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Biochemical and Biophysical Research Communications 321 (2004) 65–71
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C-reactive protein impairs angiogenic functions and decreases the secretion of arteriogenic chemo-cytokines in human endothelial progenitor cells

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Received 11 June 2004

Abstract

C-reactive protein (CRP), a predictor of future cardiovascular diseases, has been reported to damage the vascular wall by inducing endothelial dysfunction and inflammation. This proatherogenic CRP was speculated to have a role in attenuating angiogenic functions of human endothelial progenitor cells (EPCs), possibly impairing vascular regeneration and increasing cardiovascular vulnerability to ischemic injury. Herein, we investigated the direct effect of CRP on angiogenic activity and gene expression in human EPCs. Incubation of EPCs with human recombinant CRP significantly inhibited EPC migration in response to vascular endothelial growth factor, possibly by decreasing the expression of endothelial nitric oxide synthase and subsequent nitric oxide production. In addition, CRP-treated EPCs showed the reduced adhesiveness onto an endothelial cell monolayer. When assayed for the gene expression of arteriogenic chemo-cytokines, CRP substantially decreased their expression levels in EPC, in part due to the upregulation of suppressors of cytokine signaling proteins. These results suggest that CRP directly attenuates the angiogenic and possibly arteriogenic functions of EPCs. This CRP-induced EPC dysfunction may impair the vascular regenerative capacity of EPCs, thereby leading to increased risk of cardiovascular diseases.

Keywords: Angiogenesis; Atherosclerosis; C-reactive protein; Endothelial progenitor cell

C-reactive protein (CRP) level, a biomarker of inflammation, has been reported to correlate with increased risk of future cardiovascular diseases such as myocardial infarction, stroke, sudden cardiovascular death, and peripheral vascular disease [1,2]. According to the recently issued guidelines for cardiovascular risk assessment, patients with CRP levels between 1 and 3 mg/L are considered to be at intermediate risk and those with levels above 3 mg/L at high risk [3]. In normal healthy individuals, the CRP level is usually below 1 mg/L unless there are other processes such as acute infection or trauma present. CRP has been reported to be substantially stronger cardiovascular disease indicator than other traditional risk factors, providing additional prognostic information to the currently used Framingham risk scoring and supplementing the low density lipoprotein cholesterol test. However, this predictive value of CRP was recently challenged by a report claiming that CRP measurement does not provide much extra information on coronary heart disease risk as compared with other established risk factors [4]. Although CRP

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0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.06.107

appears to be closely associated with cardiovascular risk, further research might be necessary to clarify the extent to which CRP measurement helps to predict future cardiovascular risk.

Apart from the controversial role of CRP as cardiovascular risk indicator, accumulating evidence has shown the potential direct role of CRP in inducing cardiovascular diseases, in which CRP directly causes endothelial dysfunction through reducing the expression and bioactivity of endothelial nitric oxide synthase (eNOS), and inhibiting in vitro angiogenic functions, as measured by endothelial cell migration and tube formation [5,6]. In a recent study using CRP transgenic mice, CRP was shown to accelerate aortic atherosclerosis development in apolipoprotein E-deficient mice, which proves that CRP is an active player in atherogenic process as well as cardiovascular risk marker [7].

The endothelial progenitor cell (EPC) is a type of adult stem cell important for neovascularization at injured tissues. Upon cytokine stimulation and ischemic insult, EPCs in peripheral blood recruit to the injured vasculature and play a key role in repairing vessels and maintaining vascular integrity [8,9]. Given the significant contribution of EPCs to vascular homeostasis, it may not be surprising that EPC has also emerged as another indicator of developing cardiovascular diseases. Indeed, we and others recently reported that the number of circulating EPCs and their functional activity were profoundly reduced in individuals with high cardiovascular risk [10,11]. This inverse correlation of EPC with cardiovascular disease gave us the hypothesis that there might be a cause–effect relationship between proatherogenic CRP and EPC. As CRP induces endothelial dysfunction, it could directly influence the angiogenic activity of EPCs themselves and attenuate their vascular regenerative potential. Therefore, we investigated the effect of CRP on angiogenic activity and gene expression of EPCs.

Materials and methods

Materials. Recombinant human CRP was purchased from Calbiochem (San Diego, CA, USA). Endotoxin in the CRP was removed with a Detoxigel column (Pierce, Rockford, IL, USA) and found to be less than 0.125 endotoxin units (EU)/mL by Limulus assay (BioWhittaker, Walkersville, MD, USA) [5].

EPC culture and characterization. Peripheral blood mononuclear cells from human volunteers were isolated by density gradient centrifugation and cultured on gelatin-coated plates in endothelial cell basal medium-2 (EBM-2) supplemented with EGM-2 MV Singlequot (Clonetics, Walkersville, MD, USA). After 7 days of culture, EPCs were incubated with various concentrations of CRP, ranging from 0 to 10 mg/L, for 24 h at 37 °C and then used for all assays.

The EPC phenotype was confirmed as previously described [8], by staining with DiI-labeled acetylated LDL (DiI-acLDL) (Molecular Probes, Eugene, OR, USA) and FITC-conjugated *Ulex europaeus* agglutinin (FITC-ulex-lectin) (Sigma, Saint Louis, MO, USA). Dual-staining cells positive for both DiI-acLDL and FITC-ulex-lectin were identified as EPCs. To examine the nitric oxide (NO) production of EPC, EPCs were washed twice with calcium-free phosphate-buffered saline (PBS) and stained with diamino-fluorescein-2 diacetate (DAF-DA, 0.1 μM) (Sigma) with or without pretreatment with L-N^G-nitroarginine methyl ester (L-NAME, 1 mM) (Sigma). The expression of cell-specific surface molecules was investigated by flow cytometric analysis with monoclonal antibodies against von Willebrand Factor (vWF), VE-cadherin, CD34, and CD133 (DAKO, Carpinteria, CA, USA). Two-color flow cytometric analysis was performed on a PAS instrument (PARTEC, Munster, Germany) with 20,000 events stored.

EPC migration and adhesion assays. EPC migration was examined using a modified Boyden chamber (Costar, Cambridge, MA, USA), where the lower side of the filter membrane was coated with 0.5 mg/ml of type I collagen (Sigma). EBM-2 culture media with 0.1% bovine serum albumin or 50 ng/ml VEGF were added to the lower chamber and 5×10^4 EPCs were placed in the upper chamber. After 24 h incubation at 37 °C, the membrane was stained with Giemsa solution and the upper side of the filter was gently wiped away with a cotton ball. Migrated EPCs attached to the lower side of the filter were counted and the cell numbers of CRP-treated, groups were calculated as values relative to those of corresponding untreated controls.

For the assessment of EPC adhesion onto endothelium, human umbilical vein endothelial cells (HUVEC; 2×10^5/well; passages 5–7) were plated on gelatin-coated 4-well slide chambers (Lab-Tek, Naperville, IL, USA) 2 days before experiments. EPCs pretreated with CRP were harvested and labeled with the fluorescent marker, DiI (Molecular Probe). DiI-labeled EPCs (1×10^5) were seeded on the HUVEC monolayer and incubated for 24 h at 37 °C. After washing with PBS, adherent EPCs were counted in five random microscopic fields (200×) by blinded investigators. The cell numbers of CRP-treated groups were calculated as values relative to those of corresponding untreated controls.

Measurement of NO production. Quantification of NO produced by untreated or CRP-treated EPCs was carried out by using the NO-sensitive dye, DAF-DA, that becomes strongly fluorescent in a NO concentration-dependent manner. EPCs were washed twice with calcium-free PBS and bathed in Krebs–Henseleit buffer containing L-arginine (1 mM) and DAF-DA (0.1 μM) for 15 min. After washing with PBS, images of fluorescent cells were captured with a reference image of fluorescent microbeads by fluorescence microscope equipped with an excitation (450–490 nm) and emission (515–560 nm) green filter. Then, DAF-DA fluorescence intensities from each image were processed by the Image Pro Plus (Media Cybernetics) [12]. Fluorescence intensity from EPCs pre-incubated with L-NAME was measured in the same way and subtracted from those from untreated or CRP-treated EPCs. The fluorescence intensities of CRP-treated groups were calculated as values relative to those of corresponding untreated controls.

Reverse transcriptase-polymerase chain reaction. RNA was extracted using RNeasy mini kits (Qiagen, Valencia, CA, USA) and cDNA synthesis was performed using the Superscript first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). PCR primers were designed as follows: Flt-1, forward 5′-gaagaggatgaaggtgtctatcac-3′, reverse 5′-tgctgatgcttgaaccactttt-3′; eNOS, forward 5′-gacattgagagcaaagggctgc-3′, reverse 5′-cggcttgtcacctcctgg-3′; signal transducers and activators of transcription 1 (STAT1), forward 5′-ccgttttcatgacctcctgt-3′, reverse 5′-tgaatattccccgactgagc-3′; STAT2, forward 5′-agctgctgaaggagctgaag-3′, reverse 5′-agtctcaccagcagccttgt-3′; STAT3, forward 5′-cctttggaacgaagggtaca-3′, reverse 5′-cggactggatctgggtctta-3′; suppressors of cytokine signaling 1 (SOCS1), forward 5′-ctgggatgccgtgttatttt-3′, reverse 5′- taggaggtgcgagttcaggt-3′; SOCS2, forward 5′-tgagtgatgcttccttcct-3′, reverse 5′-agtgaggcctgtgtcagctt-3′; SOCS3, forward 5′-atcctggtgacatgctcctc-3′, reverse 5′-caaatgttgcttccccctta-3′; β-actin, forward 5′-aagacattttcgggctcac-3′, reverse 5′-ggcactttagtagttctcc-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward 5′-cgtggaaggactcatgac-3′, reverse 5′-caaattcgttgtcataccag-3′.

Northern blot analysis. Total RNA (10 μg/lane) was size-fractionated through a 1% agarose-formaldehyde gel and transferred to nylon

membrane (Schleicher and Schuell, Dassel, Germany). The cDNA probes were generated by RT-PCR from total RNA of EPCs or HUVEC and labeled with [^{32}P]dCTP (Amersham) using a random-primer kit (Roche, Mannheim, Germany). Membranes were hybridized overnight at 65 °C and exposed to Kodak X AR5 film (Eastman Kodak, Rochester, NY, USA). Each membrane was stripped and reprobed with a GAPDH cDNA probe in order to confirm equal loading. The densitometric analysis of the Northern blots was performed with the use of the Image-Lab (MCM design).

Enzyme-linked immunosorbent assay (ELISA). The cytokine secretion from EPCs was evaluated by Quantikine human immunoassays (R&D, Minneapolis, MN, USA) according to the manufacturer's protocols. Equal numbers (1×10^5/ml) of EPCs were pre-incubated with CRP (7.5 mg/L) for 24 h and were switched into new culture media. After additional 24 h incubation, media were collected and analyzed for monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), interferon-inducible protein-10 (IP-10), granulocyte macrophage-colony stimulating factor (GM-CSF), platelet-derived growth factor-BB (PDGF-BB), and basic fibroblast growth factor (bFGF). The EPC culture media did not contain measurable amounts of these cytokines. Serial dilutions of recombinant proteins were used as standards.

Statistical analysis. All data are presented as means ± SEM. Paired *t* tests were used to determine differences between groups and values of $p < 0.01$ were considered significant. The number of samples examined is indicated by *n*.

Results and discussion

EPC characterization

Spindle-shaped cells cultured from human peripheral blood mononuclear cells were positive for both DiI-ac-LDL uptake and FITC-ulex-lectin binding, corresponding to the previously described EPC phenotype (Fig. 1A) [8,9]. The NO production from EPC was visualized by staining with a NO-specific fluorescence probe, DAF-DA, and the NOS-derived NO release from EPC was confirmed by faint DAF-DA fluorescence after pretreatment with NOS inhibitor, L-NAME. Flow cytometric analysis demonstrated that the EPCs expressed the endothelial specific markers, vWF (53.3% positive) and VE-cadherin (47.1% positive) (Fig. 1B), whereas EPCs were negative for hematopoietic stem/progenitor cell markers after 7 days of in vitro culture [13].

Effect of CRP on migratory activity of EPCs

Recruitment of EPCs to injured vessels appears to be a multifactorial process that might be regulated by various cytokines and chemokines, such as VEGF and stromal-derived growth factor-1 [14,15]. Among these factors, VEGF, highly induced in ischemic tissue due to hypoxia-inducible transcription, promotes the proliferation and differentiation of EPCs, and its chemotactic property makes it possible for EPCs to enter and migrate into the sites of tissue injury [16,17]. Given the significance of VEGF-induced migration in the EPC homing process, we assessed the migratory capacity of EPCs in response to VEGF using a Boyden chamber migration assay. Fig. 2A shows that CRP treatment significantly inhibited the migratory activity of EPCs, at a concentration of 2.5 mg/L (34 ± 5% reduction, $^{*}p < 0.01$ vs. control), a plasma level known to predict moderate cardiovascular risk. A further decrease in migration was observed at CRP concentration for high cardiovascular risk, 5 mg/L (51 ± 11% reduction, $^{**}p < 0.05$ vs. 2.5 mg/L). It suggests that circulating EPCs in blood with elevated CRP level might exhibit low responsiveness to VEGF secreted by ischemic tissue and become impaired in their trafficking from the peripheral circulation into target sites.

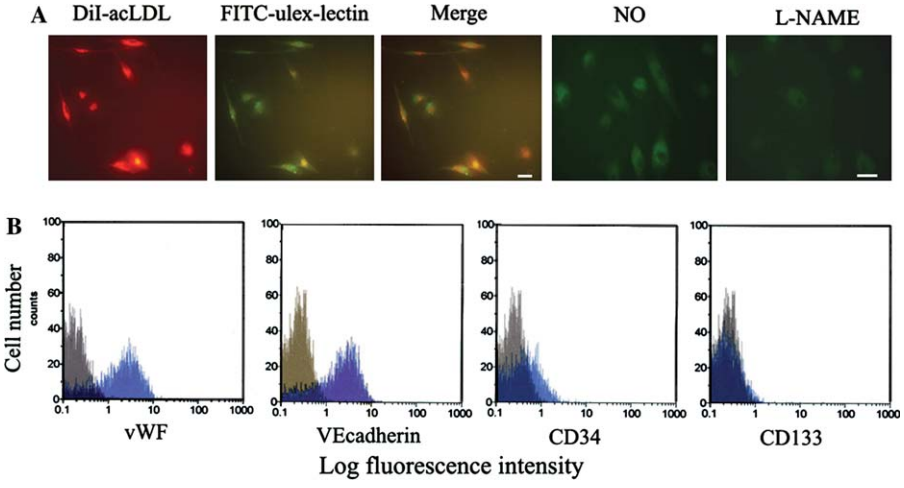

Fig. 1. EPC characterization. (A) Confocal images show that adherent cells are positive for both DiI-acLDL (red) uptake and FITC-ulex-lectin (green) binding, corresponding with the current definition of EPCs. In addition, EPCs release the NOS-derived NO, as assayed by DAF-DA fluorimetry with or without pretreatment with NOS inhibitor, L-NAME. The white bar indicates 50 μm. (B) Representative histograms of FACS analysis of cultured EPCs after 1 week, revealing that EPCs express endothelial cell-specific antigens, vWF and VE-cadherin. The majority of EPCs are negative for hematopoietic stem/progenitor cell markers (CD34 and CD133). Plots show the isotype control (gray) and specific antibody staining (blue).

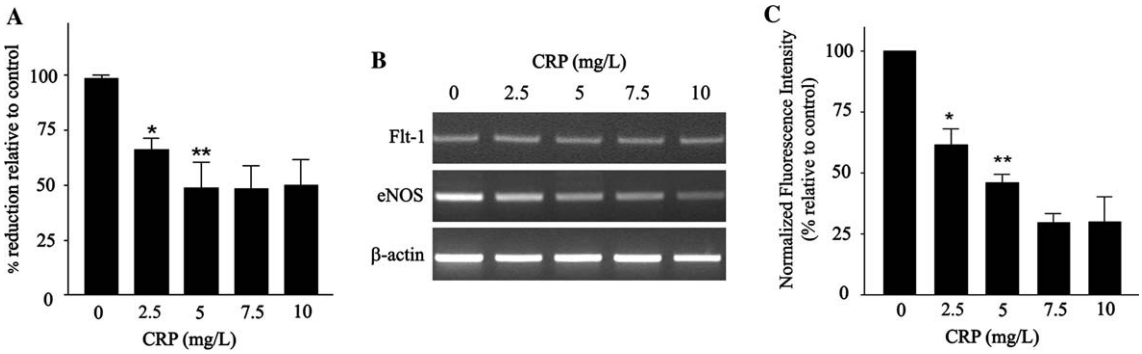

Fig. 2. CRP inhibits VEGF-mediated EPC migration. EPCs cultured until day 7 were treated with various concentrations of CRP for 24 h at 37 °C. The migratory response toward VEGF and the gene expression in EPCs were measured by a modified Boyden chamber migration assay and RT-PCR, respectively. (A) CRP-pretreated EPCs show marked reduction in migratory activity in response to VEGF (50 ng/ml), compared with untreated EPCs ($^{*}p < 0.01$ vs. control, $^{**}p < 0.05$ vs. 2.5 mg/L of CRP). Data are means ± SEM; $n = 6$. (B) The expression of eNOS, but not VEGF receptor, was downregulated in EPCs by CRP treatment. RT-PCR for Flt1 and eNOS mRNA was performed as described in Materials and methods. β-Actin served as a loading control. Similar results were obtained in three additional experiments. (C) CRP-pretreated EPCs have reduced NO production, compared with untreated EPCs ($^{*}p < 0.01$ vs. control, $^{**}p < 0.05$ vs. 2.5 mg/L of CRP). The intracellular NO generation was assessed by using the NO indicator dye, DAF-DA, as described in Materials and methods. Data are means ± SEM; $n = 4$.

Since the reduced migratory activity of EPC in response to VEGF might be due to a decrease in expression of VEGF receptor and/or downstream gene, we performed RT-PCR analysis to determine the mRNA levels of Flt-1 and constitutive eNOS that produces NO, a downstream mediator for VEGF-induced endothelial migration [18,19]. As shown in Figs. 2B and C, VEGF receptor, Flt-1, remained unaltered, whereas basal eNOS expression was significantly downregulated by CRP, possibly leading to the decrease in NO production (39 ± 6% reduction at 2.5 mg/L, $^{*}p < 0.01$ vs. control; 54 ± 3% reduction at 5 mg/L, $^{**}p < 0.05$ vs. 2.5 mg/L). The association between CRP effects on NO level and EPC migration suggests that CRP might attenuate migratory activity of EPCs by reducing NO production. Along with a role in endothelial migration, NO is known to modulate VEGF-induced angiogenesis and plays a protective role in the vasculature, by relaxing vascular tone and promoting neovascularization at ischemic tissue [20,21]. In this regard, endogenous NO level in EPCs would be crucial in determining the responsiveness to VEGF and following VEGF-mediated angiogenic activities, implying that EPCs exposed to CRP might exhibit limited vascular regenerative potential at injured sites.

Effect of CRP on adhesion of EPCs onto HUVEC monolayer

We next evaluated the effect of CRP treatment on the interaction of EPCs with endothelial cells, which is also important for EPC homing and incorporation into neovascularization sites [13]. As shown in Fig. 3A,

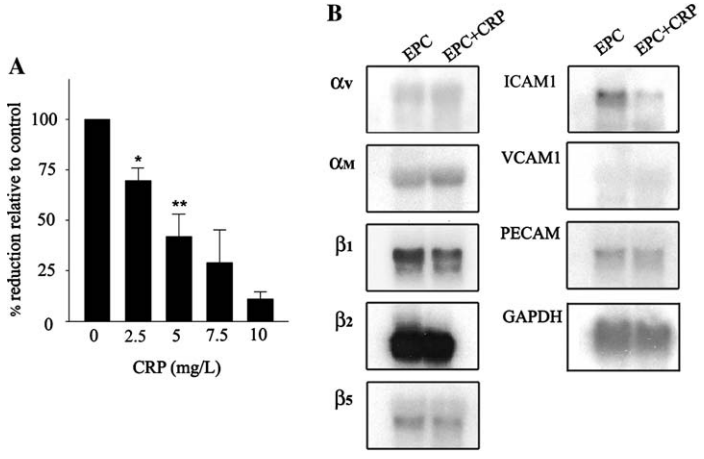

Fig. 3. CRP reduces the adhesiveness of EPC to HUVEC monolayers. (A) EPCs incorporated onto HUVEC monolayers are much fewer in CRP-treated groups than in untreated groups ($^{*}p < 0.01$ vs. control, $^{**}p < 0.01$ vs. 2.5 mg/L of CRP). DiI-labeled EPCs, pretreated with CRP or PBS, were incubated with confluent HUVEC monolayer for 24 h at 37 °C. Adhesive activities of EPCs were quantified by counting the number of red fluorescent EPCs in five random microscopic fields. Data are means ± SEM; $n = 4$. (B) CRP decreased the gene expression of adhesion-related molecules, ICAM1 and β_2 integrin subunits, on EPCs. Total RNA samples (10 μg) from EPCs with or without CRP pretreatment (7.5 mg/L) were analyzed by Northern blotting using the indicated probes. GAPDH was used as a loading control.

CRP-treated EPCs were significantly impaired in their ability to adhere to HUVEC monolayers at as low as 2.5 mg/L CRP (30 ± 3% reduction, $p < 0.01$). At higher concentration (≥5 mg/L), CRP showed more obvious inhibitory effect on EPC adhesion onto HUVEC monolayers. In conjunction with previous EPC migration data, this result implies that EPCs exposed to high CRP level (>3 mg/L) exhibited a further decrease in angiogenic functionality than those treated at moderate CRP level (1–3 mg/L).

This reduced adhesiveness of EPCs could result from the direct effect of CRP on the gene expression of vascular adhesion molecules in EPCs. Therefore, Northern blotting analysis was performed with CRP-treated and untreated EPCs to compare the mRNA level of several adhesion-related genes that are important for the hematopoietic stem cell homing pathway and leukocyte adhesion cascade [22,23]. As shown in Fig. 3B, CRP induced the substantial downregulation of intercellular adhesion molecule 1 (ICAM1; 64% reduction vs. control) and β_1 integrin subunit, which composes very late antigen-4 (20% reduction vs. control) on human EPCs. However, CRP had little effect on the expression of other adhesion-related molecules [α_V, α_M, β_2, and β_5 integrin subunits, vascular cell adhesion molecule 1 (VCAM1), and platelet/endothelial cell adhesion molecule (PECAM)]. Besides the inhibitory effect on the gene expression of ICAM1 and β_2 integrin, CRP may interfere with integrin activation by inhibiting the conformational change from the bent structure (low affinity) into the stretched structure (high affinity). According to several studies, CC chemokines such as MCP-1, MCP-3, and MIP-1 induce functionally active neoepitopes in β_1 and β_2 integrins and facilitate firm adhesion of monocytes to activated endothelium [24,25]. These findings imply that reduced adhesiveness of CRP-treated EPCs might be in part due to a decreased secretion of MCP-1 and MIP-1, as noted below in our study. However, further study is necessary to elucidate the precise mechanism of CRP effect on EPC adhesion.

Effect of CRP on the expression of arteriogenic chemocytokines in EPCs

Human EPCs have been recently reported to possess two different subpopulations that are distinct from each other in terms of cell growth pattern and phenotype [12,26]. In detail, late outgrowth EPCs exhibit typical endothelial characteristics with great proliferation potential and intrinsic angiogenicity, whereas early outgrowth EPCs express monocyte markers as well as endothelial-specific antigens, and also secrete proangiogenic cytokines including VEGF, hepatocyte growth factor, G-CSF, and interleukin-8 [12,27]. In accordance

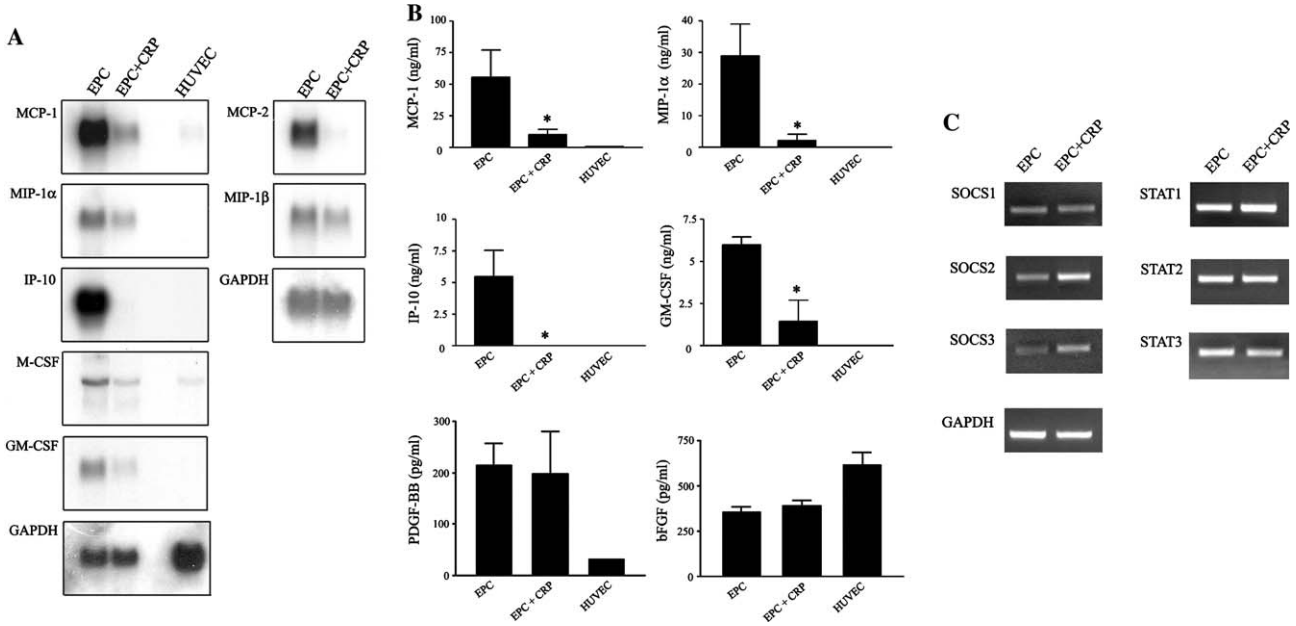

Fig. 4. CRP decreases the expression of arteriogenic chemokines and cytokines in EPCs, in part by upregulating SOCS 2 and 3 genes. (A) CRP downregulates the mRNA levels of several chemokines and cytokines, such as monocyte/macrophage and T-lymphocyte recruiting chemokines (MCP, MIP-1, and IP-10), and monocyte/macrophage surviving factors (M-CSF, GM-CSF). Total RNA samples (10 μg) from EPCs with or without CRP pretreatment (7.5 mg/L, 24 h at 37 °C) were analyzed by Northern blotting using the indicated probes. GAPDH was used as a loading control. (B) CRP reduces the secretion of MCP-1, MIP-1α, IP-10, and GM-CSF from EPCs. EPCs, pretreated with PBS or CRP (7.5 mg/L), were cultured for an additional 24 h at a concentration of 1×10^5 cells/ml of medium. Conditioned media were harvested and analyzed by ELISA. Data are expressed as means ± SEM. ($n = 4$) and asterisks indicate a significant difference from untreated EPC control ($^*p < 0.05$). (C) The expression of SOCS 2 and 3 genes was upregulated in EPCs by CRP treatment. RT-PCR for SOCS and STAT mRNA was performed as described in Materials and methods. GAPDH served as a loading control. Similar results were obtained in three additional experiments.

with monocytic phenotypes of early outgrowth cells, EPCs used in the present study also highly expressed various chemokines and cytokines, such as MCP-1, MIP-1α, IP-10, and GM-CSF (Figs. 4A and B). The potential biological significance of this expression in EPCs is interesting, as those endogenously expressed chemokines chemoattract monocytes/macrophages and T-lymphocytes that are necessary for arteriogenesis, particularly the formation of large conductance collaterals [28,29]. In addition, colony stimulating factors are known to improve the survival of monocytes/macrophages, providing an environment for stable monocyte function during the arteriogenic process [30]. However, in CRP-treated EPCs, these arteriogenic chemokines and cytokines were markedly downregulated at CRP concentration of 7.5 mg/L (Fig. 4A). In particular, the mRNA expression of MCP-1, MCP-2, and IP-10 in EPCs was almost completely blocked by CRP. MIP-1 and colony stimulating factors showed an approximately twofold reduction of mRNA level in CRP-treated EPCs. These data were further supported by ELISA (Fig. 4B), which revealed that CRP treatment significantly reduced the secretion of MCP-1, MIP-1α, IP-10, and GM-CSF in human EPCs. However, when assayed for arteriogenic growth factors such as bFGF and PDGF-BB, their protein levels in EPCs were not affected by CRP treatment, indicating that CRP modulates the expression of specific chemo-cytokines.

In an attempt to elucidate this inhibitory effect of CRP on chemo-cytokine secretion, we looked through the cDNA microarray result (data not shown), where several SOCS genes were significantly upregulated in CRP-treated EPCs. SOCS proteins are recently identified as inhibitor of the Janus kinase (JAK)/STAT pathway that regulates important biological responses, such as immune function, cellular growth, differentiation, and hematopoiesis [31]. In particular, JAK/STAT signaling is involved in transcriptional induction of MCP-1, IP-10, ICAM1, and NOS genes, which possess STAT-binding elements in their promoter regions [32]. In Fig. 4C, RT-PCR analysis was performed to examine the change in mRNA levels of several SOCS and STAT proteins upon CRP treatment, demonstrating that SOCS 2 and 3 were highly upregulated in CRP-treated EPCs (SOCS2; 2.3-fold induction, SOCS3; 3.7-fold induction). This CRP-induced upregulation of SOCS proteins may lead to the inhibition of JAK/STAT pathway, possibly resulting in a decrease in the expression of arteriogenic chemo-cytokines in CRP-treated EPCs. In addition, CRP-mediated induction of SOCS proteins might explain our observation that CRP decreased the gene expression of ICAM1 and eNOS in EPCs, thereby attenuating angiogenic activity of EPCs.

Based on previous reports and our current findings, EPCs are shown to be not only directly involved in postnatal vasculogenesis as a substrate of new endothelial cells, but also might secrete arteriogenic chemo-cytokines at EPC-incorporated foci, which in turn contributes to the development of mature conductance collateral vessels. However, upon exposure to CRP, these chemo-cytokines were drastically downregulated in EPCs, which might account for why individuals with high CRP levels exhibit high sensitivity to ischemic injury and possess a high risk of developing cardiovascular diseases. In addition, our present study may provide some insight into the unresolved mechanism underlying CRP-induced EPC dysfunction, possibly contributing to the better understanding of proatherogenic functions of CRP.

In conclusion, our findings suggest that CRP directly inhibits the angiogenic activity of EPCs and decreases the secretion of various arteriogenesis-related chemo-cytokines. Given the significant contribution of EPCs to vascular homeostasis, EPC dysfunction at physiological concentrations of CRP might lead to limited vascular regenerative capacity at injured sites by impaired angiogenesis and arteriogenesis. This functional impairment of EPCs may explain, in part, the proatherogenicity and increased incidence of cardiovascular diseases that are associated with high levels of CRP. In this regard, CRP-lowering therapy would be useful in restoring the vascular regenerative capacity of EPCs as well as by reducing damage to the vascular wall.

Acknowledgments

This work was supported by National Research Laboratory Grants from Korea Institute of Science and Technology Evaluation and Planning (M1-0203-00-0048) to D.K. Kim.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2004.06.107.

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