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Antibody Spectrum to Membrane Phospholipids in Women with Recurrent Miscarriages

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Immunoenzyme assay is developed for determination of IgM- and IgG-antibodies to 6 major human blood phospholipids. The antiphospholipid antibody spectra in pregnant and nonpregnant women with recurrent miscarriages and the dynamics of these antibodies during pregnancy are studied. Antibodies to phosphatidylserine, a phospholipid expressed on the surface of cytotrophoblast cells, are identified. It is demonstrated that the antiphospholipid antibody spectrum is important for clinical evaluation and prognosis of pregnancy.

Key Words: *antiphospholipid syndrome; antiphospholipid antibodies; immunoenzyme assay; recurrent miscarriages; phospholipids*

It is known that production of autoantibodies to membrane phospholipids is often associated with recurrent abortions, late toxemia, fetal growth retardation or intrauterine death, and thromboembolic complications.

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The incidence of pregnancy loss in patients with high titers of antiphospholipid antibodies (aPL) is 80-90% [1,4].

Antiphospholipid antibodies represent an antibody population heterogeneous by immunochemical specificity, which is related to the presence of several classes of membrane phospholipids (PL) with different structure and immunogenicity. The major membrane PL include neutral (phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin) and ne-

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gatively charged (phosphatidylserine, phosphatidylinositol, and cardiolipin) PL. It has been hypothesized that various pathological states are characterized by specific quantitative and qualitative peculiarities of the aPL spectra [1,4,6].

Phospholipid determinants are present on platelets and endothelial cells, which are the target cells for aPL. Recent investigations have demonstrated expression of phosphatidylserine on the cytotrophoblast cells. This implies that the damage to the trophoblast can be caused not only by thrombosis but also by a direct cytopathic effect of aPL [4,6].

The aim of the present study was to develop a method allowing determination and investigation of the aPL spectrum in women with recurrent miscarriages.

MATERIALS AND METHODS

For determination of antibodies to membrane PL an immunoenzyme assay (IEA) system was developed. Six PL were isolated from different animal sources by preparative chromatography: phosphatidylserine and sphingomyelin from bovine brain, phosphatidylethanolamine and phosphatidylcholine from egg yolk, cardiolipin from bovine heart, and phosphatidylinositol from the yeast. Purified PL (170 μg/ml) were suspended in distilled water containing 340 μg/ml sucrose (in case of phosphatidylethanolamine 1% acetic acid was added) using an ultrasonic desintegrator. The suspension was transferred to polystyrene microtitration plates (50 μl/well) and incubated for 18±2 h at 37°C. The plates were washed 4 times with 0.01 M phosphate buffer saline, pH 7.4±0.2. Free binding sites were then blocked with 0.5% gelatine in phosphate buffered saline (100 μl/well, 20±2°C, 1.5 h). The same solution was used for dilution of the test sera and monoclonal mouse anti-human immunoglobulin antibodies conjugated to horseradish peroxidase. The test sera (diluted 1:50) were transferred (75 μl/well) to the plates and incubated for 80 min at 20±2°C with constant shaking. Peroxidase-conjugated monoclonal anti-human IgM and anti-human IgG antibodies (1:100,000 and 1:50,000, respectively) were added in a volume of 50 μl/well, and the mixture was incubated for 1 h min at 20±2°C with constant shaking. A substrate-chromogen solution containing o-phenylenediamine and hydrogen peroxide was added to the wells, and optical density at 492 nm was measured after 10 min in a Multiskan MCC/340 photometer (Labsystems). The results were regarded as positive when the mean optical density of the test sample exceeded the mean optical density of negative controls plus 3 standard deviations.

Seventy patients with recurrent miscarriages (36 pregnant and 34 nonpregnant) were examined for the presence of aPL. All patients had 3-5 spontaneous abortions in the first trimester and 9 patients had pregnancy losses in the second trimester of pregnancy. The tests were carried out in the first (10 patients, 27.8%), second (18 patients, 50%), and third (8 patients, 22.2%) trimesters, and 10 pregnant women were followed up until delivery. The control group comprised 20 healthy nonpregnant women and 20 pregnant women without pathology. The sera were stored at -20°C before study.

RESULTS

Test conditions for IEA were chosen after comparing the reproduction and effectiveness of previously described methods of aPL determination [2,3,5] and supplemented by some new experimental approaches.

Examination of 20 patients with normal pregnancy revealed a polyclonal activation of the anti-phospholipid IgM-antibody production in the first and second trimester followed by a decrease in the IgM level in the third trimester. It is assumed that antibody production during pregnancy is normally induced by increased blood level of PL [3]. Healthy nonpregnant women showed negative IEA results.

Of 70 patients with recurrent pregnancy loss, 65 women (92.9%) had IgM and/or IgG to one or

TABLE 1. Occurrence of IgM- and IgG-antibodies to PL in Blood Serum of Women with Recurrent Miscarriages Measured by IEA

Groups	Number of positive results, abs. number (%)					
	Cardiolipin	Phosphatidylserine	Phosphatidylinositol	Phosphatidylethanolamine	Phosphatidylcholine	Sphingomyelin
IgM-antibodies						
Pregnant (*n*=36)	26 (72.2)	24 (66.7)	14 (38.9)	24 (66.7)	17 (47.2)	26 (72.2)
Nonpregnant (*n*=34)	22 (64.7)	20 (58.8)	16 (47.1)	21 (61.8)	15 (44.1)	20 (58.8)
IgG-antibodies						
Pregnant (*n*=36)	13 (36.1)	12 (33.3)	14 (38.9)	12 (33.3)	13 (36.1)	11 (30.6)
Nonpregnant (*n*=34)	19 (55.9)	24 (70.6)	22 (64.7)	20 (58.8)	22 (64.7)	19 (55.9)

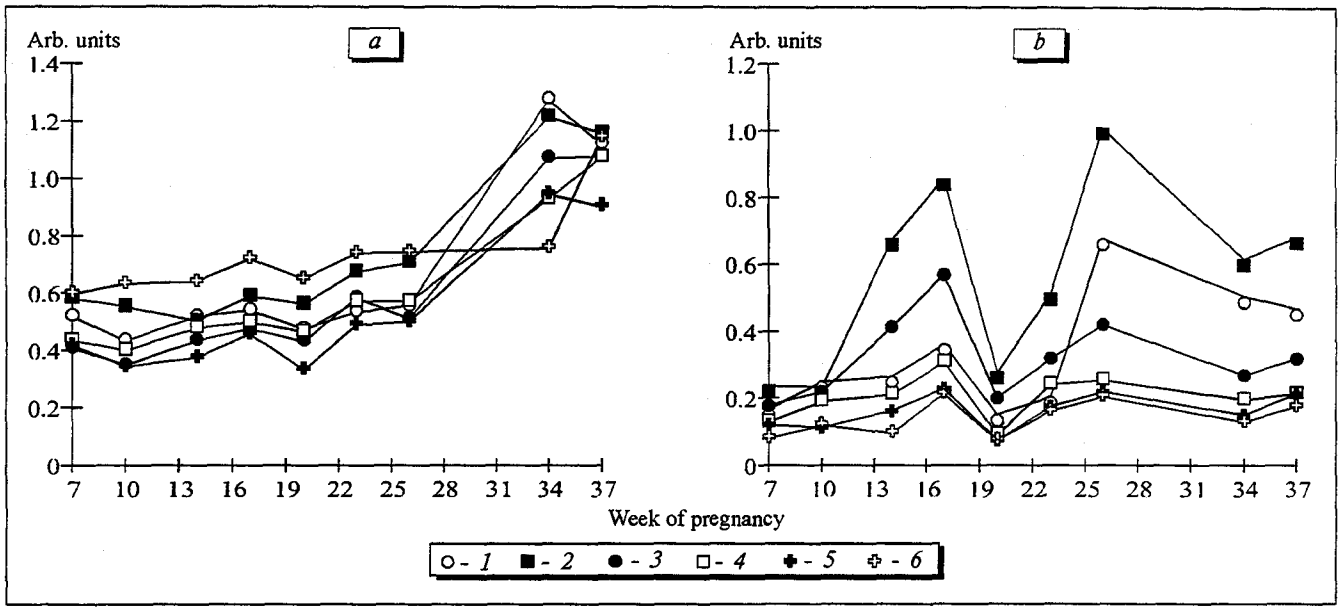

Fig. 1. Changes in blood contents of IgM (*a*) and IgG (*b*) autoantibodies to cardiolipin (*1*), phosphatidylserine (*2*), phosphatidylinositol (*3*), phosphatidylethanolamine (*4*), phosphatidylcholine (*5*), and sphingomyelin (*6*) in women with repeated miscarriages. Each point represents optical density at 492 nm upon immunoenzyme analysis of sera from 10 patients minus this parameter measured in the control group.

several PL: IgM were found in 15 (21.4%) and IgG in 11 (15.7%) patients, and 18 (25.7%) patients had both IgG and IgM to the entire PL spectrum. Of particular interest are different patterns of antibody formation in the patients: some of them had antibodies to six PL, presumably due to wide cross-reactivity, while others had more specific antibodies.

In pregnant women aPL to different PL were unequally distributed: the occurrence of IgM-antibodies to cardiolipin, sphingomyelin, phosphatidylserine, and phosphatidylethanolamine, and IgG antibodies to phosphatidylinositol, cardiolipin, and phosphatidylcholine was the highest (Table 1). It has been previously reported that IgG-antibodies to PL more frequently than IgM are associated with pregnancy losses. The presence of IgG-antibodies to two or more PL is considered to be a stronger prognostic criterion in comparison with monospecific IgG-antibodies [2]. In the examined patients with IgG-antibodies of various specificity and/or with a high level of IgM-antibodies to PL (2- to 3-fold surpassing that of healthy pregnant women) threatened abortions were diagnosed in the first trimester and then the symptoms of placental insufficiency were noted against the background of coagulation disturbances.

Repeated tests in 10 women during pregnancy showed that, unlike in women with normal pregnancy, in the patients with recurrent abortions a sharp rise of IgM-antibodies to PL, primarily to cardiolipin and phosphatidylserine, occurs in the third trimester (Fig. 1). The level of IgG-antibodies in these patients increased by the end of the second trimester and remained at this level until delivery.

In the nonpregnant women with bad obstetrics history IgG-antibodies occurred more frequently than IgM-antibodies, IgM-antibodies to cardiolipin and phosphatidylethanolamine and IgG-antibodies to phosphatidylserine, phosphatidylinositol, and phosphatidylcholine predominating (Table 1).

The high occurrence and high level of anti-phosphatidylserine antibodies in patients suggest that direct damage to the trophoblasts caused by aPL may be a possible mechanism of spontaneous abortions.

Thus, using an IEA system for the determination of antibodies to different PL, we have shown a high occurrence of aPL and antibodies to phosphatidylserine expressed on the surface of cytotrophoblast cells in patients with recurrent miscarriages. Antibody spectrum and dynamics during pregnancy were also studied. The high level of IgM- and IgG-antibodies is an important clinical and prognostic criterion for evaluating the course of pregnancy.

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