Thrombosis Research (2007) **121**, 319–325

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THROMBOSIS RESEARCH

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REGULAR ARTICLE

Platelet activation rather than endothelial injury identifies risk of thrombosis in subjects positive for antiphospholipid antibodies ☆

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Received 24 October 2006; received in revised form 25 April 2007; accepted 26 April 2007
Available online 20 June 2007

KEYWORDS
Antiphospholipid antibodies;
Thrombosis;
Platelet microparticles;
Endothelial microparticles;
P-selectin

Abstract

Background: Anti-phospholipid antibodies (APLA) are often associated with thrombosis, defining the antiphospholipid syndrome (APS) but it remains unclear why many subjects who are positive for APLA chiefly anti-cardiolipin (aCL) or anti-β2GPI (aβ2GPI) do not develop thrombosis. A related question addressed in this study is whether the target of cellular injury in APS is predominately platelets or endothelial cells (EC).
Methods: aCL and aβ2GPI were determined by ELISA in 88 patients, 60 of whom were thrombotic and 28 non-thrombotic. Platelet activation was measured by CD62P and by concentration of platelet microparticles (PMP) and EC activation was assessed by endothelial microparticles (EMP), both by flow cytometry. Lupus anticoagulant (LAC) was measured in the hospital laboratory.
Results: There was no difference in frequency of aCL or aβ2GPI, neither IgG or IgM, between the thrombotic and non-thrombotic groups. Both groups showed elevated EMP compared to controls but this did not differ between thrombotic and non-thrombotic groups. In contrast, PMP were not significantly elevated in non-thrombotic but were elevated in thrombotic compared to non-thrombotic ($p=0.03$) and controls. CD62P, an independent marker of platelet activation, was also elevated in thrombotic vs. non-thrombotic. There was a trend for increased LAC in the thrombotic group but not significant.
Conclusion: Although all subjects had evidence of endothelial activation, only platelet activation differed between thrombotic and non-thrombotic. This supports the hypothesis that platelet activation predisposes to thrombosis in the presence of

☆ This work is supported largely by the Wallace H. Coulter Foundation.
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0049-3848/$ - see front matter
doi:10.1016/j.thromres.2007.04.014

chronic EC activation. These data also raise the possibility of distinguishing risk-prone APLA-positive individuals.

Introduction

The antiphospholipid syndrome (APS) is defined by one or more episodes of thrombosis or unexplained pregnancy loss in association with persisting positive antiphospholipid antibodies (APLA), either anti-cardiolipin (aCL), anti-β2GPI (aβ2GPI), and/or lupus anticoagulant (LAC) [1–6]. APS is frequently associated with underlying autoimmune disorders, most commonly systemic lupus erythematosus (SLE), known as secondary APS, but no underlying disorder is identified in primary APS. In its most severe and life-threatening form, it is called catastrophic APS.

Ever since the identification of APS by Hughes in 1985 [7], the mechanism or target of injury underlying the thrombotic events has been vigorously debated. The leading candidates for target of APLA-induced injury have been either platelets [8–12] or the vascular endothelial cells (EC) [13–26], or both [27–32]. Among the many mechanisms proposed, interference with the protein C anticoagulant system has often been proposed [30,33–36], along with activation of complement [37,38], and other hypotheses in the references cited above. However, the ultimate effect responsible for thrombosis in most scenarios is believed to be platelet activation and/or endothelial injury [31]. Each of these and other proposed mechanisms of APLA-induced thrombosis (or targets of tissue injury) is supported by some clinical and laboratory evidence but no broad consensus has yet been reached. Closely related to these unsolved problems are why so many patients who are chronically positive for APLA or LAC remain free of thrombosis.

The present study was undertaken with the aim of evaluating the relative extents of platelet and endothelial activation in APLA-positive patients, with and without thrombosis. In this study, activation of platelets is measured by marker CD62P as well as by platelet microparticles (PMP), while endothelial activation is measured by endothelial microparticles (EMP) [39,40].

Materials and methods

Patient population

Under a protocol approved by the Institutional Review Board at this medical center, a total of 88 patients were consecutively identified during a two-year period who were persistently APLA-positive (on two or more occasions at least 6 weeks apart) by the methods given below. Of this total, 60 had a history of thrombosis (24 with venous thrombosis, 30 with arterial thrombosis, and 6 with both). Patients with history of miscarriage were excluded. All blood samples were collected at least 8 weeks after recent thrombosis to avoid effects due to acute thrombosis. The remaining 28 patients with persisting APLA had no history of thrombotic episodes. Most of these 28 cases were referred to our hematology clinic on suspicion of hypercoagulable state, autoimmune diseases, connective tissue disorders, or other reasons and the workup included APLA assay.

Table 1 lists data on the patient population. Normal healthy controls consisted of 39 volunteer donors recruited for a small fee from members of the staff. Their mean age was 48.2 years and consisted of 27/12 females/males. Normal donors with recent aspirin or other NSAIDS were excluded. There were no significant differences in the distribution of age and gender between patient and control groups.

Measurement of APLA

IgG and IgM APLA were assayed by ELISA for anti-β2-glycoprotein-I (aβ2GPI) and anti-cardiolipin (aCL) as

Table 1 Features of patient population

	Without thrombosis (non-T)	With thrombosis (T)
Total n analyzed	28	60
Mean age	55.6 yr	49.4 yr
Males/females	9 / 19	19 / 41
Site of thrombosis		
Venous	–	24
Arterial	–	30
Both	–	6
Other condtions		
Diabetes	1 (3.8%)	2 (6.7%)
Hypertension	3 (10.6%)	4 (17.4%)
Dyslipidemia	7 (25%)	13 (22%)
Smoking	8 (28.6%)	9 (14.5%)
Anti-thrombotic medications		
Aspirin	–	28 (47%)
Plavix	–	10 (17%)
Coumadin	–	30 (50%)
Lovenox	–	2 (3%)
Heparin	–	1 (2%)