C-Reactive Protein Decreases Tissue Plasminogen Activator Activity in Human Aortic Endothelial Cells
Evidence that C-Reactive Protein Is a Procoagulant

Uma Singh, Sridevi Devaraj, Ishwarlal Jialal

Objective—C-reactive protein (CRP) can promote atherothrombosis by decreasing endothelial nitric oxide synthase and prostacyclin, and by stimulating both plasminogen activator inhibitor-1 in endothelial cells and tissue factor in mononuclear cells. Plasminogen activator-1, a marker of fibrinolysis, is the primary inhibitor of tissue plasminogen activator (tPA). Thus, we tested the effect of CRP on tPA in human aortic endothelial cells.

Methods and Results—Incubation of human aortic endothelial cells with CRP (≥12.5 μg/mL) significantly decreased tPA antigen and activity. Adenyl cyclase inhibitors, an endothelin receptor antagonist, superoxide dismutase, and a nitric oxide donor failed to reverse the effect of CRP on tPA. CRP increased interleukin (IL)-β and tumor necrosis factor (TNF)-α. Neutralization of both IL-1β and TNFα reversed the inhibition of tPA by CRP. Furthermore, in volunteers that have high CRP levels, euglobulin clot lysis time was significantly increased compared with those that have low CRP levels, providing further evidence that high CRP levels are associated with a procoagulant state.

Conclusions—CRP inhibits tPA activity via generation of proinflammatory cytokines (IL-1β and TNFα). This study provides additional novel data that CRP is a procoagulant and has implications for atherothrombosis. (Arterioscler Thromb Vasc Biol. 2005;25:2216-2221.)

Key Words: inflammation ■ endothelial cells ■ C-reactive protein ■ tissue plasminogen activator ■ procoagulant

Inflammation plays a critical role in all stages of atherosclerosis.1 C-reactive protein (CRP), a prototypic marker of inflammation, has been shown to predict cardiovascular events.2 In addition, evolving data suggest that CRP could actively participate in atherothrombosis.3–7 Evidence that CRP could promote atherothrombosis includes inhibition of endothelial nitric oxide synthase (eNOS), prostacyclin (PGI2),5 increase of plasminogen activator inhibitor (PAI)-16 in human aortic endothelial cells (HAEC), and stimulation of tissue factor in mononuclear cells.7 Furthermore, support for its procoagulant activity comes from human CRP transgenic (Tg) mice, in which increased CRP levels resulted in arterial thrombosis after femoral injury.8

Thrombus formation after plaque rupture is the generally accepted cause of acute coronary syndromes. Activation of the endogenous fibrinolytic system is a function of the endothelial cells of the vessel wall.9 The key enzyme in the onset of fibrinolysis is tissue plasminogen activator (tPA), which converts plasminogen to plasmin resulting in degradation of fibrin.10 Therefore, the speed and extent of local delivery of tPA during thrombus formation is important in enhancing thrombolysis.11 Recently, it was shown that postmyocardial infarction, endothelial fibrinolytic response to venous occlusion was impaired in patients with increased CRP levels.12 We previously reported that CRP induces the expression and activity of PAI-1, a marker of impaired fibrinolysis and atherothrombosis, whose action is mediated by inhibition of tPA.6 Because there is no data examining the direct effect of CRP on tPA in HAECS, we tested the effect of CRP on tPA expression and activity in HAECs.

Methods

For all the experiments, HAECS (Clonetics) were maintained in endothelial growth medium-2MV and used within 3 to 5 passages. CRP (from human plasma, Sigma Chem), supplied in 20 mmol/L Tris pH 7.5, 140 mmol/L NaCl, 2 mmol/L CaCl2, and 0.1% NaN3 (Buffer A) was purified of endotoxin by passing through a detoxigel column that absorbs endotoxin (Pierce Biochemicals) and then eluted with Buffer A without NaN3 (Buffer B). Lipopolysaccharide (LPS) contamination was 0.125 endotoxin units/mL (=12.5 pg/mL) by the Limulus assay (Cambrex). CRP was then dialyzed extensively against Buffer B at 4°C to remove azide contamination using dialysis tubing with a cut off of 10 kDa. NaN3 was prepared in Buffer B at 0.1%, then passed through detoxigel column that absorbs endotoxin (Pierce Biochemicals) and then eluted with Buffer A without NaN3, then passed through detoxigel column followed by dialysis to mimic the preparation of CRP. The cells were treated with the same volume of the resultant azide solution as used for CRP. Also, we examined the effect of LPS on tPA in HAECS at and above the concentration of LPS present in the CRP preparation to rule out a LPS-mediated effect. 2’,5’-dideoxyadenosine, NOC-18 (nitric oxide donor), SQ22536 were purchased from Calbiochem.

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From the Laboratory for Atherosclerosis and Metabolic Research, University of California, Davis Medical Center, Sacramento, Calif.

Correspondence to Ishwarlal Jialal, Director of the Laboratory for Atherosclerosis and Metabolic Research, University of California, Davis Medical Center, 4635, 2nd Ave, Sacramento, CA 95817. E-mail ishwarlal.jialal@ucdmc.ucdavis.edu

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Tumor necrosis factor (TNF)-α neutralizing antibody and interleukin (IL)-1 receptor antagonist were obtained from R&D systems.

**Cells Treatment**

Seventy percent to 80% confluent endothelial cells (ECs) grown in 12-well plates were washed with serum-free medium (EBM-2) and incubated for different time periods: 3, 6, 12, and 24 hours with CRP (0 to 50 μg/mL) at 37°C. Cells were treated with various inhibitors (1 hour at 37°C) before CRP exposure. Cell viability was >95% in all experiments.

**Measurement of tPA Antigen and Activity**

The secreted tPA antigen levels in the cell supernates were measured by sandwich ELISA (Diagnostica Stago). This assay measures total circulating tPA (ie, free and complexed tPA). Also, tPA activity was assessed in culture supernatants using Chromализ tPA reagents (BioPool International) after acidification of samples to neutralize PAI-1. The inter- and intra-assay coefficient of variation for these assays was <10%. tPA antigen or activity is expressed as ng or IU/mg protein, respectively.

**Western Blot for tPA**

Cells were lysed with Mammalian Protein Extraction Reagent (Pierce Biotech) and 20 μg of protein per well was loaded and transferred to membranes. Membranes were blocked with 5% milk and then incubated with either anti-human tPA rabbit antibody (1:100; Santa Cruz Biotechnology) or anti-human β-actin monoclonal antibody as control. After washing and incubating with specific horseradish peroxidase-conjugated secondary antibodies, membranes were developed with enhanced chemiluminescence.

**tPA mRNA Levels**

RNA at 3-, 6-, and 12-hour time points was isolated using Trizol (Invitrogen), and 1 μg of RNA was used for first strand cDNA synthesis (Invitrogen). cDNA (100 ng) was amplified for 35 cycles using primers (Integrated DNA Technologies) specific for tPA and GAPDH as specific for tPA and GAPDH, respectively. The results from various strategies used to delineate a CRP-specific effect are shown in Table 1. LPS (50 and 1000 pg/mL) alone had no effect on tPA activity. Furthermore, polymyxin B did not affect CRP-mediated tPA activity inhibition. Boiling and trypsinization abrogated this effect, indicating that the tPA inhibition is CRP-specific and not attributed to some contaminant. The treatment of cells with the same volume of azide solution as our CRP preparation did not alter tPA activity compared with control. Also, tPA activity was unaltered in cells treated with CRP that had been preabsorbed to anti-CRP IgG–coated plates but not to plates without anti-CRP IgG. Furthermore, the treatment of cells with CD32 mAb reversed the inhibitory effect of CRP (Figure 1B).

**Cytokine Measurements**

tPA is known to be regulated by proinflammatory cytokines (IL-1β and TNFα). To gain mechanistic insights on the effect of CRP on tPA, we measured the secretion of these cytokines in culture. The supernates were concentrated 4X and assayed by ELISA (R&D Systems). The intra- and inter-assay coefficient of variation was <10%. Cytokine levels were expressed as pg per mg protein. All experiments were performed at least 3 times. Data are presented as mean±SD. ANOVA was conducted to determine dose response effects. Paired t tests were used to compute differences in the variables, and the level of significance was set at P<0.05.

**Results**

Incubation of HAECs with CRP (0 to 50 μg/mL) for a duration of 3, 6, 12, and 24 hours resulted in a significant decrease in secreted tPA antigen at 12 hours (P<0.04 at doses ≥12.5 μg/mL; Figure 1A). Hence, all further experiments were performed after a 12-hour incubation of HAEC with CRP. In addition, tPA activity was significantly decreased (P<0.03) at all doses of CRP used (Figure 1B).

The results from various strategies used to delineate a CRP-specific effect are shown in Table 1. LPS (50 and 1000 pg/mL) alone had no effect on tPA activity. Furthermore, polymyxin B did not affect CRP-mediated tPA activity inhibition. Boiling and trypsinization abrogated this effect, indicating that the tPA inhibition is CRP-specific and not attributed to some contaminant. The treatment of cells with the same volume of azide solution as our CRP preparation did not alter tPA activity compared with control. Also, tPA activity was unaltered in cells treated with CRP that had been preabsorbed to anti-CRP IgG–coated plates but not to plates without anti-CRP IgG. Furthermore, the treatment of cells with CD32 mAb reversed the inhibitory effect of CRP (Figure 1B).

Incubation of HAECs with CRP resulted in a significant (P<0.02) decrease in tPA protein (Figure 2A). However, RT-PCR for tPA mRNA expression at either 3 hours (data not shown) or 6 hours (Figure 2B) did not reveal any change in its expression with CRP treatment. These results were confirmed by real-time RT-PCR which revealed no significant change in ΔCt value for tPA mRNA of CRP-treated cells as compared with control (ΔCt for Control: 11.84±1.21 versus CRP 25 μg/mL: 12.01±1.23). These data suggest that the inhibitory effect of CRP on tPA is posttranscriptional.

Because it has previously been shown that tPA is regulated by cAMP levels,endothelin-1, reactive oxygen species, NO levels, and proinflammatory cytokines (IL-1β and TNFα), we explored these mechanisms. Pretreatment of cells with adenyl cyclase inhibitors (SQ22536 or 2',5'-
dideoxyadenosine), an ET-1 receptor blocker (bosentan), an NO donor (NOC-18), or polyethylene glycol (PEG)-SOD had no effect on CRP-mediated tPA inhibition (Table 2). The doses of the inhibitors used were based on the published literature.19–22

CRP treatment resulted in a significant (P value for trend <0.005) increase in the secretion of IL-1 and TNF from HAEC (Table 3). Pretreatment with IL-1 receptor antagonist (IL-1 RA) and neutralizing antibody to TNF-α alone resulted in a significant (P<0.05) increase in tPA activity as compared with CRP-treated cells (Figure 3). The combination of IL-1 RA and TNF-α neutralizing antibody resulted in the greatest increase compared with CRP alone (85% of control), whereas isotope control antibody had no effect (Figure 3). The combination of IL-1 RA with soluble TNF receptor (sTNFRI-p60) reversed tPA activity to 90% of control (data not shown).

Lastly, we examined the effect of CRP on the fibrinolytic activity of plasma samples from volunteers with high CRP levels versus those with low CRP levels. The fibrinolytic activity of plasma was significantly decreased in samples with high CRP compared with samples with low CRP as

**TABLE 1. Experiments Undertaken to Determine Whether the Inhibition of tPA by CRP Is Attributed to a Contaminant**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>tPA Activity (IU/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No treatment)</td>
<td>5.67±0.98</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>5.02±1.89</td>
</tr>
<tr>
<td>CRP, 25 µg/ml</td>
<td>2.35±0.79*</td>
</tr>
<tr>
<td>Trypsinized CRP</td>
<td>4.65±0.93</td>
</tr>
<tr>
<td>Boiled CRP</td>
<td>4.31±0.79</td>
</tr>
<tr>
<td>LPS, 50 pg/ml</td>
<td>6.13±0.89</td>
</tr>
<tr>
<td>LPS, 1000 pg/ml</td>
<td>5.01±0.69</td>
</tr>
<tr>
<td>Polymyxin B+ CRP, 25 µg/ml</td>
<td>2.19±0.86*</td>
</tr>
<tr>
<td>Azide solution passed through column and dialyzed</td>
<td>5.69±1.21</td>
</tr>
<tr>
<td>CRP preabsorbed to anti-IgG coated plate</td>
<td>4.99±1.02</td>
</tr>
<tr>
<td>CRP incubated with regular 96-well plate</td>
<td>3.06±1.28*</td>
</tr>
</tbody>
</table>

The values are mean±SD from 3 different experiments run in duplicates. *P<0.04 vs control.

**TABLE 2. Effect of CRP and Various Inhibitors on tPA Activity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>tPA Activity (IU/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.06±0.65</td>
</tr>
<tr>
<td>CRP, 25 µg/ml</td>
<td>1.76±1.28*</td>
</tr>
<tr>
<td>CRP + Bosentan, 10 µM</td>
<td>1.31±0.89</td>
</tr>
<tr>
<td>CRP + 2’,5’-dideoxyadenosine, 10 µM</td>
<td>1.50±0.59</td>
</tr>
<tr>
<td>CRP + SQ22536, 100 µM</td>
<td>1.47±0.39</td>
</tr>
<tr>
<td>CRP + PEG-SOD, 125 U/ml</td>
<td>1.21±0.60</td>
</tr>
<tr>
<td>CRP + NOC-18, 250 µM</td>
<td>1.46±0.42</td>
</tr>
</tbody>
</table>

The values are mean±SD from 3 different experiments run in duplicates. *P<0.028 vs control.
associated with an increased risk of thrombosis. Thus, fibrinolytic activity owing to a decreased release of tPA is evidenced by the ECLT (927±565 minutes versus 394±195 minutes, respectively; *P<0.001).

**Discussion**

CRP promotes tissue factor expression and activity in mononuclear cells and induces the release of thrombomodulin in human umbilical vein ECs. In addition to augmenting PAI-1 expression and activity, CRP decreased PGI2 production and eNOS in HAECs. Furthermore, CRP significantly increases the ratio of thromboxane B2/PGI2. Danenberg et al demonstrated that human CRP transgenic mice develop increased thrombosis after arterial injury. All the above data support a procoagulant role for CRP. Although recent reports indicate no effect of CRP on atherosclerosis in human/rabbit CRP Tg mice, it is important to note that CRP is not a major acute phase protein in mice. Delivery of human and rabbit CRP to mice infected with pathogens/endotoxin resulted in decreased lethality; this protection appeared to be attributable to a paradoxical antiinflammatory effect in mice that needs to be further elucidated. Furthermore, human CRP increases myocardial and cerebral infarction in rats. The importance of tPA has been demonstrated with successful therapeutic outcomes after thrombolytic therapy in human atherosclerotic lesions and vascular cells has been the focus of much research. Recent reports suggest that CRP-induced effects are artifacts attributable to the presence of contaminants (LPS and azide) in commercial CRP. Recently, Ridker and Cook have shown that CRP levels >20 µg/mL predict future cardiovascular events. CRP mRNA levels in atherosclerotic plaques are 10-fold higher than in normal arteries, suggesting that levels in the atheroma could be much higher than in serum. We showed that secreted CRP concentration increases 100-fold in HAECs exposed to macrophage conditioned medium. Therefore, in atherosclerotic lesions via paracrine/autocrine loops, CRP levels could be very high in certain microdomains. Furthermore, CRP levels increased from 18 to 56 µg/mL in human Tg mice after femoral wire injury resulting in a procoagulant phenotype. CRP infusion in humans raised the levels of CRP to 28.1 µg/mL and induced a procoagulant phenotype. CRP levels up to 50 µg/mL have also been reported in patients with myocardial infarction. Thus, the levels of CRP used in this study can clearly be attained in patients.

The mechanistic link between CRP and atherosclerosis has been the focus of much research. Recent reports suggest that CRP-mediated effects are artifacts attributable to the presence of contaminants (LPS and azide) in commercial CRP. Because we have previously shown that endotoxin-purified CRP inhibits NO release via eNOS downregulation, we were prompted to reexamine the effect of endotoxin-purified azide-free CRP on eNOS. We show that in HAEC, this CRP (25 µg/mL) decreased cGMP release (72% ↓, *P<0.05) and increased IL-8 (2.1-fold ↑, *P<0.01). Furthermore, in this article, using different strategies, we carefully rule out possible contamination by LPS or NaN3 and clearly show that tPA activity inhibition is CRP-specific. Our experiments document that mAb to CD32 significantly reverses CRP-mediated tPA inhibition further supporting our hypothesis that CRP, per se, inhibits tPA. Furthermore, evidence showing the link between CRP and atherosclerosis is not limited to cell culture because the presence of CRP mRNA and protein in human atherosclerotic lesions and vascular cells has been demonstrated.

Mechanistically, the endothelial tPA:Ag release is known to be upregulated by several physiological substances: tPA is downregulated by IL-1β as well as TNF-α, cAMP levels, endothelin-1, and reactive oxygen species. The data in this article show that an NO donor, adenyl cyclase inhibitors, an ET-1R blocker as well as PEG-SOD do not affect CRP-mediated inhibition of tPA, hence ruling out these pathways.

CRP treatment significantly increased IL-1β and TNF-α in the present study. Previously, CRP has been shown to

**TABLE 3. Effect of CRP on IL-1β and TNF-α in HAECs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1β (pg/mg Protein)</th>
<th>TNF-α (pg/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>CRP, 12.5 µg/ml</td>
<td>16.5±5.9 *</td>
<td>1.56±1.3 *</td>
</tr>
<tr>
<td>CRP, 25 µg/ml</td>
<td>47.5±6.4 *</td>
<td>37.5±8.5 *</td>
</tr>
<tr>
<td>CRP, 50 µg/ml</td>
<td>37.6±3.6 *</td>
<td>48.6±9.4 *</td>
</tr>
</tbody>
</table>

*P value for trend <0.005 by ANOVA for IL-1β and TNF-α.
increase IL-1β and TNF-α in peripheral blood mononuclear cells. Importantly, both IL-1 and TNF inhibit tPA. Whereas both IL-1 RA and neutralizing antibody to TNF partially reversed the inhibition of tPA by CRP, the combination of both resulted in tPA activity that was similar to control. Similar reversal of tPA activity was obtained with the combination of IL-1 RA and soluble TNFR1. We have previously shown that endotoxin-purified CRP increases IL-8 through NF-κB, and this mechanism may be relevant to CRP-induced increase in IL-1 and TNF. Future studies will be directed to elucidate these mechanisms because it is beyond the scope of this report.

In patients admitted to the intensive care unit, CRP correlates with ECLT, an index of plasma fibrinolytic activity. Importantly, we report significantly increased ECLT and hence decreased fibrinolytic capacity in plasma from volunteers with high CRP levels. Overall, we clearly show that CRP decreases intracellular tPA and secreted tPA antigen and activity without any alteration of tPA mRNA, suggesting that CRP-mediated tPA inhibition is a posttranscriptional event. However, whether it is a translational or posttranslational event will be investigated in future studies.

Based on our findings, we postulate that CRP is a procoagulant. By inhibiting tPA activity and stimulating PAI-1 in aortic ECs, CRP attenuates the fibrinolytic capacity. In the present study, we go further in making a novel observation that high CRP levels are associated with a procoagulant phenotype and impaired fibrinolysis. In this context, it is important to note that in the Physician Health Study, the greatest reduction in myocardial infarction with aspirin therapy (325 mg on alternate days) was obtained in patients with the highest CRP levels, suggesting that aspirin ameliorated the procoagulant phenotype associated with high CRP because aspirin at this dose does not decrease CRP but is a potent antiplatelet agent.

To conclude, the present study makes the novel observation that CRP inhibits tPA expression and activity via stimulation of IL-1β and TNF-α. This is additional evidence supporting the previous reports—(induction of PAI-1, tissue factor, and inhibition of PGI2 and eNOS) that CRP should be classified as a procoagulant.

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References

19. Francis RB Jr., Neely S. Inhibition of endothelial secretion of tPA and its rapid inhibitor by agents which increase intracellular cyclic AMP. Biochim Biophys Acta. 1989;1012:207–213.
27. Xia D, Samols D. Transgenic mice expressing rabbit CRP are resistant to endotoxemia. Proc Natl Acad Sci U S A. 1997;94:2575–2580.
28. Szalai AJ, VanCott JL, McGhee JR, Volanakis JE, Benjamin WH Jr. Human CRP is protective against fatal Salmonella enterica serovar typhi-
29. Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, Pepys MB. CRP and complement are important mediators of tissue
30. Gill R, Kemp JA, Sabin C, Pepys MB. Human CRP increases cerebral
infarct size after middle cerebral artery occlusion in adult rats. *J Cereb
31. Stroke rt-PA Stroke Study Group: The National Institute of Neurological
1581–1587.
32. Devaraj S, Du Clos TW, Jialal I. Binding and internalization of CRP by Fc
gamma receptors on HAEC mediates biological effects. *Arterioscler
33. Ridker PM, Cook N. Clinical usefulness of very high and very low levels
of CRP across the full range of Framingham Risk Scores. *Circulation.*
35. Venugopal SK, Devaraj S, Jialal I. Macrophage conditioned medium
induces the expression of CRP in HAEC: potential for paracrine/autocrine
36. Bisendial RJ, Kastelein JJ, Levels JH, Zwaginga JJ, van den Bogaard B,
Reitsma PH, Meijers JC, Hartman D, Levi M, Stroes ES. Activation of
inflammation and coagulation after infusion of CRP in humans. *Circ Res.*
37. Pietila KO, Harmoinen AP, Jokinty J, Pasternack AI. Serum CRP
concentration in acute myocardial infarction and its relationship to mor-
tality during 24 months of follow-up in patients under thrombolytic
38. Van den Berg CW, Taylor KE, Lang D. CRP-induced in vitro vasore-
laxation is an artefact caused by the presence of sodium azide in com-
mercial preparations. *Arterioscler Thromb Vasc Biol.* 2004;24:
e168–e171.
39. Taylor KE, Giddings JC, van den Berg CW. CRP-induced in vitro
endothelial cell activation is an artefact caused by azide and LPS. *Arte-
NM. Proapoptotic, antimigratory, antiproliferative, and antiangiogenic
effects of commercial CRP on various human endothelial cell types in
vitro. Implications of Contaminating Presence of Sodium Azide in Com-
41. Rydholm H, Bostrom S, Eriksson E, Risberg B. Complex intracellular
signal transduction regulates tPA and PAI-1 synthesis in cultured
42. Ballou CP, and Lozanski G. Induction of inflammatory cytokine release
43. Devaraj S, Kumaresan PR, Jialal I. Effect of C-reactive protein on
chemokine expression in human aortic endothelial cells. *J Mol Cell
44. Ridker PM, Cushman M, Stamper MJ, Tracy RP, Hennekens CH.
Inflammation, aspirin, and the risk of cardiovascular disease in apparently
45. Feldman M, Jialal I, Devaraj S, Cryer B. Effects of low-dose aspirin on
serum CRP and thromboxane B2 concentrations: a placebo-controlled
study using a highly sensitive CRP assay. *J Am Coll Cardiol.*
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