disposition to cancer. Type C particles were found in the cells of affected organs. These diseases affect mainly the lymphoid tissue of animals and they run a chronic course, in aleukemic and subleukemic forms.

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PRODUCTION OF A FACTOR INHIBITING MACROPHAGE MIGRATION AND GROWTH OF MELANOMA B16 UNDER THE INFLUENCE OF BCG

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KEY WORDS: melanoma B16; BCG; macrophage migration inhibiting factor.

Growth of malignant tumors has now been shown to be accompanied by immunologic changes and a disturbance of individual stages of immunopoiesis [5, 15]. BCG vaccine is widely used in clinical and experimental medicine as a nonspecific immunologic stimulator [8, 9, 12]. BCG is known to activate macrophage function and to stimulate the production of mediators of cellular immunity by lymphocytes [10]. Meanwhile the character of the effect of BCG on growth of melanoma B16 has not yet been explained. Contradictory data are to be found in the literature on this subject [4, 7, 11, 14].

The object of this investigation was to study the action of BCG on growth of melanoma B16 and on the production of macrophage migration inhibiting factor (MMIF) in C57BL/6 mice.

EXPERIMENTAL METHOD

Cells of a melanoma B16 were transplanted subcutaneously into C67BL/6 mice in a dose of 2×10^6. Two series of experiments were then undertaken.

In series I BCG was injected subcutaneously or intraperitoneally into the animals of one group in a dose of 1 mg simultaneously with melanoma cells. The mice of the other group received BCG subcutaneously in the same dose (on the side opposite to that of injection of the tumor) or directly into the tumor 14 days after transplantation of the melanoma cells. Growth of the tumor was determined 12 days after BCG immunization by counting the number of cells, and production of MMIF by the spleen cells was estimated in the direct capillary tube test [2] with modifications [1]. Phytohemagglutinin (PHA) and tuberculin (TB), in nontoxic doses for cells (2 and 100 μg/ml respectively) were used as antigen. The quantitative index of MMIF production was the migration index (MI), determined by the equation:

$$MI = \frac{\text{area of migration with antigen}}{\text{area of migration without antigen}} \cdot 100\%$$

In the experiments of series II, simultaneously with injection of the melanoma cells, 2×10^7 spleen cells were transplanted intravenously from syngeneic donors. The donors of

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TABLE 1. Growth of Melanoma B16 and Values of MI after Simultaneous Injection of BCG and of Tumor in C57BL Mice (M ± m)

Mode of injection of BCG	Number of cells in tumor, $\cdot 10^6$	MI %	
		to PHA	to TB
Intraperitoneally	175,0±20,4*	95,8±3,4*	128±5,2
Subcutaneously	144,7±15,6*	33,4±2,2*	108,0±3,2
Control	57,3±4,2	53,8±6,8	117,1±11,6

Legend. Here and in Table 2 asterisk indicates that differences are significant at $P < 0.05$.

TABLE 2. Growth of Melanoma B16 and Values of MI when BCG was Injected 14 Days after Transplantation of Tumor (M ± m)

Mode of injection of BCG	Number of cells in tumor, $\cdot 10^6$	MI %	
		to PHA	to TB
Subcutaneously	379,2±52,4*	55,1±3,9	110,2±4,0
Into tumor	325,0±70,7	86,8±6,7	122,5±6,8
Control	249,7±42,4	64,5±6,0	120,4±7,2

the spleen cells were: intact animals; mice with melanoma B16 (on the 3rd or 11th days after transplantation of the tumor); mice immunized with BCG in a dose of 1 mg intraperitoneally three days before transplantation. Animals with melanoma B16 served as the control. The mice were killed 15 days after transplantation of the tumor, the number of cells in the tumor was counted, and MMIF production by spleen cells in response to PHA and TB was estimated.

EXPERIMENTAL RESULTS

The results of the study of growth of the tumor and MMIF production in response to TB after injection of 1 mg BCG intraperitoneally or subcutaneously, simultaneously with transplantation of the melanoma, are given in Table 1.

Injection of 1 mg BCG led to activation of growth of the melanoma B16. Intraperitoneal injection of BCG led to complete cessation of MMIF production of PHA. After subcutaneous injection of BCG growth of the tumor was stimulated by a lesser degree than after intraperitoneal injection, and hyperproduction of MMIF in response to PHA was noted (MI = 33.4 ± 2.2%). Lymphokine production in response to the specific TB antigen was absent in all groups.

A similar effect was observed when 1 mg BCG was injected 14 days after transplantation of the tumor (Table 2). When BCG was injected into the tumor, growth of the melanoma was not significantly changed, whereas MMIF production was slightly reduced compared with the control ($P < 0.05$). Subcutaneous injection of BCG 14 days after transplantation of the melanoma led to an increase of about 1.5 times in the rate of growth of the tumor.

To study the mechanism of the stimulating action of BCG on growth of melanoma B16 experiments were carried out with transplantation of syngeneic spleen cells and simultaneous transplantation of the tumor. The results are given in Fig. 1. It was shown that transplantation of 2×10^7 spleen cells from intact mice does not affect growth of the tumor or MMIF production. A similar effect was observed when spleen cells obtained on the third day after injection of the melanoma cells were transplanted. Marked inhibition of tumor growth was produced by transplantation of spleen cells from mice with melanoma on the 11th day after inoculation of the tumor. MMIF production by the lymphocytes under these circumstances did not differ significantly from the control. It is important to note that marked stimulation of tumor growth was observed when cells were transplanted from mice immunized with BCG. Complete cessation of MMIF production to PHA was observed in this group of animals (MI = 97.3 ± 10.4%.

The data on cell transplantation suggests that after injection of BCG in high doses, cells suppressing production of lymphokines and, in particular, of MMIF, accumulate in the

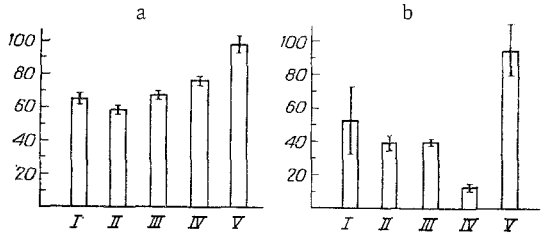

Fig. 1. Effect of transplantation of syngeneic spleen cells on MMIF production (A) and growth of melanoma B16 (B) in C57BL/6 mice. Ordinate: A) MI (in %) b) number of cells in tumor ($\times 10^6$). I) Mice with melanoma B16 without transplantation of spleen cells; II) transplantation of spleen cells of mice immunized with BCG in a dose of 0.5 mg intraperitoneally 3 days before experiment; III) transplantation of spleen cells of mice with tumors (on 3rd day after injection of melanoma B16 cells into donors); IV) transplantation of spleen cells of mice with tumors (on 11th day after injection of melanoma B16 cells into donors); V) transplantation of spleen cells of mice immunized with BCG in a dose of 1 mg intraperitoneally 3 days before experiment.

spleen, and this leads to more rapid growth of the tumor, as is confirmed by data in the literature [3, 6, 13].

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