BASIC SCIENCE: OBSTETRICS

Resistance to annexin A5 anticoagulant activity in women with histories for obstetric antiphospholipid syndrome

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OBJECTIVE: The objective of the study was to investigate whether resistance to annexin A5 anticoagulant activity (AnxA5) occurs in women with histories for obstetric complications of antiphospholipid syndrome (Obs-APS) and whether this correlates with antibody recognition of domain 1 of β2-glycoprotein.

STUDY DESIGN: One hundred thirty-six women with antiphospholipid antibodies, including 70 with histories for Obs-APS and 30 controls, were investigated.

RESULTS: Women with Obs-APS showed resistance to AnxA5 activity (median, 216%; range, 130–282% vs controls; median, 247%; range, 217–283%; $P < .0001$) and elevated levels of anti-domain I immunoglobulin (Ig) G (optical density: median, 0.056; range, 0.021–0.489 vs median, 0.042; range, 0.020–0.323; $P = .002$). Those in the lowest tertile of AnxA5 anticoagulant ratios had an odds ratio for Obs-APS of 58.0 (95% confidence interval, 3.3–1021.5). There was an inverse correlation between levels of annexin A5 anticoagulant activity and anti-domain I IgG.

CONCLUSION: Resistance to AnxA5 anticoagulant activity is associated with antibody recognition of domain I of β2-glycoprotein I and identifies a subset of women with histories for Obs-APS.

Key words: annexin A5, antiphospholipid antibodies, antiphospholipid syndrome, obstetric, β2-glycoprotein I, pregnancy loss

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The antiphospholipid syndrome (APS) is defined by the association of a persistently abnormal antiphospholipid antibody (aPL) assays (ie, elevated immunoassays anticardiolipin and/or anti-β2glycoprotein I immunoglobulin (Ig) G or IgM antibodies or a positive lupus anticoagulant test) with a history of thrombosis or specific pregnancy complications.[1]

The currently available antiphospholipid assays are empirically derived tests that do not measure a disease mechanism; the immunoassays were derived from the biological false-positive syphilis phenomenon and the lupus anticoagulant from the observation an inhibitor to the activated partial thromboplastin time, both described more than 50 years ago. The pathogenic mechanism for obstetric APS has remained enigmatic.

The syndrome is referred to as primary APS (PAPS) when it occurs without other autoimmune disease and secondary APS when it is associated with another autoimmune disease, usually systemic lupus erythematosus. In this paper, the term obstetric APS applies to aPL associated with the pregnancy complications that were defined by consensus diagnostic criteria; these include a previous unexplained recurrent first trimester loss and/or midtrimester and third-trimester intrauterine death and/or severe preeclampsia, placental abruption, or intrauterine growth retardation.[1]

The purpose of this study was to investigate whether women with histories of obstetric APS might have evidence for resistance to annexin A5 (AnxA5) anticoagulant activity in their blood. AnxA5 is a placental anticoagulant protein that is highly expressed on the apical surfaces of syncytiotrophoblasts[2] in which the protein is in an anatomic position to play a thrombomodulatory role and contribute to the fluidity of the maternal circulation through the intervillous space.

The protein is also expressed in a number of other cell types including, among others, vascular endothelial cells, renal tubular epithelial cells, and bile duct epithelial cells. The protein's potent anticoagulant activities result from its forming 2-dimensional crystals over anionic phospholipids that shield the phospholipids from contributing to critical phospholipid-dependent coagulation enzyme reactions. The aPL an-

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tibodies have been shown to reduce the quantity of AnxA5 on cultured placental trophoblasts[3,4] and accelerate the coagulation of plasma that is exposed to these cells[5]. Furthermore, aPL antibodies reduce the binding of AnxA5 to phospholipid bilayers[6-9] and create significant defects in the ordered crystallization of this protein[10,11] that expose unshielded phospholipids, thereby accelerating coagulation enzyme reactions.[10]

We previously reported that patients with APS-associated vascular thrombosis had resistance to AnxA5 anticoagulant activity[7,12] and that this reduced AnxA5 anticoagulant activity correlated strongly with antibody-mediated displacement of AnxA5 from binding to phospholipids[7] and with antibody recognition of a specific epitope on domain 1 of β_2-glycoprotein I (β2GPI).[13]

We also previously reported that women with a history of recurrent spontaneous pregnancy losses, not screened for aPL antibodies, had reduced AnxA5 anticoagulant activity.[14] However, the specific question of whether there may be evidence for resistance to annexin A5 anticoagulant activity in the blood of women with aPL-associated pregnancy complications has never been previously investigated. Nor has the question of whether anti-domain 1 IgG antibodies might correlate with obstetric APS been previously investigated.

Therefore, the aim of this study was to measure these specific parameters in women with histories of obstetric APS. Because of the inflammatory state induced by systemic lupus erythematosus, the study was confined to patients with PAPS.

Materials and Methods

Patients

After obtaining local ethical committee approval at Guy's and St Thomas's Trust, blood specimens were collected with informed consent from healthy, nonpregnant women who had a history of obstetric PAPS and both men and women with a diagnosis of thrombotic PAPS or isolated aPL antibodies. All PAPS patients satisfied the Miyakis criteria for the diagnosis of aPL and APS.[1]

In total, 136 patients with aPL antibodies were classified into 3 groups: (1) women, not currently pregnant but with a past history of obstetric PAPS (n = 70); (2) subjects without obstetric APS but with a history of thrombotic PAPS, with their last thrombotic event more than 6 months previously (n = 50); and (3) subjects with isolated aPL antibodies who had not sustained any thrombotic or pregnancy events (n = 16).

The demographic, aPL characteristics including types of obstetric APS and treatment details are summarized in Table 1. There was no significant difference in ages between groups, but obviously, those with obstetric PAPS were all female, and the majority of the other groups were also female. As described in Table 1, 29 of the 70 women with obstetric APS had histories for 3 or more spontaneous first-trimester losses, 39 of the women had a history for intrauterine fetal demise, and 26 had histories for placental insufficiency.

A minority of the obstetric PAPS group (n = 23, 30%) also had a thrombotic history. Those in the thrombotic PAPS group had similar rates of venous and arterial previous events with 9 (18%) having had both venous and arterial thrombotic events.

In addition, 30 plasmas from disease-free, nonpregnant women (group D) were obtained from a commercial vendor (George King Bio-Medical Inc, Overland Park, KS) as normal healthy controls. The plasma samples were sent as coded samples to the Pathology Department of the Montefiore Medical Center for AnxA5 resistance assay and to the Hematology Department of Utrecht University Hospital for the anti-domain I immunoassays.

Annexin A5 resistance assay

AnxA5 was purified from human placentas as previously described.[15] The effects of patient plasmas on AnxA5 anticoagulant activity were determined using a 2-stage assay as previously described.[13] Briefly, ethylenediaminetetraacetic acid (EDTA) (0.5 M) was added to recombinant human tissue factor (Innovin; Dade Behring Inc, Newark, DE) to a final concentration of 10 mM. The Innovin-EDTA was then mixed with activated partial thromboplastin time reagent-phospholipids (Actin FSL; Dade Behring Inc) at 1:1 ratio. The mixture of Innovin-EDTA–actin FSL (200 μL) was incubated with citrated test plasma (50 μL) for 5 minutes at room temperature.

The plasma-treated mixture was then centrifuged with a microcentrifuge (Eppendorf centrifuge 5417R; Brinkmann Instruments, Westbury, NY) for 15 minutes at 20 800 $\times$ g at 25°C. The pellets were washed once in N-2-hydroxyethylpiperazine-N-2-thane sulfonic acid (HEPES) buffer saline (HBS; 0.01 M HEPES, 0.14 M NaCl [pH 7.5]) and resuspended in HBS (220 μL). The suspension (50 μL) was incubated with pooled normal plasma (50 μL) at 37°C in a ST4 coagulation instrument (American Bioproducts, Parsippany, NJ) for 30 seconds.

The plasma was then recalcified with 50 μL of 0.02 M calcium or 0.02 M calcium containing AnxA5 (30 μg/mL). The coagulation times, in the presence and absence of AnxA5, were determined and the mean times of duplicate tests were recorded. The anticoagulant activity of AnxA5 was calculated as follows: AnxA5 anticoagulant ratio = (coagulation time in the presence of AnxA5/coagulation time in the absence of AnxA5) $\times$ 100%.

Plasma samples were considered to demonstrate resistance to AnxA5 anticoagulant activity when the ratios were below the mean minus 2 SD of the 30 normal healthy controls.

Anti-domain I IgG enzyme-linked immunosorbent assay (ELISA)

Anti-β2GPI IgG antibodies with reactivity toward domain I were assayed as previously described.[13,16] Briefly, hydrophobic microtiter plates (catalog no. 2595; Costar, New York, NY) were coated with domain I IgG of β2-GPI (10 μg/ml in Tris-buffered saline [TBS] consisting of 50 mM Tris and 100 mM NaCl) for 1 hour at 37°C. The plates were blocked with 150 μL of blocking solution (4% bovine serum albumin/TBS/0.1% Tween) for 1 hour at 37°C and subsequently incubated with patient plasma (diluted 1:100 in the