EXPERIMENTAL RESULTS

The experimental results given in Table 1 show that a marked antitumor and, in particular, antimetastatic action was observed after intravascular laser irradiation of the blood. Characteristically the antitumor effect of two irradiations of the blood was appreciably greater than that of a single irradiation.

The action of red (λ = 633 nm) and green (λ = 510 nm) laser radiations was approximately the same. To judge by the results, irradiation of the blood acted as a stimulus for rapid resorption of the tumor, which was reflected in a sharp reduction in its weight and disappearance of metastases. Metastases of Pliss lymphosarcoma, as we know, begin to appear on the 6th-8th day after transplantation of the tumor, and for that reason, since irradiation began so late, existing metastases were undergoing resorption.

The study of blood films from the irradiated animals showed an increase in the absolute number of lymphocytes on the 16th and 22nd days after tumor transplantation.

According to data in the literature, during intravascular laser irradiation of the blood, cellular immunity is stimulated [4, 5]. The immunostimulating properties of laser irradiation of the blood evidently explain the antitumor effects obtained in these experiments.

LITERATURE CITED

1. V. S. Zemskov, N. F. Gamaleya, Z. M. Rudykh, and V. Ya. Stadnik, Klin. Khir., No. 11, 69 (1984).
2. V. S. Zemskov, N. F. Gamaleya, and Z. M. Rudykh, Clin. Khir., No. 12, 17 (1984).
3. E. V. Gubler, Computational Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1973).
4. V. I. Kupin, A. V. Ivanov, and A. G. Gavrilov, The Use of Lasers in Medicine [in Russian], Moscow (1984), p. 95.
5. E. N. Meshalkin, V. S. Sergievskii, and G. V. Dorozhko, Zh. Ushn. Nos. Gorl. Bol., No. 5, 27 (1981).

CONDITIONS OF FORMATION OF ANTITUMOR CYTOTOXIC T LYMPHOCYTES AND INHIBITION OF THEIR ACTIVITY BY SUPPRESSOR T CELLS IN MIXED HUMAN LYMPHOCYTE AND TUMOR CELL CULTURE

I. F. Abronina and V. S. Anan'ev

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KEY WORDS: T lymphocytes; tumor cells; suppressor cells.

A mixed culture of lymphocytes and tumor cells (MCLTC) is an experimental model used to study antitumor immunity. In MCLTC cytotoxic T lymphocytes (CTL) are formed against human and mouse tumor cells [2, 6, 7, 12, 13]. As a rule CTL activity is depressed in cancer patients and animals with tumors, as a result of the action of suppressors of macrophagal and T-cell nature [4, 5, 9, 11, 14].

The aim of this investigation was to study the conditions of formation of antitumor CTL in MCLTC and depression of their activity by suppressor T cells, using fractions of peripheral blood lymphocytes, isolated in a Percoll gradient, for culture.

EXPERIMENTAL METHOD

Mononuclear cells were isolated [3] from the blood of patients with colorectal carcinoma (CRC). The isolated cells were incubated for 45 min at 37°C on plastic Petri dishes to remove

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All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Trapeznikov.)

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TABLE 1. CA of Antitumor Killer Cells (in %) Generated in MCLTC from Peripheral Blood Lymphocytes of Patients with CRC, Fractionated in a Percoll Gradient (M±m)

No. of patient	Autologous tumor			Allogeneic tumor
	Fraction			
	1-	2-	1+2-	2-
2	—2,3±1,2	11,5±3,3*	—0,6±0,8	0,0±0,8
4	0,7±0,1	15,3±1,5*	0,0±1,8	1,3±0,2
5	3,0±0,5	17,9±2,3*	1,8±0,3	—0,1±0,1
6	4,3±2,3	32,2±0,8*	14,8±2,8	0,5±3,4
8	1,1±0,4	15,2±0,9*	2,7±1,1	—7,7±2,3
10	2,9±1,5	30,8±7,3	32,1±5,8	6,5±1,7
14	—1,1±0,5	26,3±2,2	28,3±1,3	2,9±1,4
24	—1,0±0,8	24,4±1,9	29,1±8,2	3,9±1,3
26	0,5±1,2	31,0±2,1	25,6±3,9	3,2±1,3
27	0,9±0,4	18,9±1,0*	0,0±2,1	3,2±0,9
28	—0,6±1,4	11,3±1,8	9,5±1,1	0,6±0,2
29	0,6±0,4	16,9±0,4*	0,3±0,1	2,2±0,2
33	—1,0±0,3	20,6±4,8*	—6,1±1,4	0,9±0,3
34	4,5±0,4	17,0±2,6*	—1,4±1,0	—1,3±0,5
37	0,7±0,2	17,4±0,9*	0,5±0,1	0,0±0,9
27	4,5±0,5	44,2±4,1*	1,5±0,7	52,7±4,9
28	0,3±1,1	46,2±2,6*	29,4±1,1	38,0±3,4
29	4,7±1,6	42,8±4,7*	1,8±0,6	45,5±4,3
30	—2,3±1,2	50,5±3,3**	35,2±5,8	33,0±1,9
31	—0,1±0,2	41,8±12,9	32,9±5,6	31,5±2,3
32	1,9±1,1	44,9±2,1*	0,5±0,1	41,4±0,3
33	0,6±0,4	48,2±5,0*	0,4±0,2	67,9±2,6
34	0,4±0,1	45,3±1,9*	9,0±2,0	51,9±7,4
36	1,9±0,1	39,4±2,0	46,9±1,5	39,6±2,4
37	0,0±1,6	42,0±1,9*	2,0±1,3	35,3±2,7

Legend. A) MCLTC, stimulated by patient's tumor cells; B) MCLTC stimulated by a mixture of cells of five strains of CC. *$p < 0.01$, **$p < 0.05$ compared with mixture of lymphocytes of fractions 1 and 2 in the ratio of 1:1. Lymphocytes were added to tumor cells in ratio of 30:1.

adherent cells. Lymphocytes not adhering to plastic (25×10^6 cells) were layered above a stepwise Percoll gradient (1.077, 1.067, 1.056 g/ml, from "Sigma," USA). Interphases of cells isolated in the gradient and located on the boundary between layers 1.056-1.067 and 1.067-1.077 g/ml were described as fractions 1 and 2 [8].

The material from which the tumor cells were obtained consisted of tumors removed surgically from patients with CRC and five strains [1, 8, 9, 10, 12] of human carcinoma of the colon (CC), maintained by passage through nude mice [1]. A suspension of cells from the solid tumors was obtained by incubating fragments of tumor tissue in a mixture of enzymes: trypsin, collagenase, and deoxyribonuclease [10]. For use in MCLTC the suspension thus obtained was enriched up to 90% with tumor cells by sedimentation in a gradient of embryonic serum [10]. Some of the isolated tumor cells were frozen in liquid nitrogen for subsequent use in cytotoxic tests as target cells.

To obtain MCLTC, 4×10^5 tumor cells, treated with mitomycin C (50 μg/ml, 30 min, 37°C) were mixed with 2×10^6 cells of fraction 1 or fraction 2, isolated in a Percoll gradient, or a mixture of the two in the ratio of 1:1 in 2 ml of complete medium (RPMI 1640, 5% human serum, 2×10^{-3} M L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 5×10^{-3} M HEPES, 100 U/ml of antibiotics). The mixture was incubated for 6 days in 24-well "Linbro" plates at 37°C in an atmosphere with 5% CO_2. On the 3rd day of culture, 10% of chromatographically purified interleukin-2, provided by N. N. Voitenok (Laboratory of Cellular Immunology, Belorussian Blood Transfusion Research Institute, Minsk), was added to the medium. For treatment with "Ortomune" monoclonal antibodies and complement, 2×10^6 cells were incubated with 1 ml of antibodies in a dilution of 1:20 for 30 min at 4°C and then with 1 ml of complement for 60 min at 37°C.

Cytotoxic activity of killer cells generated in MCLTC was determined after incubation for 16-18 h in a microtest of ^{51}Cr-labeled tumor target cells with cultural lymphocytes [2].

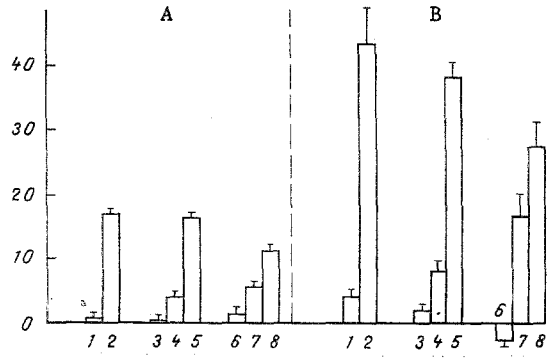

Fig. 1

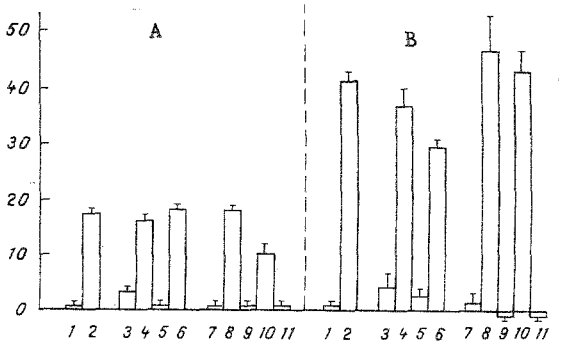

Fig. 2

Fig. 1. CA of mixture of lymphocytes of fractions 1 and 2 of Percoll gradient after treatment of fraction 1 cells with mitomycin C. Ordinate, CA (in %) of lymphocytes of fractions 1 (1) and 2 (2) of patient No. 27 against autologous tumor cells; of mixture of lymphocytes of fractions 1 and 2 containing 50, 25, and 12% of fraction 1 cells, untreated (3-5) and treated (6-8) with mitomycin C; A) MCLTC stimulated by patient's tumor cells; B) MCLTC stimulated by mixture of cells of five strains of CC.

Fig. 2. CA of lymphocytes of fraction 2 and mixture of lymphocytes of fractions 1 and 2 of Percoll gradient after treatment with monoclonal antibodies and complement. Ordinate, CA (in %) of untreated lymphocytes of fractions 1 (1) and 2 (2) of patient No. 37 against autologous tumor cells; of cells of fraction 2 treated before beginning of culture with OKT3 (3), OKT4 (4), OKT8 (5), and OKB7 (6) antibodies; fraction 1 cells untreated (7) and treated with OKT3 (8), OKT4 (9), OKT8 (10), and OKB7 (11) antibodies before beginning of culture with fraction 2 cells in the ratio of 1:1; A) MCLTC stimulated by patient's tumor cells; B) MCLTC stimulated by mixture of cells of five strains of CC.

Cytotoxic activity (CA) was determined by the formula: $[(a-b)/(c-b)]\cdot 100$, where a denotes the amount of ^{51}Cr label released (ALR) in the medium after incubation with lymphocytes; b denotes ALR in medium without lymphocytes; c denotes ALR in medium with tumor cells, disintegrated by a 1% solution of sodium dodecylsulfate.

EXPERIMENTAL RESULTS

CA of peripheral blood lymphocytes of 37 patients with CRC was studied in MCLTC after fractionation in a Percoll gradient. CA against autologous tumor cells was observed after the use of fraction 2 of lymphocytes for culture in MCLTC (Table 1). In 15 cases, in the autologous MCLTC, stimulated by patients' tumor cells, cytotoxic cells destroying autologous but not allogeneic tumor cells were formed from the lymphocytes of fraction 2. During culture of lymphocytes of fraction 1 in autologous MCLTC no cytotoxic cells were formed. In 10 of 15 cases CA of fraction 2 lymphocytes depressed the lymphocytes of fraction 1, added to cells of fraction 2 in the ratio of 1:1 at the beginning of culture. Cytotoxic cells also were generated during culture of fraction 2 lymphocytes from patients with CRC in MCLTC, stimulated by a mixture of cells of five strains of CC. CA against cells of autologous and allogeneic tumors was observed in all 10 cases studied. In eight of 10 cases CA of the lymphocytes of fraction 2 was inhibited by lymphocytes of fraction 1, as was described above. During culture of fraction 1 lymphocytes, as in the autologous MCLTC, no cytotoxic cells were formed.

Inhibition of formation of cytotoxic cells in MCLTC was evidence that suppressor cells were present in the fraction 1, isolated in a Percoll gradient. This conclusion was confirmed by dependence of inhibition of cytotoxic cell formation from lymphocytes of fraction 2 on the dose of fraction 1 cells added to the fraction 2 lymphocytes at the beginning of culture, and by the resistance of the fraction 1 cells to mitomycin C treatment (Fig. 1). When fraction 1 cells accounted for 50% of the mixture with fraction 2 lymphocytes the maximal effect of inhibition of cytotoxic cell formation was observed in the two types of MCLTC, and the effect diminished with a decrease in the dose of fraction 1 cells to 25 and 12%. When lymphocytes of fraction 1 were treated with mitomycin C and added in different doses to the fraction 2 lymphocytes at the beginning of culture, the effect of inhibition of cytotoxic cell formation in MCLTC likewise was not abolished.

The results of treatment of cells of fractions 1 and 2 by monoclonal antibodies and complement demonstrated the T-cell nature of the cytotoxic and suppressor cells and showed that

they belonged to the common suppressor-cytotoxic population ($OKT8^+$) of human lymphocytes (Fig. 2). Inhibition of cytotoxic cell formation in the two types of MCLTC was observed after treatment of the fraction 2 cells before the beginning of culture with OKT3 and OKT8 (T-cell markers) antibodies, but not by OKT4 (T-cell marker) and OKB7 (B-cell marker) antibodies in the presence of complement. Abolition of the suppression effect took place after treatment of the fraction 1 cells (before the beginning of their combined culture in MCLTC with fraction 2 lymphocytes) with OKT3 and OKT8 antibodies, but not with OKT4 and OKB7 antibodies in the presence of complement, just as in the case of treatment of the fraction 2 cells.

The formation of CTL against autologous tumor cells in MCLTC stimulated by tumor cells from patients with CRC is evidently linked with the use of fraction 2 lymphocytes, enriched up to 90% with T cells after fractionation in a Percoll gradient [8], for culture. A similar result was obtained previously with patients with malignant pleural effusion, when a population of small T cells was used with high density for culture in MCLTC [12]. Formation of CTL against autologous and allogeneic tumor cells in MCLTC, stimulated by a mixture of cells of five strains of CC, can be explained by the use of tumor cells from patients with CC, carrying different individual HLA antigens, for culture of the stimulators. Suppressor T cells inhibiting CTL formation in MCLTC from fraction 2 lymphocytes, consisting mainly of T cells, were found in fraction 1 lymphocytes isolated in a Percoll gradient. Similarly, T-cell suppressors of natural killer cells, isolated from the high-density fraction of the gradient [14], were found in the low-density fraction of lymphocytes isolated in a bovine serum albumin gradient.

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LITERATURE CITED

1. N. G. Blokhina, Yu. N. Solov'ev, A. V. Ozherel'ev, et al., Byull. Éksp. Biol. Med., No. 3, 330 (1985).
2. S. N. Bykovskaya, M. S. Iobadze, T. A. Kurpiyanova, et al., Byull. Éksp. Biol. Med., No. 1, 86 (1987).
3. A. Böyum, Scand. J. Clin. Lab. Invest., 21, Suppl. 92, 77 (1968).
4. S. Fujimoto, F. Matsuura, K. Nakagama, et al., Cell. Immunol., 38, 378 (1978).
5. T. Kataoka and F. Oh-hashi, Cancer Res., 45, 2139 (1985).
6. E. Kedar, E. Chrigui-Leira, and S. Metelman, Cancer Immunol. Immunother., 18, 126 (1984).
7. H. Kimura, Y. Yamaguchi, and T. Fujisawa, Gann, 75, 1006 (1984).
8. J. T. Kurnick, L. Ostberg, M. Stegagno, et al., Scand. J. Immunol., 10, 563 (1979).
9. R. J. North and E. S. Dye, J. Exp. Med., 155, 1063 (1982).
10. T. G. Pretlow and T. P. Pretlow, 'Cell Separation Methods and Selected Applications, Vol. 3, New York (1984), p. 53.
11. R. S. Schulof, R. W. Michitsch, P. O. Livingston, et al., Cell. Immunol., 57, 529 (1981).
12. A. Ushida and M. Moore, J. Natl. Cancer. Inst., 73, 1265 (1984).
13. F. Vanky, P. Gorsky, Y. Gorsky, et al., J. Exp. Med., 155, 83 (1982).
14. A. Yu, H. Watts, N. Jaffle, et al., New Engl. J. Med., 297, 121 (1977).