of the MAC.

Through the interaction with complement factors, CRP exerts a direct effect on arterial endothelial cells, by increasing the expression of complement inhibitory factors on the endothelial cells. This suggests that CRP-mediated complement activation is a system set to regulate the inflammatory reaction, because it will result in promoting the removal of debris from tissues and the deleterious effects of complement activation in patients with CVD [57]. However, since complement activation also leads to the production of a variety of pro-inflammatory molecules, this mechanism of CRP-mediated complement regulation might also aggravate the inflammatory status in the entire body as well as in the atherosclerotic plaque. Therefore, the direct interaction between CRP and complement can both activate and inhibit inflammation in atherosclerotic lesions.
5.2. Interaction with cell surface receptors

The close proximity of CRP to monocytic cells [58,59] in the arterial intima, attenuates its possibilities for a direct contribution to the progression of atherosclerosis. The observation that CRP is localized between monocytes underlines the possibility of a direct interaction of CRP with these cells and with monocyte-derived macrophages via binding to a specific receptor. CRP binds to several receptors on human monocytes; to FcR\(_{\text{gIIa}}\) (CD32) with high affinity and to FcR\(_{\text{gI}}\) (CD64) with lower affinity [60], increasing phagocytosis and the release of inflammatory cytokines [61]. The Fc receptors have been described to mediate the effect of CRP on human aortic endothelial cells [62]. FcR\(_{\text{gIIa}}\) is known as the putative CRP receptor for leukocytes [63] and also has been found on bovine aortic endothelial cells [64]. CRP also binds to the inhibitory receptor, FcgammaRIIb, blocking activating signals [61]. The binding of CRP to a receptor suggests its capacity to induce a specific biological effect [42], such as direct involvement in cell-mediation and opsonization. Addition of CRP with and without anti-CD32-antibody to endothelial cells demonstrated the partial mediation of CRP in regulation of cell surface protein expression, such as the endothelial protein C receptor, by CD32 [65]. However, the downstream effects of CRP binding have not yet been elucidated. The interaction of CRP with CD36, a scavenger receptor which is expressed by macrophages and is involved in uptake of low-density lipoprotein particles (LDL), demonstrates a direct role of CRP through its interference with the binding of LDL to CD36 [32].

5.3. Thrombosis

Thrombosis contributes to the progression of the atherosclerotic lesion and to the precipitation of the cardiovascular event. Direct actions of CRP which contribute to the induction of a prothrombotic state may be the enhancement of the procoagulant activity [66,67] or the reduction of fibrinolysis [68,69]. CRP has been suggested to induce a prothrombotic state via induction of tissue factor expression in human monocytes [70,71], but only in the presence of and through direct interaction with other blood cells as T-lymphocytes, B-lymphocytes and natural killer cells [72].

In transgenic mice expressing human CRP (hCRP), the injury-induced occlusion of the femoral artery (75% after 28 days) was enhanced compared to the amount of occlusion observed in wild-type mice (17% after 28 days) [73] indicating a prothrombotic effect of hCRP. A direct effect of CRP on hemostasis was shown in a recent study, where recombinant human CRP was infused into human volunteers, which resulted in the stimulation of both hemostasis and inflammation [20].

CRP may also inhibit fibrinolysis by increasing the expression and activity of the main inhibitor of fibrinolysis, plasminogen activator inhibitor-1 (PAI-1) in human aortic endothelial cells (HAEC) [74]. Since PAI-1 promotes atherothrombosis and progression of acute coronary syn-

---

Fig. 1. Schematic representation of CRP-mediated complement regulation. Binding of CRP to microbial polysaccharides or ligands exposed on damaged activates the classical pathway of complement. Activation is however limited to C1, C4, C2 and C3 with little consumption of C5–C9. Surface-bound CRP recruits fH, which regulates complement activation at the level of the alternate pathway C3 convertase and C5 convertase of both the classical and alternate pathways. fH is therefore thought to be responsible for low consumption of terminal pathway components during the CRP-initiated complement activation (adapted from Giannakis et al. [56]). fH: factor H, MBL: mannose-binding lectin pathway of complement, MAC: membrane attack complex.
dromes, this effect of CRP may also affect CVD [75]. Also, besides this effect on PAI-1, recently CRP has been demonstrated to directly decrease antigen levels and the activity of tissue plasminogen activator (tPa) in HAEC. tPa is the substance normally inhibited by PAI-1. In this study, the direct and specific role of CRP is demonstrated by using CRP that was free from sodium azide and LPS contamination [21].

5.4. Cellular modulation, recruitment and activation

CRP contributes to an arterial pro-inflammatory and pro-atherosclerotic phenotype by directly upregulating adhesion molecules and chemoattractant chemokines in endothelial cells, vascular SMCs and monocytes. On the endothelial cell surface, expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin is upregulated by CRP [76]. Via these processes, CRP induces platelet adhesion to endothelial cells [52], CRP stimulates endothelial cell dysfunction and the recruitment of monocytes and T-lymphocytes towards the endothelial wall. These findings were reported by several groups, who also showed that CRP induced monocyte chemoattractant chemokine-1 (MCP-1) production. This upregulation of adhesion molecules is partly mediated via the production of endothelin-1, a potent endothelium-derived vasoactive factor, and by the production of the inflammatory cytokines interleukin-6 and interleukin-8. As to the effects of CRP on MCP-1 expression, aortic endothelial cells seem to be unresponsive whereas venous endothelial cells [77] or monocytes [78] show increased expression of this chemoattractant. Since atherosclerosis mainly develops in the arteries, the clinical significance of the effect of CRP on venous cells is not clear.

CRP is also known to activate the NF-κB signaling pathway in saphenous vein endothelial cells [79], which, in recent light of possible azide contamination [18], might be an artefact. Also in vascular SMCs, CRP has been indicated to activate NF-κB [80]. Therefore, CRP has been suggested to mediate proliferation and activation of vascular SMCs, causing the accumulation of these cells in the vascular intima, which is a key event in the development of arterial lesions. Another manner in CRP directly affects the activation and proliferation of vascular SMCs, via upregulation of mRNA and protein and increased cell surface expression of the angiotensin type 1 receptor (AT1-R). This was demonstrated in vitro in human vascular SMCs and in vivo in a rat carotid artery angioplasty model [81].

CRP also appears to be involved in the infiltration of monocytes into the vessel wall and their subsequent development into foam cells. The deposition of CRP in the arterial wall precedes monocyte infiltration and direct involvement of CRP in recruitment of blood monocytes has been demonstrated in vitro, suggesting CRP to be chemotactic for human blood monocytes [59]. CRP also promotes MCP-1 mediated chemotaxis through upregulation of CC chemokine receptor 2 expression in human monocytes [82].

The effect of CRP on T-lymphocytes is indirect. T-lymphocytes are recruited to the atherosclerotic lesion as a result of the ongoing inflammatory process. Through the stimulation of cytokine production and secretion by macrophages, CRP exerts an indirect effect on T-lymphocytes present in the atherosclerotic lesion. CRP induces macrophages to express interleukin-12, which contributes to the development of CD4⁺ T-helper cells [83]. In turn, these cells express interferon-γ, which is synergistic with CRP in the execution of many functions contributing to the pro-atherosclerotic phenotype. In contrast, during CRP induced activation of complement and opsonization of apoptotic cells, the actively phagocytic macrophages reduce expression of IL-12 and thereby suppress T-lymphocytes [84].

5.5. Expression of inflammatory mediators: cytokines, chemokines and adhesive molecules

CRP induces inflammatory cytokines in a dose-dependent way [85], which provides further support for the hypothesis that interaction with mononuclear phagocytes constitutes an important biological role for this acute phase protein. Quantitative analysis of the CRP-induced release of interleukin-6, interleukin-1 and tumor necrosis factor-α by freshly isolated normal human monocytes, revealed slight differences in time courses. All three cytokines were detected 4h after CRP addition in vitro, with maximal levels of TNFα at 8h and of interleukin-1 and interleukin-6 at 16h [86].

Interleukin-8 (IL-8), a member of the CXC chemokines promotes monocyte–endothelial cell adhesion and arrest and is abundant in atherosclerotic plaques. In human aortic endothelial cells in vitro, CRP increases IL-8 protein and mRNA expression in a time- and dose-dependent manner via specific upregulation of NF-κB activity [87].

CRP induces production and secretion of MCP-1 in human umbilical vein endothelial cells [77], but not in aortic endothelial cells [87]. MCP-1 present in the atherosclerotic lesion [88] can also originate from monocytes. CRP induces a 7-fold increase in the production of monocyte MCP-1 in purified peripheral monocytes [72]. In patients with acute coronary syndromes, baseline level of this chemoattractant was elevated. This elevated expression is associated with both traditional risk factors for atherosclerosis as well as increased risk of myocardial infarction, independent of baseline variables target [89].

In atherosclerotic lesions, CRP directly upregulates mRNA expression of the macrophage markers CD11b and HLA-DR, as well as their protein products [37].

Monocyte expression of CD11b increased significantly up to twofold when exposed to CRP, while no significant difference in CD32 expression was observed, whereas CRP exposure decreases CD31 expression. CRP can affect
monocyte activation ex vivo and induce phenotypic changes that result in an altered recruitment to endothelial cells [90].

Another mechanism by which CRP influences the development and maintenance of the atherosclerotic lesion is its involvement in the CD40–CD40Ligand (CD40L or CD154) interaction. CD40L, a 33-kDa activation-induced T-lymphocyte surface glycoprotein, binds to CD40, a phosphorylated glycoprotein expressed on B-lymphocytes, vascular endothelial cells, monocytes, macrophages and fibroblasts. Like CRP, the amount of soluble CD40 increases during inflammation and in the atherosclerotic lesion. Therefore CD40L has been suggested to be a marker for inflammation and involved in risk of cardiovascular events as well [91]. CRP upregulates the cell surface expression of CD40 and CD40L on human umbilical endothelial cells in time [92]. CD40L is shed into the vasculature. Elevated levels of this soluble CD40L (sCD40L) identify patients with acute coronary syndromes at increased risk of recurrent MI and death, independent of other variables as cardiac troponine T or CRP [93].

5.6. Nitric oxide expression

CRP has been described to decrease the expression and bioactivity of endothelial nitric oxide synthase (eNOS or NOS3) [94], which results in reduced bioavailability of nitric monoxide (NO) and a subsequent effect of vasodilatation. It was demonstrated recently that this effect can be caused by sodium azide as well [95]; however, it is not clear whether there remains a role for CRP. It is therefore uncertain whether there is a causal role for CRP in the regulation of expression of NO and involvement in vascular reactivity [96]. Nevertheless, CRP has been suggested to exert a specific effect on endothelial eNOS expression through binding to the CRP receptor FcRγIIa [64]. In HAEcs and human coronary artery endothelial cells (HCAEcs), CRP contributes to a proatherogenic and prothrombotic state by decreasing the release of NO and of the vasodilator and inhibitor of platelet aggregation prostacyclin (PGI2), through directly increasing both superoxide and inducible NO synthase [97].

In vascular smooth muscle cells, CRP reduces expression of the inducible variant of nitric oxide synthase (iNOS) [98] and subsequent NO-synthesis as well. But again, this might be an artefact caused by sodium azide [99]. In these vascular SMCs, CRP also has been described to induce activation of the iNOS promoter [80], which, despite the fact that CRP seems to be no more than a weak inducer of NO-production, contradicts the study of Ikeda and coworkers. Nevertheless, both studies demonstrate that CRP is likely to be involved in the regulation of cellular NO-levels. Furthermore, interaction between CRP and interferon-gamma appears to enhance the effect of CRP on NO-regulation, which indicates that there may be a direct effect of CRP because contaminants will not be able to exert these specific interactions [80].

5.7. Apoptosis

CRP is directly involved in the process of apoptosis [100]. It binds to apoptotic cells in a Ca²⁺-dependent manner and augments the classical pathway of complement activation but protects the cells from assembling the terminal complement components (C5–C9). Furthermore, CRP enhances opsonization and phagocytosis of apoptotic cells by macrophages associated with the expression of the anti-inflammatory cytokine transforming growth factor-β. CRP and the classical complement components act in concert to promote non-inflammatory clearance of apoptotic cells [101]. The inhibitory effect of CRP on the NO expression of endothelial progenitor cells directly inhibits their mobilization and differentiation, survival and function, whereby it facilitates EC apoptosis and blocks the process of angiogenesis [39]. Apoptosis of vascular SMCs also plays an important role in progression of atherosclerotic lesions and contributes to increased plaque vulnerability. Silencing the CRP-regulated GADD153 gene in vascular SMCs indicated that CRP plays an essential role in induced apoptosis of vascular SMCs [100].

CRP also binds to phosphatidylcholines, by which it participates directly in activation of macrophages and neutrophils in the clearance of apoptotic and necrotic cells [102,103]. However, neutrophils are not present in the atherosclerotic plaque [23].

5.8. Lipids

The interaction between lipids and CRP is diverse. It has been suggested that CRP could be the factor that links lipoprotein-deposition and complement activation in atherosclerotic plaques. Binding of tissue-deposited CRP to enzymatically degraded LDL enhances complement activation, which may be relevant to the development and progression of the atherosclerotic lesion, particularly at early stages of atherosclerosis when low concentrations of enzymatically degraded LDL are present [31]. And, although direct involvement of CRP has not been demonstrated, through this binding of CRP to enzymatically degraded LDL, CRP may be involved in the massive release of MCP-1 from macrophages described to be caused by enzymatically degraded LDL [104].

Although the reports on interaction between CRP and ox-LDL are conflicting, complement activation as a result of this interaction is generally considered unlikely. Nevertheless, CRP has been described to directly induce lectin-like ox-LDL receptor-1 expression (LOX-1) in human aortic ECs, because this could be reduced with antibodies against CD32/CD64, ET-1 or IL-6 [105]. Via LOX-1, CRP is suggested to regulate monocytes adhesion to ECs and uptake of ox-LDL by ECs.

The majority of sub-endothelial foam cells show positive staining for CRP. Zwaka et al. demonstrated that native LDL that was co-incubated with CRP was taken up
by macrophages via macroinocytosis. It was concluded that foam cell formation in human atherogenesis might be caused in part by uptake of CRP-opsonized native LDL [32].

High levels of high-density lipoprotein (HDL) are atheroprotective since HDL is involved in transporting cholesterol from the periphery to the liver. HDL might also protect the endothelium since the CRP-induced upregulation of inflammatory adhesion molecules in HUVECs was completely blocked by HDL. So, HDL neutralizes CRP induced proinflammatory activity [106]. HDL also inhibits atherosclerosis through prevention of oxidation of LDL. It is not known whether CRP has an effect on the oxidative status of LDL.

6. Discussion and conclusions

CRP may contribute to development of the atherosclerotic lesion and the subsequent acute cardiovascular events via its role in a large number of biological pathways. And although a number of the effects of CRP may be clouded by contamination, we demonstrated that a direct role of CRP in many inflammatory processes is probable. We reviewed the roles of CRP in complement activation, cell adhesion and recruitment, thrombosis, the expression of regulatory cytokines, apoptosis and lipids. All these mechanisms are part of or are compromised by the process of inflammation. CRP may thus contribute to the development of the atherosclerotic lesion via a direct pro-inflammatory effect. CRP increases the release of endothelin-1 and upregulates adhesion molecules and chemotactic chemokines in endothelial cells and vascular SMC. Most studies focus on the effects of CRP on aortic endothelial cells but a few studies have also determined the effect on venous endothelial cells as HUVECs and it was observed that CRP induced the expression of MCP-1 [77], CD40 [92] and increased activity of NF-κB [106]. This suggests that CRP exerts different and specific effect on different cell types.

However, it is important to realize that CRP also exerts activity in anti-inflammatory or so-called protective mechanisms, such as suppressing the formation of the C5–C9 complex. Thereby CRP is capable of maintaining a certain balance in the inflammatory process and stability of the atherosclerotic lesion. In the lesion, CRP, which stimulates immunity as well as inflammation, contributes to the prolongation of the stability of the plaque.

A low level of chronic inflammation is represented by CRP levels that are only slightly, but for a prolonged period, increased and these levels characterize CRP as a predictor of a cardiovascular event. On the other hand, a high concentration of CRP (>10mg/L) can be measured for a shorter period during the acute phase where it is involved in the inflammatory defense process. It has been suggested that, because of this expression of high levels of CRP, a direct biological or a causal function in the atherosclerotic process was not possible [107], but we discussed above that one thing does not exclude the other.

Recently, several studies have reported conflicting results on the direct contribution of CRP to atherosclerosis in mice that transgenically expressed CRP. Since CRP is expressed only at a very low concentration in mice and does not show an acute phase behavior, it is possible to study the role of transgenic CRP in mice. The first report was from Paul and coworkers who observed larger aortic atherosclerotic lesions in human CRP transgenic apolipoprotein (apo) E-knockout mice than in control mice [108], but in this study the CRP levels were very high. Recently, we did not see a difference in lesion size or severity of atherosclerosis at the aortic root between apoE*3-Leiden mice that did express human CRP (<10mg/L) and controls [109]. Reifenberg and coworkers [110] reported similar observations in apoE-knockout mice that expressed rabbit CRP and subsequently discussed whether transgenic apoE-knockout mice would provide an answer about the role of CRP in atherogenesis at all. In our opinion, we believe that more studies are needed to elucidate the differences, and we cannot ignore previous studies in which mouse models were used to clearly demonstrate a direct role of CRP in atherogenesis [73].

In conclusion, it is now an established fact that elevated CRP levels are associated with a worse prognosis for CVD, such as myocardial infarction, stroke and unstable angina. But it is important to distinguish between a role as a marker and a factor that directly causes a biological effect because this will determine the optimal therapeutic intervention.

CRP may be a causal factor as well as a marker for inflammation, depending on the concentration. This concentration of CRP depends on the rates of production and clearance. The fact that CRP is a very stable protein, which is not consumed to a significant extent in any process, and the clearance of which is not influenced by any known condition [111], is in agreement with its functioning as a causal factor, attempting to prolong the stability of the atherosclerotic lesion.

In the atherosclerotic lesion, many processes involving CRP and many different cell types have been described but there is also concern that part of the observed effects are not mediated by CRP itself, but are the result of contamination of the preparations with endotoxin or azide. Therefore, currently the exact role of CRP in the initiation and progression of atherosclerosis is still unclear and needs to be further studied.

Acknowledgement

We thank Prof. R. M. Bertina for critical reading of the manuscript.

References


aortic endothelial cells than modified C-reactive protein, Atherosclerosis; in press, Corrected Proof, Available online 13 May 2005.


[66] Penn MS, Topol EJ. Tissue factor, the emerging link between inflammation, thrombosis, and vascular remodeling. Circ Res 2001; 89:1–2.


