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Antiphospholipid Antibodies Induce Monocyte Chemoattractant Protein-1 in Endothelial Cells

Chul-Soo Cho,2* Mi-La Cho,* Pojen P. Chen,† So-Youn Min,* Sue-Yun Hwang,* Kyung-Soo Park,* Wan-Uk Kim,* Do-June Min,* Jun-Ki Min,* Sung-Hwan Park,* and Ho-Youm Kim*

The presence of antiphospholipid Ab is associated with increased risk of thrombosis. The monocyte-endothelial cell interaction has been suggested to play a key role at the site of vascular injury during thrombosis. Therefore, we tested the effect of anticardiolipin Abs (aCL) on the production of monocyte chemoattractant protein-1 (MCP-1) in HUVEC. We found that monoclonal aCL as well as IgG fractions from patients with antiphospholipid syndrome (APS-IgG) could induce the production of MCP-1 in HUVEC. The ability of IgG aCL to induce MCP-1 production could be abrogated by preabsorption with cardiolipin liposomes. Simultaneous addition of either monoclonal aCL or APS-IgG with IL-1β resulted in synergistic increase in MCP-1 production, whereas the addition of control IgG lacking aCL activity did not alter IL-1β-induced levels of MCP-1. MCP-1 mRNA expression was also up-regulated when HUVEC were incubated with either APS-IgG or monoclonal aCL, and down-regulated by the treatment of dexamethasone. In addition, we found that serum levels of MCP-1 in 76 systemic lupus erythematosus patients correlated well with the titers of IgG aCL. Collectively, these results indicate that aCL could promote endothelial cell-monocyte cross-talk by enhancing the endothelial production of MCP-1, thereby shifting the hemostatic balance toward the prothrombotic state of APS. The Journal of Immunology, 2002, 168: 4209–4215.

Antiphospholipid Abs (aPL)3 are a family of autoantibodies recognizing negatively charged phospholipids that are complexed with phospholipid binding proteins (1–3). The presence of aPL is associated with thrombosis, thrombocytophenia, intrauterine fetal loss, and a variety of neurological syndromes (4). These clinical manifestations accompanied by the presence of aPL are recognized as the antiphospholipid syndrome (APS). Many studies were performed to determine how aPL might induce thrombus formation in vitro, and it has been suggested that inhibition of fibrinolysis (5, 6) and proteins C and S (7–10), as well as activation of endothelial cells (11, 12), platelets (13–15), and monocytes (14, 16–18), may contribute to the induction process.

Monocyte-endothelial cell interactions play a key role in the development of thrombosis, inflammation, and atherosclerosis (19–23). The initial step in these interactions involves local generation of soluble chemoattractant associated with activation of endothelium juxtaposed to the site of vascular injury. This event causes increased recruitment of monocytes along endothelium and then induces subsequent attachment of monocytes to endothelial cells via a set of adhesion molecules (22). The exposure of monocytes to endothelial cells may provide further stimuli for monocytes to induce synthesis and release of inflammatory mediators such as IL-1γ and TNF-α. These cytokines, in turn, could enhance local inflammatory response by promoting further monocyte attachment through increased endothelial expression of adhesion molecules and simultaneously inducing the release of IL-1β and TNF-α from endothelium (23). Finally, the effects of this adhesion and cytokine production are culminated into the enhancement of procoagulant activity, via the induction of tissue factor expression (20, 23, 24). These events might occur at the same time, leading to activation of coagulation followed by thrombus formation.

Monocyte chemoattractant protein-1 (MCP-1) belongs to the β C-C subfamily of chemokines, which stimulate the migration of monocytes (25). MCP-1 exerts various effects on monocytes, including the induction of integrin and tissue factor and the release of proinflammatory cytokines and arachidonic acid (26–29). MCP-1 is produced in human endothelial cells, mononuclear phagocytes, and fibroblasts in response to a variety of stimuli such as TNF-α, IL-1β, IL-4, LPS, leukemia inhibitory factor, and IFN-γ (25, 30–34). The overexpression of MCP-1 has been implicated in several pathologic conditions including atherosclerosis, thrombosis, and inflammatory disease (35–40).

It has been demonstrated that aPL induce endothelial cell activation, which enhances the expression of adhesion molecules, thus promoting the binding of monocytes to stressed endothelium (41). In this study, we hypothesized that aPL could enhance the production of MCP-1 by endothelial cells to facilitate trafficking of monocytes to endothelium, based on the fact that cross-talk between endothelial cells and monocytes could play an important role in thrombosis. To this end, we examined whether polyclonal and monoclonal IgG anticardiolipin Abs (IgG aCL) derived from APS patients could induce MCP-1 production from endothelial

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4Abbreviations used in this paper: aPL, antiphospholipid Ab; CL, cardiolipin; aCL, anti-CL Ab; MCP-1, monocyte chemoattractant protein-1; SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; NC, normal control; β2gpI, β2-glycoprotein I; DEX, dexamethasone; LAC, lupus anticoagulant.

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cells. We found that the IgG aCL was able to enhance the expression of MCP-1 in both protein and mRNA levels. IL-1β displayed a synergistic effect on aPL-induced MCP-1 production, while the enhanced induction of MCP-1 was suppressed by treatment of dexamethasone (DEX). Moreover, circulating MCP-1 levels correlated well with the titers of IgG aCL in sera of 76 systemic lupus erythematosus (SLE) patients. Taken together, the increased endothelial release of MCP-1 by aPL might play an important role in thrombus formation by enhancing the influx of monocytes in concert with an endothelial activator such as IL-1.

Materials and Methods

Patients, blood samples, and isolation of IgG

Five SLE patients with APS seen at the Lupus Clinic of Kangnam St. Mary’s Hospital (Seoul, Korea) were chosen for this study based on the presence of aPL and thrombosis. All patients fulfilled American College of Rheumatology criteria for the classification of APS (42). Control sera were obtained from five SLE patients and five healthy subjects lacking antiphospholipid Ab (aCL), all of whom were Korean. The serologic and clinical characteristics of SLE patients are summarized in Table I. IgG fractions from APS patients (APS-IgG) and control sera of SLE patients without APS (SLE-IgG) and healthy subjects (normal controls (NC-IgG)) were purified on protein G columns (HRTrap Protein G; Pharmacia Biotech, Uppsala, Sweden). Two mAbs against cardiolipin (CL) (CL15 and IS4) were derived from EBV-transformed cell lines from two APS patients, whose clinical and serologic characteristics have previously been described (43). CL15 and IS4 were generated as the conventional aCL (by being screened against CL in the presence of bovine serum). Characterization of these mAbs shows that CL15 and IS4 bind to CL in the presence of bovine serum (43). Purification of IS4 was performed as previously described (43).

Isolation and culture of endothelial cells

HUVEC were isolated from normal-term umbilical cord vein by collagenase digestion (45) and then grown to confluence in 75-cm² flasks containing M199 medium (Life Technologies, Grand Island, NY) supplemented with 20% FBS (Life Technologies), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. Cultures were kept at 37°C in a CO₂ incubator and medium was changed every 2–3 days until confluence was reached. HUVEC were passaged with 0.2% collagenase and 0.02% EDTA (Life Technologies); cells from passages 2–3 were used in these studies.

MCP-1 production by aPL or cytokines

HUVEC were plated on a 24-well plate containing M199 medium supplemented with 10% FBS at densities of 1 × 10⁵ cells/ml, allowed to grow at 37°C for 1 day, and then washed once with M199. Cells were incubated for 24 h with medium alone, different concentrations of APS-IgG (10–1,000 µg/ml), or aCL-mAb (1–50 µg/ml). IL-1β (Endogen, Woburn, MA) was added to selected wells at the beginning of culture. To determine the specificity of APS-IgG on MCP-1 production, absorption experiments were performed as previously described (46). Briefly, the sera were mixed with CL liposomes and incubated overnight at 4°C. The mixtures were then centrifuged at 30,000 × g for 15 min at 4°C, and the supernatants were collected and kept as absorbed sera. In some experiments, HUVEC grown in M199 medium containing 10% FBS were washed three times with HBSS (Life Technologies) to remove adherent serum proteins such as β₂gpI, and then cultured in a serum-free medium supplemented with insulin-transferrin-selenium-A (Life Technologies). Thereafter, purified human β₂gpI (10 µg/ml; Crystal Chem, Chicago, IL) was added to the serum-free HUVEC cultures. All cultures were incubated for 24 h (unless otherwise stated), and cell-free supernatant was collected and stored at −20°C until assay. All cultures were set up in triplicates and the results are expressed as mean ± SD.

Quantitative analysis of MCP-1 by ELISA

MCP-1 levels in culture supernatants and patients’ sera were measured by sandwich ELISA. Briefly, microtiter wells were coated with 100 µl per well of 2 µg/ml mouse anti-human MCP-1 (R&D Systems, Minneapolis, MN) in 50 mM sodium carbonate (pH 9.6). After incubation overnight at 4°C, wells were blocked with 1% BSA in PBS for 1 h at room temperature. The human rMCP-1 (R&D Systems) or test samples were added to the wells and incubated for 2 h at room temperature. Bound MCP-1 was detected with peroxidase-labeled extravidin (Sigma-Aldrich, St. Louis, MO), and substrate (TMB/H₂O₂). An automated microplate reader was used to measure the OD at a wavelength of 450 nm. Between each step, the plate was washed four times with PBS containing 0.1% Tween 20. Human rMCP-1, diluted in culture medium ranging from 30 to 2500 pg/ml, was used as a calibration standard. A standard curve was drawn by plotting OD vs the log of the rMCP-1 concentration.

Quantitative analysis of MCP-1 mRNA by RT-PCR

Confluent HUVEC were incubated with either APS-IgG or aCL-mAb in 1 cm² tissue culture dishes. After 6 h of incubation, total RNA was extracted using RNAzol B according to the manufacturer’s instructions.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex/Age</th>
<th>Clinical Manifestation</th>
<th>aCL (GPL)</th>
<th>IgG-Anti-β₂gpI</th>
<th>LAC</th>
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<td>SLE with APS</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<td>91</td>
<td>+</td>
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<td>3</td>
<td>F/42</td>
<td>IVC thrombosis, DVT</td>
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<td>102</td>
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<tr>
<td>4</td>
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<td>69</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>LN, PE</td>
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<td>&lt;5</td>
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<td>APS/SLE, DVT</td>
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<td>72</td>
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Table I. Clinical and laboratory profiles of patients in this study

a GPL, IgG phospholipid; IVC, inferior vena cava; DVT, deep vein thrombosis; HA, hemolytic anemia; LN, lupus nephritis; PE, pleural effusion; NP, neuropsychiatric; PAPS, primary antiphospholipid syndrome; F, female; M, male.
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(Biotex Laboratories, Houston, TX). One microgram of RNA was reverse-transcribed at 42°C using the Superscript reverse transcription system (Life Technologies) by adding 2.5 mM dNTPs, 2.5 U Taq DNA polymerase (Takara Shuzo, Shiga, Japan), and 0.25 µM sense and antisense primers. The following primers were used: sense (5'-CAATAGGAAGATCT CAGTG-3') and antisense (5'-GTGTTCAGTTTCAGGAT-3') for MCP-1 (47), and sense (5'-CCATGGAGAACCTGAGG-3') and antisense (5'-CAAAGTTCTACCGATGAC-3') for GAPDH. Reactions were processed in a DNA thermal cycler (PerkinElmer/Cetus, Norwalk, CT) through cycles of 30 s of denaturation at 94°C and 45 s of annealing at 60°C, followed by 60 s of extension at 72°C. Amplifications were preceded by a denaturation of 90 s at 94°C and followed by a final extension of 7 min at 72°C. PCR rounds were repeated for 32 cycles with MCP-1 and for 28 cycles with GAPDH. Amplified products were analyzed by 2% agarose gel electrophoresis and the band intensity of products was measured by densitometer. Results were expressed as a ratio of quantified MCP-1 product over GAPDH product.

Results

aPL induces MCP-1 in endothelial cells

To study the effects of APS-IgG on MCP-1 production by endothelial cells, HUVEC were cultured with 500 µg/ml individual IgG preparations from five SLE patients with APS (APS-IgG), five SLE patients lacking aCL activity (SLE-IgG), and five normal controls (NC-IgG). The results showed that APS-IgG-treated HUVEC secreted significantly more MCP-1 than either untreated HUVEC or HUVEC cultured with either NC-IgG or SLE-IgG. The MCP-1 levels (mean ± SD) were 201.2 ± 29.7 pg/ml for five APS-IgG-treated HUVEC and 112.6 ± 7.3 pg/ml for five NC-IgG-treated HUVEC. The MCP-1 induced by either SLE-IgG or NC-IgG was not significantly different from that in untreated cultures (Fig. 1).

The specificity of APS-IgG on MCP-1 production was examined by absorption study with CL liposome. Preabsorption of sera from three APS patients with CL liposome almost completely depleted aCL in the remaining preparation (data not shown). When HUVEC were incubated with these absorbed sera, there was no increase in MCP-1 production (Fig. 2). Subsequently, we examined the effect of patient-derived aCL-mAb on the endothelial production of MCP-1. HUVEC were incubated separately with two aCL-mAb at 10 µg/ml. The results showed that these aCL-mAb increased MCP-1 compared with untreated HUVEC (Fig. 3). In particular, MCP-1 induced by CL15 was comparable to that induced by 10 ng/ml IL-1β. To determine the concentration dependence of aCL-enhanced MCP-1 production, HUVEC were incubated with varying concentrations of APS-IgG, SLE-IgG, NC-IgG, and aCL-mAb. The results showed that the enhanced MCP-1 production by APS-IgG or aCL-mAb was concentration dependent. In contrast, IgG from both SLE patients and healthy subjects did not increase MCP-1 production even at the concentration of 1 mg/ml (Fig. 4A). Next, we studied the time course of aCL-induced MCP-1 production. Fig. 4B shows that increased MCP-1 production was detectable 6 h after incubation and the increase continued up to 24 h. The biologic activity of aCL-induced MCP-1 was confirmed by measuring its ability to induce monocyte chemotaxis. Supernatants from APS-IgG-treated HUVEC exhibited 2.3-fold higher chemotactic activity for monocytes than from NC-IgG-treated HUVEC. Moreover, enhanced chemotactic activity was significantly abrogated by preincubation of 10 µg/ml anti-MCP-1 Ab (~45% inhibition, data not shown). To exclude the possibility that aCL-induced MCP-1 production was due to the endotoxin contamination, HUVEC were incubated with test IgG aCL and polymyxin B (5 µg/ml). No reduction in aCL-induced MCP-1 production was observed (data not shown), suggesting that endotoxin contamination did not play a significant role in the enhancement of MCP-1 production by aCL.

Induction of MCP-1 by aPL is dependent on β2,gp1

It has been known that binding of aCL to anionic phospholipid, as well as the thrombotic effects of aCL, are dependent on the presence of serum cofactor protein β2,gp1 (2, 48, 49). To determine whether aCL-induced MCP-1 production is dependent on β2,gp1, HUVEC were cultured with test aCL in serum-free medium with...
or without β2-gp1. As shown in Fig. 5, the aCL-induced MCP-1 production in serum-free medium was significantly lower than that in regular culture medium. Importantly, the addition of β2-gp1 (10 μg/ml) restored the aCL-induced enhancement of MCP-1 production. In contrast, the addition of β2-gp1 had no effect on the MCP-1 production by HUVEC cultured with SLE-IgG. These findings indicate that the effect of aCL on increasing MCP-1 production is dependent on the presence of β2-gp1.

**Effect of cytokines on MCP-1 production induced by aCL**

Proinflammatory cytokines such as IL-1β and TNF-α have been shown to induce synthesis and secretion of MCP-1 by endothelial cells (30–32). Thus, we studied possible synergistic or antagonistic interaction(s) between aCL and IL-1β on MCP-1 production. To this end, HUVEC were cultured with a suboptimal concentration of IL-1β (1 ng/ml), together with APS-IgG (500 μg/ml) or aCL-mAb (10 μg/ml). The results showed that IL-1β synergized with aCL in enhancing MCP-1 production by endothelial cells (Fig. 6).

*aCL induces the expression of MCP-1 mRNA in endothelial cells*

To determine whether the increased level of MCP-1 protein are reflected at the mRNA level, we examined the effect of aCL on the

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**FIGURE 5.** Induction of MCP-1 by aCL is dependent on β2-gp1. HUVEC were incubated with indicated IgG samples in serum-free medium in the presence (filled bars) or absence (open bars) of β2-gp1 (10 μg/ml). The MCP-1 level in culture supernatant was then measured and is given.

**FIGURE 6.** Synergistic effect of IL-1β on MCP-1 induction by aCL. HUVEC were cultured with the indicated IgG samples in the presence (filled bars) or absence (hatched bars) of IL-1β. Other conditions are same as in Fig. 1.
endothelial expression of MCP-1 mRNA by semiquantitative RT-PCR analysis. Representative results from three independent experiments are shown in Fig. 7. Untreated HUVEC had a minimal expression of MCP-1 mRNA (Fig. 7, lane 1), which increased substantially after incubation with APS-IgG (500 μg/ml; Fig. 7, lane 2), aCL-mAb (10 μg/ml; Fig. 7, lane 5), or IL-1β (1 ng/ml; Fig. 7, lane 3) for 6 h. Simultaneous stimulation of HUVEC with IL-1β (1 ng/ml) and APS-IgG (Fig. 7, lane 4) or aCL-mAb (Fig. 7, lane 6) strongly enhanced the MCP-1 mRNA expression in a synergistic fashion. These results were consistent with the data of MCP-1 production at the protein level.

Suppressive effect of DEX on aCL-induced MCP-1 production

It has been reported that induction of MCP-1 can be suppressed by an anti-inflammatory agent such as glucocorticoid hormone (50). Accordingly, we examined the effect of DEX on aCL-induced MCP-1 production in endothelial cells. As shown in Fig. 8, DEX inhibited both constitutive and aCL-induced MCP-1 production in a dose-dependent manner. The maximum effect was achieved at a concentration of 1 μM DEX (the highest dose tested).

Correlation between IgG aCL and MCP-1 in sera of SLE patients

To ascertain the clinical relevance of above findings in APS patients, we quantified MCP-1 and IgG aCL in serum samples from 76 SLE patients and 99 healthy controls. Levels of MCP-1 correlated well with the titers of IgG aCL in SLE patients (r = 0.62 and p < 0.001 by Spearman’s rank correlation test) (Fig. 9A). Furthermore, we examined serial serum samples from an APS patient collected over 36 mo who had a high titer of IgG aCL and received initial plasmapheresis and subsequent i.v. Ig treatment for recurrent abortion. The levels of MCP-1 and IgG aCL rose and fell concomitantly during follow-up periods (Fig. 9B).

FIGURE 7. Induction of MCP-1 mRNA by aCL. HUVEC were cultured for 6 h in medium alone (lane 1), or with 500 μg/ml APS-IgG (lane 2), 1 ng/ml IL-1β (lane 3), APS-IgG plus IL-1β (lane 4), CL15 mAb (lane 5), and CL15 mAb plus IL-1β (lane 6). Total RNA was extracted and subjected to RT-PCR analysis. The levels of mRNA are expressed as the fold increase relative to mRNA from untreated cells, adjusted for the levels of GAPDH signal. Representative results from three similar experiments are shown.

FIGURE 8. Suppression of aCL-induced MCP-1 production by treatment of DEX. HUVEC were cultured with indicated IgG samples in the absence or presence of various concentrations of DEX (1–1000 nM).

FIGURE 9. A, Correlation of circulating MCP-1 and IgG aCL levels in SLE patients. B, Serial samples from an APS patient who was given initial plasmapheresis (PP) and subsequent Ig (IVIG) treatment for 6 mo due to previous poor obstetric outcome.
aPLs INDUCE MCP-1 IN ENDOTHELIAL CELLS

Discussion

In this study, we demonstrate that polyclonal and monoclonal aCL derived from patients with APS induce the endothelial production of MCP-1 in both protein and mRNA levels and exhibit a synergism with IL-1β on MCP-1 production. The effect of aCL on MCP-1 production is β2-gp1 dependent. In addition, circulating levels of MCP-1 correlate well with levels of IgG aCL in patients with SLE and are significantly higher in patients with thrombosis than in those without (data not shown). Combined, the results of this study show that aCL-induced MCP-1 production in endothelial cells may represent a novel mechanism for aCL-mediated thrombosis in APS.

Once aCL activate endothelial cells to increase their MCP-1 production, the increased amount of MCP-1 is able to attract more monocytes to the activated endothelial cells. The recruited monocytes in turn may augment the further activation of endothelial cells by secreting IL-1β and TNF-α. Ultimately, cytokines released by activated monocytes and endothelial cells lead to the hemostatic balance of monocyte and endothelial cells toward a prothrombotic state by inducing tissue factor procoagulant (22–24). In contrast, MCP-1 may directly promote prothrombotic states by inducing the tissue factor expression in monocytes (27, 28).

Such a hypothesis is supported by previous reports of high circulating levels of MCP-1 during thrombosis (38–40) and positive correlation between plasma levels of MCP-1 and tissue factor in patients with acute myocardial infarction (40).

It has been reported that aPL act synergistically with TNF-α in the induction of endothelial cell extracellular matrix procoagulant activity (51). In our study about the effect of IL-1 on aCL-induced MCP-1 production, a synergistic induction of MCP-1 was noted (Figs. 6 and 7). Although the underlying mechanism of this synergistic effect is unknown, it may be of clinical relevance for explaining the intermittent occurrence of thrombotic events in APS patients with high titers of aCL for prolonged periods of time. The latter observation implies that an additional factor is required for clinical expression of thrombosis, and aPL may serve to enhance the thrombotic process when initiated by a triggering factor (such as IL-1 or TNF-α), which may be conferred by SLE- or non-SLE-related endothelial cell injury.

Although corticosteroids are not generally recommended in the treatment of APS, their use may be justified in patients with desperate situations, such as catastrophic APS with undefined therapeutic mechanism(s) (52, 53). In the present study, DEX was shown to exhibit a dose-dependent inhibitory effect on both the basal and aCL-induced MCP-1 production (Fig. 8). These data suggest that the therapeutic effect of corticosteroids in the treatment of catastrophic APS might be explained, in part, by the inhibitory effect on MCP-1 production.

The intracellular signal pathway by which aPL is involved in MCP-1 up-regulation is unclear at this point. The functional NF-κB binding site can be found in the promoter of the MCP-1 gene, and cytokines associated with increased MCP-1 expression have been shown to activate endothelial cells through this element (reviewed in Ref. 54). Also, Meroni et al. (55) recently demonstrated that enhanced E-selectin expression by anti-β2-gp1 Abs in HUVEC is associated with NF-κB activation. In our preliminary study, we observed that treatment with antioxidant pyrrolidine dithiocarbamate, a well-known inhibitor of NF-κB, significantly abrogated the aPL-induced up-regulation of MCP-1 (data not shown). Given that the negative regulation of glucocorticoids and pyrrolidine dithiocarbamate on NF-κB activation is well documented (56), these data, along with inhibitory effect by DEX, underscore the involvement of NF-κB activation in aPL-mediated MCP-1 induction in endothelial cells. The effect of aPL on NF-κB activation and other signal molecules is now under investigation. In summary, we report that aCL induces MCP-1 production in HUVEC at the protein and mRNA levels. Moreover, MCP-1 levels are correlated with the titers of IgG aCL in SLE patients; aCL and IL-1β act synergistically to augment endothelial production of MCP-1; and DEX inhibited both the basal and aCL-enhanced MCP-1 production. Combined, these data represent a novel mechanism for aCL-mediated thrombosis in APS patients and may provide potential bases for MCP-1 blockade in the treatment of APS.

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References


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