Inhibitor of Indoleamine-2,3-Dioxygenase 1-Methyl-D-Tryptophan Can Stimulate the Growth of Immunogenic Tumors

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We studied the effect of 1-methyl-D-tryptophan, an inhibitor of indoleamine-2,3-dioxygenase, on the growth of transplanted hepatocarcinoma-29 in C3HA mice. Hepatocarcinoma-29 transplanted into the thigh muscles undergoes immunological rejection in more than 50% non-syngeneic recipients. Chronic local administration of 1-methyl-D-tryptophan promotes progressive growth of the tumor in recipient mice leading to 100% animal death. The stimulating effect of 1-methyl-D-tryptophan on tumor growth is discussed.

Key Words: *mice; hepatocarcinoma-29; indoleamine-2,3-dioxygenase; 1-methyl-D-tryptophan; stimulation of tumor growth*

After the role of tryptophan catabolism in non-rejection of allogeneic conceptus by maternal organism was demonstrated [9], numerous reports devoted to elucidation of the mechanism of this phenomenon appeared. It was found that indoleamine-2,3-dioxygenase (IDO), an enzyme present in trophoblast cells and catabolizing tryptophan by the kynurenine pathway, creates local tryptophan deficiency against the background of excess of tryptophan catabolism products toxic for T cells [3,7]. Experimental inhibition of IDO in pregnant mice leads to rejection of allogeneic, but not syngeneic conceptuses [9]. For oncologists, this phenomenon helps to understand how deliberately immunogenic tumors can avoid immunological attack of the organism. Indeed, IDO is expressed in cells of various tumors and macrophages infiltrating them; high activity of the enzyme in these cases is usually associated with poor prognosis [4,8,11].

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Inhibitors of IDO reduce growth rate of these tumors [12] and in combination with chemotherapeutic drugs they sometimes potentiate their effects [5]. Synthetic tryptophan analog 1-methyl-D-tryptophan (1-MT) is usually used as IDO inhibitor; it is daily injected intraperitoneally or is subcutaneously transplanted in special capsules ensuring gradual release of the preparation into the blood. Daily dose of 1-MT in these cases attains 800 mg/kg body weight [5]. To overcome IDO-related immunoresistance of the tumor, activity of the enzyme only in tumor cells and infiltrating macrophages should be suppressed; therefore, we hypothesized that this can be attained by considerably lower doses of the inhibitor due to its local administration into the region of tumor location.

Here we verified this assumption on the model of deliberately immunogenic transplantable murine tumor hepatocarcinoma-29 (H-29). This tumor originates from CBA mice [1], but can be transplanted to 100% C3HA mice; in the majority of them the tumor soon stops grow and regresses. We expected that inhibition of IDO by regular administration of 1-MT into the tumor will increase the number of mice with tumor regression.

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MATERIALS AND METHODS

We performed transplantation of 5×10^5 H-29 tumor cells in 0.1 ml physiological saline to 2-month-old male C3HA mice (n=16) into thigh muscles. One day after transplantation, 2 groups were formed, each included 8 mice. Experimental group mice received injections of 1-MT (1 mg in 0.1 ml physiological saline with Tween-80) into the site of transplantation. Controls received an equivalent volume of physiological saline with Tween-80 according to the same scheme. A total of 13 injections were made. The tumors appearing in the thigh muscles were regularly measured with a caliper and the tumor volume was calculated. The animals were observed until their death and the lifespan from the moment of tumor cell transplantation and tumor weight were recorded.

The results were processed using standard methods of variation statistics, significance of differences was determined using Student t test.

RESULTS

In both groups, the tumors simultaneously appeared in all animals and on day 7 after transplantation their mean volumes in the control and experimental groups were 0.320±0.044 and 0.270±0.033 cm^3, respectively. During the next week, the tumors grew with the same rate in both groups and on day 13 after transplantation their volumes were 0.640±0.073 and 0.63±0.14 cm^3, respectively. In 6 control mice, the tumor growth was arrested (their mean volume 1 month after transplantation was 0.50±0.22 cm^3), while in 2 mice the tumors continued to grow and led to animal death on days 47 and 65 after transplantation (tumor weight by the moment of death was 4.3 and 6.5 g, respectively). Of 6 mice with tumor growth arrest, one mouse died on day 27; an ulcerated necrotized tumor node (evidently, at the stage of regression) weighing 0.7 g was found.

Intramuscularly transplanted H-29 tumor, nonsyngeneic for 8 control mice, progressively grew and caused death of 3 animals (37.5%), while in 5 mice (62.5%) the tumor completely regressed after a short-term growth. Immunological mechanism of regression is confirmed by the absence of tumor growth in mice after repeated transplantation of H-29 cells. In contrast, transplanted tumors progressively grew in mice of the experimental group receiving 1-MT and after 1 month attained a volume of 6.00±1.53 cm^3 *vs*. 0.5 cm^3 in the control. All experimental mice died 41.0±2.7 days after transplantation (Fig. 1), the mean tumor volume by the moment of death was 6.90±0.93 cm^3.

This effect, in particular, stimulation of tumor growth under conditions of chronic local treatment with 1-MT cannot be caused by general or selective toxic effect of the preparation on tumor cells. The daily dose of 1-MT in our experiment was 20-fold lower than in systemic administration [5]. After 9 injections of 1-MT or physiological saline (day 13 of the experiment), the tumor weight in mice of the control and experimental groups were similar and body weight in both groups decreased by less than 10% (7.7 and 8.1%, respectively) from the initial value. The selective toxic effect of 1-MT on tumor cells also should be excluded, because we observed stimulation of tumor growth in mice of the experimental group. Experiment on IDO-knockout animals showed that 1-MT had a selective effect on certain cells of the lymphoid system (1-MT protects these cells from IDO-related deficit of tryptophan and accumulation of its toxic metabolites) [6].

The effect of 1-MT on tumor growth observed in our experiment is a result of protection of lymphoid cells determined by IDO inhibition. At the first glance it seems paradoxically that 1-MT does not inhibit, but even stimulates tumor growth. However, it is known that the tumor develops under conditions of the so-called "pathological immunological preference" [2]; the immune response to tumor is complex and apart from inhibitory influence can include a stimulating component (the well-known phenomenon of immunological stimulation of tumor growth) [2,10]. Therefore, stimulation of the immunity in tumor carriers can led to both suppression and stimulation of tumor growth. IDO suppresses not only T-effectors, but also T-regulators promoting tumor growth and also acts as an antagonist of other mechanisms regulating antitumor immunity [2]. Therefore, inhibition of IDO, as is seen from our results, can sometimes stimulate, but not inhibit tumor growth.

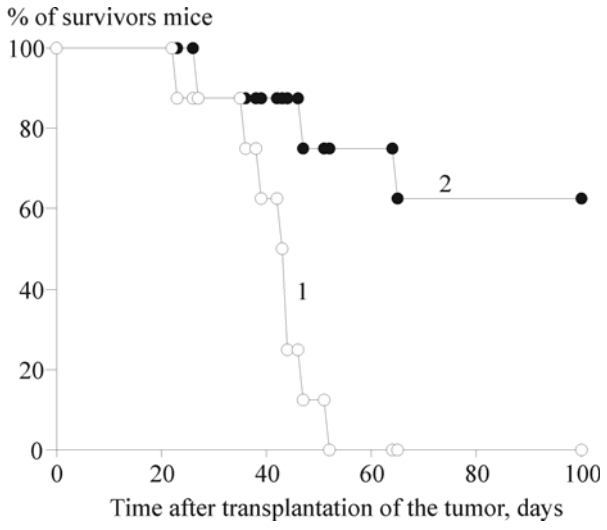

Fig. 1. Survival of C3HA mice with transplanted non-syngeneic tumor H-29 after local administration of IDO inhibitor 1-MT (*1*) or physiological saline (*2*).

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