Determination of TNF activity can thus be used as a test of function of the immune system in patients with neoplastic conditions, but the mechanisms of regulation of TNF activity require further study.

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DETERMINATION OF TROPHOBLASTIC β_1-GLYCOPROTEIN IN TUMOR TISSUE AND BLOOD SERUM IN OVARIAN CANCER

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The specific protein of pregnancy, trophoblastic β_1-glycoprotein (TBG), is known as a biological marker of trophoblastic tumors [7]. In some cases, however, TBG can be found in the blood of patients with nontrophoblastic tumors [12, 13]. There is also evidence of detection of TBG by the immunoperoxidase method in the tissue of an ovarian adenocarcinoma [11]. There is no general agreement on the serum TBG concentration in ovarian cancer. An increase in the seruin TBG concentration to 3-10 μg/liter in ovarian cancer has been found by radioimmunoassay in 16.7% [12] of cases, and up to 12-100 μg/liter in 75% [13].

This paper gives the results of immunochemical identification of TBG in tumor tissue and blood serum in ovarian cancer.

EXPERIMENTAL METHOD

Extracts from tumor and normal tissues were prepared under standard conditions: Tris-glycine buffer (pH 8.3) was added to a weighed sample of tissue in the ratio (*v/w*) of 2:1. Various fractions were obtained from tumor extracts by precipitation with

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TABLE 1. Serum TBG Concentration in Patients with Tumors of the Reproductive System

Disease	Number of samples	TBG concn., μg/liter: number of samples with TBG concentration			
		<1.94	2–3	3–5	10
Carcinoma of the ovary	24	16	5	2	1
Benign ovarian tumors	19	9	9	1	0
Carcinoma of uterus	31	20	9	2	0
Healthy blood donors					
Women	30	27	2	1	0
Men	16	16	0	0	0

TABLE 2. Results of EIA of TBG in Tissues and Biological Fluids in Tumors of the Reproductive System

Material	Number of samples	Number of samples containing TBG in a concntration (μg/liter) of				
		<1.94	2–3	3–10	10–30	30–60
Carcinoma of the ovary	22	14	4	3	0	1
Tissue of metastases of ovarian carcinoma in omentum	13	10	3	0	0	0
Normal uterus	6	2	2	2	0	0
Carcinoma of the uterus	7	3	3	1	0	0
Normal mother	3	0	2	1	0	0
Ascites fluid from patient with ovarian carcinoma	12	4	3	3	1	1

neutral salts, soluble in organic acids, and isolated according to their molecular weight during chromatography, etc. On immunization of rabbits with the fractions antisera were obtained and were exhausted with blood serum from normal blood donors and a mixture of extracts from normal organs. Known methods of immunochemical analysis were used: the agar precipitation test with a standard test system, immunoelectrophoresis, polyacrylamide gel disk electrophoresis, gel-filtration, ion-exchange, hydrophobic, and affinity chromatography, immunofluorescence, and enzyme immunoassay [1, 2, 4, 6, 9].

The method of obtaining TBG was described by the writers previously [5]. A standard preparation of TBG for enzyme immunoassay (EIA), on electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate, revealed two subunits: the major component had a molecular weight of 60 kD, the minor – 42 kD.

As the solid phase for EIA polystyrene plates ("Dynatek," Switzerland) were used. The following substances were tested for planting on the solid phase in EIA: the γ-globulin fraction of antiserum to TBG, obtained by precipitation with ammonium sulfate, the immunoglobulin G fraction obtained by ion-exchange chromatography on DEAE-Sephadex in 0.0175 M phosphate buffer (pH 6.3) from the γ-globulin fraction of anti-TBG, and pure antibodies to TBG isolated by affinity chromatography from a precipitation of TBG, activated by trichlorotriazine, and immobilized on sepharose 6B. Hexamethylene-diamine was used as the spacer, and the length of the bridge was 31 A. Analysis of the reliable pure antibodies gave the following results: the sensitivity of the system was 1.94 μg/liter, the standard curve from 0.97 to 125 μg/liter of TBG had a coefficient of variation of under 10%, and the coefficient of variation in "discovery" and "parallelism" tests did not exceed 10%.

The TBG concentration was determined by EIA in blood serum from 76 patients and 46 normal individuals (Table 1), and also in extracts of tumor and normal tissues and ascites fluid of patients with ovarian cancer (Table 2).

EXPERIMENTAL RESULTS

Of the 12 adsorbed antisera, two antisera obtained by immunizing rabbits with the glycoprotein fraction of ovarian adenocarcinomas (AO-77), soluble in 0.6% sulfosalicylic acid, revealed a β_1-glycoprotein with mol. wt. of 110 kD in the blood serum of pregnant women and in the placenta, which was not found by the agar diffusion test in the blood serum of healthy donors, in extracts from normal organs, and extracts from ovarian tumors. Immunochemical identification of the a β_1-glycoprotein thus revealed with TBG in the precipitation test demonstrated its complete identity with ovarian adenocarcinoma and TBG.

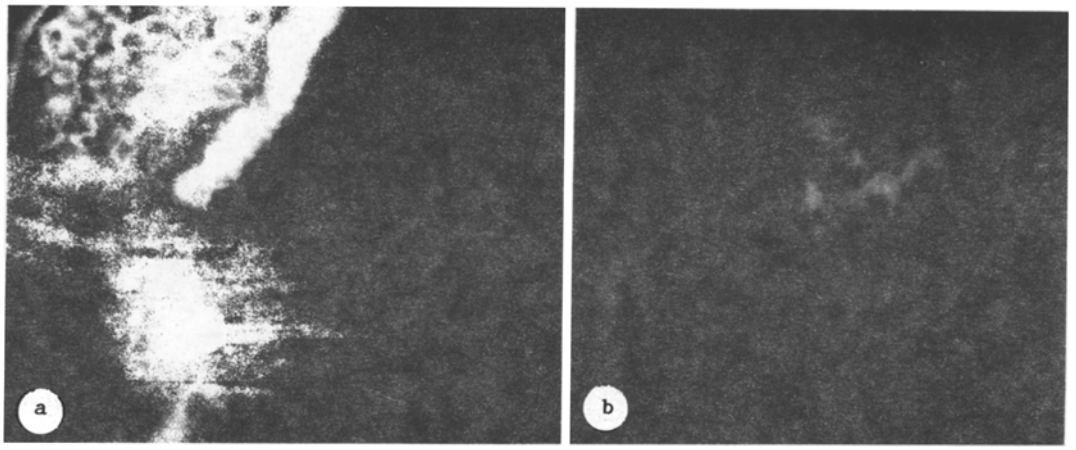

Fig. 1. Immunofluorescence analysis of TBG in tissue sections of ovarian adenocarcinoma AO-77. 630×. a) Experiment. Section treated with: 1) anti-TBG, 2) donkey antirabbit globulin labeled with FITC; b) control. Section treated with: 1) anti-TBG adsorbed with TBG, 2) donkey antirabbit globulin labeled with FITC.

The results of EIA showed that when the γ-globulin fraction was planted on the solid phase anti-TBG gave a high nonspecific background level and the sensitivity of the method was 7.8 μg/liter. The sensitivity of the method was increased (3.9 μg/liter) when the IgG fraction from anti-TBG was used in EIA, but the nonspecific background was preserved. A false positive reaction was observed in 35 and 15% of cases in the first and second variants of EIA respectively. When pure antibodies were used in EIA the nonspecific background was absent, false positive reactions were not observed in the donors, and the sensitivity of the method was 1.94 μg/liter.

Analysis of the results of EIA of TBG in the tissues and biological fluids showed (Table 2) that the TBG level in 18% of cases (in 4 of 22) exceeded 3 μg/liter in extracts from ovarian adenocarcinoma. Moreover, the highest level (30 μg/liter) was found in ovarian adenocarcinoma AO-77, on immunization with which (glycoprotein fraction soluble in 0.6 M sulfocalicylic acid) antibodies were obtained to TBG. A raised TBG level also was found in the ascites fluid of patients with ovarian cancer, and in 41.6% of cases it exceeded 3 μg/liter.

A TBG level above 3 μg/liter was found (Table 1) in the blood serum of 3 of 24 patients with ovarian cancer (12.5%) and of 2 of 31 patients with carcinoma of the uterus (6.5%). The TBG concentration in the blood serum of a patient with ovarian adenocarcinoma AO-77 was 10 μg/liter.

The increase in the TBG concentration in tumor tissue of ovarian adenocarcinoma AO-77 and in the blood serum of a patient with this tumor suggests the presence of morphological structures in the tumor which synthesize TBG. A marked degree of fluorescence of follicular structure composed of stratified squamous epithelium with moderate fluorescence of the cytoplasm was discovered by an immunofluorescence method in paraffin sections through tissue of ovarian adenocarcinoma AO-77 (the histological picture of adenocarcinoma with regions of granulosa-cell carcinoma), but part of the inner wall of the follicle was lined with arightly fluorescent cylindrical epithelium. On the whole this follicular structure had some degree of similarity with the syncytiotrophoblast [3] (Fig. 1).

The results relating to the TBG content in tumor tissue and blood serum in ovarian cancer are thus indirect confirmation of TBG biosynthesis as ovarian tumors. The following evidence has been found of the possible synthesis of TBG in ovarian tumors. First, on immunization of rabbits with extract of ovarian adenocarcinoma (glycoprotein fraction) antibodies identical with antibodies to serum TBG from retroplacental blood were obtained. Second, the TBG concentration is raised in ovarian cancer: in the tumors in 18% of cases, in the blood serum in 12.5%, and in ascites fluid in 41.6%. Third, fluorescent structures resembling the trophoblast, which normally synthesizes TBG [8], have been found by the immunofluorescence method in the tissues of an ovarian adenocarcinoma. Some investigators consider that structures of trophoblast type (cyto- and syncytiotrophoblast) in ovarian tumors are derived from normal sex cells [3]. As a result of amitotic division of their nuclei they form multinuclear sex cells which can differentiate into cells similar to the trophoblast [3]. These structures of trophoblast type may evidently be responsible for TBG biosynthesis by ovarian tumors.

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