C-reactive protein is a mediator of cardiovascular disease
R. J. Bisogni, S. M. Boekholdt, M. Vergeer, E. S. G. Stroes and J. J. P. Kastelein
[Abstract] [Full Text] [PDF]

The macrophage: the intersection between HIV infection and atherosclerosis
S. M. Crowe, C. L. V. Westhorpe, N. Mukhamedova, A. Jaworowski, D. Sviridov and M. Bukrinsky
[Abstract] [Full Text] [PDF]

C-reactive protein impairs the endothelial glyocalyx resulting in endothelial dysfunction
S. Devaraj, J.-M. Yun, G. Adamson, J. Galvez and I. Jialal
[Abstract] [Full Text] [PDF]

Dissociation of Pentameric to Monomeric C-Reactive Protein on Activated Platelets Localizes Inflammation to Atherosclerotic Plaques
[Abstract] [Full Text] [PDF]

CRP is a novel ligand for the oxidized LDL receptor LOX-1
H. H. Shih, S. Zhang, W. Cao, A. Hahn, J. Wang, J. E. Paulsen and D. C. Harnish
[Abstract] [Full Text] [PDF]

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CRP promotes monocyte-endothelial cell adhesion via Fcγ receptors in human aortic endothelial cells under static and shear flow conditions

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Monocyte-endothelial cell adhesion is a key early event in atherogenesis (16, 19). After CRP mediates its biological effects in HAEC via upregulation of the Fcγ receptors CD32 and CD64 in HAEC. Thus, in the study, we tested whether native, azide, and endotoxin-free CRP augments monocyte-endothelial cell adhesion via the Fcγ receptor-mediated pathway in HAEC and explored mechanisms under both static and shear flow conditions to better mimic the in vivo milieu.

METHODS

The investigation conforms with the principles outlined in the Declaration of Helsinki. HAEC (Cambrex) were grown in endothelial basal medium-2 media containing growth supplements and used between passages 2–5. CRP was obtained from pleural/ascites fluid and purified as described previously (25). The average endotoxin concentration in the final preparation was <0.125 EU/ml using the Limulus Amoebocyte Lysate test (Cambrex). Because of the concern raised recently with regard to the effect of azide in CRP preparations, we dialyzed our CRP extensively at 4°C to remove azide using dialysis tubing with a cutoff of 10 kDa. The isolated CRP migrated as a single band on a nonreducing gel.

SB-203580 (5 μM), U-0126 (1 μM), BAY 11–7082 (10 μM), caffeic acid phenethyl ester (CAPE; 5 μM), and piceatannol (10 μM) were purchased from Calbiochem; sodium azide (s-2002) was from Sigma. 1-β-NK1 was obtained from Alexis Pharmaceuticals. Anti-VCAM-1, anti-ICAM-1, anti-p65, and anti-IKK antibodies were purchased from Santa Cruz Biotechnology. Antibodies to CD32 and CD64 or transient transfection with small interference RNA to CD32 attenuated CRP-induced NF-κB activity, ICAM, VCAM, and monocyte-endothelial cell adhesion under static conditions. Also, the Syk kinase inhibitor piceatannol and MG-132, a proteasome degradation inhibitor, produced similar attenuation in NF-κB activity, ICAM, VCAM, and adhesion. Furthermore, CRP-activated endothelial cells supported monocyte rolling, arrest, and transmigration in shear flow (2 dyn/cm²), and this was also inhibited by preincubation with antibodies to CD32 and CD64. Thus, in HAEC, CRP upregulates monocyte-endothelial adhesion by activation of NF-κB through engaging the Fcγ receptors CD32 and CD64.

C-reactive protein: human aortic endothelial cells; tissue-type plasminogen activator; plasminogen activator inhibitor

Inflammation is pivotal in all stages of atherosclerosis (15). Numerous prospective studies have shown that high levels of C-reactive protein (CRP) predict cardiovascular events, and several recent studies (11, 20, 28) have documented a proatherogenic, prothrombotic role for CRP. In endothelial cells, CRP has been shown to decrease endothelial nitric oxide synthase (eNOS), prostacyclin, and tissue-type plasminogen activator (tPA) and to upregulate plasminogen activator inhibitor-1 (PAI-1) and IL-8 (11). Monocyte-endothelial cell adhesion is a key early event in atherogenesis (16, 19). After endothelial cell dysfunction, mononuclear cells, such as monocytes and T lymphocytes, transition from rolling to firm arrest and then diapedese into the subendothelial space. The rolling and tethering of leukocytes on the endothelium are orchestrated by adhesion molecules, such as selectins (E-selectin and P-selectin), cell adhesion molecules (ICAM-1 and VCAM-1), and integrins (1, 2). It has previously been shown that CRP induces the expression of ICAM and VCAM in human aortic endothelial cells (HAEC) and stimulates monocyte-endothelial cell adhesion under static conditions (18, 25, 26). In a recent study, Kawanami et al. (12) have shown that CRP upregulates VCAM-1 expression in bovine aortic endothelial cells via upregulation of NF-κB. Our group (7) has recently shown that CRP mediates its biological effects in HAEC via upregulation of the Fcγ receptors CD32 and CD64. In this study, we examined the mechanisms by which native CRP promotes monocyte-endothelial cell adhesion under static conditions and tested the effect of CRP on adhesion under shear flow. Incubation of HAEC with CRP (>25 μg/ml) upregulated NF-κB activity, and this resulted in a significant increase in ICAM (54% increase, P < 0.001), VCAM (41% increase, P < 0.01), and monocyte-endothelial cell adhesion (44% increase, P < 0.02) compared with those of control. Preincubation with antibodies to CD32 and CD64, but not CD16, effectively inhibited this activation. Blocking NF-κB activity with inhibitors or a dominant negative inhibitor of NF-κB significantly decreased ICAM, VCAM upregulation, and subsequent monocyte-endothelial cell adhesion. Preincubation with antibodies to CD32 and CD64 or transient transfection with small interference RNA to CD32 attenuated CRP-induced NF-κB activity, ICAM, VCAM, and monocyte-endothelial cell adhesion under static conditions. Also, the Syk kinase inhibitor piceatannol and MG-132, a proteasome degradation inhibitor, produced similar attenuation in NF-κB activity, ICAM, VCAM, and adhesion. Furthermore, CRP-activated endothelial cells supported monocyte rolling, arrest, and transmigration in shear flow (2 dyn/cm²), and this was also inhibited by preincubation with antibodies to CD32 and CD64. Thus, in HAEC, CRP upregulates monocyte-endothelial adhesion by activation of NF-κB through engaging the Fcγ receptors CD32 and CD64.
transfection was measured using an ELISA (R&D Systems, Minneapolis, MN), and values were expressed as cell protein (in ng/ml) (25).

Monocyte-HAEC adhesion assay: static. Monocytes were obtained from normal, healthy volunteers using negative magnetic separation (25), loaded with the fluorescent dye CFDA-SE, as described previously (25), for 30 min at 37°C. Labeled monocytes were washed with PBS and added (5 × 10^6/ml) to monolayers of HAECs. After cells were incubated for 60 min and gently washed, a number of bound cells were assayed by fluorescence excitation (485 nm) and emission (535 nm) and %bound monocytes were calculated. LPS (100 ng/ml) and TNF (10 ng/ml) were used as a positive control.

Specificity of effects of CRP. Three strategies were used: 1) CRP was incubated in a boiling water bath for 1 h, 2) CRP was preadsorbed to plates coated with anti-CRP IgG (Alpco Diagnostics) or irrelevant antibody and supernatant was used, and 3) cells were pretreated with polymyxin B (10 μg/ml) for 30 min before CRP challenge.

Western blot analysis. Nuclear and cytoplasmic extracts were prepared using NE-PER reagents from Pierce Biotechnologies. Nuclear, cytosolic, or total protein samples (from cell lysates) were mixed with sample buffer, boiled for 10 min, separated by 10% SDS-PAGE under denaturing conditions, and electroblotted to nitrocellulose membranes (Amersham Pharmacia Biotech). The nitrocellulose membranes were blocked in nonfat milk incubated with appropriate primary antibody (ICAM, VCAM, IKK, or p65). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody. Signals were visualized by enhanced chemiluminescence as described previously (25).

RT-PCR for ICAM and VCAM mRNA. RNA was isolated from HAEC using TRIzol. RNA (1 μg) was used for first-strand cDNA synthesis (Invitrogen). cDNA (100 ng) was amplified for 35 cycles using primers (Integrated DNA Technologies) specific for ICAM, VCAM, and GAPDH, respectively, and PCR products were run on an agarose gel (25).

NF-κB activity. NF-κB activity was assessed by using the NF-κB luciferase reporter gene assay as well as the p65 ELISA assay in the nuclear extracts using reagents from Active Motif. Briefly, for the reporter gene assay, the phospho-NF-κB-Luc reporter plasmid (BD Biosciences), containing 5 NF-κB binding sites as enhancer and the firefly luciferase reporter gene (1 μg), was used to transfet HAEC using FuGene transfection reagents (Roche) for 24 h. After transfection, cells were stimulated with CRP for 6–8 h, and cells were then lysed, and luciferase activity was measured in a luminometer using a Luciferase Assay system (Promega).

RNA interference. Inhibition of ICAM and VCAM as well as CD32 was achieved with the use of commercially available small interference RNA (siRNA, Ambion, no. 105997, 138777, and 202328, respectively). Briefly, HAECs were transfected with 100 nM/ml annealed siRNA against ICAM, VCAM, or CD32 or respective negative controls using siRNA transfection reagents from Ambion. After incubation with siRNA at 37°C overnight, HAEC were incubated with CRP (25 μg/ml) for 6–8 h and subjected to further analysis. Also, dominant negative inhibitory κB (IκB), mutant, and phospho-β-galactosidase (pβ-Gal) vectors were obtained from BD Biosciences/Clontech, and HAEC were transfected using FuGene 6 transfection reagents for 24 h before activation with CRP. Transfection efficiency, which was 70%, was determined by cotransfecting with pβ-Gal vector and assessing β-Gal activity using reagents from Promega.

Monocyte-HAEC adhesion assay: shear flow. For shear flow studies (9), HAEC were grown to confluence in the six-well plates before treatment and were utilized at passages 5–6. Medium was changed to 1.5 ml/well of fresh endothelial growth medium-2 on the day of the experiment. HAEC were treated with 0.03 ng/ml TNF-α for 4 h, 50 μg/ml CRP for 8 h, or both 50 μg/ml CRP for 8 h and 0.03 ng/ml TNF-α for 4 h. In experiments in which antibody blocking was applied, antibodies (5–10 μg/ml) were added 1 h before the addition of CRP. Monocytes (2 × 10^6 monocytes/ml) were layered on anti-body-treated HAEC monolayers, treated with 10 μg/ml CD16, CD32, or CD64 or isotype control (IgG1) for 60 min before use.

To assess monocyte adhesion under shear flow, HAEC monolayers were doubly washed with HBSS with Ca^2+ and Mg^2+. Polyethylene (PE) tubing was then inserted into the vacuum seals of a custom parallel plate silicone rubber flow chamber that is placed into a single well of a six-well plate above a monolayer of aortic endothelium. Once in contact with the monolayer, a vacuum seal is applied, a syringe pump is used to pull the buffer through a PE tubing and across the flow channel, and substrate (Harvard Apparatus, Holliston, MS) is attached with one end inserted into the outlet of the flow chamber. A small reservoir inserted in the inlet is used to add buffer and monocyte suspension at a shear stress of 2 dyn/cm^2. Monocyte rolling, arrest, and transmigration were video recorded on the inverted-phase contrast microscope. Monocytes were observed for 30- to 60-s digital image sequences captured over individual fields of view every minute. Adhesion was imaged using a Nikon TE200 inverted microscope equipped with a Plan Fluor 20X (numerical aperture = 0.45) phase-contrast objective, an analog CCD camera (Dage-MTI, Michigan City, MD), and a digital frame grabber (Scion, Frederick, MD). Image sequences were captured at a frame rate of 30 sequences/s and analyzed by using Image-Pro Plus v 4.5 (Media Cybernetics, Silver Spring, MD). Arrest was defined as a cell that did not translate more than one cell diameter in 30 s. Transmigration was confirmed by observing cells transition from phase bright above the monolayer to phase dark upon diapedesis by phase contrast microscopy as previously described (1, 13, 14, 23, 29).

Statistical analyses. Analysis of data was performed using GraphPad Prism v 4.0 software (Graphpad Software, San Diego, CA). The data were expressed as means (SD). ANOVA was used to assess dose-response effects followed by paired-tests for parametric data and Wilcoxon’s signed rank tests for nonparametric data. A P value of <0.05 was considered statistically significant. All experiments were performed at least three times in duplicate.

RESULTS

Time-course experiments with different doses of native azide-free and endotoxin-free CRP revealed that CRP induced ICAM and VCAM release from 6 h to a maximum at 12 h of incubation (data not shown), and this time point was used to study upregulation in subsequent experiments. As shown in Fig. 1, A–C, CRP (≥25 μg/ml) upregulated ICAM-1 and VCAM-1 expression as evidenced by ELISA, flow cytometry, and Western blot analysis. In addition, CRP upregulated adhesion of human monocytes to HAEC at 8 h (Fig. 1D). It is important to note that these effects were not abrogated by the addition of polymyxin B and were abolished when CRP was preadsorbed on anti-CRP-coated plates or with the use of boiled CRP. We also tested the effect of azide (0.05%) on ICAM, VCAM, and adhesion, and azide failed to have an effect (Table 1). Furthermore, ICAM-1 and VCAM-1 mRNA expressions were induced after incubation with CRP for 6–8 h (Fig. 1E). Transient transfection with either VCAM siRNA or ICAM siRNA resulted in a 70–80% knockdown of VCAM and ICAM expression, respectively, and resulted in a similar inhibition as in CRP-induced adhesion [control, 24.3% (SD 8.7]; CRP (25 μg/ml), 54.7% (SD 14.1)]; CRP (25 μg/ml) + ICAM siRNA, 29.8% (SD 11.1)]; and CRP (25 μg/ml) + VCAM siRNA, 24.6% (SD 7.7), n = 3).

Because both ICAM and VCAM are induced by NF-κB and we have previously shown that CRP induction of IL-8 in HAEC is through activation of NF-κB (7), we tested the effect of native azide-free and endotoxin-free CRP on NF-κB as shown by increased IKK in cytosol as well as p65 in nuclear
extracts by Western blot analysis and NF-κB p65 activity in nuclear extracts by ELISA (Fig. 2). Also, NF-κB-mediated reporter gene expression was enhanced after stimulation with CRP [Luciferase reporter activity (in relative luminescence units): control, 188; CRP (25 μg/ml), 294; and CRP (50 μg/ml), 336]. Inhibitors of NF-κB, BAY 11, SN-50, and CAPE or addition of MG-132, the proteasome degradation inhibitor, significantly reversed CRP-induced ICAM, VCAM, and adhesion (Fig. 3, A and B). Furthermore, the p38 MAPK inhibitor, but not the ERK or JNK inhibitor, significantly reversed CRP-induced ICAM, VCAM, and adhesion. Also, transient transfection with dominant negative IκB resulted in a mean 86% decrease in NF-κB activity compared with the mutant when incubated with CRP (data not shown). Furthermore, the dominant negative IκB, but not the mutant, significantly attenuated CRP-induced increase in ICAM, VCAM, and adhesion (Fig. 3, C and D). These data indicate that CRP signals inflammation through p38 MAPK and nuclear translocation of NF-κB.

Our group (7) has shown previously that several of the proatherogenic effects of CRP in HAEC are orchestrated via the Fcγ receptors CD32 and CD64. Preincubation of HAEC with antibodies to CD32 and CD64, but not CD16 or IgG1 isotype control, effectively inhibited the activation of NF-κB, ICAM, VCAM, and adhesion (Fig. 4). Furthermore, transient transfection with siRNA to CD32 resulted in a 70–80% knock-down of CD32 expression.

Table 1. Specificity of the effects of CRP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ICAM, ng/mg protein</th>
<th>VCAM, ng/mg protein</th>
<th>Adhesion, % bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6 (SD 0.8)</td>
<td>4.1 (SD 0.9)</td>
<td>24 (SD 11)</td>
</tr>
<tr>
<td>CRP, 50 μg/ml</td>
<td>6.1 (SD 1.1)*</td>
<td>8.9 (SD 1.7)*</td>
<td>67 (SD 14)*</td>
</tr>
<tr>
<td>CRP + azide</td>
<td>6.3 (SD 0.9)*</td>
<td>7.7 (SD 1.4)*</td>
<td>64 (SD 11)*</td>
</tr>
<tr>
<td>CRP + polymixin</td>
<td>6.4 (SD 1.4)*</td>
<td>8.5 (SD 1.8)*</td>
<td>68 (SD 11)*</td>
</tr>
<tr>
<td>CRP + anti-CRPAb</td>
<td>3.9 (SD 0.7)†</td>
<td>4.6 (SD 1.4)†</td>
<td>23 (SD 13)†</td>
</tr>
</tbody>
</table>

Values are means (SD) of 3 different experiments in duplicate. CRPAb, C-reactive protein (CRP) antibody. *P < 0.05 compared with control; †P < 0.03 compared with CRP (50 μg/ml).

Fig. 1. Effect of C-reactive protein (CRP) on ICAM, VCAM, and monocyte-endothelial cell adhesion. Human aortic endothelial cells (HAEC) were incubated with CRP (0, 12.5, 25, or 50 μg/ml) for 12 h for soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) levels as determined by ELISA (A), flow cytometry (B), and Western blot analysis (C) as described in METHODS. B, inset: histogram for ICAM and VCAM. HAEC were incubated for 6–8 h with or without CRP, followed by addition of monocytes, and adhesion was monitored as described in METHODS (D), and mRNA for ICAM and VCAM was assessed by RT-PCR (E). *P < 0.05 by ANOVA; all values were significantly different at doses ≥25 μg/ml CRP. Data are means of 4–7 experiments in duplicate.
down of mRNA for CD32 (Fig. 4C), CRP-induced NF-κB activity, ICAM, VCAM, and monocyte-endothelial cell adhesion under static conditions, whereas the negative control siRNA had no effect (Fig. 4). We were unable to obtain siRNA to CD64 from commercial sources that could effectively block CD64 expression in HAEC. Because both Fcγ receptors signal through SYK kinase activation, we tested the effect of the SYK kinase inhibitor piceatannol, which also produced similar attenuation in NF-κB activity, ICAM, VCAM, and adhesion (Fig. 4). Thus it appears that, under static conditions, inhibition of CRP via blockade of the Fcγ receptors CD32 and CD64, via inhibition of NF-κB, results in decreased ICAM and VCAM production and decreased monocyte-endothelial cell adhesion.

Because in vivo adhesion is mediated by upregulation of vascular adhesion molecules under conditions of fluid shear flow, we tested the effect of CRP under conditions at shear stress of 2 dyn/cm². Preincubation of HAEC with CRP for 8 h also resulted in significant upregulation of E-selectin (control, 159 mfi (SD 78); and CRP (50 μg/ml), 394 mfi (SD 116), P < 0.05), ICAM, and VCAM. Human monocytes were freshly isolated from human blood and placed in a custom parallel-plate flow channel. Monocytes were observed to roll, arrest, and transmigrate on TNF-stimulated HAEC (Fig. 5A) as previously described (1, 13, 14, 23, 29). In response to activation with CRP, monocytes were recruited at a rate that was 70% commensurate to TNF-α stimulation and similarly rolled, arrested, and transmigrated. Costimulation with TNF-α significantly increased the number of monocytes that eventually transmigrated as detected by the characteristic transition from a phase bright to dark cell upon diapedesis. Enhanced monocyte recruitment in response to CRP was specific for Fcγ receptors CD32 and CD64, as preincubation in the presence of blocking monoclonal antibodies significantly inhibited rolling and arrest compared with treatment with antibodies to CD16 (Fig. 5B).

**DISCUSSION**

Several studies (20, 21) have shown that CRP, the prototypic marker of inflammation, predicts cardiovascular events. Recent lines of evidence point to a proinflammatory, procoagulant role for CRP (11). In HAEC, CRP has several proatherogenic effects, such as a decrease in eNOS, prostacyclin, and tPA and upregulation of IL-8, PAI-1, and adhesion of monocytes to endothelium (11). The Fcγ receptors CD32 and CD64 have been shown to mediate CRP-induced activation of IL-8 and inhibition of eNOS and prostacyclin (7, 11, 17, 28). In this study, we make the novel observation that native azide-free CRP upregulates ICAM and VCAM via activation of the NF-κB pathway, resulting in increased adhesion of monocytes to HAEC under static conditions. Also, we show for the first time that CRP upregulates monocyte adhesion to HAEC under shear flow conditions, which is more relevant to the in vivo milieu. In addition, we demonstrate that these events are mediated by engaging the Fcγ receptors CD32 and CD64 in both static and shear flow conditions.

It is important to note that these effects of CRP are not due to contamination with either lipopolysaccharide or azide, because this CRP was purified from pleural/ascites fluid and had extremely low levels of endotoxin and was extensively dialyzed. Also, boiled CRP failed to have these effects, and the effects of CRP were not abrogated with polymixin B. Furthermore, using CRP preincubated with anti-CRP-coated plates abrogates the effects of CRP. These data collectively support the notion that the effects are due to native CRP per se. Furthermore, the concentrations of CRP that we have used have been reported in patients with myocardial infarction, and, recently, Ridker and Cooke have shown that even CRP >20 mg/l predicts cardiovascular events (5, 20, 21). Also, 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 (30).
Previously, Pasceri et al. (18) have shown increased expression of ICAM, VCAM, and E-selectin in human umbilical vein and human coronary arterial endothelial cells with CRP and in saphenous vein endothelial cells. Verma et al. (26) showed upregulation of ICAM and VCAM with CRP; however, these previous groups did not explore cellular mechanisms. Subsequently, we showed in HAEC that endotoxin-purified CRP, in addition to decreasing eNOS, upregulated ICAM and VCAM and monocyte-endothelial cells adhesion (7, 11, 25, 28). In the present study, we show that native azide-free CRP upregulates ICAM, VCAM, and adhesion under static and shear flow conditions and goes further in elucidating the mechanisms.

The transcription factor NF-κB plays a key role in inflammation and atherosclerosis (24). Verma et al. (27) demonstrated that CRP induces NF-κB activity in saphenous vein endothelial cells. We have shown earlier that CRP activates IL-8 release from HAEC via activation of NF-κB (6). Because...
both ICAM and VCAM have NF-κB binding elements in their promoter, we examined the effect of NF-κB inhibitors and dominant negative IκB on ICAM and VCAM expression in HAEC and also its effects on monocyte-endothelial cell adhesion. Very recently, Kawanami et al. (12) have demonstrated that VCAM-1 expression induced by recombinant CRP in bovine aortic endothelial cells was mediated by activation of the NF-κB p65 subunit. However, they did not purify their CRP of endotoxin and azide or examine ICAM-1 release and failed to examine its effects on the biological end point, i.e., monocyte-endothelial cell adhesion. In our study, we further demonstrate that CRP induces both soluble ICAM and VCAM by ELISA as well as flow cytometry and that inhibition of either of these using siRNA results in similar attenuation of monocyte-HAEC adhesion. Furthermore, we were able to decrease CRP-induced adhesion by blocking CD32 and CD64. These results point to a common pathway initiated by binding of CRP to CD32 and CD64 and endocytosis or direct signaling transmembrane. Previously, Fernandez et al. (8) have shown that cross-linking of either CD32 or CD64 but not CD16 resulted in activation of LPS-induced cytokines in macrophages, and Banki et al. (3) have demonstrated that cross-linking of CD32 induced maturation of human monocyte-derived dendritic cells via NF-κB signaling. Thus we speculate that these receptors converge on a common pathway at the level of transcription, such as NF-κB. In support of this, we have shown that downregulation of NF-κB activity, using either soluble inhibitors of NF-κB or a dominant negative IκB or the proteasomal degradation inhibitor, resulted in significant reversal of CRP-induced ICAM, VCAM, and monocyte-HAEC adhesion. Furthermore, we show that this activation of NF-κB induced by CRP is dependent on activation of the Fcγ receptors CD32 and CD64. In our studies, we show convincingly, using specific antibodies to CD32 and CD64, as well as using siRNA to CD32, that NF-κB activation induced by CRP was markedly attenuated, resulting in decreased ICAM, VCAM, and adhesion. Our group (7) has previously shown that the decrease in prostacyclin and upregulation of IL-8 by CRP is mediated via the Fcγ receptors CD32 and CD64. Recently, Mineo et al. (17) have shown that eNOS downregulation by CRP appears to be via Fcγ receptor IIb (CD32).

CRP has been shown to activate NF-κB and the MAPK pathway in vascular smooth muscle cells (10). Furthermore, Kawanami et al. (12) have shown that CRP-induced VCAM is attenuated in presence of the p38 MAPK inhibitor in BAEC. These data support the findings in our study of decreased adhesion observed with the p38 MAPK inhibitor but not after inhibition of ERK or JNK pathways. In addition, CD32 appears to signal via Syk kinase activation (22). We also show that the Syk kinase inhibitor piceatannol attenuated the increase in adhesion observed with CRP, further implicating the role of Fcγ receptors in mediating the effects of CRP. Thus CRP appears to promote monocyte-endothelial cell adhesion via upregulation of CD32. CD32, via activation of Syk and NF-κB, results in upregulation of ICAM and VCAM as well as adhesion.

Shear stress, especially when blood flow is disturbed and/or is nonlaminar, plays an important role in the pathogenesis of the atherosclerotic plaque (4). The combination of altered arterial hemodynamics around curvatures, arterial branch ostia, and bifurcations, where secondary flows occur, and systemic risk factors promote atherosclerotic lesion initiation, progression, and, ultimately, development of complicated plaques. Analysis of vascular regions prone to atherogenesis due to disturbed blood flow in vivo has revealed that altered shear stress can prime the endothelium to respond to proatherogenic stimuli through upregulation of the proinflammatory transcription factor NF-κB. Furthermore, low shear stress influences adhesion of leukocytes to activated endothelium through enhanced expression of adhesion molecules, such as monocyte chemotactic protein-1, E-selectin, ICAM, and VCAM (4). In the present study, we show for the first time that pretreatment of HAEC with CRP increased rolling, arrest, and transmigration under low shear flow conditions. Activation through these pathways was observed to superpose, resulting in enhanced recruitment and monocyte transmigration. It is also interesting to note that the effects of CRP on adhesion under shear flow are also mediated via the Fcγ receptors CD32 and CD64 because antibodies to these significantly attenuated CRP-induced adhesion under flow conditions.

In conclusion, we convincingly show that native CRP augments monocyte-endothelial cell adhesion in HAEC under static and shear flow conditions via the Fcγ receptors CD32 and CD64 by upregulation of NF-κB activity, ICAM, VCAM, and E-selectin. Thus strategies aimed at downregulating CRP, CD32, and CD64 and cellular adhesion molecules in HAEC may prove to be beneficial in downregulating monocyte recruitment into the artery wall and in reducing atherosclerosis.

GRANTS
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