Autoantibodies to Human Chorionic Gonadotropin in Habitual Abortions

I. V. Ponomareva, G. T. Sukhikh, E. B. Nikitin, P. A. Kiryushchenkov, V. M. Sidel'nikova, and L. V. Van'ko

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An enzyme immunoassay is developed for detecting autoantibodies to human chorionic gonadotropin in the blood serum and a high incidence of these antibodies in women with a history of habitual abortions is demonstrated. A high level of autoantibodies is associated with threatened abortion, placental dysfunction, and hemostasis disorders. The results confirm the hypothesis regarding the production of autoantibodies to human chorionic gonadotropin as a possible autoimmune mechanism underlying habitual abortion.

Key Words: *autoantibodies; enzyme immunoassay; habitual abortion; human chorionic gonadotropin*

Reports about the production of antibodies directed toward the homologous gonadotropic hormones in humans are rare. There are documented cases of the production of antibodies in response to parenteral human chorionic gonadotropin (HCG) and human pituitary gonadotropic hormones (luteinizing and follicle-stimulating hormones — LH and FSH), administered for diagnostic and therapeutic purposes to men with reproductive disorders [2,4,6] or for stimulating ovulation in women [1]. HCG-based contraceptive vaccines have been designed [7,8], whose mechanism of action manifests itself not only in the prevention of HCG binding to receptors of corpus luteum cells and intensive hormone release from the blood, but also in the direct effect of antibodies on the cells of the embryonal trophectoderm [3].

Reports about the production of autoantibodies to endogenous HCG and LH in women with a history of spontaneous abortions [5] are especially interesting. These autoantibodies were shown to possess high affinity and a capacity for neutralizing the biological activity of both hormones *in vivo*. Theoretically, autoantibodies to HCG are regarded as a possible autoimmune cause of spontaneous abortions [9]. Hence, our aim was to develop a method for detecting antibodies to HCG in human blood serum and to elucidate their role in the pathogenesis of miscarriages.

Laboratory of Clinical Immunology, Department for the Prevention of Miscarriages, Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow (Presented by V. I. Kulakov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

An enzyme immunoassay (EIA) has been developed for detecting antibodies to HCG. The test is carried out with Sigma high-purity HCG, murine monoclonal antibodies (MAb) to HCG α-subunit (Center for Molecular Diagnosis and Treatment, Moscow), and conjugates of MAb to human immunoglobulins M and G (IgM and IgG) with horseradish peroxidase (Institute of Viral Preparations, Russian Academy of Medical Sciences). In addition, we used MAb to HCG β-subunit, high-purity preparations of luteinizing hormone (Sigma), and HCG α- and β-subunits (Center for Molecular Diagnosis and Treatment).

MAb to HCG α-subunit in 0.1 M carbonate-bicarbonate buffer solution at a concentration of 3 μg/ml were put in the wells of a polystyrene microplate,

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100 μl per well, and incubated at 20±2°C for 18±2 h. After each step of the analysis the plates were washed 4 times with phosphate buffer saline, pH 7.4, with 0.5 g/liter Tween-20. Wells with adsorbed MAb were treated with 0.5% solution of bovine serum albumin (Serva) in 0.1 M carbonate-bicarbonate buffer solution and pipetted 200 μl per well, after which they were left for 1.5 h incubation at 20±2°C. The HCG preparation, blood sera, and conjugates were diluted with phosphate buffer saline containing 0.5 g/liter Tween-20 and 0.5% bovine serum albumin. The reagents were pipetted 100 ml per well and incubation was carried out for 1 h on a shaker at 20±2°C. The hormone preparation was used in a concentration of 6000 U/liter, test sera in 1:100 dilution, and conjugates against human IgM and IgG in dilutions 1:100,000 and 1:50,000, respectively. The substrate chromogenous mixture containing o-phenylenediamine and H_2O_2 was added to wells in a dose of 100 μl and incubated for 15 min in the dark at 20±2°C. Optical density was measured with a Labsystems Multiskan MCC/340 photometer at 492 nm.

Results of analysis were considered positive if the optical density of a sample surpassed the sum of the mean optical density of negative controls and three mean square deviations.

The test group consisted of 120 women with a history of habitual abortions, 70 of them pregnant and 50 not pregnant. All the women were healthy, without a history of clinical manifestations of autoimmune diseases, and none of them had ever been prescribed human gonadotropic hormones. The control group consisted of 30 pregnant women with normal gestation, 35 healthy women with a history of normal deliveries (no miscarriages), and 50 healthy donors: women who had never been pregnant and men. Sera were stored at -20°C before investigation.

RESULTS

The described variant of EIA was used in studies of the clinical material, because in comparison with the variant with adsorption of HCG preparation directly on the solid phase it permits a 1.5-2 times higher binding of antibodies at the same level of negative control signals in both variants of the analysis.

Replacement of the native HCG preparation with the α- or β-subunits of the hormone revealed that the test sera contained heterogeneous antibodies characterized by different specificities towards each of the hormone subunits.

Study of possible cross-reactions of sera positive for anti-HCG antibodies with human pituitary gonadotropic hormones close to them in antigenic structure was particularly interesting. For these studies LH or FSH was used in EIA instead of HCG, and microplates with MAb to the α-subunit of the hormones were adsorbed on it. Testing of 30 sera for IgM and IgG showed that 22 (73.3%) sera reacted with LH and 14 (46.6%) with FSH. Eight (26.7%) sera did not react with the pituitary gonadotropic hormones. The sera most frequently cross-reacted with LH. This could be due to the close antigenic similarity between the molecules of HCG and LH in comparison with FSH.

Table 1 presents the results of screening of 70 pregnant and 50 nonpregnant women suffering from habitual abortions for autoantibodies to HCG. IgM antibodies were detected in 56 (80%) of the pregnant women, IgG in 31 (44%). In 5 (7%) women only IgG antibodies were detected. Follow-up of 20 pregnant women showed that the level of antibodies changed in a wavelike fashion in the course of pregnancy, in accordance with the physiological fluctuations in the serum levels of HCG, lagging behind by 2-3 weeks (Fig. 1). Three characteristic peaks of IgM levels were observed: during weeks 10-14, 20-24, and

TABLE 1. Detection of IgM and IgG Autoantibodies to Human Chorionic Gonadotropin in the Blood Sera of Women with a History of Habitual Abortions and in Controls by Enzyme Immunoassay (EIA)

Group of women		Total	Results of EIA detecting			
			IgM		IgG	
			positive	negative	positive	negative
Group examined:						
	pregnant	70	56 (80)	14 (20)	31 (44)	39 (56)
	nonpregnant	50	34 (68)	16 (32)	26 (52)	24 (48)
Control group:						
	pregnant	30	4 (13)	26 (87)	-	30 (100)
	nonpregnant	35	-	35 (100)	-	35 (100)

Note. In parentheses: percent of total number of patients in the group.

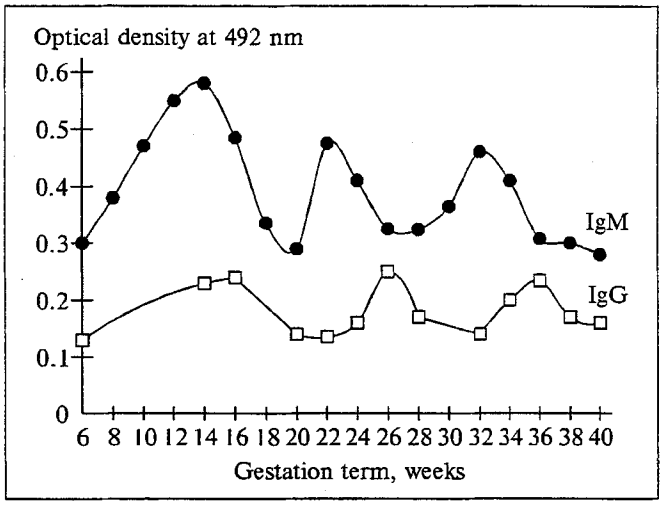

Fig. 1. Changes in the level of IgM and IgG autoantibodies to human chorionic gonadotropin in the course of pregnancy in women with a history of habitual abortions. Each point corresponds to a mean value of optical density obtained in enzyme immunoassay with sera of 10 patients minus the analogous value for the control group.

30-34 of gestation. Changes in the IgG level repeated the fluctuations of the IgM level, lagging behind them by 1-2 weeks. Threatened abortion during the first trimester was observed in 58 (95%) women. The development of hemostasis disorders was typical. Forty-six (75%) women developed placental dysfunction. Timely prednisolone therapy with adjustment of the dose depending on the level of autoantibodies in combination with low aspirin doses and/or heparin injections was effective in 95% of cases. In the control group IgM antibodies were detected in negligible amounts during the characteristic gestation periods only in 4 (13%) pregnant women; in the other cases the results of analysis were negative.

Screening of nonpregnant women revealed IgM antibodies in 34 (68%) and IgG antibodies in 26 (52%) women. A high level of IgG antibodies was observed in women with the most problematic obstetric history. The production of both IgM and IgG antibodies persisted for 1-2 years of the follow-up. Examinations of 10 women during several menstrual cycles in various phases thereof showed that the level of antibodies increased 1.5-2 times during the ovulatory period. Since the cross-reactivity of the sera with LH is high, it is possible that the production of autoantibodies in nonpregnant women is maintained for a long time by release of this hormone during the ovulatory period of the cycle.

Hence, our data confirm that the production of autoantibodies to HCG is a possible autoimmune cause of habitual abortions. This hypothesis is confirmed by the high incidence of autoantibodies to HCG in women with this abnormality and by the close correlation between the presence of antibodies and threatened abortion, placental dysfunction, and hemostasis disorders.

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