Experimental Study of Antiblastic Activity in Potentiated Antibodies to Tumor Necrosis Factor-α

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In mice with Lewis lung carcinoma and melanoma B-16 administration of potentiated antibodies to tumor necrosis factor-α started 1 day after transplantation of a small number of tumor cells (10^6) produced an antiblastic effect.

Key Words: *transplanted tumors; tumor necrosis factor-α*

The possibility of using tumor necrosis factor-α (TNF) in oncology attracts much recent attention. This cytokine possesses antiblastic activity: it produces a direct cytotoxic effect on tumor cells, causes hemorrhagic necrosis of tumors, and activates the immune system [5]. The therapeutic and toxic doses of TNF are very close and it often causes side effects, including hyperthermia, headache, hypotension, and blood changes.

Previous experiments demonstrated the efficiency of substances in ultralow doses [2,3]. Regulatory peptides exhibit biological activity in dilutions of 10^{-12}-10^{-20}. Here we studied the effect of homeopathically prepared TNF in ultralow doses on the development of experimental tumors.

MATERIALS AND METHODS

Experiments were performed with potentiated antibodies to TNF (PAB-TNF). A mixture of homeopathic dilutions C12+C30+C200 was prepared. The preparation in a dose of 0.3 ml was administered through a gastric tube for 14-17 days starting from the first (series I and III) or fifth day (series II) after transplantation of tumor cells. Control animals received an equivalent volume of potentiated distilled water (C12+C30+C200, PDW).

The effects of PAB-TNF on the development of tumors were studied on 118 C57Bl/6 mice with transplanted Lewis lung carcinoma and melanoma B-16. The animals weighing 20-25 g were obtained from the Laboratory of Biological Models (Institute of Pharmacology)

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Tumor cells (10^6 or 4-6×10^6 cells in 1 ml physiological saline) were routinely transplanted into thigh muscles [6]. The efficiency of therapy was evaluated on day 19 (series I) or 21 (series II and III) by the weight of tumors (difference between the weights of experimental and contralateral hindlimbs). The index of suppression (IS) of tumor growth was calculated. The intensity of metastatic process was evaluated by the number of lung metastases in each animal and the mean number of metastases in the group. The area of metastases was calculated by the equation of a circle. The incidence of metastasizing was calculated as the percentage of animals with metastases from the total number of animals in group. The index of suppression of metastasizing (ISM) was calculated by the formula [1]:

$$ISM = [(A_C \times B_C - A_E \times B_E)/(A_C \times B_C)] \times 100\%,$$

where A_C and A_E are the incidence of metastasizing in lungs in the control and experimental groups, respectively; and B_C and B_E are the mean number of lung metastases in control and experimental groups, respectively.

The results were analyzed by *U* test, Mann-Whitney test, and Fischer angular transform.

RESULTS

Treatment with PAB-TNF started 1 day after transplantation of 10^6 tumor cells markedly inhibited the growth of primary tumor node in mice with Lewis lung carcinoma. The weight of tumors in these mice was 1.3-fold lower than in control animals (Table 1). PAB-TNF 2.3-fold decreased the number of lung metastases (Table 1). This preparation had no effect on the development of tumors when the therapy started 5 days after transplantation of 4-6×10^6 cells (Table 1).

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TABLE 1. Effect of PAB-TNF on Development of Tumors in Mice with Lewis Lung Carcinoma ($X \pm m$)

Parameter	Females, 10^6 tumor cells		Males, $4\text{-}6 \times 10^6$ tumor cells	
	PDW (n=11)	PAB-TNF (n=11)	PDW (n=14)	PAB-TNF (n=10)
Tumor weight, g	4.43±0.19	3.40±0.47*	6.82±0.24	6.97±0.24
IS, %	—	23	—	-2
Incidence of metastasizing, %	100	91	100	100
Number of metastases	10.36±1.85	4.55±0.89*	23.71±1.01	22.70±2.51
Area of metastases, mm^2	2.42±0.57	2.17±0.87	35.45±7.36	32.05±11.79
ISM, %	—	73	—	4

Note. Here and in Table 2: *p<0.05 compared to PDW.

TABLE 2. Effect of PAB-TNF on the Development of Tumors in Male Mice with Melanoma B-16 ($X \pm m$)

Parameter	10^6 tumor cells		$4\text{-}6 \times 10^6$ tumor cells	
	PDW (n=18)	PAB-TNF (n=17)	PDW (n=18)	PAB-TNF (n=19)
Tumor weight, g	3.53±0.24	3.64±0.24	5.56±0.22	5.24±0.13
IS, %	—	-3	—	6
Incidence of metastasizing, %	50	24*	50	53
Number of metastases per mouse	1.61±0.60	0.76±0.48	0.89±0.28	1.53±0.48
Area of metastases, mm^2	0.20±0.08	0.10±0.06	0.07±0.02	0.25±0.09
ISM, %	—	79	—	-82

In mice with melanoma B-16, administration of PAB-TNF suppressed the formation of metastases after transplantation of 10^6 tumor cells (Table 2), but was ineffective in mice receiving higher doses of tumor cells (similarly to mice with Lewis lung carcinoma, Table 2). The rate of growth of the primary tumor node remained unchanged.

Thus, experiments with two metastasizing tumors showed that PAB-TNF inhibit metastasizing dissemination in animals after transplantation with a low number of tumor cells. The preparation suppressed the growth of the primary tumor node only in mice with Lewis lung carcinoma.

The ability of PAB-TNF to improve the antimetastatic resistance of the organisms is of considerable importance, because the life of patients with malignant tumors often depends on the presence and development of metastases in vital organs. TNF not only produces a direct cytotoxic effect on tumor cells, but also interacts with specific receptors expressed on most cells and activates antitumor immune reactions [5]. Our previous experiments showed that potentiated antibodies to γ-interferon stimulate the synthesis of this cytokine in mice [7]. The data suggest that PAB-TNF also stimulate secretion of endogenous TNF, which improves the antitumor resistance of the organisms.

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