EFFECT OF COMBINED ADMINISTRATION OF BCG AND ANTITHYMOCYTIC SERUM ON SYNGENEIC TUMOR GROWTH IN MICE

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UDC 616.345-006.66-02:615.371:579.873.21]-092.9

KEY WORDS: BCG; antithymocytic serum; natural killer cells; activated macrophages; weakly immunogenic tumor.

Numerous investigations in recent years on different models and on animals of different species and man have shown that tumor growth is accompanied by the formation of suppressor T lymphocytes [3, 5]. T suppressors inhibit nonspecific and specific immunoreactivity of the host [2, 7] and so facilitate tumor growth. However, this situation is often ignored during the immunotherapy of cancer patients. Moreover, many of the preparations used for immunostimulation increase the antitumor reactivity of the recipient, on the one hand, but stimulate suppressor formation, on the other hand, i.e., they raise the level of dynamic balance of host—tumor interaction, so that the final result is difficult to predict. In particular, BCG, which considerably increases the activity of antitumor defense factors (macrophages, natural killer cells) [9], in some cases potentiates tumor growth as a result of stimulation of T suppressor formation [8]. It has thus become an urgent problem to seek a way of acting on the immune system of the host which would enhance immunoreactivity but, at the same time, would not stimulate (or would even inhibit) the action of suppressor cells.

The object of this investigation was to study the effect of combined administration of BCG and antithymocytic serum (ATS) on growth of syngeneic tumors in mice and also to identify the cell population producing the antitumor effect in such a system.

EXPERIMENTAL METHOD

Experiments were carried out in a syngeneic system on male BALB/c mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. An "Akatol" tumor (adenocarcinoma of the large intestine of BALB/c mice) was obtained from the Laboratory of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. A suspension of tumor cells (TC) was prepared by means of a mechanical mincer and filtered through three layers of gauze; the percentage of living cells was determined by staining with trypan blue. The TC were injected subcutaneously into mice in the dorsal region. Rabbit ATS was obtained by immunizing rabbits five times with brain tissue from CBA mice [4]. The serum was inactivated by heating for 30 min at 56°C, and adsorbed with liver cells, erythrocytes, serum, and bone marrow cells from CBA and BALB/c mice until complete disappearance of activity in the hemagglutination test with mouse erythrocytes and the gel-precipitation test with serum from intact mice. Exhaustion of activity against B lymphocytes was verified by investigation of the serum for inhibition of antibody-forming cells in Jerne's test with sheep's erythrocytes. Normal rabbit serum (NRS) was obtained from the same rabbits before the beginning of immunization and treated in the same way as ATS. The BCG preparation, in the form of a suspension containing 600-900 million living bacteria in 1 ml, was obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. At each injection the mouse received 0.1 ml of suspension subcutaneously in the region of injection of TC. A suspension of spleen cells (SC) was prepared in a glass homogenizer of Potter type in medium 199, filtered through two layers of gauze, and washed three times by centrifugation for 10 min at 1000 rpm. All manipulations with the cells were carried out under sterile conditions in an ice bath. The SC suspension was freed from T lymphocytes by incubation of 25×10^6 SC in a volume of 2.5 ml of medium 199 with 5 ml ATS

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 2, pp. 68-70, February, 1983. Original article submitted July 9, 1982.

0007-4888/83/9502-0247$07.50

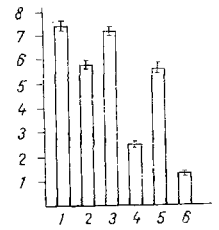

Fig. 1

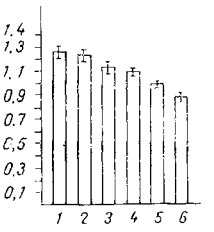

Fig. 2

Fig. 1. Effect of combined administration of ATS and BCG on growth of syngeneic Akatol tumor in mice. 1) Control (growth of tumor alone). Mice treated with: 2) BCG, 3) NRS, 4) ATS, 5) BCG + NRS, 6) BCG + ATS. Here and in Figs. 2 and 3: ordinate, weight of tumor (in g).

Fig. 2. Effect of adoptive transfer of SC from mice treated with ATS and BCG on growth of Akatol tumor in syngeneic mice. 1) Control (growth of tumor alone). Mice inoculated with: 2) intact SC_{int}; 3) SC_{tum}; 4) SC_{tum} BCG; 5) SC_{tum} ATS; 6) SC_{tum} (ATS + BCG). Explanation in text.

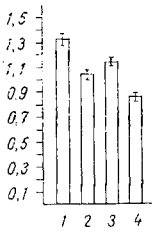

Fig. 3. Determination of type of cells giving antitumor effect during adoptive transfer of SC from mice treated with ATS + BCG. 1) Control (growth of tumor alone). Mice inoculated with: 2) SC_{tum} (ATS + BCG); 3) SC_{tum} (ATS + BCG) from which PC had been removed; 4) SC_{tum} (ATS + BCG) treated *in vitro* with ATS and complement.

in a dilution of 1 : 10 for 30 min at room temperature, after which 2.5 ml of guinea pig complement was added and the mixture was incubated for 40 min at 37°C. The remaining cells were washed by centrifugation three times. Phagocytic cells (PC) were removed with iron carbonyl [10] (particle size 1 × 1 × 1.5 μ, from the All-Union Research Institute of Ferrous Metallurgy). The cells (40 million in a volume of 5 ml) were treated with 400 mg of the sterile iron powder and incubated for 1 h at 37°C. The PC were removed by means of a magnet and the remaining cells were washed twice. The efficiency of this procedure was verified by staining for nonspecific esterase [6]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of combined administration of ATS and BCG on tumor growth was compared with the action of BCG, ATS, and NRS alone or of a combination of BCG with NRS, and also with growth of the tumor in mice receiving the TC only. TC were injected subcutaneously in a dose of 10^7 cells, BCG was given as two injections on the 1st and 10th days of the experiment subcutaneously into the region of injection of TC, and the ATS or NRS was injected into the retro-orbital sinus in a dose of 0.2 ml on alternate days from the 3rd through the 15th days of the experiment. Each group consisted of 10 mice. On the 20th day the mice were killed and the weight of the tumor determined. The results of three experiments are shown in Fig. 1.

The strongest inhibition of tumor growth compared with the control was observed with combined administration of ATS and BCG ($P < 0.001$). BCG vaccine ($P < 0.001$) or ATS ($P < 0.001$) separately also inhibited tumor growth, but the weight of the tumor in the animals of these groups was greater than in mice treated with ATS and BCG ($P < 0.001$). NRS did not affect tumor growth itself and did not potentiate the action of BCG.

In the experiments of series II the effect of syngeneic SC from mice with tumors, treated with both ATS and BCG [SC_{tum} (ATS + BCG)] on tumor growth was compared with the effect of SC from intact mice (SC_{int}), from mice with tumors (SC_{tum}), or SC from mice treated with ATS alone (SC_{tum} ATS) or with BCG alone (SC_{tum} BCG). On the same day the recipients (BALB/c mice) received 3×10^6 TC subcutaneously and 5×10^7 SC from the donors into the caudal vein. Each group consisted of 10 mice. The animals were killed on the 15th day of the experiment. The results of these experiments are given in Fig. 2.

The most marked delay of tumor growth was observed in mice receiving SC from animals with tumors treated with a combination of ATS and BCG ($P < 0.001$). To determine the type of cells responsible for this effect, a suspension of SC of treated donors, before injection into recipients, was treated with ATS and complement or with iron carbonyl and the magnet. Treatment of SC_{tum} (ATS + BCG) with iron carbonyl reduced their inhibitory effect only slightly compared with untreated cells ($P > 0.05$), whereas treatment of SC with ATS and complement actually potentiated this effect ($P < 0.001$; Fig. 3).

The data described above show that the principal cells responsible for inhibition of tumor growth in the model used (the weakly immunogenic Akatol tumor [1]) during treatment with BCG are neither T lymphocytes nor macrophages since, first, the use of ATS increased this inhibition and, second, elimination of T lymphocytes and phagocytic cells from the suspension of SC from treated animals with tumors before adoptive transfer did not reduce the ability of the transplanted cells to inhibit tumor growth in the recipients. It can be tentatively suggested that the effector cells are K cells and natural killer cells. Finally, enhancement of the antitumor action of BCG by injection of ATS *in vivo* and the greater effectiveness of adoptive transfer by treatment with ATS *in vitro* may be evidence that in this case T lymphocytes exert a predominantly suppressor action. These results indicate that the search for effective methods of two-way action on the immune system of tumor carriers suitable for clinical use is promising.

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