ONCOLOGY

Antimetastatic Effect of Thymus-Dependent Antigen (Sheep Erythrocytes) in C57Bl/6 Mice with Lewis Carcinoma

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We revealed an antimetastatic effect of thymus-dependent corpuscular antigen (sheep erythrocytes) injected intravenously or intraperitoneally in sensitizing or high doses alone or in a complex with a course dose of cyclophosphamide to C57Bl/6 mice with Lewis carcinoma. Injection of the antigen appreciably reduced the number and volume of Lewis carcinoma metastases in the lungs, notably increased the therapeutic effect of cyclophosphamide, and restored hemopoiesis, particularly, the red blood stem suppressed by the tumor process and cytostatic treatment. The growth of primary tumors virtually did not change. High dose of sheep erythrocytes was more effective.

Key Words: *sheep erythrocytes; thymus-dependent antigen; Lewis carcinoma; metastases; cyclophosphamide*

Sheep erythrocytes (SE) are widely used in experimental immunological studies as the corpuscular thymus-dependent antigen (AG). Division of AG into T-independent and T-dependent is based on their capacity to induce immune response *in vivo* in thymectomized or nude mice. Realization of the humoral immune response to T-dependent AG requires cooperation between thymic (T cells) and bone marrow lymphocytes (B cells) [1,9]. SE are used for evaluation of agglutinin titer, number of antibody-producing cells in the spleen, delayed-type hypersensitivity (DTH) reaction, and other parameters of the immune response. SE receptors are present on human T-lymphocytes as their specific markers; this phenomenon is widely used in clinical practice for the diagnosis of immunodeficiency of different origin, including that caused by malignant tumors [7].

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We previously revealed a significant decrease in the number of lung metastases after injection of SE to C57Bl/6 mice with Lewis carcinoma in comparison with animals not receiving SE (SE was injected for evaluation of DTH reaction). The difference in the growth of primary tumors was negligible. This result was reproduced in subsequent experiments, when we also observed a 15.1% prolongation of the life span of these mice ($p<0.05$). Various cells (fetal, tumorous, somatic) are used in experiments and clinical practice for immunotherapy of malignant tumors [4,8,11,12]. We studied the effect of xenogenic peripheral blood cells (SE) on the growth and metastases of Lewis carcinoma in C57Bl/6 mice.

MATERIALS AND METHODS

Experiments were carried out on 48 female C57Bl/6 mice (20-22 g, 2.5-3 months) bred at the vivarium of R. E. Kavetskii Institute. Transplantable Lewis pul-

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monary carcinoma, SE, cyclophosphamide (CP), and peripheral blood were used in the study.

Cyclophosphamide (Kievmedprom Company) was injected intraperitoneally in a total dose of 150 mg/kg. Lewis carcinoma was transplanted intramuscularly into the right hind paw (2.5×10^5 trypsin-treated cells in 0.1 ml Hanks' medium).

SE suspension was injected intravenously into the retroorbital sinus in a single sensitizing dose of 2×10^5 cells/0.1 ml normal saline or intraperitoneally in a dose of 10^8 cells/0.3 ml normal saline on day 7 after tumor transplantation. The animals were divided into 6 groups (8 mice per group): group 1 were controls (normal saline), group 2 mice received CP, group 3 mice received 2×10^5 SE intravenously; group 4 mice received SE in the same dose+CP, group 5 mice were intraperitoneally injected with 10^8 SE, and group 6 mice received SE in the same dose+CP. On day 28 after tumor transplantation the animals were sacrificed by cervical dislocation and parameters of tumor growth were evaluated: primary tumor weight, number and volume of lung metastases, weights of the thymus and spleen. Absolute counts of peripheral blood leukocytes and erythrocytes were determined before and at the end of experiment using a Goryaev chamber. The results were processed using Student's *t* test.

RESULTS

Injection of CP significantly ($p<0.05$) decreased the weight of primary tumors and volume of Lewis carcinoma metastases in the lungs in comparison with controls by 47.2 and 67.2%, respectively; the number of metastases tended to decrease (Table 1).

After intraperitoneal immunization with SE in a dose of 10^8 the inhibition of the number and volume of metastatic involvement was 47.1 and 71.1% lower, respectively ($p<0.05$). After a lower dose of SE no statistically significant results, but just a trend to antimetastatic effect was observed. Immunization with SE in the highest dose produced the same effect as CP, but without side effects characteristic of drug therapy (body weight loss and hemotoxicity). Immunization with SE in both doses did not stimulate the growth of primary tumor; a trend to its decrease was observed even after a low dose of SE. Injection of SE in a dose of 10^8 significantly ($p<0.01$) potentiated the antimetastatic effect of CP and decreased the number of metastases by 25.6% and volume of metastatic involvement of the lungs by 71.7% in comparison with the cytostatic alone. A lower dose of SE in combination with CP treatment did not lead to statistically significant results, but decrease the volume of metastases. Immunization of mice with SE in both doses did not change the antitumor effect of CP.

Injection of CP to tumor-bearing mice significantly decreased the counts of peripheral blood leukocytes and erythrocytes (by 49.4 and 48%, respectively, $p<0.01$, Table 1). Tumor growth even without CP treatment was associated with appreciable suppression of both hemopoietic stems: the counts of the peripheral blood leukocytes and erythrocytes in tumor-bearing mice were lower than in intact animals by 35.6 and 22.8%, respectively ($p<0.001$). Immunization with SE in both doses in parallel with CP treatment abolished the toxic effect of the cytostatic on the red blood stem: erythrocyte counts in these animals virtually did not differ from those in controls. The capacity of SE to reduce or completely abolish the toxic effect of CP on erythrocytes is very important, because anemia develops in virtually all cancer patients. We observed no significant effect of immunization with SE alone or in combination with CP on white blood in mice during the growth of Lewis carcinoma. However, injection of SE in a higher dose slightly increased the leukocyte count, which was in line with previous reports [5] on the capacity of high doses of SE (15% suspension) to restore impaired

TABLE 1. Effects of SE and CP on Peripheral Blood Morphology, Growth and Metastases of Lewis Carcinoma in C57Bl/6 Mice ($M\pm m$)

Treatment	Incidence of metastases, %	Weight of primary tumors, g	Metastases		Peripheral blood values	
			number	weight, mg	leukocytes, $\times10^9$/liter	erythrocytes, $\times10^{12}$/liter
Control	100	3.62±0.26	15.50±3.22	115.1±40.2	8.30±1.47	7.24±0.16
CP	100	1.91±0.37*	9.00±0.84	37.7±10.7*	4.20±0.35*	3.76±0.93*
SE, 2×10^5	100	2.54±0.52	11.50±1.94	65.1±29.9	6.25±1.31	6.92±0.54
CP+SE, 2×10^5	100	1.90±0.18*	8.00±1.25*	23.4±13.2*	4.42±0.29*	7.62±0.55$^+$
SE, 10^8	100	3.73±0.14	8.20±1.21*	33.2±10.0*	7.92±1.27	6.84±0.11
CP+SE, 10^8	75.6	1.94±0.42*	6.7±0.2*$^+$	10.70±8.32*$^+$	5.24±0.64	6.88±0.16$^+$

Note. *$p<0.05$ compared to the control, $^+p<0.05$ compared to CP.

hemo- and lymphopoiesis in mice receiving CP and platidiam.

Hence, a new effect of corpuscular thymus-dependent AG (SE) is detected: appreciable inhibition of Lewis carcinoma metastases in C57Bl/6 mice, potentiation of the therapeutic effect of CP cytostatic, and protection of the red blood stem from the toxic effect of the cytostatic (most pronounced in rapidly proliferating blood and intestinal epithelial cells) often limiting the use of CP. The antimetastatic effect of SE is largely due to the immunological mechanisms, which are well studied in experiments with SE used as a thymus-dependent AG. Low doses of SE (10^5) injected intravenously induce DTH reaction, whose material substrates are effector T cells responsible for defense in bacterial (tuberculosis), viral, and parasitic infections and immune diseases; these cells determine the transplantation and antitumor immunity. High doses of SE (10^8-10^9) injected intraperitoneally activate T suppressors. CP and some other cytostatics can suppress and eliminate T suppressors [2]. A relationship between the capacity to DTH reaction to bacterial AG and efficiency of antitumor therapy was detected [3]. C57Bl/6 mice are a low-reactive strain (judging from their response to thymus-dependent AG (activity of antigen-presenting cells, capacity to produce agglutinins, form antibody-producing, nuclear, and rosette-forming cells in the spleen) [6,10], which is explained by their genetic characteristics, but this fact did not change their capacity to inhibit Lewis carcinoma metastases.

Many scientists consider biotherapy of malignant tumors a promising trend [8,11], and the search for new protocols and means of immunotherapy including T-dependent and T-independent antigens is now in progress.

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