

Characterization of Antiphospholipid Antibodies in Women with Recurrent Spontaneous Abortions

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Antiphospholipid antibodies are important in the etiology of recurrent pregnancy loss. To date, most studies have concentrated on antibodies to cardiolipin specifically. In this study, the serum of 352 women with recurrent pregnancy loss was studied by enzyme-linked immunosorbent assay for antibodies to six phospholipid epitopes: cardiolipin, phosphoserine, phosphoglycerol, phosphoethanolamine, phosphatidic acid and phosphoinositol. Of these women, 59.1% had either an IgG or IgM antibody to one of the six phospholipids. This compared to only 4.6% in the control group. Approximately 75% of the isotypes were IgM. The most common phospholipid epitope was phosphoserine. However, in patients with antibodies to only one phospholipid, phosphoethanolamine was the most common. These findings support recent evidence that antiphospholipid antibodies may interfere with the formation of syncytiotrophoblasts in the placenta. In addition, antiphospholipid antibodies occur more frequently in patients who suffer recurrent miscarriages than was previously thought.

Introduction

It is estimated that approximately 40% of women with systemic lupus erythematosus (SLE) will have antibodies to negatively charged phospholipid,¹ with reported ranges of 30% to 70% having suffered thrombotic events. In 1986 a group of women without known autoimmune diseases who had recurrent spontaneous abortions (RSA) or vascular thrombosis was described.³ These women had antibodies to a negatively charged phospholipid, cardiolipin, with high titers primarily of the IgG isotype. The mechanisms of action defined were platelet membrane

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damage, endothelial wall injury, inhibition of prostacyclin⁴ and inability to activate protein C⁵; thus, RSA would occur via placental vascular insufficiency. In 1985 Harris showed that anticardiolipin antibody (aCL) bound equally to all negatively charged phospholipids.⁶ Subsequent studies that addressed issues of antiphospholipid antibodies (aPLs) therefore assumed nondifferential binding or concentrated only on aCL.

In 1985 Lockshin et al demonstrated that the level of aCL was useful as an early predictor of fetal distress or death in patients with SLE.⁷ Recent work has implicated antiphosphoserine antibody, predominantly of the IgM isotype, as an inhibitor of placental formation and a cause of RSA.⁸

In the study described below, aPLs were characterized in a group of women who have suffered recurrent fetal loss.

Materials and Methods

Patients

Three hundred fifty-two patients with a history of two or more consecutive spontaneous pregnancy losses were evaluated. They were premenopausal (aged 21-45; mean, 39), and all were recruited between March 1991 and May 1992. Women with collagen vascular disease were excluded. Phospholipid antibodies of the IgM and IgG isotypes to cardiolipin, phosphoethanolamine, phosphoinositol, phosphoserine, phosphatidic acid and phosphoglycerol were measured.

Controls

The control group consisted of 43 people without known immunologic or rheumatologic diseases. None of the women in the control group had suffered RSA or any other thromboembolic phenomena.

aPL Assay

The assay used has been described previously. Briefly, six purified phospholipids were coated separately on Immulon 2 96-well enzyme-linked immunosorbent assay plates overnight. The plates were blocked the next day with phosphate-buffered saline (PBS) and 10% newborn calf serum for two hours and then washed (Biotech BT500) in PBS.

Fifty microliters of patient serum was added to the appropriate wells and incubated for one hour followed by a

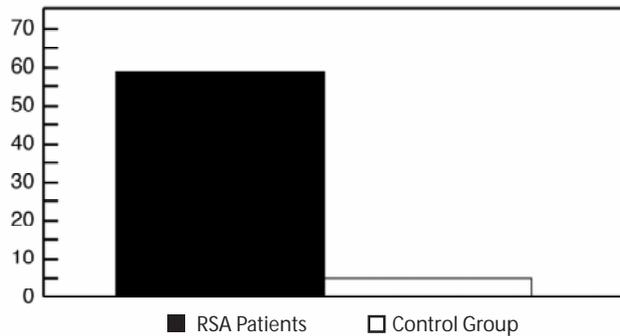


Figure 1
Prevalence of aPLs in patients with recurrent spontaneous abortion.

second wash with PBS. Alkaline phosphatase-conjugated goat antihuman IgG and IgM was incubated for an hour, then washed in PBS. Sigma Substrate 104 in diethanolamine buffer was added to the wells and incubated for 30 minutes at 37°C, and the reaction was stopped with NaOH. The trays were read in a BioTech BT2000 microtiter reader at 405 nm. Delta optical densities were calculated by subtracting out the background (wells without the phospholipid antigens). The results were compared to the mean of the delta optical densities for the control group. Positivity was defined as 3 SD above the mean of the controls.

Results

The prevalence of one or more antibodies to any of the six phospholipids was 59.1% (208/352) in the study population. In the control group, only 4.6% (2/43) had a positive aPL (Figure 1).

Two hundred eight patients had 439 antibodies of the IgG or IgM isotype to the phospholipids. The most frequently identified aPLs in order of decreasing frequency were to phosphoserine, 20.5% (90/439);

Percent of Positive Epitopes

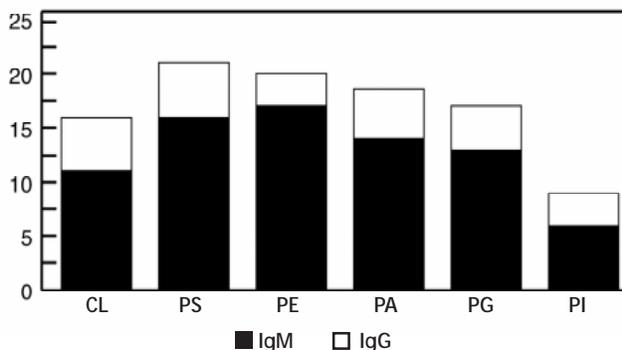


Figure 2
Frequencies of aPL in patients with recurrent spontaneous abortion. CL = cardiolipin, PS= phosphoserine, PE= phosphoethanolamine, PA= phosphatidic acid, PG= phosphoglycerol, PI= phosphoinositol.

phosphoethanolamine, 19.1% (84/439); phosphatidic acid, 18.7% (82/439); cardiolipin, 16.4% (72/439); phosphoglycerol, 16.2% (71/439); and phosphoinositol, 9.1% (40/439) (Figure 2). In the two positive controls there were 2 antibodies, both of the IgG isotype, 1 each to cardiolipin and phosphatidic acid. In the RSA patients, 75.2% (330/439) of the antibodies were of the IgM isotype, and 24.8% (109/439) were of the IgG isotype (Figure 3).

Of all of the patients studied, 18.2% (64/352) had aCL versus 40.9% (144/352) with any other combination excluding aCL. Of all the patients with any aPL, 30.8% (64/208) were to cardiolipin (Figure 4). Eighty-one patients had antibodies to only one epitope: 37.0% (30/81) were to phosphoethanolamine, 14.8% (12/81) to phosphatidic acid and 16.0% (13/81) to cardiolipin (Figure 5). Of this group, 83.9% (68) were IgM and 16.1% (13) were IgG isotypes (Figure 3).

The remainder of the patients (132) had antibodies to multiple epitopes. They had 358 antibodies of the IgG or IgM isotype. In this group, in order of decreasing frequency, were antibodies to phosphoserine, 22.9% (82/358); phosphatidic acid, 19.6% (70/358); phosphoglycerol, 18.2% (65/358); cardiolipin, 16.5% (59/358); phosphoethanolamine, 15.1% (54/358); and phosphoinositol, 7.8% (28/358) (Figure 6). In these patients, 73.2% (262/358) of the antibodies were of the IgM and 26.8% (96/358) of the IgG isotype (Figure 3).

Discussion

The incidence of antiphospholipid antibodies in controls as compared to RSA patients was significantly different, 4.9% vs. 59.1%, respectively. Reported ranges in the general population are 0-17%.⁹ Clearly, antiphospholipid antibodies can be implicated in miscarriage. There are other reasons for immunologically mediated miscarriages, including inadequate alloimmune response and other autoimmune processes, which may explain the absence of aPLs in 39.9% of our patients, in whom immune

Percent

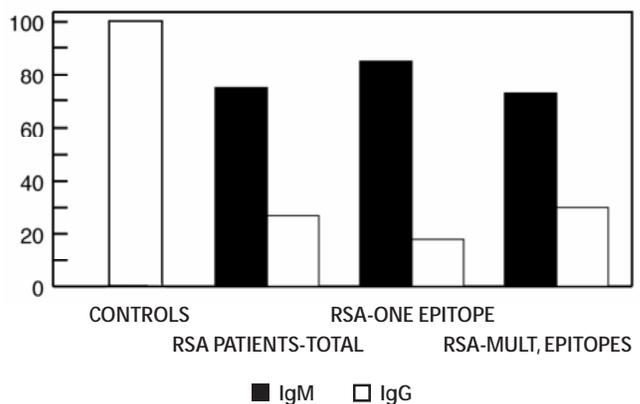


Figure 3
Distribution of isotypes in patients with aPLs.

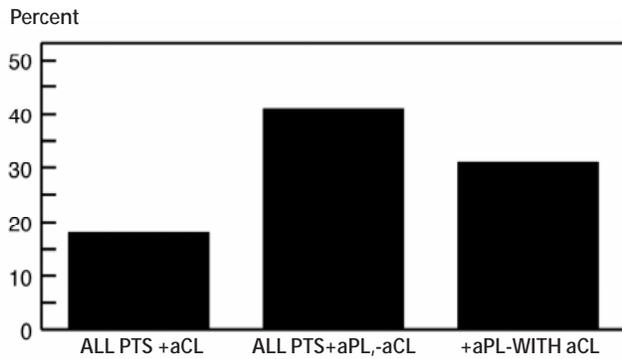


Figure 4
Prevalence of aPLs in patients with recurrent spontaneous abortion.

mechanisms were identified. (All other etiologies had been excluded.)

Earlier studies have shown that the prevalence of aCL in patients with aPL syndrome was 5-15%. This is consistent with the incidence of aCL in our patients (18.2%) who suffered recurrent fetal loss. The prevalence of aPLs, excluding aCL, in our population was significantly greater (40.9%). aPL alone, as a marker for recurrent fetal loss, is inadequate.

In order of decreasing frequency, the aPLs identified in our RSA patients were to phosphoserine, phosphoethanolamine, phosphatidic acid, phosphoglycerol, cardiolipin and phosphoinositol. However, the frequencies were not statistically different. Patients with only one aPL had more antiphosphoethanolamine than any other aPL. It has been shown that phosphoethanolamine and phosphoserine are adhesion molecules that are necessary for the formation of syncytia from myoblasts.¹⁰ Evidence in support of this is interference with the formation of syncytiotrophoblasts in the presence of antibodies to phosphoserine.⁸ IgM aPLs, primarily antiphosphoserine, from patients with RSA react with

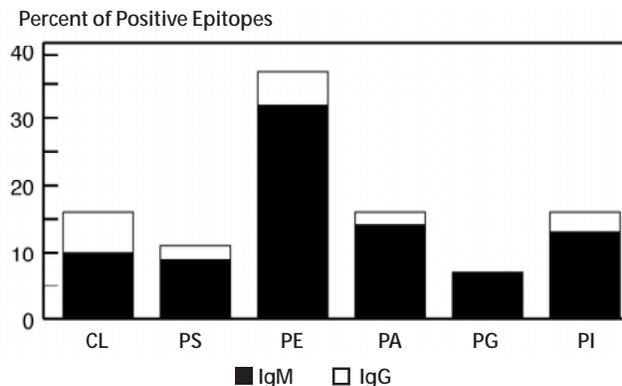


Figure 5
aPL profile in patients with an antibody to only one epitope. CL = cardiolipin, PS= phosphoserine, PE= phosphoethanolamine, PA= phosphatidic acid, PG= phosphoglycerol, PI= phosphoinositol.

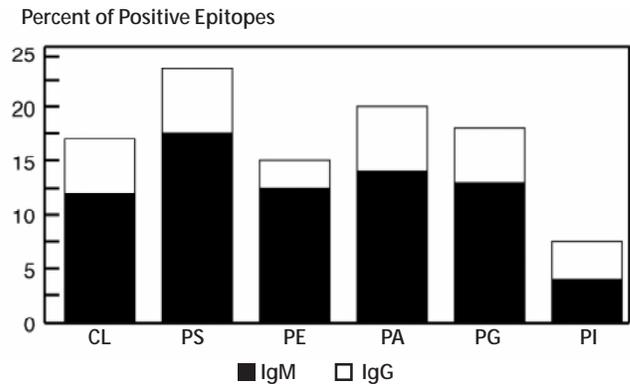


Figure 6
aPL profile in patients with antibodies to multiple epitopes. CL = cardiolipin, PS= phosphoserine, PE= phosphoethanolamine, PA= phosphatidic acid, PG= phosphoglycerol, PI= phosphoinositol.

syncytia in second-trimester human placentae.¹¹ Therefore, in addition to the hypercoagulable milieu that aPLs create in mature endovascularature, these antibodies may cause damage that predates the formation of the placenta.

Approximately 75% of the isotypes were of the IgM class. This is consistent with the findings of Vogt et al¹¹ and Kwak et al¹² but differs from Harris's conclusion that IgG aPLs are the most clinically significant. It is conceivable that two mechanisms are operative: IgG aPLs affect the clotting cascade, and IgM aPLs affect the formation of syncytiotrophoblasts.

Treatment of aPLs in recurrent pregnancy loss has included combinations of aspirin and prednisone¹³ or aspirin and heparin¹⁴ with overall equal efficacy. The theoretical advantage of heparin is its ability to interfere with the binding of aPLs early on, with minimal side effects. We must end our overdependence on aCL and concentrate on those aPLs that appear to operate against the trophoblast at the cellular level.

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