EVALUATION OF SPECIFICITY OF THE TEST FOR β_1-G-GLOBULIN IN TROPHOBLASTIC TUMORS BY A QUANTITATIVE IMMUNOENZYMIC METHOD

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A method of quantitative immunoenzymic determinaton of β_1-G-globulin (TBG) in blood serum has been developed. The sensitivity of the method is about 6 ng/ml TBG. An increased concentration (12-100 ng/ml or more) of TBG is observed as a rule in trophoblastic tumors of the uterus. Ectopic TBG synthesis is found in certain tumors of the gastrointestinal tract and in teratoblastomas of the ovary.

KEY WORDS: immunoenzymic determination; trophoblastic β_1-G-globulin; chorionepithelioma; trophoblastic tumor; teratoblastoma of the ovary.

The immunodiffusion and immunoautoradiographic tests for trophoblast-specific β_1-G-glycoprotein (TBG) are nowadays used to establish and evaluate the course of pregnancy [1-3, 6] and also for the diagnosis and monitoring of chorionepithelioma of the uterus [4, 9, 11, 12]. The highly sensitive radioimmunologic determination of TBG has shown that in certain cases this protein can be found in the blood serum of patients with nontrophoblastic tumors [10].

The object of this investigation was to determine the specificity of the test for TBG in trophoblastic tumors by means of a quantitative immunoenzymic method (IEM) developed by the writers, on the basis of the test suggested previously for the determination of other antigens [7, 13, 14].

EXPERIMENTAL METHOD

The TBG was prepared as follows. A semipurified sample of TBG obtained from the blood serum of parturient women was used as the material for immunization. To one volume of serum 2.5 volumes of 0.5% rivanol solution was added. The mixture was carefully stirred on a magnetic mixer for 30 min, after which the residue was separated by centrifugation at 6000 rpm. The rivanol was removed from the supernatant by adsorption on activated charcoal. Ammonium sulfate was added to the supernatnat to 50% saturation, the mixture was incubated at room temperature for 1 h, and it was then centrifuged at 6000 rpm for 30 min. The residue was dissolved in a minimal quantity of distilled water and dialyzed against water for 5 days at 4°C. The resulting material was lyophilized and used for immunization and for preparation of an immunosorbent for TBG.

To obtain immune sera against TBG, chinchilla rabbits weighing 3-5 kg were immunized with small doses of antigen for 1.5 months. The first injection (100 mg protein) was given as a mixture with Freund's complete adjuvant into the zone of the popliteal lymph nodes. After a period of 15 days, three cycles of immunization were given, with weekly intervals between them; in the course of 3 days, 20 mg protein mixed with Freund's complete adjuvant was injected daily for 3 days, with alternation of the site of injection (subcutaneously, intramuscularly, and intravenously without the adjuvant). The total quantity of protein per rabbit was about 300 mg. Blood was taken on the 7th, 9th, and 12th days after the last injection. A single reimmunization was given 1 month after the blood was taken, when 100 mg of the preparation were injected intravenously. The resulting antiserum was absorbed with lyophilized human plasma (20 mg plasma to 1 ml antiserum). Completeness of absorption and specificity of the antisera were verified by the immunodiffusion method with a standard test system for TBG.

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TABLE 1. Immunoenzymic Determination of TBG in Blood Serum of Cancer Patients and Blood Donors

Diagnosis	No. of patients	No. of determinations	Concentration of TBG, ng/ml					
			under 6,25	6,25—12,5	12,5—25	25—50	50—100	over 100
Chorionepithelioma of uterus	73							
before treatment	39	39					2	37
during treatment	25	122	19	9	10	6	2	76
after treatment	9	32	21	2	2	1	2	4
Destructive hydatidiform mole	23							
before treatment	18	18						18
during treatment	5	32	1		4	2	2	23
after treatment	0	3	2					1
Testicular tumors	33	33	28	2	2			1
Tumors of nontrophoblastic type (of breast, lung, uterus, ovary, gastrointestinal tract)	117	117	113	4*				
Blood donors (men and women)	127	127	125	2†				
Total . . .	373	523	309	19	18	9	8	160

*Tumors of gastrointestinal tract.
†Women donors.

Monospecific antibodies against TBG were isolated from the antisera thus obtained on an immunosorbent prepared on ACA-34 ultragel, on which TBG was immobilized with the aid of glutaraldehyde [8]. The sample of TBG which was used for immunization was also used for fixation of the immunosorbent. The isolated antibodies were concentrated by ultrafiltration on an XM-100A membrane (from Amicon) and used for the subsequent experiments.

To prepare the conjugate, horseradish peroxidase type VI (from Sigma) was used. Conjugation of the antibodies with the enzyme was carried out with the aid of glutaraldehyde (from Merck) [5, 14].

The technique of the IEM was as follows. Type 3040-11tm polystyrene tissue culture plates (from Falcon Plastics) were used as the solid phase. Antibodies were fixed on the solid phase in carbonate buffer, pH 9.6, with an antibody concentration of 10 μg/ml (0.3 ml in each compartment) overnight at 4°C. Unbound antibodies were washed off with buffered physiological saline (BPS) with the addition of 0.05% Tween-20. All the reagents were diluted with BPS with Tween. The order of conduct of the IEM and analysis of its results were described previously [14].

To plot the calibration curve, a sample of TBG titrated with the aid of the standard test system for TBG was used. The minimal quantity of TBG capable of "matching" the standard test system was 5 μg/ml. These data were verified by means of the standard TBG prepared for WHO.

The material for testing (blood serum of cancer patients) was obtained from the Oncologic Research Center, Academy of Medical Sciences of the USSR, and from the National Cancer Institute, Bethesda (USA) in accordance with the plan for Soviet—American collaboration for 1976-1978. Donors' blood sera was obtained from the Moscow Blood Transfusion Station.

EXPERIMENTAL RESULTS

Immunochemical analysis of the antibody—enzyme conjugate showed that only 4% of antibodies retained their immunochemical activity. The conjugate was used in the experiments in dilutions of 1 : 100-1 : 200. These dilutions enabled the work to be done with TBG in solutions within the concentration range from 6.25 to 100 ng/ml.

The results of determination of TBG in serum from blood donors and patients with various malignant neoplasms are given in Table 1. They show that the TBG level in the donors' blood serum did not exceed 6.25 ng/ml, except in two women in whom it was increased to 10 mg/ml. This can probably be explained by the onset of pregnancy or by the use of contraceptives.

In patients with various tumors of nontrophoblastic type the TBG concentration as a rule did not exceed 6.25 ng/ml. Patients with tumors of the gastrointestinal tract were exceptions. The TBG concentration in the blood serum of these patients in 19% of cases was about 10 ng/ml. In patients with testicular tumors an increase in the TBG concentration to 100 ng/ml was observed. In three of five patients with a TBG concentration of over 6.25 ng/ml, elements of a chorionepithelioma were found in the tumor tissues.

In patients with different types of trophoblastic tumors, the TBG concentration before treatment was over 50 ng/ml in 100% of cases. The TBG concentration in the blood serum, it must be noted, fell appreciably after surgical and chemotherapeutic treatment. However, patients with chorionepithelioma of the uterus were observed in whom the blood TBG level remained high even after treatment (about 28% of cases with a TBG level of 12.5 ng/ml). This fact probably confirms once again the view that incompletely cured forms of trophoblastic tumors may exist.

The results of clinical trials of the test for TBG by the IEM demonstrated the high sensitivity and specificity of this method, in agreement with the results obtained previously by immunoautoradiographic and radioimmune methods. The test now suggested for determination of TBG may be of great importance not only for the specific diagnosis and prognosis of treatment of patients with trophoblastic tumors, but also for the epidemiological studies of the population aimed at the early detection of patients with trophoblastic tumors after hydatidiform mole.

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