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Time Course of the Binding of Some Prostanoids in the Serum of Mice with Lewis Lung Carcinoma with Spontaneous Metastases. Effect of Exogenous Prostaglandins on Tumor Dissemination

L. L. Mikhailevskaya, V. G. Vavilova, L. A. Selezneva, and A. S. Kinzirskii

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It is shown that the ability of serum to bind some prostanoids changes depending on the stage of development of the primary tumor node. Surgical removal of the tumor modulates the binding of TxB_2. When the PGE_2 content increases in the organism, the PGE_2 binding in the serum is stepped up, and dissemination is enhanced.

Key Words: *Lewis lung carcinoma; metastasis; prostaglandins*

Prostaglandins (PG) are involved in the primary and secondary neoplastic processes [6], but the specific mechanisms of their action are not always clear.

The aim of the present study was to investigate the effect of prostaglandins E_2 and $F_{2\alpha}$ on the growth and spontaneous dissemination of Lewis lung carcinoma (LLC), as well as the organism's competence with respect to these prostanoids and TxB_2.

MATERIALS AND METHODS

The experiments were carried out on C57Bl/6 and BDF_1 mice of both sexes, with an initial weight of 19-23 g. Transplantation of tumor material from LLC was performed subcutaneously or intramuscularly by the routine method [3]. The primary tumor node was surgically removed on day 8 after transplantation. The mice were killed (diethyl ether) on days 21-25 of tumor carriership. After the mice had been killed, the weight of the primary tumor was recorded and the lungs were excised, divided into lobes, and placed in Bouin's fluid (time of exposure not less than 24 h). After fixation, the number of surface metastatic nodes was counted using an MBS-9 microscope (8×2). The rate of metastasis was assessed in terms of its incidence (the number of animals with metastases in % of the total number of mice in the group) and its severity (the mean number of metastatic nodes per mouse). The index of metastasis inhibition (IMI) was calculated by the formula:

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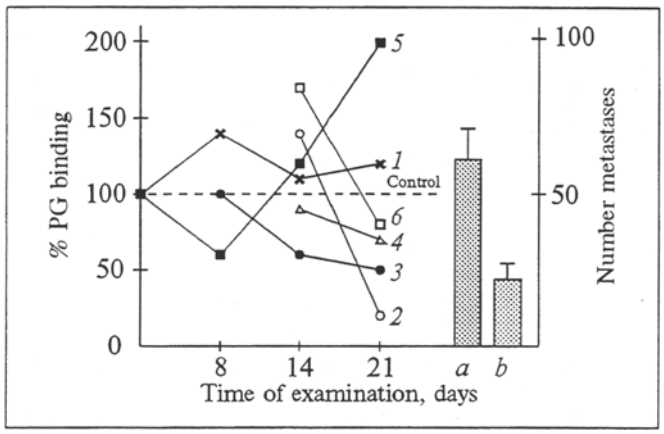

Fig. 1. Binding of PGE_2, $PGF_{2\alpha}$, and TxB_2 in serum of tumor-bearing mice with LLC at different times of tumor growth and development and after its surgical removal. *1*) PGE_2 in normal tumor carriers; *2*) the same in operated carriers; *3*) $PGF_{2\alpha}$ in normal tumor carriers; *4*) the same in operated carriers; *5*) TxB_2 in normal tumor carriers; *6*) the same in operated carriers. *a*: number of metastases per animal on day 21 of tumor carriership; *b*: the same in operated animals on day 12 postoperation.

$$IMI = \frac{(A_c B_c) - (A_c B_c)}{A_c B_c} \times 100\% \qquad [1],$$

where A_c and A_c are the incidence of metastases in the experiment and in the control, while B_c and B_c, respectively, are the mean number of metastases per mouse for the same groups.

The effect of PGE_2 and $PGF_{2\alpha}$ on the tumor growth and metastasis of LLC was studied under conditions of repeated intraperitoneal injections of the substances in doses of 30-300 μg/kg. The protocol of injections was as follows: from day 1 to day 8, from day 8 to day 17, and from day 1 to day 21 of tumor carriership, twice a day, the interval between injections being 6 h.

The level of spontaneous binding of PGE_2, $PGF_{2\alpha}$, and TxB_2 was measured in the serum on days 8, 14, and 21 posttransplantation. For this purpose, blood was taken from the retroorbital sinus, the serum was obtained, and the binding was assayed radioimmunologically [2]. The serum was used as the source of antibodies. Aliquots of serum (100 μl) were incubated with ^3H-labeled PG (10,000 cpm, 100 μl) in 50 mM Tris-HCl buffer, pH 7.8, containing 0.25% gelatin. The exposure time was 2 h. The total volume of the sample was 400 μl. After incubation at room temperature, a suspension of dextran-coated carbon (Tris-HCl + 400 mg carbon + 80 mg dextran) was added in a volume of 200 μl to each sample, and the samples were shaken in a vortex (10 min) and centrifuged for 15 min at 4°C (5000 g). Aliquots (400 μl) of the supernant obtained were transferred to vials each containing 5 ml of ZhS-8 scintillation liquid, and radiation was then determined with a counter.

The results were statistically processed using Student's *t* test; the differences were regarded as reliable at a probability level of 95%.

RESULTS

The experiments showed that at early or late stages of tumor carriership metastasis was slightly stimulated by PGE_2 in doses of 100-300 μg/kg (Table 1). However, no reliable differences were found for these doses, which attests to a stable tendency for metastasis to increase under the influence of PGE_2 in high doses. This phenomenon may be associated with the immunodepressive effect of PGE_2, as well as with the direct effect of the prostanoid on the cells of LLC. These findings are in accord with data of Young *et al.* [7-9], who found that metastasis of LLC is aggravated against the background of 16,16-dimethyl PGE_2, a stabilized PGE_2 analog. Cells of LLC with a high metastatic status have been shown to migrate more intensively in the presence of PGE_2 in the culture medium.

The results presented in the table show that PGE_2 exerted no marked effect on the spontaneous metastasis of LLC. Evidently, this substance plays a less important role in the invasion of LLC.

It was established that under these conditions neither prostanoid affected the growth of the primary tumor node, irrespective of whether the tumor material was transplanted subcutaneously or intramuscularly.

The binding of PGE_2 rose slightly on day 8 after tumor transplantation and then dropped almost to the control level (Fig. 1). Surgical removal of the tumor resulted on day 21 in a sharp drop of the PGE_2 binding, although on postoperative day 6 the amount of bound PG was markedly higher than that in the control. Days 7-8 are known to be critical for spontaneous metastasis of LLC, since it is during this period that tumor cells are released from the primary node [5]. The findings of Young *et al.* showed that there is a relationship between LLC and the PGE_2 content in the organism [9]. LLC cells which produce an appreciable amount of PGE_2, as well as cells for which this prostanoid is an attractant, were discovered [7]. Our results corroborate the relationship between the level of PGE_2 binding and the severity of tumor invasion. Removal of the primary tumor node of LLC lowered the number of metastases and reduced the amount of bound PGE_2

TABLE 1. Effect of Repeated Intraperitoneal Injections of Exogenous PG on Growth and Spontaneous Metastasis of LLC in Mice ($M \pm m$)

Group	Dose, μg/kg	Protocol of injection, days	Tumor growth		Metastasis	
			weight of primary tumor, g	% inhibition of tumor growth	number of metastases per animal	IMI, %
LLC*	–	−8.3+0.6 (12)	–	58+12 (12)	–	
PGE_2	300	1−8	8.0+0.5 (8)	3.6	93+19 (8)	−60
	100	1−8	6.5+0.6 (8)	22.0	59+11 (8)	−2
	30	1−8	7.4+0.7 (8)	11.0	61+7 (8)	−5
PGE_2	300	8−21	8.4+0.7 (8)	−1.0	99+11 (8)	−71
	100	8−21	8.0+0.3 (8)	3.6	72+32 (8)	−24
	30	8−21	7.8+0.7 (8)	6.0	78+15 (8)	−34
LLC*	–	–	11.1+0.2 (15)	–	67+12 (14)	
PGE_2	30	1−21	10.2+0.4 (15)	8.0	57+14 (15)	15
LLC**	–	–	9.5+2.5 (10)	–	36+6 (10)	
$PGF_{2\alpha}$	30	1−8	9.9+2.6 (8)	−4.0	42+6 (8)	−17
	30	8−21	9.7+3.5	−2.0	28+5 (9)	22
LLC*	–	–	11.1+0.2 (15)	–	67+12 (15)	
$PGF_{2\alpha}$	30	1−21	11.2+0.3 (15)	−1.0	47+7 (15)	30

Note. One and two asterisks, respectively, indicate experiments performed on male C57Bl/6 mice (tumor inoculated subcutaneously) and on female BDF_1 mice (tumor inoculated intramuscularly).

in the serum. This leads to the conclusion that the titer of autoantibodies against PG drops and the PGE_2 concentration in the blood decreases. These results are in line with the findings of Chiabrando *et al.* [4], who demonstrated that by day 23 of LLC carriership the PGE_2 concentration had risen almost 4-fold.

It was shown that the binding of $PGF_{2\alpha}$ by the serum of mice with LLC and of the animals in which the tumor was removed is lower than that in the case of intact animals. Probably, the number of antibodies against this prostanoid is reduced in the serum of tumor carriers. It seems likely that, in contrast to TxB_2, $PGF_{2\alpha}$ does not play an important role in the biology of LLC development.

It is worthy of note that during the critical period of metastasis, the TxB_2 binding was markedly lower than in the control, whereas later it rose sharply. Reportedly, there is a direct relationship between the thromboxane level in the organism and the metastasis of Lewis carcinoma [4]. Removal of the tumor markedly lowered the TxB_2 binding, which was indicative of a drop in the anti-TxB_2-antibody titer. This in turn suggested that the TxB_2 concentration in the blood was also reduced.

Thus, our studies demonstrated that LLC is sensitive to the PGE_2 level in the organism. The PGE_2 concentration, the titer of anti-PGE_2-antibodies, and the severity of metastasis correlate with each other: as the PGE_2 concentration increases, the antibody titer rises and metastasis is aggravated. Similar regularities are possible in the case of TxB_2, but not in the case of $PGF_{2\alpha}$.

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