Activation of Inflammation and Coagulation After Infusion of C-Reactive Protein in Humans

Radjesh J. Bisendial, John J.P. Kastelein, Johannes H.M. Levels, Jaap J. Zwagina, Bas van den Bogaard, Pieter H. Reitsma, Joost C.M. Meijers, Daniel Hartman, Marcel Levi, Erik S.G. Stroes

C-reactive protein (CRP) has been postulated to play a causal part in atherosclerosis and its acute complications. We assessed the effects of CRP-infusion on coagulation and inflammatory pathways to determine its role in atherothrombotic disease. Seven male volunteers received an infusion on two occasions, containing 1.25 mg/kg recombinant human CRP (rhCRP) or diluent, respectively. CRP-concentrations rose after rhCRP-infusion from 1.9 (0.3 to 8.5) to 23.9 (20.5 to 28.1) mg/L, and subsequently both inflammation and coagulation were activated. This sequence of events suggests that CRP is not only a well known marker of cardiovascular disease, but is also probably a mediator of atherothrombotic disease.

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C-reactive protein (CRP) has emerged as an independent predictor of cardiovascular risk in various clinical settings. Evidence showing direct prothrombotic and inflammatory effects of CRP in vitro has led to the concept that CRP might be an active mediator of atherothrombotic events. Although direct pathophysiological functions of CRP itself are a matter of debate, experimental observations from mice transgenic for human CRP have suggested a contributive role of CRP in the development of cardiovascular complications. To date, no in vivo data in humans exist to support a contributive role. Transgenic for human CRP have suggested a contributive role. To date, no in vivo data in humans exist to support a contributive role. Experimental observations from mice transgenic for human CRP have suggested a contributive role. To date, no in vivo data in humans exist to support a contributive role. To date, no in vivo data in humans exist to support a contributive role. To date, no in vivo data in humans exist to support a contributive role.

Materials and Methods

Seven healthy, nonsmoking men, aged 33 (26 to 51) years, were included in this study after written informed consent was obtained.

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CRP-concentrations rose on infusion of rhCRP from 1.9 mg/L (0.3 to 8.5) to 23.9 mg/L (20.5 to 28.1). After an initial fall, concentrations more than four times higher than peak concentrations were observed in humans, we observed no direct effects of the purified rhCRP solution on temperature, blood pressure, or heart rate. In analogy to the findings in humans, a minor cytokine response was observed on purified rhCRP infusion (data not shown). The rhCRP was stored in a CaCl₂-containing buffer (pH 8.5) at 0 to 4°C, and all experiments were performed within 4 weeks after rhCRP preparation.

CRP concentrations were measured with high-sensitivity and immunonephelometric assays (Roche Diagnostics Corporation). Tumor necrosis factor-α (TNF-α), IL-6, and IL-8 were assayed by cytokometric bead array analysis (BD Biosciences). We measured concentrations of soluble E-selectin, (R&D Systems), von Willebrand factor (vWF:Ag; Dako), prothrombin F1+2 (Dade Behring), plasminogen activator inhibitor type-1 (Monozyme), and D-dimer (Asserachrom D-dimer, Roche) using ELISAs. Additionally, serum amyloid A protein (SAA; Anogen) and type II secretory phospholipase A₂ (sPLA₂; CLB) were measured with this technique. Monocytic expression of CD11b and CD14 was quantified using a fluorescence-activated cell sorter Vantage flow cytometer (Becton Dickinson) at baseline, and at 4 and 24 hours after infusion. Monocytes were gated by their specific forward and side-scatter pattern and further identified by high CD14 expression.

Data are medians and ranges. Differences between treatment groups were tested by analysis of variance for repeated measures. Comparisons within groups were done with the Wilcoxon signed rank test. A probability value less than 0.05 was regarded significant.
ng/mL (19.1 to 69.0) to 67.7 ng/mL (25.8 to 115.7) ($P < 0.05$), respectively. After 4 hours, IL-6 increased significantly from less than 1.6 pg/mL (<1.6 to 14.7) to 99.6 pg/mL (5.0 to 709.5, $P < 0.05$ versus baseline) and so did IL-8 from 14.1 pg/mL (6.4 to 29.2) to 106.0 pg/mL (37.7 to 243.0; $P < 0.05$) (Figure 1). TNF-α concentrations remained unaltered; furthermore, a trend toward monocytic CD11b and CD18 upregulation was recorded. After 8 hours, both serum amyloid A protein [4.7 mg/L (0.3 to 27.9) to 206.2 mg/L (27.8 to 2099.2), $P < 0.05$ versus baseline] and sPLA$_2$ [2.0 ng/mL (2.0 to 6.0) to 22.0 ng/mL (9.0 to 60.0), $P < 0.05$] concentrations rose significantly (Figure 1).

Coagulation activation was associated with a 3-fold increase in prothrombin F1 +2 ng/mL concentrations 4 hours after rhCRP-infusion ($P < 0.05$), and a 3.5-fold increase of D-dimer concentrations ($P < 0.05$) (Figure 2). Additionally, plasminogen activator inhibitor type-1 was significantly enhanced [35.0 ng/mL (14.0 to 109.0) to 71.0 ng/mL (18.0 to 83.0); $P < 0.05$].

**Discussion**

Our findings strongly suggest that CRP activates several pathways with known consequences for cardiovascular events. Even a short-term increase from a single bolus, obtaining concentrations that are pathophysiologically relevant, induces endothelial cell activation, elicits an acute systemic inflammatory response, and activates the coagulation cascade. This striking sequence of events indicates that CRP, beyond its predictive value, probably also has a causal relation to the occurrence of cardiovascular events.

Although CRP concentrations greater than 3 mg/L already denote heightened cardiovascular risk, patients are usually exposed to these raised concentrations for many years before potential onset of cardiovascular events. Of note, mechanisms behind this association between modestly elevated CRP levels and cardiovascular events may not necessarily reflect the effects observed in this study. In view of the acute nature of the present study, CRP concentrations targeted at 25 mg/L, in accordance with previous in vitro studies, seemed appropriate to assess potential direct effects of CRP in vivo. The induction of a proinflammatory state in response to CRP is illustrated by IL-6 and IL-8 increases at 4 hours, followed by significant rises in the acute phase reactants SAA and sPLA$_2$ from 8 hours onwards. Accordingly, a second peak of endogenous CRP release was noted after 24 hours.
A previously reported drawback of inflammatory activation by CRP is the potential lipopolysaccharide-contamination in commercially available rhCRP solutions. Using extensively purified rhCRP, several details preclude that possibility in our study. First, we did not note an increase in TNF-α, which is an established hallmark of lipopolysaccharide-induced inflammatory activation. Second, the calculated quantity of lipopolysaccharide given during rhCRP infusion was at most 1.75 EU/kg, whereas the critical amount to induce activation of coagulation and/or TNF-α release exceeds 20 EU/kg.9 Last, there was lack of a febrile response in any of the subjects, whereas the kinetics of the observed cytokine responses subsequent to rhCRP infusion differ profoundly from that evoked by lipopolysaccharide infusion. Activation of coagulation induced by CRP might result from enhanced monocytic tissue-factor activity, because in vitro CRP has been shown to induce monocytic tissue-factor expression.10 Alternative mechanisms might include downregulation of the anticoagulant protein C pathway secondary to the inflammatory response. The precise mechanisms underlying the CRP-mediated activation of the inflammatory and coagulation pathways need further investigation. Although we evaluated only seven individuals, the consistency of the observations clearly lend further support to the development of compounds that specifically block CRP bioactivity in vivo.

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References

Key Words: inflammation ■ endothelium ■ atherosclerosis ■ thrombosis
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