Dynamics of Antitumor Resistance after Cyclophosphamide Injection

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Antitumor resistance decreased in mice 24 h after injection of cyclophosphamide in a dose of 100 mg/kg. This was seen from more rapid growth of Ehrlich's ascitic carcinoma transplanted intraperitoneally 24 h after cyclophosphamide injection and 17% reduction of the lifespan of mice with tumors. Three, 7, 10, 14, and 22 days after cyclophosphamide injection, the antitumor resistance increased and the lifespan of animals with Ehrlich's ascitic carcinoma transplanted at the corresponding periods increased by 20, 14, 42, 29, and 36%, respectively, in comparison with mice with transplanted tumor not injected with the drug. Injection of cyclophosphamide 1 day after tumor transplantation prolonged of the lifespan of animals with tumors by 76%.

Key Words: *antitumor resistance; antitumor immunity; cyclophosphamide*

It is known that the effect cyclophosphamide (CP) is determined by not only its direct cytostatic and cytotoxic activities of its active metabolites (*e.g.* bis(β-chlorethyl)-amine) on tumor cells, but also its immunomodulatory effects [1,5-7]. Enhanced immune response to the tumor after CP treatment is primarily attributed to selective exhaustion [6] and functional inhibition [7] of $CD4^+CD25^+$ regulatory T cells suppressing the adaptive and innate antitumor reactions and causing the formation of tumor tolerance. In addition, CP suppressing proliferation of lymphocyte clones involved in the immune response is more active towards B cells [1,3] stimulating the growth of malignant tumors [2,8]. Reduced count of macrophages after CP treatment of experimental animals [5] and induction of thrombospondine-1 (potent inhibitor of angiogenesis [4]) gene and expression of its protein in endothelial cells can contribute to the antitumor effect of CP injected to animals.

Injection of CP 24 h before transplantation of a xenogenic tumor stimulated tumor formation and metastases in nude mice [10] and rats [9], *i.e.* CP promoted reduction of antitumor immunity not linked with the T cell system. It remains unclear to what measure the CP antitumor effect depends on its effects on the innate and adaptive immunity cells involved in tumor-associated immune reaction. One more aspect deserving research is the time course of antitumor resistance after CP injection.

We studied changes in antitumor resistance after CP injection to animals which had no tumor by the moment of drug injection. The antitumor reaction in this case was induced primarily by mechanisms of nonspecific immunity (as there were no tumor-associated antigens and the adaptive response did not develop by the moment of CP injection) and the possible effects of CP on antigen-specific lymphocyte clone precursor cells.

MATERIALS AND METHODS

The study was carried out on 2-month-old female Af mice (21.0±0.15 g, n=160). The animals were kept in vivarium at 20±1°C with free access to water and food. The lifespan of animals after intraperitoneal transplantation of Ehrlich's ascitic carcinoma (EAC) in a

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dose of 0.5×10^6 cell/mouse was evaluated. Animals of experimental groups were intraperitoneally injected with CP in a single dose of 100 mg/kg 1, 3, 7, 10, 14, and 22 days before tumor transplantation, or received two doses 7 and 1 days before EAC transplantation, or were injected with CP 1 day after tumor cell transplantation (17 mice were used in each series). Controls (n=24) received no CP. Due to injection of CP 1 day and more before tumor transplantation, the tumor cells were not directly exposed to active metabolites of the drug, because its half-life in rodent plasma was 17 min [9,10]. Hence, CP effect on EAC under our experimental conditions was due to its immunomodulatory activity.

RESULTS

The lifespan of animals with tumors after intraperitoneal transplantation of 0.5×10^6 EAC cells was 25.0±1.0 days. Injection of CP 1 day before tumor transplantation reduced mouse lifespan: they survived 20.5±0.5 days after intraperitoneal transplantation of EAC (17% less than the controls; p<0.05; Fig. 1). Injection of CP 3, 7, 10, 14, and 22 days before EAC transplantation prolonged the lifespan of tumor-bearing animals, which lived for 29.9±2.3, 28.6±1.1, 35.4±1.7, 32.2±1.8, and 32.9±1.1 days, respectively (20, 14, 42, 29, and 36% longer than controls with EAC, p<0.05 in all cases). Two injections of CP (7 and 1 days before tumor transplantation) leveled the lifespan prolongation effect observed after CP injection 7 days before the tumor transplantation: the lifespan of animals with tumors slightly decreased in comparison with the control (by 11%; p>0.05) and was 22.3±1.1 days. Tumor growth after transplantation was noted in all experimental series. More rapid growth of tumors transplanted 1 day after CP injection was presumably due to impairment of the immune system and reduction of the counts and/or functional activity of effectors of innate antitumor immunity, the main of which being NK and NKT cells [6,11]. One more possible cause of this shift could be damage or suppression of naive T cells (cytotoxic T cell precursors), which could inhibit the development of the adaptive response. The increase of antitumor immunity after CP injection could be due to slower restoration of cells responsible for the formation of oncotolerance or their higher sensitivity to CP than of cells with antitumor functions.

The lifespan was maximum (44.1±5.2 days; 76% longer than in the control; p<0.05) in animals injected with CP 1 day after tumor transplantation. The tumors did not develop in 4 mice (incidence of tumor growth after transplantation was 71%). More pronounced inhibition of EAC growth (40-62%) in animals injected with CP 1 day after tumor cell transplantation in comparison with CP injection 3-22 days before tumor transplantation was presumably due to the direct effect of active drug metabolites on tumor cells.

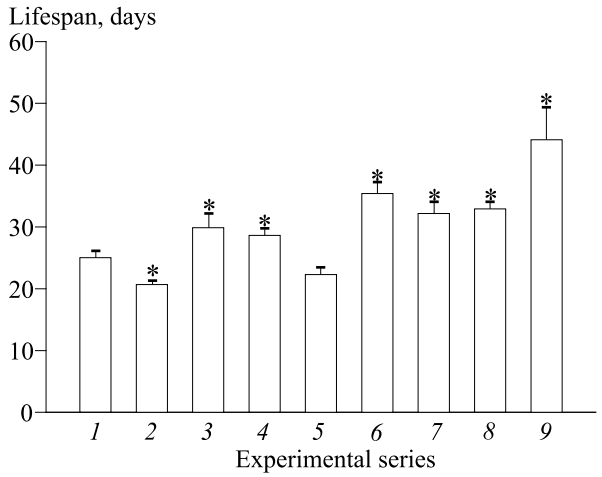

Fig. 1. Effects of CP on lifespan of mice with transplanted EAC. *1*) EAC; *2*) CP+EAC after 1 day; *3*) CP+EAC after 3 days; *4*) CP+EAC after 7 days; *5*) CP 7 days before+CP 1 day before EAC; *6*) CP+EAC after 10 days; *7*) CP+EAC after 14 days; *8*) CP+EAC after 22 days; *9*) EAC+CP after 1 day. p<0.05 in comparison with *1*.

Hence, the data indicate that CP injection to animals was followed by periods when the immunity status promoted the tumor development and periods when tumor resistance increased. The immunity was suppressed 1 day after CP injection in a dose of 100 mg/kg and was restored and increased 3-22 days after CP.

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