The p38 mitogen-activated protein kinase (MAPK) pathway mediates induction of the tissue factor gene in monocytes stimulated with human monoclonal anti-\(\beta_2\)Glycoprotein I antibodies

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Abstract

The anti-phospholipid syndrome (APS) is characterized by thrombosis and the presence of anti-phospholipid antibodies (aPL). Tissue factor (TF), the major initiator of the coagulation system, is induced on monocytes by aPL in vitro, explaining, in part, the pathophysiology in this syndrome. However, little is known regarding the nature of the aPL-induced signal transduction pathways leading to TF expression. In this study, we investigated aPL-inducible genes in PBMC using cDNA array system and real-time PCR. Our results indicated that the mitogen-activated protein kinase (MAPK) pathway was related to TF expression when PBMCs were treated, in the presence of \(\beta_2\)Glycoprotein I (\(\beta_2\)GPI), with human monoclonal anti-\(\beta_2\)GPI antibodies [\(\beta_2\)GPI-dependent anti-cardiolipin antibodies (aCL/\(\beta_2\)GPI)]. Western blotting studies using monocyte cell line (RAW264.7) demonstrated that p38 MAPK protein was phosphorylated with nuclear factor \(\kappa B\) (NF-\(\kappa B\)) activation by monoclonal aCL/\(\beta_2\)GPI treatment, and that SB203580, a specific p38 MAPK inhibitor, decreased the aCL/\(\beta_2\)GPI-induced TF mRNA expression. The p38 MAPK phosphorylation, NF-\(\kappa B\) translocation and TF mRNA expression triggered by aCL/\(\beta_2\)GPI were abolished in the absence of \(\beta_2\)GPI. These results demonstrated that the p38 MAPK signaling pathway plays an important role in aPL-induced TF expression on monocytes and suggest that the p38 MAPK may be a possible therapeutic target to modify a pro-thrombotic state in patients with APS.

Introduction

Anti-phospholipid syndrome (APS) is a clinical condition characterized by recurrent thrombotic events and/or pregnancy morbidity associated with the persistence of anti-phospholipid antibodies (aPL) (1). Anti-cardiolipin antibodies (aCL) are members of the aPL family, a large and heterogeneous group of circulating Igs arising in a wide range of infectious and autoimmune diseases, particularly systemic lupus erythematosus (1). Since the early 1980s, the interest in aCL has exponentially increased due to their association with clinical manifestations of APS (2, 3).

aCL are detected by immunological assays (radioimmunoassay and ELISA) (2, 4, 5). Many studies have indicated that antibodies against \(\beta_2\)Glycoprotein I (\(\beta_2\)GPI) are one of the predominant antibodies detected with aCL assay in APS patients (6–12). APS-related aCL recognizes the epitope(s) on the \(\beta_2\)GPI molecule when \(\beta_2\)GPI interacts with a lipid membrane composed of negatively charged phospholipids [\(\beta_2\)GPI-dependent anti-cardiolipin antibodies or antibodies against cardiolipin/\(\beta_2\)GPI complex (aCL/\(\beta_2\)GPI)] (11). In the past decade, many studies have investigated the pathophysiology of thrombosis in APS and considerable interest has focused on the role of aPL as a clue to mechanisms related to thrombosis (13). Results of intensive research works have significantly advanced understanding of the mechanisms by which these antibodies may play a direct role in clot formation. Classically, in vitro evidence suggests that aCL/\(\beta_2\)GPI are involved in hemostatic abnormality. \(\beta_2\)GPI interacts with negatively charged phospholipids involved in the coagulation process, having both pro-coagulant and anticoagulant properties. \(\beta_2\)GPI suppresses the thrombomodulin–protein C system.
p38 and anti-phospholipid antibodies

(14), factor X, XI and XII activation (15–18) and prothrombinase activity (19). Antibodies against β2GPI may modify the properties of β2GPI and favor a pro-thrombotic state.

However, individuals with β2GPI deficiency do not have a thrombotic tendency, thus aCL/β2GPI-associated thrombosis cannot be merely explained by ‘β2GPI insufficiency’ (20, 21). Investigators turned their focus on functions of endothelial or other cells which might be modified by aPL. In this scenario, β2GPI serves as a ‘co-factor’ to prepare receptors for auto-antibody binding to cells. In this case, irrespective of the functions of β2GPI itself, auto-antibodies against β2GPI may alter the bound-endothelium properties from ‘anti-thrombotic’ to ‘pro-thrombotic’, leading to the production of pro-coagulant substances such as tissue factor (TF) (22, 23), vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin (24–26), plasminogen activator inhibitor-1 or endothelin-1 (27).

TF is the major initiator of the extrinsic coagulation system (28), functioning as the protein co-factor for the plasma serine protease, activated factor VII (FVIIa) (29, 30). Induced TF forms a complex with FVIIa that triggers the blood clotting cascade by activating factors IX and X, leading to thrombin generation (29). In normal conditions, TF is not expressed on intra-vascular cells (28) but it can be induced under stimuli such as LPS (31), tumor necrosis factor-alpha (TNF-α) (32, 33) and IL-1 (34). Evidence has supported the role of TF pathway in the pathogenesis of aPL-related thrombosis (22, 23, 35–37). Preliminary experimental data showed that sera or IgG fraction containing aCL induce TF-like pro-coagulant activity in endothelial cell (38, 39) or PBMC (40, 41). We and others demonstrated the up-regulation of TF pathway in patients with APS (35, 42, 43). Patients with APS have a pro-thrombotic state as evidenced by elevated basal thrombin generation (37, 44). Increased TF expression on endothelial cell or monocytes induced by aPL could, in part, be responsible for the hyper-coagulability and explain the existence of thrombosis in both the arterial and venous circulation that characterizes those patients.

On the other hand, only a few data have been published regarding the intracellular pathway in aPL-induced expression of TF or other pro-coagulant substances. We screened gene expression of molecules involved in signal transmission in aCL/β2GPI-induced TF expression using cDNA array system and demonstrated the significance of the p38 mitogen-activated protein kinase (MAPK) phosphorylation procedure in such cell activation.

Methods

Isolation and preparation of cells

Venous blood was collected in heparin from healthy donors. PBMCs were isolated on Ficoll-Paque plus® gradient centrifugation (Amersham Biosciences Corp., Piscataway, NJ, USA). PBMCs were washed with RPMI-1640 medium (Sigma Chemical Co., St Louis, MO, USA) supplemented with 10% FCS (GIBCO BRL, Paisley, UK) containing penicillin and streptomycin (RPMI-10 medium) once at 20°C, 400 × g, for 5 min and twice at 4°C, 400 × g, for 5 min. PBMCs were then re-suspended in RPMI-10 medium and counted using the trypan blue dye exclusion method. Murine RAW264.7 (American Type Culture Collection registration no. #TIB-71) monocytes were maintained in a 5% CO2 atmosphere at 37°C in DMEM (GIBCO BRL) supplemented with 10% FCS containing penicillin and streptomycin.

Proteins

Human β2GPI was purified from normal sera, as described (11), and the purity was confirmed using SDS-PAGE. Fatty acid-free BSA was obtained from Sigma-Aldrich Inc. (A-6002; St Louis, MO, USA). Two human IgM monoclonal aCL/β2GPI (EY2C9 and TM1G2) and one control monoclonal IgM lacking aCL/β2GPI activity (TM1B9) were used in this study. EY2C9 and TM1G2 are IgM class human monoclonal aCL/β2GPI established from APS patients with high titers of aCL/β2GPI (45). The characteristic of EY2C9 and TM1G2 is that these are mAbs that bind to the cardiolipin-β2GPI complex but not to cardiolipin alone (45). In the absence of cardiolipin, they do not recognize β2GPI immobilized on the plain ELISA plate, but do bind to β2GPI coated on an oxidized ELISA plate. The epitope mapping by phage-displayed peptide library demonstrated W235 in the fourth domain of β2GPI as a key amino acid residue at the epitopic center (46). Therefore, we consider that these mAbs represent autoimmune aCL/β2GPI found in patients with APS. The mAbs, when purified from serum-free medium culture supernatant, showed a single band on SDS-PAGE. LPS were intensively removed from these antibody preparations with DetoxiGel® (Pierce, Rockford, IL, USA) and were not detected using the Limulus ameocyte lysate assay (Limulus ES-II Single Test Wako: Wako, Osaka, Japan).

RNA extraction and cDNA array analysis

Total RNAs were isolated from PBMC or RAW264.7 using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), and stored at −80°C until use. Poly(A) RNA was isolated from total RNA (100 μg) using a MagExtractor® (TOYOBO, Osaka, Japan), and poly(A) RNA (2 μg) was reverse transcribed by ReverTraAce (TOYOBO) in the presence of cDNA synthesis primers and biotin–16-deoxyuridine triphosphate (TOYOBO), according to the manufacturer’s recommendation. cDNA array analysis was performed using human cDNA expression filters [Human Immunology Filters (TOYOBO), on which 621 species of human cDNA fragments and housekeeping genes were redundantly spotted]. Genes on the filter are shown on the web site http://www.toyobo.co.jp/seihin/xr/product/genenavi/genenavigator.html. Hybridization and subsequent cDNA array analyses were done as described (47), but with some modification. Briefly, cDNA array filters were pre-hybridized with PerfectHyb® solution (TOYOBO), and then hybridized with a biotin-labeled cDNA probe overnight at 68°C. After washing under high-stringency conditions, specific signals on the filters were visualized using Phototope-Star Detection Kits (New England Biolabs, Beverly, MA, USA), according to the manufacturer’s recommendation. Fluorescence signals for mRNA expression levels were obtained using a Fluor-3 Multimager system (Nippon Bio-Rad Laboratories, Tokyo, Japan) and intensity of the signals was determined using ImageG 4.2 software (BioDiscovery, Los Angeles, CA, USA).
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control to normalize the mRNA abundance. The signal intensity among filters was compared in an E-Gene Navigator Analysis (GeneticLab, Sapporo, Japan) and expressed as mRNA expression index to the intensity of the internal GAPDH gene.

**Quantitative TaqMan real-time PCR**

Real-time PCR amplification and determination were done using the ABI PRISM 7000® Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and gene-specific sets of TaqMan Universal PCR Master Mix® and Assays-on-Demand® Gene Expression probes (Applied Biosystems). A standard curve for serial dilutions of GAPDH was generated using a standard method provided by the manufacturer (Applied Biosystems), and was used to determine the amounts of cDNA transcripts.

**Western blot analyses**

For western blot analysis of p38 phosphorylation and nuclear factor κB (NF-κB) translocation, PBMC or RAW264.7 cells were treated with monoclonal aCL/β2GPI (10 μg ml⁻¹) or control mAb in the presence/absence of β2GPI (50 μg ml⁻¹) in serum-free medium, or 10% FCS medium at 37°C, followed by a preparation of cytosolic and nuclear proteins using a proteome extraction kit (Merck, Darmstadt, Germany). The cell lysates were resolved on 10% SDS gel and then transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with PBS containing 5% non-fat dry milk (Nestle USA, Inc., Solon, OH, USA) and 0.1% Tween–PBS buffer for 1 h, and probed with the rabbit polyclonal anti-phospho-MAPK antibody using a Phospho-MAPK family antibody sampler kit (Cell Signaling Technology, Inc., Beverly, MA, USA) overnight at 4°C. For some experiments, the blots were stripped and re-probed with polyclonal anti-MAPK antibody that recognizes both activated and non-activated MAPK proteins using an MAPK family antibody sampler kit (Cell Signaling Technology, Inc.). After three washes in 0.1% Tween–PBS buffer, the membranes were exposed to HRP-conjugated goat anti-rabbit antibodies at room temperature. Immunoreactive proteins were visualized using enhanced chemiluminescence assay (Amersham Biosciences Corp.). For analysis of NF-κB, nuclear lysates from the stimulated cells were blotted and reacted with an anti-NF-κB antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), the NF-κB antigen was visualized in the same fashion. In some experiments, the p38 MAPK inhibitor, SB203580 (Calbiochem, La Jolla, CA, USA), and the negative control for p38 MAPK inhibition studies, SB202474 (Calbiochem), were dissolved in dimethyl sulfoxide before addition to the culture medium.

**Surface staining for FACS analysis**

Surface aCL/β2GPI binding on RAW264.7 was analyzed using FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) with the CellQuest program. The cultured cells were washed with FACs buffer (2% BSA, 0.1% NaN₃ and PBS), and treated with 50 μg ml⁻¹ of β2GPI at room temperature for 10 min, followed by exposure to EY2C9 or TM1G2 (final concentration, 20 μg ml⁻¹) for 30 min on ice. After washing twice with FACs buffer, cells were stained with FITC-conjugated goat anti-human IgG/IgM antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 30 min on ice. After further two washes with FACs buffer, cells were subjected to FACS analysis. For each sample, data from 10,000 volume-gated viable cells were collected.

**Results**

**Identification of monoclonal aCL/β2GPI antibody-inducible genes**

The effect of monoclonal aCL/β2GPI on PBMC was screened utilizing cDNA arrays and mRNA expression of genes associated with the human immune system, including transcription factors, effector molecules and cytokines shown in Fig. 1. As a whole, the most increased mRNA expression by aCL/β2GPI treatment in cell signaling was detected in 2-h incubated cells (Fig. 1A). In the 2-h cDNA array analysis in PBMC exposed to EY2C9 and control IgM, mRNA related to MAPK pathway such as p38 regulated/activated protein kinase, TNF receptor-associated factor 6 (TRAF6), Sp-1, SAPK4 (p38) and MAPK-activated protein kinase (MAPKAPK)-3 increased >2-fold in EY2C9-treated cells compared with those treated with control IgM (Fig. 1A and Fig. 1B, left). In contrast, the expression of other signaling pathway molecules (Fig. 1B, right), such as tyrosine kinase, protein kinase C and Akt kinases, increased <2-fold. The expression of pro-inflammatory cytokine genes such as TNF-α and IL-1β, known to be regulated by the MAPK pathway, were enhanced up to 2- to 4-fold in the cDNA array (Fig. 1C). To confirm the results of cDNA array screening and previously reported TF mRNA expression by stimulation of aCL/β2GPI, a real-time PCR method was applied. Both EY2C9 and TM1G2 stimulation increased TF mRNA expression by stimulation of aCL/β2GPI, a real-time PCR method was applied. Both EY2C9 and TM1G2 stimulation increased TF mRNA expression (Fig. 2A), TNF-α (Fig. 2B) and IL-1β (Fig. 2C) mRNA expression. Up-regulated TF mRNA expression was also detected using real-time PCR in the monocyte cell line RAW264.7 after treatment with EY2C9 or TM1G2 (data not shown).

**Identification of p38 MAPK phosphorylation as a pathway of aCL/β2GPI activation**

According to the results of cDNA array analysis, some MAPK-related molecules were up-regulated in PBMC by stimulation with aCL/β2GPI, therefore, we further asked if the cDNA array results were associated with MAPK pathway activation. Non-phosphorylated forms of MAPKs (total MAPKs) were detected in unstimulated RAW264.7 cells. Cells treated with EY2C9 showed phosphorylation of p38 MAPK, which persisted for at least 60 min (Fig. 3). In contrast with ERK and JNK phosphorylation by LPS, neither ERK nor JNK pathway was activated with EY2C9 stimulation (Fig. 3).

**Reduction of p38 MAPK activation in aCL/β2GPI-mediated up-regulation of TF expression**

To elucidate the role of p38 MAPK in TF mRNA expression, the effect of p38 MAPK inhibitors on the cells stimulated with monoclonal aCL/β2GPI was examined. The p38-specific inhibitor, SB203580, entirely hampered p38 MAPK phosphorylation in RAW264.7 treated with EY2C9 (Fig. 4A), but SB202474, the inactive analogue of SB203580, did not affect...
Fig. 1. Screening of up-regulated genes induced by monoclonal aCL/β₂GPI in PBMC: mRNA level of genes encoding various intracellular signaling transcription factors and molecules. PBMCs were stimulated with monoclonal IgM aCL/β₂GPI (EY2C9) or control antibody at 30 μg ml⁻¹ for 30 min and 2, 4, 6, and 12 h. mRNA expression levels were analyzed by cDNA array as described in Methods. A. mRNA expression level of selected genes encoding mainly those associated with the MAPK pathway. These panels show fold increases in the mRNA expression level in EY2C9-stimulated cells compared with that in control IgM-stimulated cells. Increased mRNA expression was detected in 2-h incubated cells. B. Two-hour cDNA array analysis. mRNA expression level of selected genes encoding mainly those associated with the MAPK pathway (left panel) and various other signaling pathways (right panel). Genes are put in order of mRNA expression levels in each panel. PBMCs were treated with monoclonal IgM aCL/β₂GPI (EY2C9) or control antibody at 30 μg ml⁻¹ for 2 h. mRNA expression levels were analyzed using cDNA array as described in Methods, and shows fold increase of the mRNA expression level in EY2C9-stimulated cells compared with that in control IgM-stimulated cells. MAPK-related molecule mRNA expressions are higher than other cell signal transduction molecules. C. mRNA expression level of some selected genes encoding cytokines and chemokines. This panel shows fold increases in the mRNA expression level in EY2C9-stimulated cells for 2 h compared with that in control IgM-stimulated cells for 2 h.

p38 phosphorylation. Addition of SB203580 to cells stimulated with EY2C9 or TM1G2 decreased TF mRNA expression up to 75% (Fig. 4B).

β₂GPI dependency of p38 MAPK phosphorylation and TF induction

To evaluate the β₂GPI dependency of EY2C9 stimulation, we established RAW264.7 cells adapted to serum-free medium and treated these cells with EY2C9 in the absence/presence of human β₂GPI. In the cells stimulated by EY2C9, p38 MAPK phosphorylation was observed in the presence of β₂GPI either in serum-free medium or in medium supplemented with 10% FCS, but there was no apparent effect of this monoclonal aCL/β₂GPI in the absence of β₂GPI (Fig. 5A). In addition, NF-κB was increased in the nuclear fraction after stimulation with EY2C9 in the presence of β₂GPI (Fig. 5A). Furthermore, EY2C9 or TM1G2 induced RAW264.7 TF mRNA expression in a β₂GPI-dependent manner, whereas β₂GPI had little effect on LPS-induced expression of TF mRNA (Fig. 5B). In FACS analysis, EY2C9 bound to the cells in the presence of β₂GPI, but no binding of EY2C9 was found in the absence of β₂GPI (data not shown).

Discussion

In the present study, we demonstrated that the p38 MAPK-dependent signaling pathway participates in aPL-mediated TF expression. The multi-screening using the cDNA array system combined with real-time PCR analysis indicated that the MAPK pathway was related to TF expression when cells were treated with monoclonal aCL/β₂GPI. We performed western blotting studies to confirm the result of cDNA array at protein level that p38 MAPK protein was phosphorylated. The specific p38 MAPK inhibitor decreased TF mRNA expression by aCL/β₂GPI stimulation, suggesting a crucial role of the p38 MAPK pathway in this system.

The association between aPL and the occurrence of thrombosis is widely recognized. The effect of aCL in the inhibition of natural anticoagulant systems, the impairment of fibrinolytic activity and the direct effect of these antibodies on cell functions or injury are some of the proposed mechanisms to explain the thrombotic tendency of patients with APS. Endothelial cells, monocytes and activated platelets may be a predominant target of aCL/β₂GPI associated with the procoagulant state characteristic of APS.
Pro-coagulant cell activation, accompanied with TF expression and TF pathway up-regulation, is one of the key events considered to explain the pathophysiology of thrombosis in patients with APS. We showed elevated plasma levels of soluble TF in APS patients (22, 35), and Cuadrado et al. (42) reported that monocytes prepared from APS patients had high...
TF expression. Tissue factor pathway inhibitor (TFPI), a physiological inhibitor of the extrinsic coagulation system, was also increased in plasma from patients with APS (22), suggesting up-regulation of TF and TFPI in affected patients. In vitro experiments, numerous reports show that the IgG fraction from patients with aPL induced pro-coagulant activity on cells (38–41, 48). Our previous observation that human monoclonal aCL/β2GPI induced TF mRNA and TF activity on PBMCs or endothelium was confirmed by Reverter et al. (23) using the same mAbs. Apart from the TF molecule, other pro-coagulant substances induced by aPL were extensively investigated. Del Papa et al. (24, 49–51) have reported a series of molecules associated with endothelium activation by aPL in vitro, and other groups (25, 26) have shown adhesion molecules expression induced by IgG with aPL activity in vitro and in vivo models. Thus, it is widely accepted that aPL can induce the expression of TF or other pro-coagulant substances on cells in some conditions.

Recently, the signal transduction mechanism has been explored and associated with the increased expression of pro-coagulant substances in response to aPL. Dunoyer-Geindre et al. (52) presented an indirect but essential role of NF-κB in endothelial cell activation by aPL. IgG purified from APS patients induced the nuclear translocation of NF-κB leading to the transcription of a large number of genes that have a NF-κB-responsive element in their promoter. This nuclear translocation of NF-κB, at least in part, can explain the increased expression of TF by endothelial cell.

Protein kinases are key regulators of cellular signaling that control inflammation, cell differentiation, cell growth and cell death, and thus have been attractive targets for the treatment of neoplasms and inflammatory diseases. p38 MAPK was originally identified as a target molecule for a protein kinase inhibitor SB203580, a pyridinyl imidazole derivative which inhibits the production of pro-inflammatory cytokines. Isoforms of p38 MAPK are strongly activated by environmental stress or inflammatory cytokines [for review, see ref. (53)]. MAPK/ERK kinase (MEK)3 and MEK6, MAPKKs which obtain high specificity for p38, are activated by several MAPKKs that become active by oxidative stress, ultraviolet irradiation, hypoxia, ischemia, Gram-negative bacteria-derived LPS (54, 55) or inflammatory cytokines such as TNF-α, IL-1β and IL-18. Accordingly, activation of p38 is considered to be critical for normal immune responses, and properties of the p38 pathway in inflammatory process have been investigated.
Activation of p38 MAPK increases activities of pro-inflammatory cytokines, such as TNF-α and IL-1β. Up-regulation of TNF-α, IL-1β and macrophage inflammatory protein 3β (MIP3β) was also found in the present study (Figs 1C and 2B and C). Downstream of activated p38 MAPK, MAPKAPK-2/3 is a substrate for p38 that undergoes post-transcriptional regulation of TNF-α. p38 also activates transcriptional factors such as activating transcriptional factor-2, which forms a heterodimer with JUN family transcriptional factors and associates with the activator protein-1 (AP-1)-binding site. After LPS stimulation of dendritic cells, NH2-termini of histone H3 undergo structural alteration in a p38-dependent pathway, which results in enhancement of accessibility of the cryptic NF-κB-binding sites (56). The promoter region of the TF gene contains two AP-1-binding sites and one NF-κB-binding site, and these transcription factors are proven required for maximal induction of TF gene transcription. Moreover, p38 MAPK pathway has been implicated in the regulation of TF expression in monocytes, endothelial cells and smooth muscle cells (57–61).

In the present study, we have shown that stimulation by monoclonal aCL/β2GPI-induced phosphorylation of p38, locational shift of NF-κB into the nucleus and up-regulation in TF expression. TF expression induced by aCL/β2GPI occurred only in the presence of β2GPI, suggesting that perturbation of monocytes by aCL/β2GPI is initiated by interaction between the cell and the auto-antibody-bound β2GPI. It remains to be determined how aCL/β2GPI bind β2GPI on the cell surface and how signal transduction events occur upstream of p38. Using endothelial cells, Raschi et al. (62) reported that the dominant negative construct of TRAF6 and that of myeloid differentiation protein 88 (MyD88) abrogated the NF-κB activation induced by monoclonal aCL/β2GPI as well as that induced by IL-1 or LPS. They proposed that aCL/β2GPI react with β2GPI likely associated to a member of the Toll-like receptor (TLR)/IL-1 receptor family. The results of our study are compatible with this report; firstly, in our cDNA array experiment, the expression levels of the members of the MyD88 signaling pathway such as interleukin-1 receptor associated kinase 1 (IRAK-1), TRAF6 and IκB kinase α, β and γ were up-regulated after treatment with monoclonal aCL/β2GPI (Fig. 1) and, secondly, TRAF6 activates MEK3 and MEK6 which are the kinases upstream of p38 and JNK via activation of an MAPKKK called transforming growth factor-β-activated kinase. The different
behaviors in p38, ERK and JUN activation between aPL and IL-1/TLR suggest that the signal/receptor structure of aCL/β2-GPI be different from that of IL-1/TLR.

Several inhibitors for p38 have been developed and investigated in animal models of inflammatory diseases, and recently some of these inhibitors, such as BIRB796 or RWJ67657, are under clinical trials. Administration of SB203580 was beneficial in a murine model of endotoxin-induced shock and collagen-induced arthritis in mice. BIRB796 inhibited endothelial activation after administration of LPS in humans (63, 64), and RWJ67657 inhibited TNF-α, IL-8 and IL-6 in humans without significant adverse effects (65).

Strategies are focused on preventing the induction of procoagulant substances by aPL. New lipophilic statins, such as fluvastatin and simvastatin, can inhibit endothelial cell activation induced by aCL/β2-GPI (66, 67) and also the TF up-regulation on endothelial cells can be inhibited by these drugs, providing an additional therapeutic tool for treatment of thrombosis in APS. Recently, dilazep, an anti-platelet agent, was reported to reduce TF induction by aPL in vitro (48). In addition, our findings give a clue to establish more specific treatments by down-regulating the p38 MAPK pathway, presumably contributing to better management of the affected patients.

Acknowledgements

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>aCL</td>
<td>anti-cardiolipin antibodies</td>
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<tr>
<td>aCL/β2GPI</td>
<td>β2GPI-dependent anti-cardiolipin antibodies</td>
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<td>AP-1</td>
<td>activator protein-1</td>
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<td>aPL</td>
<td>anti-phospholipid antibodies</td>
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<td>FVIIa</td>
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<td>glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>β2GPI</td>
<td>β2Glycoprotein I</td>
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<td>TF</td>
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References


