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DETECTION OF ANTIBODIES TO CROSS-REACTING BACTERIAL ANTIGENS IN CANCER PATIENTS

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UDC 612.017.1:616-006-097

KEY WORDS: antibodies; tumor-associated glycoprotein; cancer

Microorganisms belonging to different taxa contain a wide range of antigens whose epitopes cross-react with antibodies to differential antigens of normal tissues and also with antibodies to tumor-associated antigens [4]. For instance, cross reactions have been found between BCG antigens and experimental human hepatocarcinomas and melanomas [9], and between *Streptococcus pyogenes* antigens and hamster fibrosarcomas and human basal-cell carcinomas [1, 7]. Data showing a high level of homology of products of oncogenes of the ras type with proteins of microorganisms, whose synthesis is coded by analogous sequences, are particularly interesting [10]. The most extensive studies in the field of cross reactions between microorganisms and tumors have been undertaken with the saprophytic microorganism *Bacillus megaterium* H, whose cells have been shown by immunofluorescence and immunoelectron microscopy and by other methods to contain an antigen cross-reacting with monospecific sera against various human tumors [4].

The facts described above served as a basis for the present investigation, with the aim of studying the serologic activity of serum from patients with cancerous and noncancerous diseases of the gastrointestinal tract and from animals with tumors toward *B. megaterium* H antigen, using enzyme immunoassay as the test.

EXPERIMENTAL METHOD

The test object consisted of blood serum from 217 persons, of whom 30 were healthy blood donors, 160 were patients in the Gastroenterology Department of Novosibirsk No. 18 Hospital, and 27 were patients with malignant tumors under treatment at the Novosibirsk No. 1 City General Hospital. Sera from 16 A/Sn mice, into which $1 \cdot 10^5$ ascites syngeneic B-lymphoma cells had been transplanted intraperitoneally, and sera from 6 Balb/c mice in which fibrosarcoma formation had been induced by intramuscular application of methylcholanthrene (1 mg), also were tested.

A glycoprotein with mol. wt. 65-70 kD, isolated by preparative electrophoresis from growth medium of *B. megaterium* H, homogeneous in polyacrylamide gel density gradients and cross-reacting with monospecific rabbit serum against a human gastric tumor, was used as antigen.

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TABLE 1. Probability of Occurrence of OD Interval among Groups of Patients

Interval of OD values	Healthy donors (n = 30)	Patients in Gastroentero-logy Department (n = 160)	Patients with tumors (n = 27)
0,000—0,91	0,700	0,281	0,148
0,092—0,182	0,167	0,231	0,111
0,183—0,273	0,067	0,163	0,185
0,274—0,364	0,066	0,150	0,111
0,365—0,455	0,000	0,075	0,074
0,456—0,546	0,000	0,081	0,075
0,547—0,637	0,000	0,013	0,148
0,638 and higher	0,000	0,006	0,148

Enzyme immunoassay (EIA) with the human sera was carried out on microplates (USSR, Leningrad "Medpolimer" Factory), and with mouse sera on microplates from the firm "Nunc," Denmark. Serum was obtained by centrifugation of the material at 1500 rpm for 30 min. To each well of the Soviet flat-bottomed microplates was added 100 μl of 1% glutaraldehyde in buffered physiological saline (BPS), pH 7.4, and the sample was kept for 1 h at 37°C. The liquid was expelled from the wells by shaking and was replaced by 100 μl of a solution of the antigen (tumor-associated glycoprotein — TAGA) in bicarbonate buffer, pH 9.2, in a concentration of 20 μg/ml, and the plates were kept in a refrigerator for 18 h at 4°C. They were then washed with BPS, containing 0.05% Tween 21. To prevent nonspecific sorption, 400 μl of a 1% solution of bovine serum albumin (BSA) in BPS, pH 7.4, was added to each well, and the samples were kept for 1 h at room temperature. Washing with BPS containing 0.05% Tween 21 was repeated three times. The test serum (100 μl) was then added to the wells, which were washed out six times 1 h later, after which 100 μl of antibodies against human immunoglobulins, labled with horseradish peroxidase (from the N. F. Gamaleya Research Institute of Immunology, Epidemiology, and Microbiology), in BPS, pH 7.4, containing 0.05% Tween 21 and 1% BSA, in a dilution of 1:250 or 1:1000, was added and the samples were kept for 1 h at room temperature and then again washed six times. To each well of the microplate were added 100 μl of substrate, containing 5 μg orthophenylenediamine in 50 ml of 20 mM citrate buffer, pH 4.7, and 5 μl of a 30% solution of hydrogen peroxide. With the appearance of a color after 15 min, 50 μl of a 50% solution of sulfuric acid was added to each well. The results were read photometrically on a "Titertek Multiscan MCC/340" instrument. Control measurements were made in wells in which one of the components (TAGA, serum, peroxidase conjugate) was omitted. Each determination was repeated three times. The sera were adsorbed for 2 h by incubating 0.3 ml serum with $1 \cdot 10^5$ normal or tumor cells. Before adsorption the serum was heated for 1 h at 56°C. EIA was carried out by the usual method [11], and antibodies adsorbed on the tumor cells were eluted with citrate or Tris-glycine buffer, pH 3.2. Blood for preparing the mouse sera during tumor development was obtained from the retro-orbital sinus.

EXPERIMENTAL RESULTS

Testing the human sera showed that the optical density in 29 patients with malignant tumors of the gastrointestinal tract (two additional cases were discovered among the group with noncancerous disease) in EIA averaged 0.348 ± 0.044 and differed significantly from the optical density (OD) in the healthy donor group, where it was 0.082 ± 0.014 ($p < 0.05$). Among the patients with malignant tumors the lesion was gastric (OD 0.353 ± 0.065) in 15 cases and intestinal (OD 0.385 ± 0.073) in 14 cases. As the disease progressed the values of OD rose: 0.272 ± 0.056 in 16 patients with stage III and 0.442 ± 0.064 in 13 patients with stage IV ($p < 0.05$).

Of 158 patients under treatment in the Gastroenterology Department 19 had a concomitant tumor: rectal polp in 6 patients, fibromyoma of the body of the uterus in 7, prostatic adenoma in 4, lipoma of the lumbar region in 1, and gastric polyp in 1 case. OD in this group of patients was 0.215 ± 0.035. Six patients had previously undergone surgery for benign tumors, but only two had OD below 0.091, whereas in the rest it was above 0.365.

Twenty patients had a hereditary predisposition to cancer. OD in these patients was 0.244 ± 0.034, significantly higher than in the healthy blood donors ($p < 0.05$).

TABLE 2. Values of OD and EIA and Serum Titers in IFT for Mice with Transplanted Tumors with Glycoprotein from *B. megaterium* H

Tumor	Number of sera	Time of determination of serologic activity						
		OD in EIA				titers in IFT		
		intact animals	3/45	7/68	12/80	7	12	18th day
OH-2	10	0,54±0,05	0,75±0,08	1,50±0,15	1,60±0,10	1:32	1:32	1:6
OH-3	6	0,47±0,05	0,69±0,03	1,7±0,22	1,59±0,09	1:64	1:40	1:12
Fibrosarcoma	6	0,24±0,05	0,75±0,11	0,98±0,2	1,4±0,25	1:32	1:24	1:16

Legend. Numerator — days of determination of OD of sera from mice with transplanted tumors; denominator — with fibrosarcoma.

TABLE 3. Serologic Activity of Sera of Tumor-Bearing Mice toward *B. megaterium* H Glycoprotein after Adsorption by Syngeneic and Allogeneic Lymphocytes and by Tumor Cells

Sera of mice with tumors	Values of EIA after adsoprtion of sera				
	by lymphocytes		OH-2	OH-3	fibrosarcoma
	A/Sn	Balb/c			
OH-2	1,40±0,13	1,50±0,16	0,39±0,05	0,45±0,08	0,53±0,03
OH-3	1,38±0,14	1,28±0,08	0,24±0,05	0,44±0,03	0,50±0,06
Fibrosarcoma	1,37±0,12	1,42±0,09	0,32±0,06	0,28±0,07	0,42±0,04

Table 1 gives the distribution of OD intervals among healthy donors and patients. Most values of OD in the donors fall in the 0.000-0.091 interval, whereas in the patients in the Gastroenterology Department half of all values of OD fall in intervals below 0.182, compared with only a quarter in cancer patients. Meanwhile values of OD of 0.365 and above were found in half of the patients with tumors but in one-sixth of patients in the Gastroenterology Department. On the basis of these results two groups of people were distinguished: 1) with OD values up to 0.182, which included 86.7% of the healthy subjects, and 2) with OD values of 0.183 and above, including the patients with tumors and some from the Gastroenterology Department. Patients with chronic atrophic and hyperplastic gastritis had higher values of OD, close to those for patients with tumors. Patients with duodenal ulcer, chronic cholecystopancreatitis, chronic hepatitis, cholelithiasis, and some patients with gastric ulcer also had raised or high values of OD (over 0.183). Average values of OD for groups of patients in the Gastroenterology Department were: chronic gastritis (5 patients) 0.114 ± 0.041, chronic atrophic gastritis (5) 0.196 ± 0.023, chronic hyperplastic gastritis (7) 0.217 ± 0.043, chronic gastroduodenitis (8) 0.232 ± 0.043, gastric ulcer (6) 0.188 ± 0.077, duodenal ulcer (40) 0.215 ± 0.022, chronic cholecystitis (22) 0.151 ± 0.031, cholelithiasis (5) 0.224 ± 0.031, chronic hepatitis (18) 0.238 ± 0.045, opisthorchiasis (23) 0.180 ± 0.029), and lambliasis (22) 0.188 ± 0.031. No significant differences compared with the group of healthy blood donors were observed only in patients with chronic gastritis, cholecystitis, and gastric ulcer. Since raised values of OD in EIA with TAGA were observed in chronic atrophic and hyperplastic gastritis, duodenal ulcer, and cholelithiasis, which some authorities regard as precancerous states [2, 3], the possibility cannot be ruled out that detection of antibodies to the tumor-associated antigen of *B. megaterium* H can be used as a test of identification of groups of individuals with precancerous states among patients with noncancerous diseases also.

Results similar to those described above were obtained in an investigation of sera of animals with transplanted or induced tumors. OD of the sera of such animals with TAGA was significantly higher than OD of sera of intact animals (Table 2) and correlated with the rate of tumor growth. For instance, in A/Sn and Balb/c mice the mean value of OD with TAGA was significantly higher by the 3rd day after transplantation than OD of the intact animals. it reached a maximum on the 7th-12th day, and then fell a little in the terminal stages of tumor development (18th day). Lowering of serologic activity at these times was evidently caused by an increase in the rate of release of tumor antigens into the circulation and by their neutralizing antitumor antibodies. This is shown by the results of a parallel immunofluorescence test (IFT). It will be clear from Table 2 that the titers of autologous sera in the reaction with tumor membrane antigens also were depressed at these same times. Increased serologic activity of sera also could be observed on a model of methylcholanthrene sarcoma, induced in Balb/c mice. OD of sera of the mice differed significantly as early as on the 45th day.

To elucidate the causes of appearance of antibodies reacting with the microbial antigen in neoplastic disease, the antigenic determinant inducing their formation must be found. There is no compound so far discovered that is characteristic of tumor tissue alone, for all tumor antibodies or markers also exist in the normal body tissues, and their appearance in the tumor cell is the result of extra expression of the tumor genome [5]. Our discovery that the microbial antigen can cross-react with the sera of humans and animals with tumors arising from different cells and tissues does not contradict modern views on the immunology of the tumor cell, for there is the possibility of expression of cryptoantigens, characteristic of the normal cell, during malignant growth.

Evidence in support of this possibility is given by the discovery, with the aid of monoclonal antibodies, of carbohydrate epitopes of glycoproteins and glycolipids found in many types of cells, and expressed on them during embryogenesis, differentiation, and oncogenesis [8]. The serologic activity of the sera of humans and animals with tumors toward the TAGA of *B. megaterium* H may perhaps be connected with the presence of carbohydrate sequences in its composition, expressed during oncogenesis and functioning as oncofetal antigens. The study of the serologic activity of mouse sera after their adsorption by syngeneic and allogeneic lymphocytes and tumor cells (Table 3) showed that neutralization of activity was observed only when tumor cells were used; moreover, they possessed neutralizing activity not only in syngeneic, but also in allogeneic systems.

Similar results also were obtained in tests of the serologic specificity of antibodies eluted from membranes of autologous tumor cells, when they reacted with the bacterial antigen; activity of the eluted antitumor antibodies, moreover, was similar to activity of monoclonal antibodies to the cross-reacting antigen of *B. megaterium* H. [6].

The results of this investigation thus indicate that the glycoprotein isolated from *B. megaterium* H is a promising antigen for tumor immunology and it can be used for immunologic monitoring of the tumor process.

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