ANTIGENS COMMON TO HUMAN MALIGNANT TUMORS AND CERTAIN SPECIES OF MICROORGANISMS

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Investigations have shown that the somatic cells of man and animals contain antigens common with certain species of microorganisms [2, 3, 5]. This interesting fact was noted by Zhukov-Verezhnikov and co-workers as long ago as in 1944 [1]. However, many aspects of this problem are still inadequately studied. For example, the character of the species and tissue immunologic specificity of these common antigens, their immunologic properties, and so on, have not been described. Such investigations are important both theoretically and practically, especiallyin connection with the study of the possible prophylactic action of, for example, mycobacteria and mycobacterial preparations against human malignant tumors.

In the investigation described below immunologic tests were used to determine the presence of antigens common to human malignant tumors in various situations and microorganisms of several species.

EXPERIMENTAL METHODS

Lyophilized BCG vaccine, the standard preparation produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, and dried living *Listeria monocytogenes* vaccine, strain AUF, manufactured by Stavropol' Bioproducts Factory, and also human malignant tumors from various situations, normal organs, and erythrocytes of blood groups I(O) and II(A) were used. These objects were studied in the agglutination test, Ouchterlony's gel diffusion test, and by immunoelectrophoresis. Sera of chinchilla rabbits immunized with whole microorganisms of the above-mentioned species, and also with saline extracts prepared from the tissues of a human melanoma of the skin and breast carcinoma, were used as the sources of antibodies. The rabbits were immunized by the scheme mentioned in [3]. To study the immunologic identity of antigens common to malignant tumors and BCG microorganisms, a rat ovarian carcinoma (strain OYa, described by Pogosyants et al. [4]) and immune serum against antigens of that tumor also were used in the experiments. Both native immune sera and the same sera concentrated by MacErlean's method [6] were investigated.

Antigens common to microorganisms and cells isolated from the tissues of a sarcoma of the thigh, carconoma of the ovary, and kidney, liver, and erythrocytes of healthy subjects also were studied. The test antigens for the gel diffusion test were saline extracts prepared from microorganisms, human malignant tumors (melanoma, carcinomas of the breast, stomach, and ovary, sarcoma of the soft tissues of the thigh and arm, carcinoma of the large intestine, liposarcoma, carcinoma of the skin), fibroadenoma of the breast, and normal human organs — liver, kidney, stomach, thigh muscles, spleen, frozen and thawed 5 times. To study the immunologic specificity of the common antigens, immune sera against BCG were absorbed with human erythrocytes and kidney cells in the ratios of 1:1 and 1:2 for 60 min at 37°C and for the next 24 h at 4°C. The results of the tests were read macroscopically and also under the low power of the microscope. For the gel diffusion test, protein in the antigens was determined by Lowry's method. The results of the gel diffusion test and of immunoelectrophoresis were read after 24-72 h. The human malignant tumors were obtained from the Mos-

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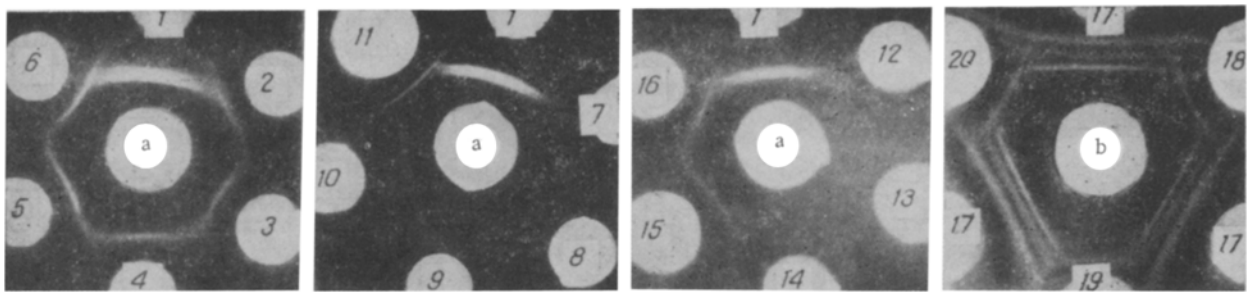

Fig. 1. Gel diffusion test on fraction IV of concentrated antisera against BCG and *L. monocytogenes* with homologous and tissue human and rat antigens. 1) BCG; 2-6) different specimens of human breast carcinoma, Nos. 4452, 1304, 2665, 8336, and 1709 respectively; 7) liver; 8) kidney; 9) muscle; 10) spleen; 11) melanoma; 12) fibroadenoma of the breast; 13) carcinoma of the breast No. 15510; 14) liposarcoma; 15) ascites carcinoma of the rat ovary, strain OYa; 16) papillary adenocarcinoma of the human ovary; 17) *L. monocytogenes*; 18) carcinoma of breast No. 2665; 19) adenoid basal-cell carcinoma of skin; 20) carcinoma of breast No. 1709. a) Serum against BCG; b) serum against *L. monocytogenes*.

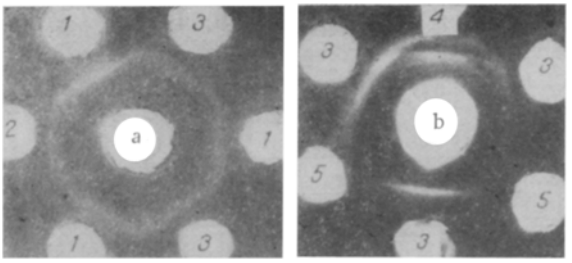

Fig. 2. Gel diffusion test with antisera against antigens of human and rat malignant tumors and antigens from homologous tissues and BCG and *L. monocytogenes* microorganisms. a) Serum against antigens of melanoma of the human skin; b) serum against antigens of ascites carcinoma of the rat ovary, strain OYa. 1) Melanoma of human skin; 2) physiological saline; 3) BCG; 4) ascites carcinoma of rat ovary, strain OYa; 5) *L. monocytogenes*.

TABLE 1. Results of Gel Diffusion Test on Sera of Rabbits Immunized with BCG and *Listeria monocytogenes* with Antigens of Human Malignant Tumors

Human tumors	No. of specimens of tumors tested	No. of specimens of tumors reacting with immune sera	
		against BCG	against L. monocytogenes
Melanoma of skin	7	5	4
Carcinoma of breast	15	10	6
Sarcoma of soft tissues	7	6	3
Carcinoma of stomach	5	3	2
Carcinoma of ovary	2	2	1
Carcinoma of intestine	1	1	0
Liposarcoma	1	0	0
Adenoid basal-cell carcinoma of skin	1	1	1

cow City Oncologic Dispensary after they had been investigated histologically, and from the Joint Laboratory of Morbid Anatomy and Cytology (Head, Candidate of Medical Sciences N. M. Otsep).

EXPERIMENTAL RESULTS

As the writers showed previously [3], normal rabbit sera can agglutinate both rat's erythrocytes and malignant cells obtained from a sarcoma from the thigh induced by dimethylbenzanthracene in animals of the same species, in low dilutions. It was accordingly decided to verify the presence of antibodies of this type against human erythrocytes of the above-mentioned group and against cells isolated from sarcomas of the soft tissues of the human thigh in normal rabbit sera.

Investigations in this direction, using sera from 10 intact rabbits, showed that six samples of these animals agglutinated malignant cells in dilutions of 1:2-1:16. The remaining sera did not agglutinate malignant cells. The same sera in every case agglutinated group II(A) erythrocytes in dilutions of 1:4-1:8. These sera contained hemolysins against group I(0) erythrocytes in titers of 1:2-1:8. Instead of hemolysis, sera heated to 60°C for 30 min caused agglutination of these erythrocytes, also in low dilutions. Thus the sera of intact rabbits contain (but not in every case) natural antibodies capable of reacting with antigens both of human erythrocytes and of human malignant cells in low dilutions.

The main experiments showed that sera of rabbits immunized with BCG vaccine (Nos. 96 and 163) agglutinated cells isolated from three specimens of sarcoma of the soft tissues of the thigh and arm, a Krukenberg tumor, and carcinoma of the human ovary in comparatively high titers, namely 1:128-1:512. The same sera agglutinated liver cells in dilutions of 1:2-1:4, kidney cells in dilutions of 1:16-1:32, and erythrocytes in a dilution of 1:16. Agglutination of sarcoma of the thigh and carcinoma of the ovary cells by the serum of a rabbit immunized with *L. monocytogenes* was observed in comparatively high dilutions. After absorption of the serum of a rabbit immunized against BCG (No. 96) or against group A erythrocytes a positive reaction was observed only with the cells of the above-mentioned malignant tumors and not with erythrocytes. Cells of malignant tumors were agglutinated by the serum in dilutions of 1:64-1:128. A similar picture was observed also during absorption of the serum of a rabbit immunized against *L. monocytogenes* (No. 82) with group II(A) erythrocytes. Sera of rabbits immune to BCG, after absorption by kidney cells, did not agglutinate these cells or sarcoma of the soft tissues of the thigh in dilutions of 1:32-1:64. Absorbed sera immune to microorganisms thus did not react in the agglutination test with erythrocytes, nor with liver and kidney cells, but they continued to agglutinate cells isolated from the human malignant tumors that were tested. This is evidence of the specificity of the common antigens present in the microorganisms of BCG and *L. monocytogenes* and in human malignant tumor cells.

The results of investigation of antigens common to human malignant tumors and microorganisms of BCG and *L. monocytogenes* by the gel diffusion test are summarized in Table 1. The results show that an antigen common with antigens of the above-mentioned microorganisms was present in most specimens of melanoma of the skin and carcinoma of the breast, stomach, and ovaries. Sera immune against the microorganisms formed no precipitation lines with extracts from normal organs and tissues of the human liver, kidney, spleen, stomach, or thigh muscles.

Antigens common to carcinoma of the human and rat ovary, carcinoma of the breast, and skin melanoma and of the microorganisms studied in these experiments were found to exhibit either partial (Fig. 1) or complete immunologic identity (Fig. 2). Complete immunologic identity was found between the common antigens contained in breast carcinomas from different patients (Fig. 1). A similar picture also was observed in relation to common antigens of carcinoma of the rat ovary and carcinoma of the human ovary, i.e., tumors of the same organ but of different species (Fig. 1). Blood sera of rabbits taken before the animals were immunized with antigens of microorganisms or of human and animal malignant tumors formed no precipitation lines with these antigens in a single case.

The immunoelectrophoretic test showed that the antigen common with microorganisms of BCG and carcinoma of the human stomach has electrophoretic mobility in the β-globulin zone. As the writers observed previously [3], an antigen common for BCG microorganisms and cells of a primary induced sarcoma of the rat thigh muscle also has electrophoretic mobility of the β-globulin zone.

It was thus shown by three immunologic reactions that BCG microorganisms and *L. monocytogenes* cells have a common antigen with human malignant tumors, characterized by either partial or complete immunologic identity. Complete immunologic identity was found between antigens of different specimens of tumors from the same organ (carcinoma of the breast, for example) or from similar tumors taken from different species (carcinoma of the human and rat ovaries, for example).

These data can be used to study how malignant cells are affected by immune sera specific not against the antigens of those cells, but against antigens which they contain in common with certain strains of microorganisms.

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INDEPENDENCE OF ALPHA-FETOPROTEIN EXPRESSION OF SERUM PROTEIN PRODUCTION IN ADULT RATS WITH HEPATOMA McA-RH7777

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Many hepatocellular carcinomas synthesize the embryo-specific serum protein alpha-fetoprotein (AFP) together with other blood serum proteins [1]. However, some hepatomas both in man and in animals do not produce AFP [1]. Moreover, even in those hepatomas which do produce AFP this protein is not synthesized by all cells [5]. Isaka et al. [7] showed that ascites rat hepatoma is genetically heterogeneous for this trait, and that clones differing by as much as 50 times in their level of AFP production can be isolated from it.

This paper describes an attempt to determine whether AFP expression in a hepatoma is connected with the production of other serum proteins or whether it is regulated discretely and is independent of the synthesis of proteins of the "adult" type.

EXPERIMENTAL METHODS

A culture of rat hepatoma McA-RH7777 cells was generously provided by Dr. Becker and Professor Van Potter (McArdle Laboratories on Cancer Research, Wisconsin, USA) under the terms of the Soviet-American agreement on collaboration on tumor immunology. The cells were cultured in L15 medium (Flow) with 10% embryonic calf serum (Gibco, USA).

Cloning was carried out in methylcellulose M-281 (Fisher, USA). The final methylcellulose concentration in the mixture was 1% and of embryonic calf serum 30%. In each tube 10,000 cells were cultured in 2 ml of the methylcellulose solution. Clones were isolated on the 10th-14th day. The clones grown in methylcellulose were separated under a "Diawerth" microscope (from Leitz, West Germany). The clones were then cultured in 96-well microplates (from Falcon Plastics, USA).

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