EFFECT OF PRELIMINARY CYCLOPHOSPHAMIDE AND PRETRANSPLANTATION IRRADIATION ON GROWTH OF A SYNGENEIC MOUSE TUMOR

A. S. Babadzhanov and E. G. Slavina

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KEY WORDS: syngeneic tumor; cyclophosphamide; whole-body irradiation; T suppressors; activity of natural killers.

T suppressors differ from other lymphocyte populations not only in their antigenic markers, but also in several properties, especially high radiosensitivity and increased sensitivity to cyclophosphamide (CP) [1]. Conversely, activated macrophages and natural killer cells (NKC), which play the principal role in the system of natural antitumor resistance, possess relatively high radioresistance [7]. Although CP, when injected in doses of 10 to 100 mg/kg, injure not only T suppressors but also other subpopulations of T lymphocytes and NKC, their activity is restored by the 5th-6th day, whereas T suppressors are inactivated for a long time [3].

In the investigations described below the effect of a single injection of CP and of pretransplantation irradiation on growth of a syngeneic tumor was studied in mice.

EXPERIMENTAL METHOD

Male BALB/c mice weighing 20-22 g were used. A syngeneic Acatol tumor was maintained by subculture in BALB/c males. CP was injected intraperitoneally in a dose of 100 mg/kg body weight 3 days before inoculation of tumor cells (TC). The mice received a single dose of whole-body irradiation of 5 Gy, 2 h before injection of TC, on a "Stebel'" apparatus (GUPOS). The source of radiation was ^{137}Cs and the mean dose rate in the working chamber was 6.3 Gy/min. A suspension of spleen cells (SC) was made up in a glass homogenizer of Potter type in medium 199, filtered through two layers of gauze, and washed 3 times by centrifugation at 1000 rpm for 10 min each time. All manipulations were carried out under sterile conditions on ice. To remove T lymphocytes, 25×10^6 SC were incubated in 2.5 ml of medium 199 with 5 ml of diluted (1:10) adsorbed rabbit antithymocyte serum (ATS) (initial titer of the serum 128) for 30 min at room temperature, followed by the addition of 2.5 ml guinea pig complement and incubation for 40 min at 37°C. The remaining cells were washed 3 times by centrifugation. Phagocytic cells (PC) were eliminated with the aid of iron carbonyl (particle size $1 \times 1 \times 1.5$ μm). To 40 million cells in a volume of 5 ml 400 mg of iron carbonyl was added, and after incubation for 1 h at 37°C cells which had taken up particles of iron were removed by means of a magnet. The effectiveness of this procedure was verified by staining the residual cells for nonspecific esterase. Tumor cells were injected subcutaneously in a dose of 3×10^6 living TC per mouse. Each group consisted of 10 mice. The animals were killed 15 days after inoculation and the weight of the tumor was determined.

Natural killer activity was determined by the usual method [5]: SC (a pool of SC from three of four mice) were incubated for 4 h with ^{51}Cr-labeled (50 μCi/ml) YAC-1 lymphoma cells in the ratio of 200:1 and 100:1, in a volume of 200 μl, in the wells of plates with 96 round-bottomed wells (from Linbro) at 37°C in an atmosphere with 5% CO_2. Before the beginning of incubation the plates with the cells were centrifuged for 5 min at 1000 rpm. At the end of incubation 100-μl samples were taken from each well for measurement of radioactivity on a gram-counter (Ber Rold).

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
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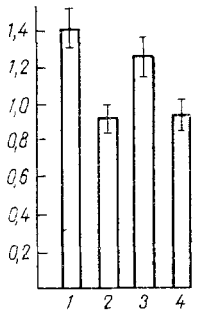

Fig. 1

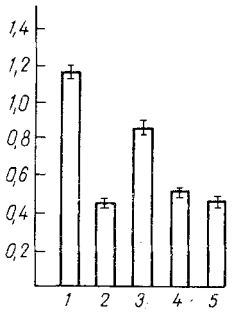

Fig. 2

Fig. 1. Effect of preliminary injection of CP on growth of Acatol tumor in BALB/c mice. Ordinate, weight of tumor (in g). 1) Control of tumor growth; 2) mice receiving CP; 3) mice receiving CP and inoculated with NSC; 4) mice receiving CP and inoculated with NSC treated *in vitro* with ATS and complement.

Fig. 2. Effect of adoptive transfer of SC of normal mice and mice receiving CP on growth of Acatol tumor in irradiated (5 Gy) mice. Ordinate, weight of tumor (in g). 1) Control of tumor growth; 2) irradiated mice; 3) irradiated mice inoculated with NSC; 4) irradiated mice inoculated with SC of mice receiving CP; 5) irradiated mice inoculated with SC treated *in vitro* with ATS and complement.

TABLE 1. Determination of MID of Acatol Tumor in Irradiated and Intact Mice (M ± m)

Parameter studied	Irradiated recipients					Intact recipients				
	dose of tumor cells									
	10^7	10^6	10^5	10^4	10^3	10^7	10^6	10^5	10^4	10^3
Time of appearance of palpable tumor, days	7	9	*	*	*	4	6	8	17	*
Weight of tumor on 30th day, mg	4033±228	354±12	—	—	—	9316±305	1020±59	304±35	147±14	—

*Tumors had not appeared in a single animal in the group by the 30th day.

EXPERIMENTAL RESULTS

As Fig. 1 shows, preliminary injection of CP caused inhibition of growth of the tumor ($P < 0.005$). Inoculation of the mice with 5×10^7 SC from intact syngeneic donors (NSC) simultaneously with inoculation of the tumor almost completely abolished the effect of CP. Injection of SC treated beforehand *in vitro* with ATS and complement, had no such action. Table 1 gives data on the effect of pretransplantation irradiation of mice in a dose of 5 Gy on success of tumor inoculation. In irradiated mice the minimal inoculation dose (MID) for Acatol tumors was increased by comparison with MID in intact animals. In mice with a developing tumor mass, at sacrifice on the 30th day it was much smaller in irradiated than in intact recipients ($P \times 0.005$). Injection of 5×10^7 NSC into the irradiated mice, just as in experiments with CP, significantly reduced inhibition of tumor growth ($P < 0.05$), but preliminary treatment of the NSC *in vitro* with ATS and complement abolished this effect of the NSC (Fig. 2). Injection of SC of mice receiving CP into irradiated mice likewise did not reduce the effect of irradiation. In our opinion, in this model the effect of a single injection of CP or of pretransplantation irradiation in inhibiting tumor growth is largely determined by its action of T suppressors.

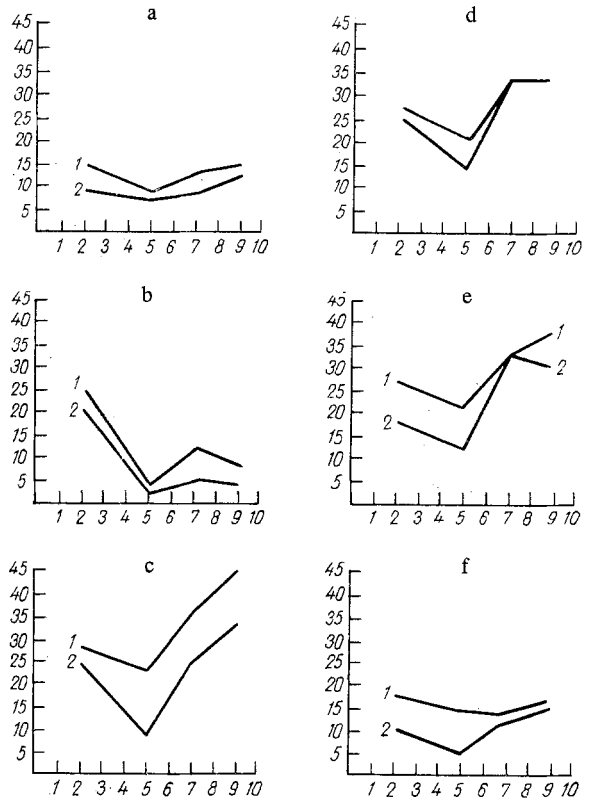

Fig. 3. Investigation of activity of NKC in SC population of mice with tumors, irradiated before transplantation in a dose of 5 Gy. Abscissa, time after inoculation of tumor (in days); ordinate, level of cytotoxicity (in %). 1) Effector–target ratio 200:1; 2) 100:1. a) Intact control (normal mice); b) control of tumor growth; c) irradiated recipients of tumor; d) irradiated recipients of tumor, inoculated with NSC; e) irradiated recipients of tumor, inoculated with NSC treated *in vitro* with ATS and complement; f) irradiated recipients of tumor inoculated with NSC from which PC had been removed.

Injection of NSC into irradiated mice, it will be noted, did not restore the level of tumor growth to the control value. This may perhaps be due to the action of irradiation on the tumor bed and, in particular, on connective tissue and blood vessels [2, 4]. Irradiation can also induce activation of macrophages [6]. In the writers' view elimination of T suppressors in these experiments lead to increased activity of NKC.

Investigation of NKC activity *in vitro* in this system showed (Fig. 3) that NKC activity was highest on every day of the investigation in the irradiated mice with tumors. Although NKC activity in the unirradiated mice was considerably increased on the 2nd day after injection of TC, on subsequent days it had a tendency to fall. Injection of 5×10^7 NSC or of SC treated with ATS and complement into the irradiated mice did not affect NKC activity in these mice. However, transfer of NSC from which macrophages had been removed significantly reduced NKC activity. This was perhaps due to the fact that the mice of this group received a larger number of T cells (an increase in their proportion in the population).

In our view, the search for methods of removal of T suppressors arising during tumor growth, on the basis of their high radiosensitivity and their selective vulnerability to CP, is promising for clinical application. The search for doses of chemotherapeutic agents suitable for clinical use and selectively depressing T suppressors is a promising development.

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EXPERIMENTAL MODEL FOR DEMONSTRATING LEUKEMIA-SPECIFIC ANTIGENS IN ACUTE HUMAN LEUKEMIAS

S. V. Danilevich, I. A. Kalinina,
and T. I. Bulycheva

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KEY WORDS: leukemia-specific antigens; leukemia-associated antigens; leukemic cells; "remission" lymphocytes; cytotoxic antibodies.

The question of the existence of leukemia-specific antigens (LSA) is patients with acute leukemia is one of the most important in cancer immunology. To detect LSA, immunization of inbred animals with human leukemia cells followed by the study of the specificity of the developing antibodies, which may be directed not only toward the sought (LSA) antigens, but also to specific, tissue, differential, and so on, antigens, not to mention histocompatibility antigens which are also present in leukemia cells, is most frequently used [3, 6, 7].

All these factors create additional difficulties in the way of final interpretation of the results of the study and demonstration of the presence of LSA in a patient's leukemic cells.

In this paper we describe a new model for the demonstration of antigenic differences between leukemia cells and lymphocytes, by means of which we attempted to avoid these shortcomings.

EXPERIMENTAL METHOD

(CBA × C57Bl/6)F_1 mice were immunized intraperitoneally by two injections (with an interval of 10 days between them) of leukemia cells from the same patient with acute lymphoblastic leukemia, in a sessional dose of 10^8 cells per mouse. Serum was obtained 10 days after the last immunization of the animals. Intact mouse serum served as the control. The sera were tested for the presence of antibodies in the C'-dependent cytotoxic test (C'-CTT) in Terasaki plates parallel with leukemia cells used for imminuzation, and with lymphocytes obtained from the peripheral blood of the same patient during a remission (the method of isolating the cells was described previously [1]). The antibody titer in the sera was expressed as its final dilution causing 50% death of target cells. Immune sera were adsorbed with leukemia cells in the acute period and with "remission" leukocytes of the same patient in doses with which complete exhaustion of the serum from the corresponding antibodies was achieved (between 5×10^7 and 4.5×10^8 cells/ml). The conditions of adsorption were: incubation of cells in serum at 37°C for 20 min with shaking of the tube every 5 min,

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