Global effects of fluvastatin on the prothrombotic status of patients with antiphospholipid syndrome

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ABSTRACT

Objective Numerous mechanisms have been proposed to explain the thrombotic/proinflammatory tendency of antiphospholipid syndrome (APS) patients. Prothrombotic monocyte activation by antiphospholipid antibodies involves numerous proteins and intracellular pathways. The anti-inflammatory, anticoagulant and immunoregulatory effects of statins have been aimed as a therapeutic tool in APS patients. This study delineates the global effects of fluvastatin on the prothrombotic tendency of monocytes from APS patients.

Methods Forty-two APS patients with thrombosis and 35 healthy donors were included in the study. APS patients received 20 mg/day fluvastatin for 1 month. Blood samples were obtained before the start, at the end and 2 months after the end of treatment.

Results After 1 month of treatment, monocytes showed a significant inhibition of tissue factor, protein activator receptors 1 and 2, vascular endothelial growth factor and Flt1 expression that was related to the inhibition of p38 mitogen-activated protein kinase (MAPK) and nuclear factor kappa B/Rel DNA-binding activity. Proteomic analysis showed proteins involved in thrombotic development (annexin II, RhoA and protein disulphide isomerase) with altered expression after fluvastatin administration. In-vitro studies indicated that the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by fluvastatin might inhibit protein prenylation and MAPK activation.

Conclusion The data from this study support the belief that fluvastatin has multiple profound effects in monocyte activity, which might contribute to thrombosis prevention in APS patients.

Monocyte activation induced by antiphospholipid antibodies (aPL) results from a complex and intricate interplay of numerous proteins and intracellular pathways, eventually responsible for the development of thrombosis in antiphospholipid syndrome (APS).[1–10] Procoagulant monocyte activation, accompanied with tissue factor (TF) expression, and TF pathway upregulation, is one of the key events in the pathophysiology of thrombosis in patients with APS.[1–6] Besides, it has been shown that TF signalling activities in APS are mainly mediated by protease activated receptors (PAR).[7 8] Accordingly, PAR1 and PAR2-induced signalling is directly involved in both the constitutive mitogen-activated protein kinase (MAPK) activation,[9] and in the increased expression of the proinflammatory cytokine vascular endothelial growth factor (VEGF).[10 11] In APS monocytes we recently showed increased levels of VEGF and its receptor Flt1 in comparison with healthy monocytes in vivo and in vitro after treatment with aPL, with p38 MAPK signalling playing an important role.[12] Therefore, increased VEGF/Flt1 activity might be responsible for the TF overexpression found in monocytes of APS.

In endothelial cells and tumour cells, a reciprocal induction of TF and VEGF exists. Thrombin is involved in the upregulation of VEGF in endothelial cells.[13] The excess of plasmatic thrombin in APS,[14] most likely induced by TF expression, and acting through the activation of PAR,[15] might also be related to the elevated VEGF production found in APS patients.

Recent proteomic studies have identified novel critical proteins that might be involved in the pathogenic mechanisms of thrombosis in the setting of APS, such as annexins I and II, RhoA proteins or protein disulphide isomerase (PDI).[16]

The newly discovered anti-inflammatory, anticoagulant, antiproliferative and immunoregulatory effects of statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) have aimed the study of these compounds as a therapeutic tool in the treatment of thrombosis in APS patients. Those effects are partly due to the interference with the mevalonate pathway and inhibition of geranylgeranylation and prenylation of guanine nucleotide-binding proteins (GTPases).[17] However, their cellular and molecular mechanisms of action are not yet fully understood.

In this study we attempted to delineate those mechanisms through both an in-vivo and a confirmatory in-vitro study, in which the global effects of fluvastatin on the prothrombotic tendency of monocytes from APS patients have been evaluated.

MATERIALS AND METHODS

Patients

Forty-two patients fulfilling the classification criteria for APS,[18] with previous thrombotic events, and 35 healthy donors were included in the study during a period of 24 months. All of the patients signed an informed consent form. We excluded all APS patients who had evidence of an underlying systemic rheumatic disease, other medical conditions or who were using drugs for any other conditions. None of the healthy controls had a history

of autoimmune disease, bleeding disorders, thrombosis or pregnancy loss.

All patients were tested for the presence of anticardiolipin and lupus anticoagulant: IgG and IgM anticardiolipin were determined by ELISA using a standardised commercial kit (Inova Diagnostics, San Diego, California, USA).

Results were expressed in standard IgG anticardiolipin units (GPL). Lupus anticoagulant was detected according to the guidelines recommended by the Subcommittee for Standardisation of the International Society on Thrombosis and Haemostasis.[19] The characteristics of the patients and the controls at the beginning of the study are shown in table 1.

APS patients were given 20 mg/day fluvastatin for 1 month. No treatment was given to healthy donors. Blood samples were obtained before the start of the treatment, at the end of the treatment and 2 months after the end of treatment. During this treatment, patients were not withdrawn from their therapy: all APS patients with thrombosis were being treated with an oral anticoagulant (acenocoumarol). APS patients without thrombosis were taking a low dosage of aspirin (125 mg/day) or received no treatment. No patients were being treated with immunomodulatory agents, because they had no other underlying systemic autoimmune disease. Additional details of patients during statin treatment are included in a supplemental file (available online only).

Monocyte isolation

The isolation of non-activated monocytes from peripheral blood was performed by the depletion of non-monocytes, by using a commercially available kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Purity was evaluated with fluorescein isothiocyanate (FITC)-conjugated anti-CD14 antibody and flow cytometry. By this method, 92.1±4% viable monocytic cells (mean±SEM) were obtained.

Flow cytometry analysis

Flow cytometric analysis was performed as previously described,[3] using a FACScan (BD Biosciences, San Jose, California, USA) and specific monoclonal antibodies to human TF (clone TF9-6B4, FITC-conjugated; American Diagnostica, Greenwich, Connecticut, USA), to PAR1 and PAR2 FITC-conjugated (Santa Cruz Biotechnology, Santa Cruz, California, USA), or to human CD14 PE-conjugated (Caltag, South San Francisco, California, USA).

Table 1 Characteristics of primary APS patients and healthy donors at the beginning of the study

	APS patients (n=42)	Healthy donors (n=35)
Age in years, mean (SD)	47.3±8.9	43.4±6.7
Men/women (n)	8/34	6/29
Arterial thrombosis (n)	28	0
Venous thrombosis (n)	14	0
Recurrent thrombosis (n)	24	0
Anticardiolipin antibodies		
IgG isotype (GPL)	57.0±11.5	6.3±4.2
IgM isotype (GPL)	22.3±4.3	11.2±8.0
β_2-GPL activity (SGU)	55.9±12.7	9.7±8.1
Lupus anticoagulant (n)	28	0
Hypercholesterolaemia (n)	6	3
Hypertension (n)	4	1
Diabetes (n)	2	0
Anticoagulants treatment (n)*	14	0
Antiplatelets treatment (n)†	22	0

*Anticoagulants were acenocoumarol and heparin.
†Antiplatelet administered was acetylsalicylic acid.
APS, antiphospholipid syndrome; GPL, standard IgG anticardiolipin units; SGU, standard GPL units..

Proteomic studies, western blotting and electrophoretic mobility shift assay

Proteomic studies (including protein extraction, two-dimensional gel electrophoresis, detection of protein spots and data analysis), were performed as previously described.[16]

VEGF, Flt1, IκBα, actin, phospho-p38 MAPK, p38 MAPK, AnxI, AnxII, PDI, ubiquitin Nedd8, RhoA, Hsp60 protein levels were determined by western blotting,[3 20] using specific antibodies (Santa Cruz Biotechnology). Nuclear extracts (20 µg) were tested for nuclear factor kappa B (NFκB)-binding activity in electrophoretic mobility shift assay analyses (consensus oligonucleotides: 5′-AGTTGAGGGGACTTTCCCAGGC-3′ and 3′-TCAACTCCCCTGAAAGGGTCCG-5′) using the digoxigenin electrophoretic mobility shift assay kit from Roche Diagnostics (Basel, Switzerland) according to the manufacturer's recommendations.

RNA isolation and quantitative real-time reverse transcriptase PCR

Total RNA from monocytic cells was extracted using TRI Reagent (Sigma, St Louis, Missouri, USA) according to the manufacturer's recommendations.

The expression levels of TF, VEGF, Flt1, PAR 1 and two genes, and glyceraldehyde-3-phosphate dehydrogenase as a housekeeping gene were measured by quantitative real-time reverse transcriptase PCR using the LightCycler thermal cycler system (Roche Diagnostics, Indianapolis, Indiana, USA), as described elsewhere.[3 8 12]

Purification of IgG and in-vitro exposure of normal monocytes to aPL in the presence or absence of fluvastatin and mevalonate

IgG from the pooled sera of seven patients with APS (characterised by high titres of anticardiolipin, ie, >120 GPL units), and from the pooled sera of seven healthy subjects (as controls) was purified by protein G-sepharose high-affinity chromatography (MAbTrap kit) according to the recommendations of the manufacturer (Amersham Biosciences, Uppsala, Sweden).

For in-vitro studies, monocytes purified from healthy donors were cultured in serum-free RPMI 1640 containing 2 mM l-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin and 250 pg/ml fungizone (BioWhittaker/MA Bioproducts, Walkersville, Maryland, USA), at 37°C in a humidified 5% carbon dioxidec atmosphere. Purified normal monocytes (1.5×10^6/ml) were incubated with normal human serum (NHS) IgG (200 µg/ml) or purified APS patient IgG (200 µg/ml) for 6 h at 37°C. To test the effect of fluvastatin on APS patient IgG-induced monocyte activation, monocytes were pretreated for 16 h at 37°C with 10 µM fluvastatin, then incubated for 6 h with purified APS patient IgG or NHS as above in the presence or absence of 100 µM mevalonate.

Statistical analysis

All data are expressed as mean±SEM. Statistical analyses were performed using SigmaStat software (Jandel Scientific GmbH, Erkrath, Germany). Following normality and equality of variance tests, comparisons were made by a parametric test (paired Student's t test) or alternatively by using a non-parametric test (Mann–Whitney rank sum test). Correlations were assessed by Pearson product–moment correlation. Differences were considered significant at $p<0.05$.

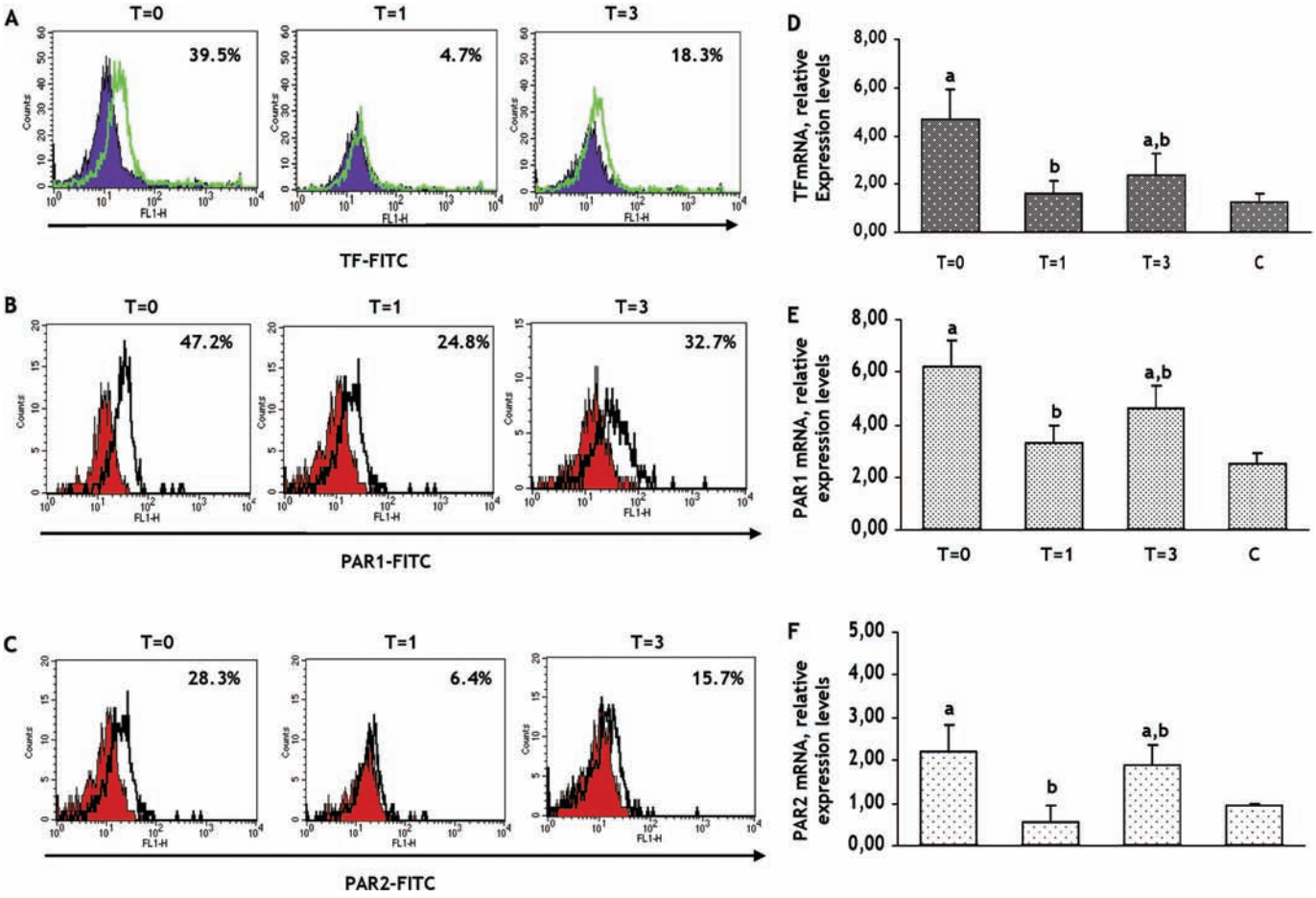

Figure 1 Downregulation of tissue factor (TF) and protein activator receptor (PAR) expression in monocytes from antiphospholipid syndrome patients after fluvastatin treatment. (A–C) FACS analysis of TF, PAR1 and PAR2 expression, respectively, before and after fluvastatin treatment. Open histograms show the specific staining, also indicated as a percentage (see text for details); closed histograms represent cells stained with isotype-matched control antibodies. (D–F) TF, PAR1 and PAR2 mRNA relative expression levels, respectively. Bar graphs show mean mRNA levels±SEM. Significant differences at $p<0.05$: (a) versus healthy donors; (b) versus values found at the beginning of the study.

RESULTS

Effects of fluvastatin on TF, PAR and VEGF/Flt1 expression in monocytes from APS patients

TF cell surface expression in monocytes from APS patients was significantly higher than in control subjects (36.6±5.9% vs 5.4±2.2%; $p<0.01$). Likewise, both PAR1 and PAR2 cell surface expression levels were found to be increased compared with healthy donors (PAR1: 43.8±3.6 vs 21.5±3.2 $p<0.05$; PAR2: 19.1±3.6 vs 4.1±2.2 $p<0.05$; figure 1A–C). Parallel data were shown by messenger RNA levels (all $p<0.05$; figure 1D–F).

Monocytes from APS patients who had been treated daily with fluvastatin, showed a significant inhibition on the protein expression of TF, PAR1 and PAR2, so that the percentages of cell surface expression after 1 month of treatment (T=1) were, respectively: TF, 7.23±3.7%; PAR1, 20.8±3.0%; and PAR2, 7.9±3.9%; all $p<0.05$. mRNA levels exhibited a similar behaviour (figure 1D–F; all $p<0.05$). These levels then had a slowly return but, although remaining significantly changed in relation to T=0, they were also found to be significantly higher than those found at T=1. Cell surface expression percentages at that point were, respectively: TF, 19.08±4.2%; PAR1, 31.6±3.5%; PAR2, 12.3±0.8%; all $p<0.05$.

Because of the known relationship between TF and the VEGF/Flt1 axis in the setting of APS, we further evaluated the effect of the in-vivo fluvastatin treatment on the expression of these cytokines. The changes promoted by fluvastatin on VEGF and its receptor Flt1 (figure 2) paralleled those of TF and PAR.

Effects of fluvastatin on both p38 MAPK phosphorylation and NFκB binding activity

The in-vivo fluvastatin treatment that inhibited TF and VEGF/Flt1 also promoted a significant inhibition of both p38 MAPK phosphorylation and NFκB/Rel DNA-binding activity after 1 month of treatment (figure 3A,B, respectively). In concordance with the results of TF, PAR and VEGF/Flt1 expression levels, NFκB activation and p38 MAPK phosphorylation levels also showed a shift to baseline levels 2 months after the end of fluvastatin administration.

Influence of fluvastatin administration on the proteomic profile of monocytes from APS

One month after fluvastatin treatment, we found six proteins with altered expression compared with their control values in monocytes from the APS patients included in the study (figure 4). Among them, three proteins were directly related to thrombosis development: annexin II (AnxII) and RhoA proteins, whose expression was significantly inhibited, and PDI, showing an increased expression (all $p<0.05$; figure 4). Then the expression levels of those proteins slowly returned to basal values (although theyremained significantly altered vs baseline levels) 2 months

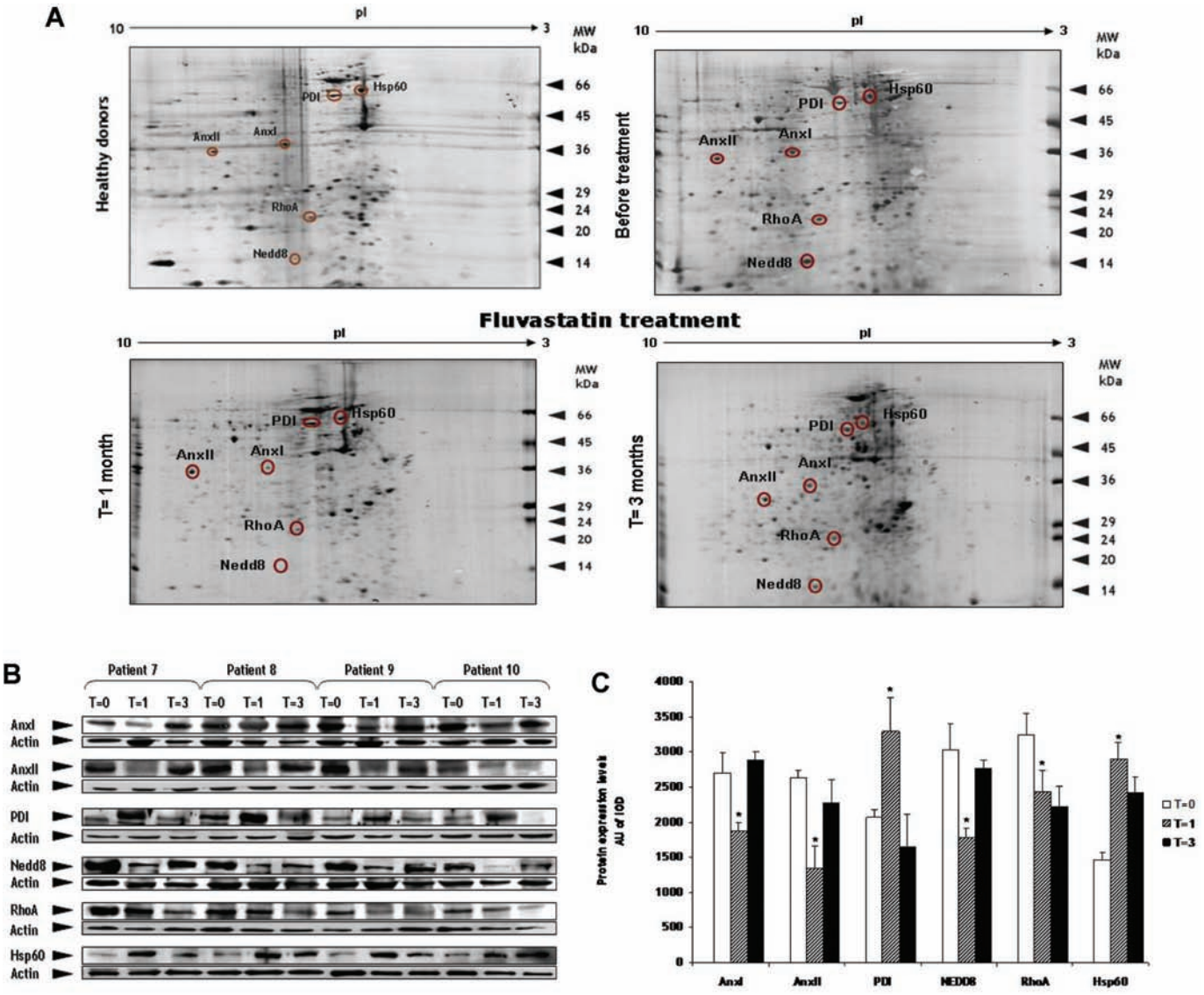

Figure 2 Fluvastatin downregulated vascular endothelial growth factor (VEGF) and Flt1 expression in monocytes from antiphospholipid syndrome (APS) patients. (A) Representative western blots of monocyte samples from five APS patients and four healthy donors performed in triplicate as described in the Materials and methods section. (B,C) The bar graphs show mean protein expression levels±SEM from all the patients and controls included in the study. (D,E) VEGF and Flt1 mRNA expression levels. The bar graphs show mean mRNA levels±SEM from all APS patients (n=42) and controls (n=35) included in the study. Significant differences at $p<0.05$: (a) versus healthy donors; (b) versus values found at the beginning of the study. AU, arbitrary units; IOD, integrated optical density.

after the end of the treatment, except for RhoA protein, which remained reduced on T=3 (the relative normalised amounts of that proteins on the three points of measurements were: AnxII, T=0: 220.6±17.3; T=1: 95.3±10.4; T=3: 157.4±3.7. PDI, T=0: 22.7±1.9; T=1: 88.7±4.3; T=3: 53.2±3.5. RhoA proteins, T=0: 378.2±16.2; T=1: 127.4±4.7; T=3: 123.1±9.8). Western blotting of the six altered proteins was performed (figure 4B,C), confirming the proteomic data.

Molecular mechanisms underlying the effects of fluvastatin in the prevention of procoagulant and proinflammatory effects of aPL

In support of the in-vivo studies, FACS experiments showed that fluvastatin in vitro prevented TF expression (figure 5A) and the increase in PAR1 and PAR2 expression (figure 5B,C) in aPL-IgG treated monocytes. However, fluvastatin treatment had no effect on TF and PAR2 expression in NHS-IgG treated or untreated monocytes (figure 5). Likewise, in-vitro studies showed a significant inhibition of the aPL-IgG-induced expression of VEGF and Flt1 after fluvastatin administration (figure 5D).

Stimulation of monocytes with aPL-IgG induced both p38 MAPK phosphorylation and NFκB DNA-binding activity, which was abolished by the pre-incubation of cells with fluvastatin (figure 5E).

Finally, we evaluated whether the aPL-IgG-induced expression of proteins identified by proteomic analysis was also regulated by fluvastatin. As shown in figure 5F, RhoA, AnxII and PDI expression levels changed significantly under the effects of fluvastatin.

As the conversion of l-mevalonate by HMG-CoA reductase is the rate-limiting step in the cholesterol synthesis pathway, which is inhibited by statins, we also investigated whether all these effects were abrogated by the addition of mevalonate. As shown in figure 5, the global inhibitory effects of fluvastatin were reversed in the presence of mevalonate.

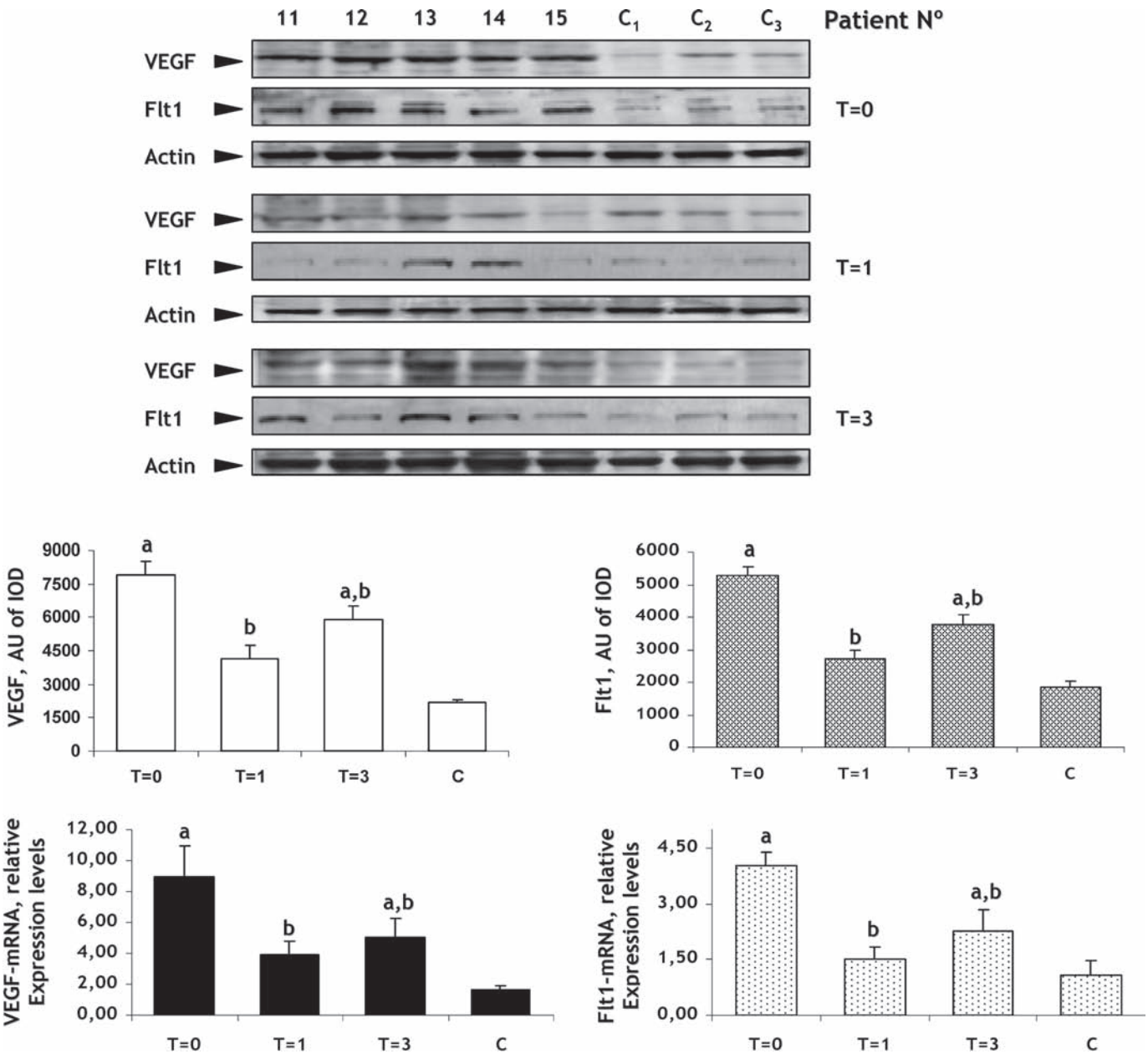

Figure 3 In-vivo fluvastatin treatment promoted a significant inhibition of both nuclear factor kappa B (NFκB)/Rel DNA-binding activity and p38 mitogen-activated protein kinase (MAPK) phosphorylation in monocytes from antiphospholipid syndrome (APS) patients. Representative (A) Electrophoretic mobility shift assay of nine APS patients and three healthy donors done using digoxigenin-labelled NFκB consensus oligonucleotide, as described in the Materials and methods section. Cytoplasmic extracts were subjected to western blot analysis using anti-IκB and antiactin antibodies. (B) Panels are representative of the western blot of nine APS patients and three controls showing phosphorylated and non-phosphorylated forms of p38 MAPK. (C) Bar graphs show mean protein expression levels±SEM from all APS patients (n=42) and controls (n=35) included in the study. Significant differences at $p<0.05$: (a) versus healthy donors; (b) versus values found at the beginning of the study.

DISCUSSION

The overall data obtained in the present study suggest that the antithrombotic properties of statins combine the downregulation of TF with the inhibition of PAR1, PAR2, VEGF and FLt1 expression. We have further demonstrated that p38 MAPK and NFκB are involved in the modulation of the global anticoagulant effect of statins. This chain of events may lead to a reduction in the thrombotic/inflammatory burden in APS patients.

It has previously been established that TF expression in various cell types, including monocytes/macrophages, endothelial cells and neutrophils, is inhibited by statins,[21–26] but so far its inhibition in monocytes from APS patients, as well as the underlying signalling pathways have not been elucidated. In the present study constitutive p38 MAPK activation found in monocytes was blocked by in-vivo treatment with fluvastatin. p38 MAPK activation by IgG-aPL treatment has been shown to be related to NFκB activation, which in turn directly induces TF expression in monocytes.[3] The observed in-vivo inhibition of both p38 MAPK and NFκB activities after fluvastatin treatment further reinforced the hypothesis of a direct inhibitory effect of fluvastatin on the molecular mechanisms of TF induction in the monocytes of APS patients.

PAR play key roles in the development of atherosclerosis, cell injury and tissue remodelling. Moreover, the association of PAR2

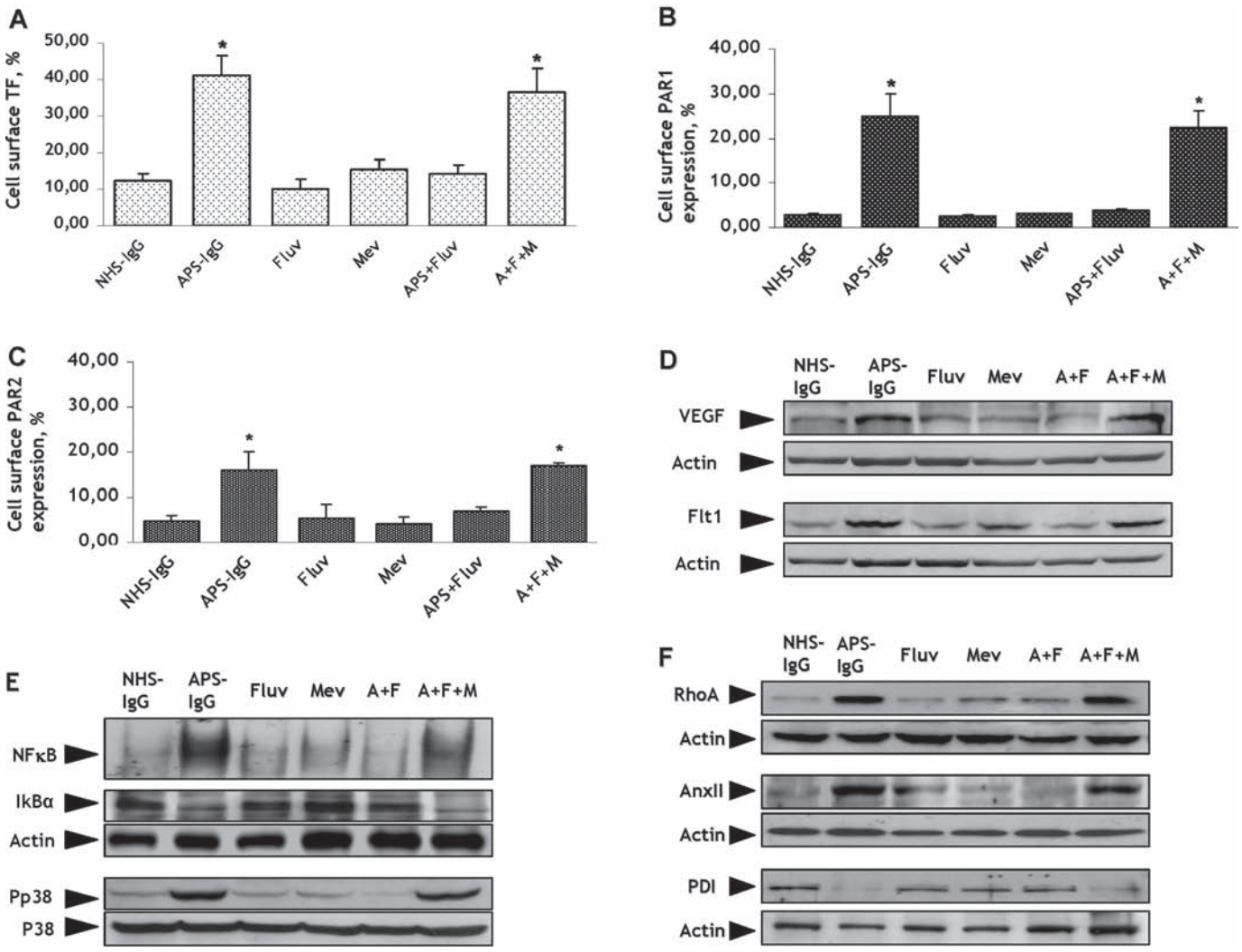

Figure 4 Proteomic analyses showed significant changes in the protein expression patterns of monocytes from antiphospholipid syndrome (APS) patients after fluvastatin treatment. (A) Representative image of the scanned images of the Sypro Ruby-stained gels used to detect and compare spots. Protein spots differentially expressed between healthy donors and APS patients before, after 1 month of treatment (T=1) and 2 months after the end of treatment (T=3), are enclosed in circles, and molecular mass standards are shown on the right. (B) Panels are representative western blots of four patients analysed at the beginning of the study (T=0), after 1 month of treatment (T=1) and 2 months after the end of treatment (T=3), all performed in triplicate as described in the Materials and methods section. (C) The bar graph shows mean protein expression levels±SEM of the six proteins identified by proteomic analysis as significantly altered after fluvastatin treatment of all the subjects included in the study. *Significant differences at $p<0.05$ versus values found at the beginning of the study. AU, arbitrary units; MW, molecular weight;PDI, protein disulphide isomerase.

with TF expression and TF-dependent signalling pathways has become increasingly apparent in many inflammatory diseases.[27] We recently showed a significant increase in PAR1 and PAR2 expression in monocytes from thrombotic APS patients, which were directly associated with TF expression both in vivo and in vitro.[8] Accordingly, our data link statins with PAR and TF inhibition, thus suggesting that TF, which is responsible for excessive thrombin generation by PAR 1 and 2, is concurrently downregulated by statins. Our data further agree with the study of Redecha *et al*,[7] which, by using a murine model, demonstrated the beneficial effects of statins in the setting of APS, showing that statins prevented neutrophil activation by downregulating TF and PAR2 and protected mouse fetuses from aPL-IgG-induced injury.

Increased TF activity and the upregulation of proinflammatory cytokines is also related to thrombosis in APS. We previously showed that VEGF might act as a regulatory factor in aPL-mediated monocyte activation and TF expression, thereby contributing to the proinflammatory/prothrombotic phenotype of APS patients.[12] In this complex setting of molecules related to aPL-induced monocyte activation, VEGF expression has been shown to be further associated with the PAR1 and PAR2 proinflammatory effects on monocytes and endothelial cells.[28] Moreover, intracellular signalling represented by aPL-induced p38 MAPK activation was further related to the regulation of VEGF/Flt1 expression.[12] Therefore, as suggested by our results, it is conceivable that the statins might exert their pleiotropic effects by also targeting these cytokines. In support of this hypothesis, a very recent study by Jajoria *et al*[29] has shown a significant decrease in the titres of VEGF in the plasma of APS subjects after 30 days of treatment with fluvastatin. Moreover, that study further addressed the beneficial effects of fluvastatin in other prothrombotic/proinflammatory markers induced by aPL in APS patients, including TF, also evaluated in this study.

All the above described the antithrombotic and anti-inflammatory effects of statins that can either result from their capacity to interfere with the mevalonate pathway and inhibit the prenylation of Rho family GTPases, or due to an effect independent

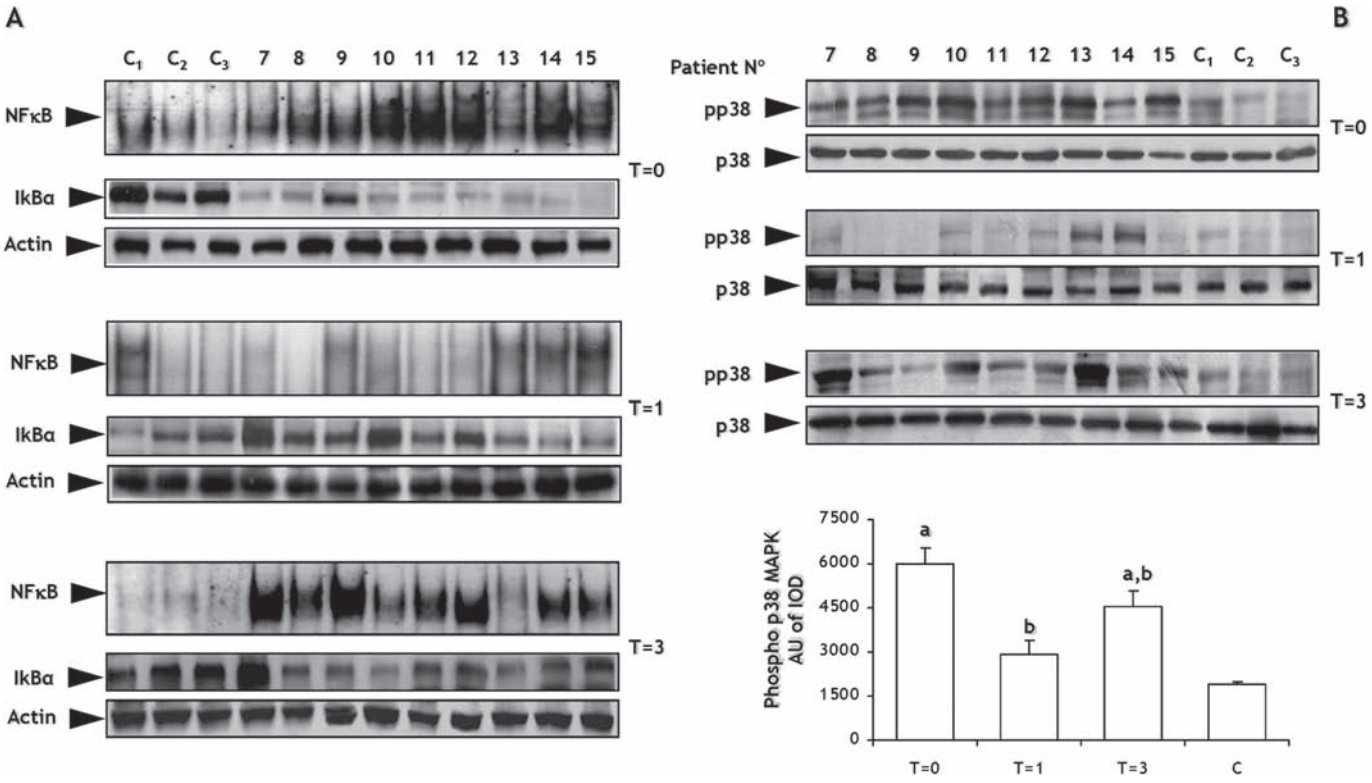

Figure 5 In-vitro studies delineated the molecular mechanisms underlying the effects of fluvastatin in the prevention of procoagulant and proinflammatory effects of antiphospholipid antibodies (aPL). (A–C) Tissue factor (TF) and protein activator receptor (PAR) expression in monocytes treated with aPL-IgG, or normal human serum (NHS)-IgG. Bar graphs show mean cell surface levels±SEM of four separate experiments. (D) Representative panels of western blots of vascular endothelial growth factor (VEGF) and Flt1 analysed in four independent experiments with similar results. (E) Panels show a representative electrophoretic mobility shift assay of nuclear factor kappa B (NFκB) and western blot analysis of four independent experiments, using anti-IκBα, antiactin, antiphosphorylated p38 mitogen-activated protein kinase (MAPK) and anti-p38 MAPK antibodies. (F) Panels show representative western blot of each protein analysed in four independent experiments with similar results. PDI, protein disulphide isomerase.

of the mevalonate pathway. Rho family GTPases have been implicated for roles in almost every aspect of vascular biology, influencing the biology of endothelial cells, smooth muscle cells, leucocytes and platelets, and regulating such diverse processes as vasoreactivity and hypertension, inflammation and wound healing, neointimal formation and thrombosis.[30] As many of the effects can be reversed by geranylgeranylpyrophosphate, but not farnesylpyrophosphate, the Rho family is implicated as a major target of statins. We previously showed, in proteomic studies, that RhoA protein expression was increased in monocytes from thrombotic APS patients, both in vivo and in vitro after aPL treatment.[16] In this study, by using proteomic and western blot studies, we demonstrated that its expression was significantly reduced after fluvastatin treatment.

RhoA protein has been shown to play a key role in p38 MAPK activation and macrophage proliferation in response to oxidised low-density lipoprotein,[31] and to promote NFκB activation in endothelial cells after lipopolysaccharide treatment.[32] Both pathways have been proved to be directly activated in aPL-activated monocytes, and are directly related to TF and VEGF/Flt1 expression in the setting of APS.[3 12] Moreover, a previous study by Park *et al*[33] has shown that simvastatin inhibits angiogenesis by interfering with the geranylgeranylation of RhoA. It might thus be hypothesised that the inhibition of the RhoA proteins after fluvastatin treatment could explain, at least partly, the reduction in thrombotic/inflammatory events by statins.

Another previously unexplored effect of fluvastatin therapy was the normalisation of PDI expression, which was minimal in thrombotic APS patients.[16] A recent study has shown that the surface accessible, extracellular Cys186–Cys209 disulphide bond of TF is critical for coagulation, and that PDI (a multifunctional protein catalysing the oxidation, reduction and isomerisation of disulphide bridges) disables coagulation by targeting this disulphide. Moreover, in that study decreased PDI expression was associated with a twofold increase in TF procoagulant activity.[34] Therefore, reduced expression of PDI, previously shown in monocytes from APS patients with thrombosis, seemed to contribute to their prothrombotic state.[35] Our data further agree with a recent study,[36] showing that atorvastatin, at doses of 80 mg/dl, induced a normalisation of PDI expression in monocytes from patients with coronary syndrome, thus pointing to PDI as a mediator of post-translational changes, and immune and inflammatory responses susceptible to being regulated by statin treatment.

A third protein involved in thrombosis development found altered in its expression in response to fluvastatin was AnxII. Previous in-vivo and in-vitro studies by others and our own group have shown that AnxII (a common receptor for aPL-induction of monocyte activation and induction of TF expression) is expressed on monocytes and is involved in monocyte activation and TF expression induced by aPL antibodies.[16 37] The present data further suggest that its expression is regulated by fluvastatin treatment, thus including TF in the setting of protective effects against thrombosis exhibited by this statin.

From this and other studies, it is clear that the inhibition of HMG-CoA reductase by fluvastatin, which is a rate-limiting

enzyme of the mevalonate pathway, might reduce the expression and activity of specific subfamilies of small GTPases, therefore inhibiting protein prenylation and MAPK activation. This inhibition is likely to have profound effects on key cellular processes, including the suppression of TF and PAR expression, and anti-inflammatory activities on macrophages through the inhibition of proinflammatory cytokines such as VEGF/Flt1.

Our study provides significant evidence that fluvastatin has profound and multiple effects in monocyte activity, which is directly related to the prevention of thrombosis in APS patients, but larger scale, randomised, double-blind trials are needed to validate the role of statins for the treatment of this autoimmune disease.

Nevertheless, elucidating the mechanisms of action of statins and their relative importance will help to rationalise the design of such alternative and/or complementary therapy in APS patients.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Ethics Committee from the Reina Sofia Hospital from Cordoba-Spain.

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Global effects of fluvastatin on the prothrombotic status of patients with antiphospholipid syndrome

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