Endothelial cell activation by immunoglobulins from patients with immune thrombocytopenic purpura or with antiphospholipid syndrome

Idiopathic thrombocytopenic purpura (ITP) is characterized by the presence of autoantibodies reacting with platelet-specific antigens resulting in thrombocytopenia. In the antiphospholipid antibody syndrome (APS), clinical manifestations are associated with the presence of antiphospholipid antibodies (APLA). There is some overlap between the two pathologies.[1,2] Indeed APLA are present in about 30% of cases with ITP and thrombocytopenia is detected in about 20% of APS. Interestingly, some ITP patients with persistent APLA may later develop thrombotic complications, particularly when platelet counts normalize after corticoid therapy or splenectomy.[3] Cell-based mechanisms may play a major role in the pathogenesis of APLA. Identified targets of these antibodies are monocytes, platelets and EC.[4] Several groups have shown that APLA are able to activate EC leading to over-expression of leukocyte adhesion molecules.[5-7] We have previously observed that EC-activating antibodies are present in about half of the patients with APS.[8] The aim of our study was to compare the EC activation ability of antibodies from patients with ITP and APS.

Serum samples were obtained from four groups of patients (Table 1). Only patients with persistent APLA were included in groups 2, 3 and 4. IgG were isolated from patient or control plasmas by affinity chromatography on Protein G Sepharose. The antibody preparations were free of endotoxin as measured by the limulus lysate assay. EC were isolated from umbilical cord veins (HUVEC).[6,8] Cells were incubated overnight with purified patient IgG (0.5 mg/mL) or with control IgG in the presence of 10 µg/mL of human β2GP1. Total RNA was extracted and reverse transcribed for quantitative real-time PCR. Negative controls were processed in parallel without reverse transcriptase. We used the non-parametric Kruskal-Wallis test followed by the Mann-Whitney test to compare the different patient groups both with each other and with the control group.

Based upon the distribution of the ratio of mRNA levels of control IgG-treated cells over non-treated cells (1.0±0.4 SD, n=14), we established a threshold value of 1.8 (defined by 1±2SD). Ratios obtained with patient IgG above 1.8 for either VCAM-1 or E-selectin mRNA (or both) were considered positive. HUVEC activation was observed for 52% of the APLA containing patient IgG samples (4/11 patients in group 2, 20/39 in group 3 and 9/14 in group 4) but for none of the samples from group 1 or the control group (Figure 1). Positive responses were respectively for group 2: 1 for VCAM, 1 for E-selectin and 2 for both; for group 3: 3 for VCAM-1, 10 for E-selectin and 7 for both; and for group 4: 1 for VCAM-1, 7 for E-selectin and 1 for both. There was a significant difference in the ability of patients' IgG to activate HUVEC between group 1 and groups 2, 3 or 4 (Figure 1). No difference was seen between IgGs from APS patients with or without thrombocytopenia. There was no difference in prevalence of EC activating IgG between patients with pregnancy morbidity, thromboembolism, or both. Out of 33 EC-activating samples, 31 were ACL positive, 16 were anti-β2GP1 positive, and 18 had lupus anticoagulant (LA) activity. Together, these results imply that none of these assays alone or in combination is sufficient to identify EC activating IgG, but that the absence of APLA is associated with an absence of EC activation. In patients with APLA, the prevalence of LA was significantly higher in the two groups with thrombocytopenia (groups 2 and 4) compared with the group without a thrombocytopenia (group 3) (p=0.041 and 0.050 respectively). The main result of our study is that in patients with ITP, EC activation is observed only in patients with APLA. As previously observed with APS patients,[8] only part of the samples containing APLA from ITP and APS patients are able to activate EC. Among the groups with APS, no difference in EC activation was observed between patients with obstetrical complications or thrombosis. A similar result using endothelial microparticles as EC activation marker was recently obtained by Jy *et al.*[9] Studies aiming to differentiate APLA profiles from patients with ITP and APS have led to conflicting results.[2,3] In our study, 10/11 patients with ITP and APLA had LA. This high proportion of LA positive samples is explained by the cross sectional design and inclusion criteria of our study, since ITP patients in group 2 were selected on the basis of clear LA and/or high antiphospholipid titers in ELISA. It is interesting to note that in APS patients the prevalence of LA was higher in patients with thrombocytopenia (12 out of 14) than in APS patients with normal platelet counts (16 out of 32 tested, 7 of whom were on antivitamin K treatment). This finding suggests that the LA-positive samples in our patient groups 2 and 4 contain antibodies that enhance platelet activation. Indeed, there is evidence for interactions between APLA and platelets. APLA can bind to platelet phospholipids and it was suggested that APLA binding could lead to the removal of platelets by the reticulo-endoplasmic system. However, platelet activation should be a

Table 1. Demographics, clinical data and endothelial cell activation of the controls and patient groups.

	Control group	*Group 1*	*Group 2*	*Group 3*	*Group 4*
n 14		11	11	39	14
Female/Male	8/6	5/6	8/3	34/5	9/5
Age (years)					
Mean	42.5	49.7	38.6	38.6	45.6
Minimum/Maximum	26/60	25/72	18/76	18/73	22/73
Platelet count (G/L)					
Mean	247	68	71[a]	246	108
Minimum/Maximum	144/288	27/109	1/120	150/391	11/289[b]
Associated autoimmune diseases					
Systemic lupus erythematosus or lupus-like disorder	0	0	5	10	1
Other autoimmune diseases	0	1	0	1	3
Not known	0	0	1	0	8
No	14	10	5	28	2
Types of APLA					
LA	0	0	10	16[c]	12
Anticardiolipin antibodies	0	0	10	37	10
Anti-β2GPI antibodies	0	0	7	19	6
Clinical events					
Venous or/and arterial thromboembolism	0	0	0	29[d]	14
Obstetrical complications	0	0	0	19[d]	1
Ability to activate EC[e]					
Positive/negative	0/14	0/11	4/7	20/19	9/5
% positive	0%	0%	36%	51%	64%

Group 1, patients with ITP without APLA; group 2, patients with ITP and APLA; group 3, patients with APS without thrombocytopenia; and group 4, patients with APS and thrombocytopenia. [a]Platelet count not available in 1 patient at the time of study inclusion (platelet count 67 g/L when ITP was diagnosed). [b]Two patients with normal platelet count: 1 patient usually thrombocytopenic but on corticotherapy at the time of inclusion; 1 patient with severe thrombocytopenia during a catastrophic APS but with a platelet count > 150 g/L at the time of inclusion. [c]LA could not be performed at the time of inclusion in 7 cases because patients were on antivitamin K therapy. [d]Nine of the 34 female patients had both a history of thromboembolism and obstetrical complications. [e]Cell activation was defined as increased E-selectin and/or VCAM-1 expression levels as compared with non stimulated EC.

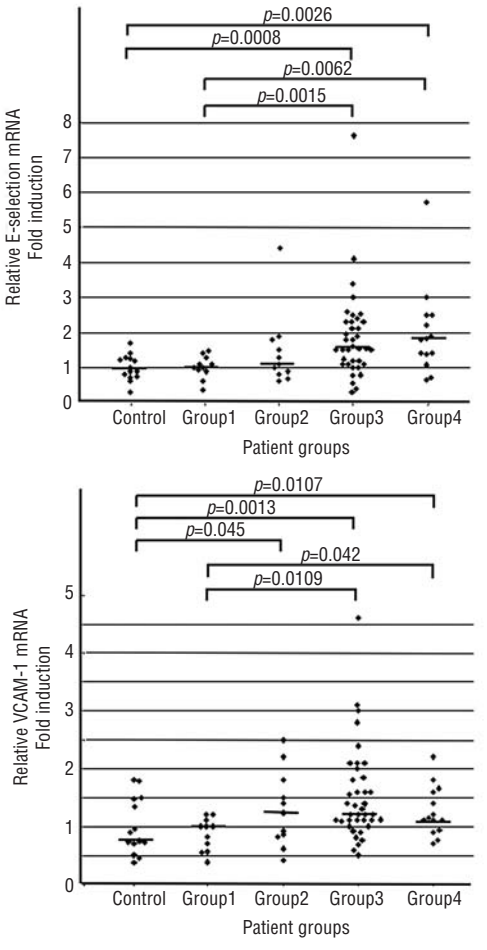

Figure 1. Changes in expression of inflammatory markers in EC stimulated with antibodies from patients and controls. Results of real-time quantitative RT-PCR for E-selectin (upper figure) and VCAM-1 (lower figure) are shown. The groups analyzed were: control group, n=14; group 1 patients with ITP but no APLA, n=11; group 2 patients with ITP and APLA, n=11; group 3 patients with APS without thrombocytopenia, n=39; group 4 patients with APS and thrombocytopenia, n=14. Data points represent relative mRNA levels in cells incubated with patient IgG compared with control IgG. Horizontal bars represent median value for each group.

pre-requisite for APLA-platelet binding because it leads to the exposure of negatively charged phospholipids on the platelet surface.[10] Nojima *et al.*[11] showed that plasma containing both anticardiolipin and LA increased platelet activation in the presence of low concentrations of ADP, suggesting that these antibodies promote platelet activation, thus contributing to the pathogenesis of arterial thrombosis and thrombocytopenia. Jy *et al.*[9] found that platelet activation predisposes to thrombosis in the presence of chronic EC activation in subjects positive for APLA. The presence of APLA in patients with ITP puts them at an increased risk of thrombosis rather than hemorrhage, even when thrombocytopenia is severe.[12] In conclusion, our data show that some APLA in patients with ITP may be able to activate EC. Such a mechanism may have clinical significance because it may increase the risk of thrombosis, particularly when correction of thrombocytopenia is obtained.

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Acknowledgments: we would like to extend special thanks to Dr G. Reber, S. Waldvogel-Abramoswki, S. Mach-Pascual, for providing patients' samples and carefully reviewing the manuscript.

Funding: this work was supported by the Fondation Dr. Dubois-Ferrière Dinu Lipatti and by the Swiss National Science Foundation, grants 3200-067746 and 3100-105844.

Key words: antiphospholipid syndrome, antiphospholipid antibodies, immune thrombocytopenic purpura, endothelial cell activation.

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Citation: Dunoyer-Geindre S, Boehlen F, Favier R, Wahl D, Musial J, Korte W, Satta N, Kruithof E, de Moerloose P. Endothelial cell activation by immunoglobulins from patients with immune thrombocytopenic purpura or with antiphospholipid syndrome. Haematologica 2008 Apr; 93(4):635-636. doi: 10.3324/haematol.12358