INCREASED IMMUNOLOGIC REACTIVITY OF LYMPHOCYTES IN ONCOLOGIC PATIENTS TREATED WITH ELEUTHEROCOCCUS EXTRACT

V.I. Kupin, E.S. Polevaya, A.N. Sorokin*
All-Union Cancer Research Center, USSR Academy of Medical Sciences, *N.F. Gamaleya Institute of Epidemiology and Microbiology, Moscow, USSR

In the last decade, a number of studies have shown more or less decreased immunologic reactivity in almost all patients suffering from malignant tumors. In this connection, particular attention is paid to the use of various methods of immunotherapy and immunocorrection for complex treatment of malignant tumors. For this purpose, various immunostimulating preparations are prescribed. Studies on the immunostimulating ability of natural adaptogens and their potential use in clinical practice are now of great interest. Eleutherococcus extract (EE) is one of such promising preparations. Extensive research in the field of pharmacological action of natural biologically active substances, primarily of plant origin, in association with investigation of mechanisms of their action and the administration of such drugs for pharmacosomation has been carried out by Prof. I.I. Brekhman.

Publications by Prof. I.I. Brekhman and his followers have shown the efficiency of EE as an agent that lowers toxicity and enhances the anti-tumor and anti-metastatic activities of some cytostatics used in conventional treatment. The immunostimulating ability of Eleutherococcus has not yet been studied.

Materials and Methods

Lyophylized EE was dissolved in medium 199 to concentrations of 0.01; 0.10 and 1.00 mg/ml. EE effect was examined in peripheral blood lymphocytes in 58 individuals (24 donors, 12 patients with skin melanoma, 10 patients with stomach carcinoma and 12 patients with mammary gland carcinoma). All tests were repeated in triplets. Lymphocytes were isolated from heparinized blood in the density gradient Lymphoprep 1077. Lymphocyte samples ($2 \cdot 10^6$ cells/ml) were incubated in medium 199 with aqueous solution of EE at 37° C for 30 min. The control was prepared in the same conditions, without adding the EE. Immunologic tests included determination of T-lymphocytes by the method of spontaneous "active" and total rosette-formation (TAc- and TEt-RFC, respectively). B-

lymphocytes were estimated by complementary rosette-formation (EAC-B-RFC). Taking into account the literature data on interferon-inducing activity of Eleutherococcus in mice (Wacker, Eilmes, 1978; Wacker, 1983), we have studied the ability of Eleutherococcus preparations to induce production of interferon by lymphocytes obtained from healthy humans. To this end, lymphocyte samples ($1 \cdot 10^6$) were incubated with Eleutherococcus preparation at the concentrations given above at 37° C for 72 hrs. After incubation, the samples were centrifuged and the supernatant was assayed for interferon.

Results

The results of immunologic tests in donors and in patients prior to Eleutherococcus administration are given in Table 1; a significant decrease in the value of TAc and TEt rosette-formation was recorded for all patients with stomach and mammary gland carcinoma and with skin melanoma.

Table 1

Immunologic reactivity indices for donors and patients with different forms of malignant tumors

Diagnosis, number of individuals examined	Immunologic indices RFC in %		
	TAc	TEt	EAC-B-rosette
Donors	50.2 ± 1.8	60.4 ± 1.3	16.4 ± 1.2
24	28 ÷ 71	46 ÷ 77	4 ÷ 32
Melanoma	43.1 ± 4.1	53.0 ± 2.5*	11.3 ± 4.1
12	19 ÷ 69	44 ÷ 74	1 ÷ 49
Stomach carcinoma	32.2 ± 2.2*	42.0 ± 3.2*	18.0 ± 1.9
10	25 ÷ 47	38 ÷ 74	11 ÷ 30
Mammary gland carcinoma	41.7 ± 3.4*	53.0 ± 3.6*	15.7 ± 1.8
12	26 ÷ 68	38 ÷ 74	8 ÷ 26

*Here and below $P<0.05$

EE significantly enhanced the number of TEa-RFC lymphocytes in donors at all the concentrations studied. The values of TEt and EAC-B-RFC had the trend to increase (Table 2).

Table 2
Immunologic indices prior to and upon the administration of EE in various concentrations

Concentration, mg/ml	Immunologic indices, RFC in %		
	TEa	TEt	EAC-B-rosette
0.01	58.0 ± 1.3*	62.2 ± 1.5	19.9 ± 1.6
	42 ÷ 73	46 ÷ 83	3 ÷ 41
0.10	55.4 ± 2.1*	64.0 ± 1.0	18.9 ± 1.4
	26 ÷ 76	53 ÷ 79	1.7 ÷ 36
1.00	57.7 ± 1.6*	61.3 ± 1.2	18.1 ± 1.2
	40 ÷ 78	40 ÷ 77	2 ÷ 30
Background	50.2 ± 1.8	60.4 ± 1.3	18.4 ± 1.2
	28 ÷ 71	46 ÷ 77	4 ÷ 32

Table 3 shows an increase of immunologic indices in donors and in patients after administration of Eleutherococcus. The difference between the rates of increase of immunologic indices for donors and patients of different groups was not found to be statistically significant. However, almost all the immunological indices showed a higher increase in patients than in donors (Table 3).

This evidence was used as a basis for studying the immunostimulating ability of Eleutherococcus. To estimate an optimal dose of EE, we have conducted a study in vitro of the preparation in concentrations of 0.01, 0.1 and 1.0 mg/ml. The results were statistically processed using the number of individuals with the maximal increase of immunological indices in response to a studied EE concentration (Table 4).

It was found that 50 % donors had a maximal increase of TEa-RFC at EE concentration of 0.01 mg/ml. An increase of the concentration from 0.01 to 1.0 mg/ml resulted in a fall of the number of donors with the maximal response of this index (21 and

Table 3
Immunologic indices in patients and donors after administration of Eleutherococcus in vitro

Diagnosis, number of individuals examined	Immunologic indices, RFC in %		
	TEa	TEt	EAC-B-rosette
Donors, 24	29.9 ± 3.4	11.0 ± 1.2	25.5 ± 2.8
	5 ÷ 38	1 ÷ 20	1 ÷ 17
Melanoma, 12	39.3 ± 5.6	8.1 ± 2.3	72.7 ± 24.4
	7 ÷ 69	1 ÷ 28	6 ÷ 250
Stomach carcinoma, 10	44.1 ± 6.7	22.2 ± 4.4	32.3 ± 7.7
	1 ÷ 67	2 ÷ 46	5 ÷ 81
Mammary gland carcinoma, 12	28.7 ± 6.1	20.1 ± 4.8	36.3 ± 7.2
	1 ÷ 68	1 ÷ 53	1 ÷ 65

Table 4
Percentage of persons with a considerable increase of immunological indices in response to administration of Eleutherococcus in different concentrations and of persons insensitive to Eleutherococcus

Diagnosis	Eleutherococcus concentration, mg/ml									No effect		
	0.01			0.10			1.00					
	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.	T-act.	T-tot.	B-comp.
Donors	50	33	29	21	37	17	29	29	21	0	1	33
Melanoma	33	33	58	17	8	33	50	33	8	0	26	1
Stomach carcinoma	20	20	20	40	30	20	30	50	60	10	0	0
Mammary gland carcinoma	18	27	27	18	27	48	54	36	36	10	10	19

29 %). Accordingly, the TEa-RFC is an only reliable index for detecting the stimulation; its maximal value was recorded at EE concentration of 0.01 mg/ml. Thus, minimal EE concentrations can be recommended for healthy people. A group of patients suffering from skin melanoma had a lower percentage of persons sensitive to minimal doses of EE (33 % against 50 % in donors). The values of EAC-RFC proved to be most sensitive to small EE concentrations (58 % in comparison to 29 % in the control). An increase of EE concentration up to 0.1 and 1.0 mg/ml resulted in a fall of the percentage of persons with a minimal immunological response. Therefore, we can conclude that maximal EE concentrations can be recommended for skin melanoma patients.

Stomach carcinoma patients should be treated with medium and high EE concentrations (0.1 and 1.0 mg/ml), which induced stimulation of immunological indices: TEa in 30-40 %, TEt in 30-50 %, and EAC-E-RFC in 20-60 % patients.

For mammary gland carcinoma patients, a high EE concentration of 1.0 mg/ml appeared to be effective. It induced maximal immunological response in 54 % patients for TEa and in 36 % for TEt and EAC-E-RFC.

Therefore, it was found that the donors are more sensitive to EE in low concentrations, while melanoma, stomach carcinoma and mammary gland carcinoma patients are more sensitive to medium and high concentrations. This evidence allows us to conclude that the EE affects human lymphocytes *in vitro* and stimulates immunological indices in healthy man and in patients. Accordingly, we believe that general biological effect of EE is realized through immunological mechanisms, and Eleutherococcus can be included into the group of preparations with a pronounced immunostimulating effect.

Study of EE revealed its ability to induce production of γ-interferon by human peripheral blood lymphocytes (Table 5).

Under similar conditions Eleutherococcus did not induce production of interferon by splenocytes of mice. At the same time, Eleutherococcus showed some protective action against the infection of mice with encephalomyocarditis virus.

There are interesting clinical observations on Eleutherococcus administration to mammary gland carcinoma patients. Immunologic indices in Eleutherococcus-treated patients did not change after

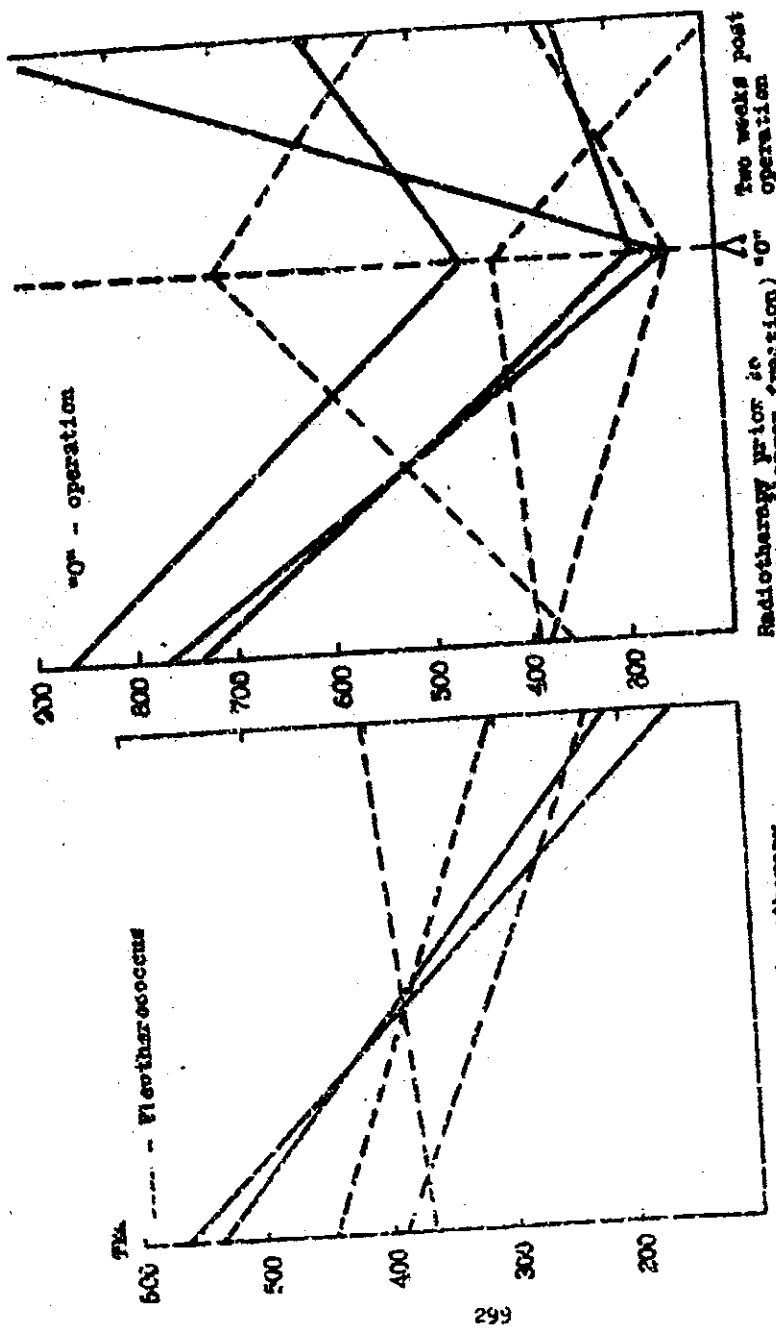

Fig. Changes in immunologic indices according to test for TEa-RFC in patients during the complex therapy of disseminated mammary gland carcinoma receiving Eleutherococcus extract.

0.0133 - 2	6(4 + 8)
4	24(8 + 64)

Note. Lymphocyte number in sample $1 \cdot 10^6$ cells/ml, after incubation for 72 hrs at 37° C.

radiotherapy or even somewhat improved. In contrast, in patients not given Eleutherococcus, immunologic indices sharply fell (c. Figure, left). In patients exposed to radiotherapy and to polychemotherapy, 2 trends were observed:

1. Patients not treated with Eleutherococcus could not endure more than one course of polychemotherapy, because of a sharp decline in the number of white blood cells. Immunologic indices in TEa also declined in absolute figures 2-4 times.

2. Patients treated with Eleutherococcus showed good tolerance to 2 courses of polychemotherapy. Leukogram of such patients did not suffer and immunologic indices changed slightly.

Thus, the studies on immunologic reactivity of oncologic patients treated with EE and the obtained results show a wide spectrum of immunostimulating activity (activation of TEa, TEt and EAC-B-RFC and induction of γ-interferon production).

Moreover, it is reasonable to use the preparation for treating patients exposed to radio- and chemotherapy.

References

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