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Link between anti-CD36 antibodies and thrombosis in the antiphospholipid syndrome

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ABSTRACT

Objective. *Some studies have previously suggested the involvement of antibodies directed against CD36 (anti-CD36) in the pathogenesis of thrombosis. The aim of this study was to evaluate the prevalence of anti-CD36 in patients with antiphospholipid antibodies (aPL) and its relationship with thrombosis.*

Methods. *Anti-CD36 were tested using an indirect MAIPA assay in 62 patients with autoimmune aPL but without SLE; there were 38 with and 24 without thrombosis. Nineteen patients with thrombosis served as an aPL(-) control group and 58 healthy subjects as the normal control group.*

Results. *15 of 62 aPL patients (24.2%) but only 1 of 58 (1.7%) normal controls had anti-CD36 ($p < 0.0005$). As compared to normal controls, the prevalence of anti-CD36 was significantly higher in aPL patients with (26.3%, $p < 0.0005$) or without thrombosis (20.8%, $p < 0.01$). Anti-CD36 were significantly more frequent in aPL patients with thrombosis than in thrombosis aPL(-) subjects (26.3% vs 0%, $p = 0.02$). The presence of anti-CD36 seems to be more frequent in aPL patients with recurrent thrombosis than in those with a single episode (36.8% vs 15.8%).*

Conclusion. *The presence of anti-CD36 is highly prevalent in patients with autoimmune aPL with a trend to being more frequent in patients with recurrent episodes of thrombosis.*

Introduction

The antiphospholipid syndrome (APS) is regarded as an autoantibody-mediated thrombotic disorder. Thromboses in venous and arterial territories as well as pregnancy morbidity are clinical features of definite APS (1). However, not all patients with antiphospholipid antibodies (aPL) develop APS, suggesting that other factors may influence the development of such clinical features.

In vivo endothelial cell and platelet activation is one of the main findings in APS (2). It is mainly related to some aPL such as autoantibodies reacting with β_2 glycoprotein I (anti-β_2GPI). Vermylen *et al.* (3) suggest that the attachment of antibodies to different proteins expressed on the surface of platelets and endothelial cells could induce cellular activation and thrombosis. CD36, also known as glycoprotein IV (GPIV), is a membrane antigen expressed on platelets and endothelial cells as well as on other cells (4). Some studies have previously demonstrated that antibodies directed against CD36 (anti-CD36) induce platelet activation (5). Furthermore, there is some evidence suggesting the involvement of anti-CD36 in the pathogenesis of thrombotic complications in patients with aPL but not systemic lupus erythematosus (SLE) (6), such as in patients with thrombotic thrombocytopenic purpura (7).

In the present study we evaluated the presence of anti-CD36 in a series of 62 patients with autoimmune aPL but without any other autoimmune disorder. Anti-CD36 were tested using a monoclonal antibody immobilization of platelet antigen (MAIPA) assay and patients were grouped according to the presence or absence of a history of thrombosis.

Patients and methods

Patients

We tested 62 patients with autoimmune aPL. None of the included patients had specific laboratory or clinical features of underlying SLE, SLE-like disease, or any other autoimmune disorder. There were 38 (17 female and 21 male; median age 43 years) aPL patients with a history of thrombosis (27 venous, 14 arterial) and 24 (15 female and 9 male; median age 35 years) without. Nineteen of the 38 patients with thrombosis had had recurrent thrombotic episodes. Eleven patients had mild or moderate thrombocytopenia (6 with and 5 without thrombosis). The remaining 19 patients belonging to the aPL group without thrombosis were referred for aPL screening because of abnormal coagulation tests (n = 6) or a history of pregnancy loss (n = 13). Nineteen (10 female and 9 male; median age 44 years) aPL(-) patients with thrombosis (16 venous, 3 arterial) served as the thrombosis control group. Fifty-eight (30 female and 28 male; median age 40 years) healthy subjects were recruited

as the normal control group.
Blood was obtained by clean venipuncture and collected into plastic tubes containing 0.11 M sodium citrate (ratio 9:1) to investigate the presence of LA. After double centrifugation at 2,500g for 15 minutes, platelet-poor plasma was immediately assayed for LA. Blood collected into glass tubes and allowed to clot at 37°C was used for sera preparation after centrifugation at 1,500g for 10 minutes. Sera samples for MAIPA were decomplemented at 56ºC for 30 minutes prior to storage at -20°C.

Preparation of washed control platelets
Venous blood from adult volunteers was anticoagulated with EDTA 0.077 M (ratio 9:1). Platelet-rich plasma was obtained by centrifugation at 120g for 10 min. Platelets were washed three times in washing buffer composed of 36 mM citric acid, 5 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 103 mM NaCl, 100 nM PGE_1, 25 µg/ml apyrase and 1% w/v bovine serum albumin pH 6.5 (8), and stored at 4°C for at least 24 hours in isotonic saline containing 0.1% sodium azide.

MAIPA assay
Platelet antibodies were detected by using a MAIPA assay as previously described (9) with some modifications (10). Briefly, target platelets were incubated for 30 min with the decomplemented serum from a patient or control donor and then, after washing, incubated with the murine monoclonal antibody anti-CD36 (FA6-152, Immunotech, France). The sensitized platelets were washed and solubilized. The trimolecular complex consisting of the human platelet-reactive antibodies, the anti-CD36 and the target platelet membrane GpIV were then fixed to microtitre plates (Nunc MaxiSorp, Kamstrup, Roskilde, Denmark) coated overnight at 4ºC with a goat anti-mouse IgG antibody (Jackson Immuno Research, West Grove, PA, USA). Binding was revealed using a peroxidase-labelled goat anti-human IgG Fcγ fragment antibody (Jackson). Three normal sera were analysed on each plate. Ratio values were obtained according to the following formula (patient OD/control OD), where the control OD represented the mean obtained in each run. Results were considered positive if the ratio exceeded the threshold of positivity calculated using the 54 controls (98th percentile 1.60).

Detection of aPL and anti-protein antibodies
The presence of lupus anticoagulant (LA) activity was investigated by means of screening tests, mixing studies and confirmatory procedures as described in detail before (11). Anticardiolipin antibodies (aCL) of both isotypes were measured using a standardised ELISA technique and international standards (Louisville APL Diagnostics, Louisville, KY). Results were expressed as standard units (u) for IgG (GPL) or IgM (MPL). Titres higher than 20 u were considered for APS diagnosis. The home-made ELISAs for anti-β_2GPI and anti-prothrombin antibodies (anti-PT) were performed as previously reported using microtitre plates (Nunc MaxiSorp) irradiated by electron beam at 100 kGy and γ-irradiated plates (Nunc MaxiSorp), respectively. The cut-off values (15 arbitrary units for IgG or IgM) were previously assessed as the 99th percentile of 95 normal sera (11).

Statistical analysis
χ^2 test with Yates correction or Fisher's exact test were used to compare proportions. Correlation analysis between antibodies titres were carried out by Spearman test. Significance was defined at $p < 0.05$.

Results
Among aPL patients, 55 had LA, and 34 and 25 had moderate or high titres of aCL of IgG and IgM isotypes, respectively. Anti-β_2GPI were detected in 37 (29 IgG and 19 IgM), while posi-

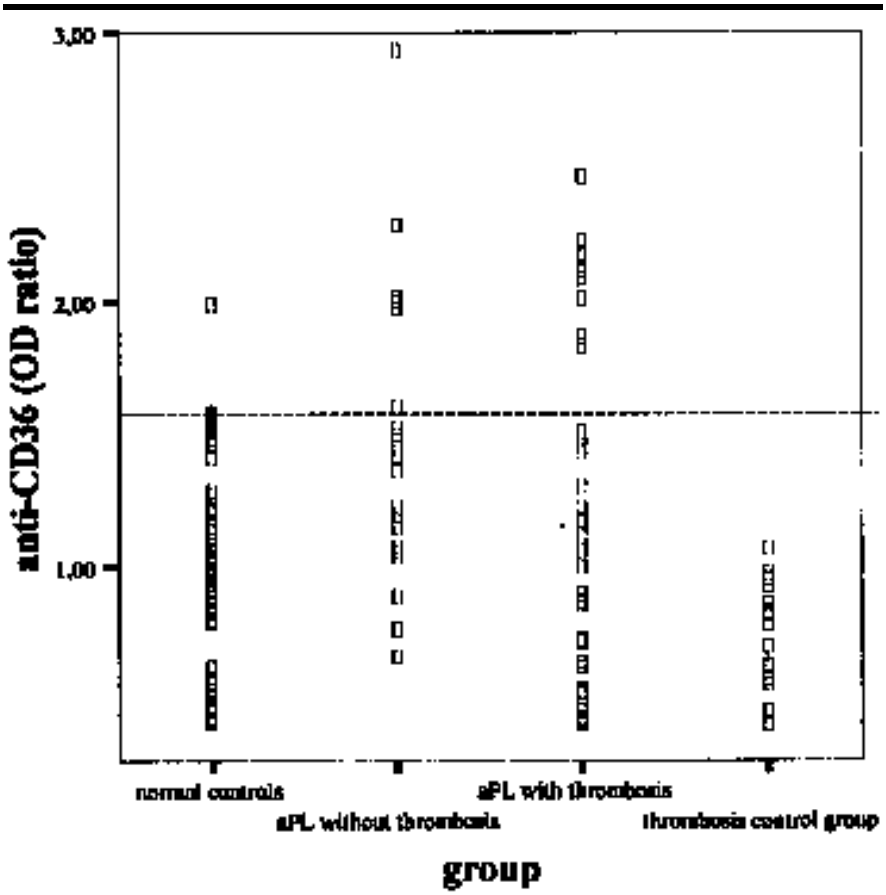

Fig. 1. Results of anti-CD36 in the different groups of patients and controls. The broken line indicates the cut-off rate.

tive anti-PT were found in 32 (25 IgG and 13 IgM) patients.

Anti-CD36 (IgG isotype) were positive in 1 out of 58 (1.7%) normal controls and in 15 of 62 (24.2%) aPL patients. The prevalence in the whole group of patients with autoimmune aPL was significantly higher than in the normal group ($p < 0.0005$). The distribution of anti-CD36 results found in different groups of patients is shown in Figure 1. As compared to normal controls, the prevalence of anti-CD36 was significantly higher in both aPL patients with (10/38, 26.3%, $p < 0.0005$) or without thrombosis (5/24, 20.8%, $p < 0.01$). But the difference between both aPL groups did not reach statistical significance.

Anti-CD36 in aPL patients with thrombosis were also significantly more prevalent than in thrombosis aPL (-) subjects (26.3% vs 0%, $p = 0.02$). Interestingly, the presence of anti-CD36 seems to be more frequent in aPL patients with recurrent thrombosis (7/19, 36.8%) than in those with a single thrombotic event (3/19, 15.8%).

There were no correlations between IgG titres of anti-CD36 with aCL (Rho = 0.078), anti-b_2GPI (Rho = 0.072) or anti-PT (Rho = 0.061). No association was also found between presence of anti-CD36 and thrombocytopenia (data not shown).

Discussion

The association of anti-CD36 with thrombotic complications in patients with LA was first described by Rock et al. (6) in a pilot study. As in that report, patients with SLE were excluded from our study because antibodies directed against several platelet membrane glycoproteins are highly prevalent in the setting of such autoimmune disease (12, 13). Our findings indicated that anti-CD36 occur in about 25% of the 62 patients with aPL. However, this high prevalence was detected in aPL patients with previous thrombosis as well as in patients without thrombosis. The difference in the prevalence of anti-CD36 (26.3% vs 20.8%) between aPL patients with or without history of thrombosis did no reach statistical significance, but probably a higher number of patients is needed to draw a definite conclusion. Our data in primary APS are in agreement with two previous reports showing anti-CD36 in 5 out of 6 (6) and 3 out of 11 (14) patients with primary APS. On the other hand, in other study (12) none of the 21 aPL patients with thrombosis had detectable anti-CD36 assayed by MAIPA. We also found that the frequency of anti-CD36 in aPL patients with recurrent thrombotic events tended to be greater than in those with a single episode. This difference (36.8% vs 15.8%) should be confirmed on larger studies in order to evaluate the potential role of anti-CD36 as a marker of recurrent thrombosis.

The comparison of the prevalence of anti-CD36 in aPL patients with or without thrombosis has not been extensively searched. There are only two studies in which these antibodies were not found, but only 6 patients without thrombosis were included (6,14). We found anti-CD36 in 5 out of 24 aPL patients without a history of thrombosis or any other APS-related clinical feature. The clinical relevance, if any, of anti-CD36 in patients with autoimmune aPL is not well understood. CD36 is a membrane antigen expressed in platelets, monocytes and microvasculature endothelial cells, and it has been implicated in haemostasis, thrombosis, etc. It has been also suggested that anti-CD36 are able to induce cellular activation (5). The finding that anti-CD36 are present either in aPL patients with or without APS might be compatible with our recent study (15) showing platelet, endothelial cell and blood coagulation activation in definite APS but also in aPL patients without APS.

The concept that thrombosis is an immune event mediated by specific antibodies has been reviewed by Vermylen *et al*. (3). The occurrence of thrombosis in patients with autoimmune diseases appears to be multifactorial, and several mechanisms contributing to the disruption of the balance between the normal procoagulant and anticoagulant pathways have been proposed. The presence of additional antibodies such as anti-CD36 may potentiate the prothrombotic activity prior to thrombosis and contribute to the pathogenesis of the APS.

In conclusion, anti-CD36 are highly prevalent in patients with aPL but they do not seem to be clearly associated with thrombosis. Nevertheless, there is a suggestion that anti-CD36 could be linked to the recurrence of thrombosis. Further studies are required to determine more precisely the contribution of these autoantibodies in the APS.

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