ANTIGENS COMMON TO Mycobacterium bovis (BCG) AND SOME MALIGNANT AND NORMAL TISSUES OF LABORATORY ANIMALS

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It was shown by agglutination, gel diffusion, and immunoelectrophoretic tests that an antigen common to Mycobacterium bovis (BCG) and some malignant and normal cells of rat tissues exists. By absorption of immune sera and the agglutination test the specificity of this antigen was demonstrated for BCG cells and cells of primary induced tumors of rat muscle tissue. This antigen has electrophoretic mobility in the β-globulin zone.

KEY WORDS: BCG vaccine; primary induced tumors; heterogeneic antigens; heterophilic antibodies.

Several workers have demonstrated the prophylactic and therapeutic action of mycobacteria and mycobacterial preparations against various malignant tumors in experimental animals and man [6, 7, 10, 12, 13]. The mechanism of this action has been explained as a nonspecific increase in the immunological reactivity of the body [9, 11]. However, according to data in the literature, the antineoplastic effect of these microorganisms could be for a different reason, namely, that they contain antigens common with the antigens of malignant tumors, and these stimulate crossed immunological reactions [4, 8]. Zhukov-Verezhnikov and co-workers first demonstrated the presence of such antigens in some species of bacteria and normal and malignant human cells [1-3]. However, it has to be pointed out that the immunotherapy of cancer patients with BCG vaccine in some cases has been accompanied by side effects, namely fever, disturbances of liver function, granulomatous hepatitis, or "BCG-itis." It is very probable that, for example, the development of hepatitis is due to the presence of antigens common both to the mycobacteria and to certain normal organ tissues, which induce the corresponding autoimmune disease.

The object of this investigation was to use immunological tests to study whether antigens common to Mycobacterium bovis (BCG) and certain malignant and normal tissues of laboratory animals could be found.

EXPERIMENTAL METHOD

A standard production batch of BCG vaccine from the N. F. Gamaleya Institute of Epidemiology and Microbiology (lyophilized and containing whole microorganisms), and various samples of tumors induced in the thigh muscles of Wistar rats by 9,10-dimethyl-1,2-benzanthracene, taken at the sixth through eighth month of carcinogenesis, and a spontaneous mammary gland tumor (fibroadenoma) of a rat of the same breed were used. These objects were studied in the agglutination test, Ouchterlony's gel-diffusion test, and by immunoelectrophoresis. Rabbits immunized intraperitoneally and intramuscularly with whole BCG microorganisms and with cells of primary induced tumors served as the source of antibodies. The animals were immunized five times at intervals of 10 days, and the immunization cycle was then repeated. Sera were taken 10 days after immunization. Native immune sera and the same sera concentrated by MacErlean's method [5] were used as fractions IV and V. The tests were set up with whole erythrocytes and cells isolated from tissues of malignant tumors and of normal liver, kidney, and spleen, and also saline extracts prepared from BCG cells,

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TABLE 1. Agglutination of Cells of Primary Induced Tumor and Erythrocytes of Wistar Rats by Normal Rabbit Sera

Dilution of sera	Rabbit No. 42		63		70		88		90		195		200		17		19		21		22		23	
	n	h	n	h	n	h	n	h	n	h	n	h	n	h	n	h	n	h	n	h	n	h	n	h
	Tumor cells																							
1:2	—	—	—	—	—	—	+	—	—	—	+	—	2+	—	2+	4+	2—	4+	3—	3+	2—	2+	—	—
1:4	—	—	—	—	—	—	3+	—	3+	—	3+	—	3+	—	4+	4+	3+	4+	4+	2+	2+	2+	3—	—
1:8	—	—	—	—	—	—	3+	—	3+	—	3+	—	3+	—	4+	3+	3+	4+	4+	2+	2+	2+	3—	—
1:16	—	—	—	—	—	—	3+	—	2+	—	2+	—	+	—	4+	3+	3+	4+	4+	2+	+	+	2+	—
1:32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:64	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Erythrocytes																							
1:2	3+	—	2+	—	+	+	2+	—	2+	—	3+	—	3+	—	+	—	—	—	—	—	—	—	—	—
1:4	2+	—	+	—	—	—	2+	—	2+	—	2+	—	—	—	—	—	—	—	—	—	—	—	—	—
1:8	—	—	—	—	—	—	+	—	+	—	2+	—	—	—	—	—	—	—	—	—	—	—	—	—
1:16	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
1:32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Legend. Here and in Table 2: agglutination carried out at 37°C for 60 min and at 4°C for 24 h. n) Native sera, h) the same sera heated to 60°C for 30 min; 4+) one large agglutinated mass, 3+) several fairly large agglutinated masses, 2+) moderate agglutination, +) weak agglutination, —) no agglutination.

TABLE 2. Agglutination of Rat Tissue Cells by Immune Serum of Rabbit No. 63 against BCG

Dilution of sera	Native serum			Serum absorbed by erythrocytes			Serum absorbed by spleen cells		
	cells								
	tumor	spleen	erythrocytes	tumor	spleen	erythrocytes	tumor	spleen	erythrocytes
1:2	4+	3+	3—	4—	3+	—	2+	—	—
1:4	4+	3—	2+	4—	2—	—	3+	—	—
1:8	4+	2+	—	4—	2+	—	3+	—	—
1:16	3+	—	—	3—	—	—	2+	—	—
1:32	+	—	—	—	—	—	—	—	—
1:64	—	—	—	—	—	—	—	—	—

malignant tumors, and normal tissues. Protein in the extracts was determined by Lowry's method on a spectrophotometer at 750 nm. To determine the specificity of the common antigens thus revealed, the immune sera were absorbed with liver and spleen cells and erythrocytes in the ratio of 1:1 and 1:2 for 60 min at 37°C and for 24 h at 4°C.

EXPERIMENTAL RESULTS

The sera of normal animals are known to contain heterophilic antierythrocytic and antimicrobial antibodies. With this fact in mind, it was decided to test for the presence of heterophilic antibodies in normal rabbit sera before carrying out the main experiment. The results of this preliminary experiment, using the agglutination test in the different forms indicated above, are given in Table 1.

These results show that the sera of normal rabbits reacted differently with erythrocytes and cells of malignant tumors. For example, the sera of rabbits Nos. 17, 19, 21, and 22 caused agglutination of malignant cells in dilutions of 1:16-1:32 but did not react with rat erythrocytes. Antibodies from normal rabbit sera which reacted with malignant tumor cells were also found to be more thermostable than heterophilic antibodies reacting with erythrocytes.

During the main experiment it was found that the sera of rabbits Nos. 42 and 63, immunized with BCG, agglutinated the cells of all six samples of primary induced tumors used in the test in a dilution of 1:8-1:64.

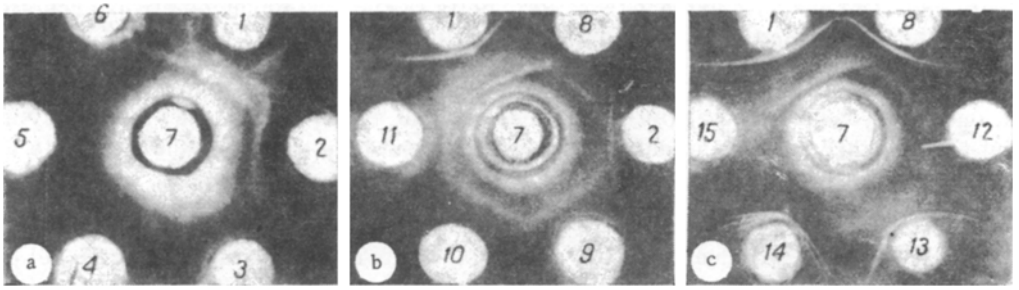

Fig. 1. Gel-diffusion test with fraction IV of concentrated immune serum against BCG cells and antigens from BCG cells and rat tissues. 1) BCG; 2) induced tumor of Nos. 13/I, 21/IV, 27/IV, 4/V, and 24/V, respectively; 7) fraction IV of concentrated immune serum No. 42; 8) liver; 9, 10) induced tumor No. 5/II, 8/V; 11) kidney; 12) muscle of rat A; 13) muscle of rat B; 14) muscle of rat C; 15) muscle of rat D.

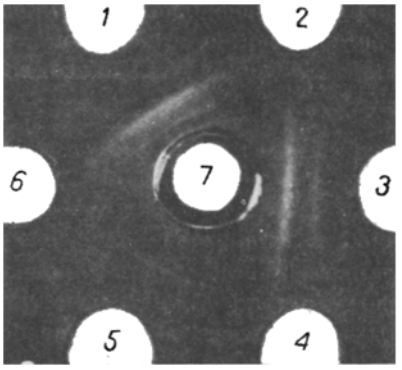

Fig. 2

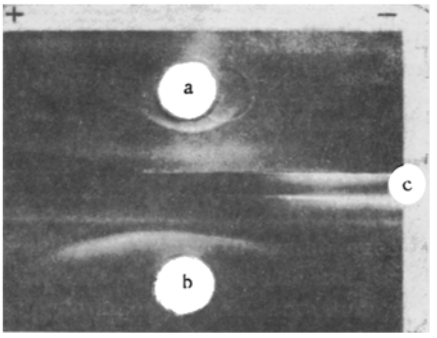

Fig. 3

Fig. 2. Gel diffusion test with fraction IV of concentrated immune serum against antigens of malignant tumor (7) and antigens from homologous tissue and from BCG cells. 1) Induced tumor of rat No. 13/I; 2, 4, 6) physiological saline; 3) spontaneous fibroadenoma of rat mammary gland; 5) antigens from BCG cells.

Fig. 3. Immunoelectrophoresis of fraction IV of concentrated immune serum against BCG and antigens from BCG cells and from tissue of induced rat tumor: a) antigen from tissue of induced tumor No. 5/II; b) antigen from BCG cells; c) fraction IV of concentrated serum in gutter.

The same sera caused agglutination of rat erythrocytes in a dilution of 1:4-1:8 and of spleen cells in a dilution of 1:8-1:16. Kidney and liver cells were not agglutinated by these sera.

Different results were obtained by absorption of immune sera of rabbits Nos. 42 and 63 by rat erythrocytes and spleen cells. As Table 2 shows, after absorption of the immune sera by erythrocytes a positive agglutination reaction was observed with cells of the malignant tumor and spleen. After absorption of the same sera by spleen cells a positive reaction was obtained only with malignant tumor cells.

For the gel diffusion test and immunoelectrophoresis fractions IV and V of sera from rabbits Nos. 42 and 63, immunized with BCG microorganisms, and of rabbits Nos. 2 and 34, immunized with whole malignant cells, were used. The results of the gel-diffusion test in one of these experiments are shown in Fig. 1. In this case fraction IV of the immune serum of rabbit No. 42 gave three precipitation bands with extract of BCG microorganisms and one or two nonidentical bands with extracts from tissues of primary induced tumors of rats Nos. 13/I and 5/II. This serum formed precipitation bands also with extracts from liver tissue and thigh muscle. However, extracts from tissues of primary induced tumors of rats Nos. 21/IV, 27/IV, 4/V, and 24/V gave negative results with this serum. The same serum did not react likewise with several samples of extracts prepared from normal muscle and liver tissues. The serum of immune rabbit No. 2/IV gave three precipitation bands with antigens from extracts of the primary induced tumor and spontaneous mammary gland

fibroadenoma of rats and one band with extract from BCG cells (Fig. 2). Immunoelectrophoresis showed that the antigen common to BCG cells and the primary induced rat tumor is located in the β-globulin zone (Fig. 3).

It can thus be concluded from these observations that normal rabbit sera contain two types of heterophilic antibodies: one type reacts in the agglutination test with rat erythrocytes, the other with malignant tumor cells but not with erythrocytes. The antibodies of the second type are more thermostable than those of the first type.

In the agglutination test the sera of rabbits immunized with BCG cells reacted in the agglutination test with all samples of cells of primary induced tumors used in the experiments, whereas in the gel diffusion test these sera gave precipitation bands only with some samples of extracts prepared from the tissues of primary induced tumors.

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