EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Immune Status of Oncological Patients During Therapy with Fetal Preparations

A. I. Autenshlyus, S. Yu. Rodionov, N. A. Pak, V. M. Stolyarova, E. S. Mikhailova, K. P. Plyaskin, S. V. Pushkarev, and N. V. Vasil'ev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 5, pp. 576-578, May, 1997
Original article submitted February 28, 1996

The parameters of humoral and cell-mediated immunity were studied in cancer patients treated with fetal preparations. The study revealed a tendency towards an increase in serum interferon and activity of tumor necrosis factor-α (in the patients with pronounced response to therapy), increased count of $CD8^+$-lymphocytes (in the patients not responding to embryonal proteins), and a tendency towards a decrease in the counts of lymphocytes with signs of apoptosis in the patients in whom the treatment was ineffective.

Key Words: *cancer; fetal therapy; immune status*

Injection of fetal proteins to patients with cancer sometimes induces degradation of tumor tissue [4]. Although the mechanism of this effect is unknown, it is probable that its key factor is the blockade of antibodies to surface receptors of tumor cells [6]. This results in demasking of tumor-associated antigens, and the cell becomes available for cytotoxic lymphocytes (ligands). In addition, fetal therapy seems to activate the immune response to oncofetal proteins [1,5].

The effect of fetal therapy on the main parameters of the immune status is unknown. Traditional methods, such as surgery, chemo- and radiotherapy, suppress the immunity [7]. The degree of depression of cell-mediated immunity in cancer patients, detected both *in vivo* and *in vitro,* directly correlates with the dissemination of the process: at the early stages the systemic parameters are, as a rule, normal [10], and change during tumor progression [9].

Our aim was to assess the main parameters of the immune status in cancer patients treated by fetal preparations.

Bioterapiya Research and Clinical Center, Novosibirsk

MATERIALS AND METHODS

Blood samples of 45 cancer patients (stages III-IV) treated with fetal preparations (Table 1) and of 13 normal subjects was analyzed. Venous blood was collected before and after treatment with water-salt extract of human embryonal material or its individual fractions. The therapy lasted 4-6 weeks. The patients were divided into 3 groups with different reactions to treatment: 1) patients with well and medium-differentiated tumors reacting to fetal preparations by degradation of tumors and metastases; 2) patients with poorly differentiated tumors in whom this effect was not observed; and 3) patients on preventive antimetastatic therapy after removal of the tumor. Control group (group 4) consisted of healthy donors. The diagnoses and efficacy of therapy were verified by cytological, histological, and instrumental methods.

For quantitative characterization of lymphocyte subpopulations we used monoclonal antibodies (Medbiospektr Research and Production Unit, Moscow) as described elsewhere [2,3,12]. Populations of $CD7^+$ lymphocytes (total count of T cells); $CD3^+$ lympho-

0007-4888/97/0005-0502$18.00

cytes (mature T cells); $CD4^+$ lymphocytes (T helpers/inducers — Th/i), $CD8^+$ lymphocytes (T suppressors/cytotoxic — Ts/c); $CD22^+$ B lymphocytes, $CD16^+$ natural killers (NK), $CD95^+$ lymphocytes with apoptosis markers (LA) were assessed separately.

Functional activity of T and B lymphocytes was assessed in the blast transformation test [13]. Cytolytic activity of NK was assessed as described elsewhere [11] with K-562 cell culture as the target.

Biological activity of tumor necrosis factor-α was studied in L-929 cells by assessing the cytolytic effect of the factor [14]. Serum interferon was measured by suppression of the cytopathic effect of mouse encephalomyocarditis virus [8].

RESULTS

Table 2 presents the relative and absolute counts of lymphocyte populations and subpopulations before and after therapy.

Significant ($p<0.05$) intergroup differences were observed for the following parameters: the relative counts of total and mature T cells were decreased in all patients (absolute in groups 1 and 2); the levels of Th/i and Ts/c (absolute and relative) were decreased only in group 2; the counts of B cells and NK (absolute and relative) were increased in all groups of patients; the count of LA (absolute and relative) was significantly increased in groups 2 and 3.

After treatment, the majority of absolute and relative values did not change. The relative content of Ts/c in group 2 significantly increased and the count of B lymphocytes in group 3 decreased. In group 1 the content of LA was nearly normal.

TABLE 1. Characteristics of Groups of Patients

Diagnosis	Number of patients		
	group 1	group 2	group 3
Breast cancer	7	10	1
Lung cancer	1	2	—
Rectal cancer	3	1	1
Gastric cancer	1	2	2
Prostatic cancer	1	1	—
Cancer of the tongue, mouth, and neck	4	—	1
Ovarian cancer	—	1	—
Lymphoid leukemia	1	2	—
Sarcoma	—	1	—
Melanoma	—	1	—
Basalioma	—	1	—
Total	18	22	5

TABLE 2. Immune Status of Patients with Cancer During Fetal Therapy (*M±m*)

Parameter	Group 1 (*n*=18)		Group 2 (*n*=22)		Group 3 (*n*=5)		Group 4 (*n*=5)
	before therapy	after therapy	before therapy	after therapy	before therapy	after therapy	control
T lymphocytes, total ($CD7^+$)	38.44±2.30	40.25±3.25	36.50±5.12	45.33±6.69	42.00±3.88	39.00±4.92	62.00±2.3
	0.67±0.08	0.61±0.07	0.56±0.23	0.51±0.07	0.88±0.21	0.33±0.25	0.98±0.09
Mature T lymphocytes ($CD3^+$)	39.00±2.14	38.50±3.03	35.00±2.60	45.33±6.69	42.40±3.51	39.50±4.60	58.00±2.3
	0.68±0.16	0.57±0.1	0.51±0.17	0.29±0.06	0.87±0.19	0.58±0.21	0.92±0.08
Th/i ($CD4^+$)	25.78±1.46	27.25±0.96	22.00±1.00	29.33±3.31	29.20±3.07	26.00±3.94	32.20±1.60
	0.48±0.05	0.41±0.09	0.29±0.06	0.26±0.03	0.59±0.13	0.39±0.17	0.56±0.04
Ts/c ($CD8^+$)	22.89±2.80	23.50±1.09	15.0±0.86*	24.6±2.37*	26.00±3.84	20.50±4.02	27.60±1.3
	0.42±0.08	0.36±0.08	0.21±0.06	0.26±0.03	0.48±0.07	0.31±0.14	0.44±0.04
B lymphocytes ($CD22^+$)	13.33±1.69	14.50±1.29	18.00±1.22	11.33±2.37	14.80±2.75	8.50±1.09	5.00±0.60
	0.25±0.04	0.20±0.03	0.22±0.06	0.14±0.03	0.32±0.03*	0.10±0.04*	0.07±0.01
NK ($CD16^+$)	18.22±2.89	18.50±1.79	17.75±3.01	17.33±3.92	17.40±1.08	17.00±2.18	13.40±1.40
	0.27±0.05	0.28±0.07	0.23±0.11	0.20±0.03	0.25±0.04	0.19±0.07	0.14±0.02
LA ($CD95^+$)	7.33±1.85	7.00±1.50	12.50±3.90	6.00±2.45	13.20±2.50	9.00±0.62	3.50±0.80
	0.12±0.03	0.08±0.07	0.37±0.16	0.06±0.02	0.22±0.03*	0.10±0.03*	0.05±0.01

Note. Numerator: relative value (% of the total count of lymphocytes); denominator: absolute ($\times 10^9$/liter) value. *$p<0.05$ in comparison with the pretreatment value.

TABLE 3. Immune Status of Cancer Patients During Fetal Therapy (*M*±*m*)

Parameter	Group 1 (*n*=18)		Group 2 (*n*=22)		Control
	before therapy	after therapy	before therapy	after therapy	
Stimulation index:					
B lymphocytes	4.84±0.29	4.83±0.60	6.11±1.74	4.75±0.27	4.80±0.25
T lymphocytes	5.07±0.66	4.13±0.71	4.82±0.29	4.55±0.42	6.50±0.70
NK cytotoxicity index	31.25±1.26	34.43±1.25	36.12±3.06	35.00±1.45	27.00±4.40
Serum interferon titer	22.44±6.54	28.00±6.41	23.67±3.28	20.62±2.79	6.21±0.74
Activity of tumor necrosis factor-α	6.44±0.80	13.25±4.42	10.59±3.46	13.83±4.74	1.91±0.64

Studies of the functional activity of lymphocytes showed that in groups 1 and 2 serum interferon titers and activity of tumor necrosis factor-α were appreciably increased in comparison with the control (Table 3). The indexes of T and B cell stimulation did not differ from the control. The studied parameters virtually did not change during therapy, although an increase in the titer of serum interferon and in the activity of tumor necrosis factor-α in group 1 may indicate activation of T lymphocytes and macrophages producing these cytokines.

The data permit a conclusion that the parameters characterizing the status of T-cell immunity are decreased in comparison with the norm in all the patients, which is in line with published data. The relative and absolute counts of B lymphocytes, NK, and LA are increased. On the whole, fetal therapy did not appreciably change the immune status of patients in group 1. In group 2 the relative content of Ts/c increased, suggesting an increase of immunosuppression, which explains the absence of induction of antitumor immunity in patients with poorly differentiated tumors. The most essential feature of group 1 (where fetal therapy was effective) was the stability of the relative and absolute counts of apoptotic lymphocytes, whose level was nearly normal.

Fetal therapy induces no pronounced changes in the immune status of cancer patients, which prompts the search for the causes of tumor degradation at the level of realization of the effector cytotoxic immune reactions directly in the foci of tumor growth and metastases.

REFERENCES

1. G. I. Abelev, *Immunologiya*, No. 4, 5 (1982).
2. A. Yu. Baryshnikov, *Gematol. Transfuziol.*, No. 12, 15 (1988).
3. A. Yu. Baryshnikov, *Ibid.*, No. 8, 4 (1990).
4. S. P. Batrak, *Vrach. Delo*, No. 10, 127 (1971).
5. N. V. Vasil'ev, V. V. Gorodilova, and E. V. Mandrik, *Problems in Tumor Immunology* [in Russian], Tomsk (1986).
6. A. N. Glushkov, *Immunologiya*, No. 5, 11 (1991).
7. V. V. Gorodilova, *Vopr. Onkol.*, **27**, No. 6, 22 (1981).
8. S. S. Grigoryan, I. A. Maiorova, A. M. Ivanova, *et al.*, *Vopr. Virusol.*, No. 4, 433 (1988).
9. Yu. A. Grinevich and L. Ya. Kamenets, *Fundamentals of Clinical Immunology of Tumors* [in Russian], Kiev (1986).
10. K. A. Lebedev and I. D. Ponyakina, *Immunogram in Clinical Practice* [in Russian], Moscow (1990).
11. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, *et al.*, *Immunologiya*, No. 3, 88 (1981).
12. S. P. Sidorenko, E. P. Vetrova, O. V. Yurchenko, *et al.*, *Gematol. Transfuziol.*, No. 4, 19 (1990).
13. H. Frimmel', *Immunological Methods* [in Russian], Moscow (1979).
14. A. Meager, H. Leung, and J. Woolley, *J. Immunol. Methods*, **116**, 1 (1989).