ENHANCEMENT OF ANTITUMOR IMMUNITY BY THYMOSIN IN HYPOCORTICALISM

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Bilateral adrenalectomy and administration of thymosin (fraction 3) after adrenalectomy inhibit to different degrees the development of mammary gland tumors induced in rats by 7,12-dimethylbenzanthracene. The maximal effect was observed from the use of thymosin in conjunction with hypocorticalism; there was a substantial reduction in the frequency of developing neoplasms, the latent period of their development was lengthened, and their growth retarded.

KEY WORDS: induced tumor; thymosin; adrenalectomy.

An important role in the pathogenesis of malignant neoplasms is ascribed to disturbances of immunity reactions mediated by T lymphocytes. Thymosin, extracted from calf thymus, has the ability to restore the activity of cell-mediated immune reactions if these are inadequate [4, 10]. This effect is due to the ability of thymosin to convert precursor T cells into T lymphocytes and to stimulate their maturation to effector cells [3, 9]. Glucocorticoids are antagonists of thymosin as regards their effect on lymphocytes and, for that reason, a fall in their body level prolongs and enhances the restorative action of thymosin on reactions of cellular immunity [1, 6].

The object of this investigation was to study the effect of thymosin on the development of induced mammary gland tumors in adrenalectomized rats.

EXPERIMENTAL METHODS

Experiments were carried out 145 noninbred female rats. Mammary gland tumors were induced by intravenous injection of 7,12-dimethylbenzanthracene (DMBA) as a specially prepared emulsion. Injection of the carcinogen (in a dose of 2 mg per rat) was repeated three times at intervals of seven days. Adrenalectomy was performed on some of the animals 14 days after the end of DMBA administration, and 10 days later a course of thymosin or bovine serum albumin (BSA) was started. Thymosin (fraction 3), isolated from the calf thymus [5], or BSA was injected intramuscularly in a dose of 10 mg per rat every 10 days for nine injections. The group of animals receiving BSA was used as the control, excluding any effect of thymosin on the development of mammary gland tumors because of its protein nature.

The dynamics of tumor development was studied for seven months after the end of DMBA injection. The time from the last injection of the carcinogen until the appearance of a palpable tumor was taken as the latent period of tumor development. The volume of the tumor was calculated by Schreck's formula [8]. The rate of growth of the neoplasms was determined from the difference between their volumes every 2 weeks. The numerical data were subjected to statistical analysis by Student's method and the method of alternative variation.

EXPERIMENTAL RESULTS

Data showing the effect of the various procedures on the frequency of appearance of mammary gland tumors and the latent period of their development are given in Table 1.

In the control series neoplasms appeared in 27 of the 34 animals (79.4%), on average 96.9 ± 11.1 days after the end of DMBA administration. Adrenalectomy shortened the number of tumors appearing by the

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TABLE 1. Dynamics of Appearance of Mammary Gland Tumors in Experimental Animals

Experimental conditions	Number of rats	Frequency of appearance of tumors, %	Distribution (in %) of developing tumors depending on duration of latent period*			Latent period, days (M ± m)
			under 3 months	under 5 months	under 7 months	
Injection of DMBA (control)	34	79,4	56,6	20,0	23,4	96,9±11,1
Injection of DMBA + adrenalectomy	29	55,2†	70,6	11,8	11,6	89,5±13,7
Injection of DMBA + thymosin	31	61,1	42,1	31,6	26,3	118,0±14,1
Injection of DMBA + adrenalectomy + thymosin	34	41,2†	46,7	0†	53,3†	128,2±24,0
Injection of DMBA + BSA	17	82,3	78,6†	21,4	0†	58,8±6,0†

*Percentage calculated relative to total number of tumors appearing in rats of that group 7 months after end of DMBA administration.
†Difference from control value statistically significant.

TABLE 2. Effect of Different Procedures on Rate of Growth of Induced Mammary Gland Tumors in Rats

Time after appearance of tumors, weeks	Increase in volume of tumors (in cm^3) in separate groups of animals (M ± m)				
	injection of DMBA	injection of DMBA + adrenalectomy	injection of DMBA + thymosin	injection of DMBA + adrenalectomy + thymosin	injection of DMBA + BSA
2	0,70±0,11 (21)	1,05±0,41 (11)	1,51±0,55 (14)	0,55±0,15 (14)	0,45±0,04 (14)
4	1,65±0,35 (20)	0,55±0,15* (11)	1,22±0,51 (13)	1,32±0,45 (13)	1,30±0,25 (14)
6	3,15±0,60 (18)	1,72±0,35* (8)	1,25±0,55* (10)	0,51±0,25* (8)	3,31±0,8 (14)
8	4,85±2,30 (15)	0,01±0,58* (6)	1,82±0,85 (9)	0,11±0,1* (5)	2,73±1,05 (11)
10	2,90±0,51 (13)	1,65±0,42* (5)	0,81±0,45* (8)	0,12±0,31* (5)	2,35±0,9 (10)
12	1,82±0,91 (9)	1,91±0,82 (5)	1,22±0,61 (7)	0,82±0,65 (5)	3,10±1,15 (9)

Legend. 1. Number of animals shown in parentheses. 2. Asterisk indicates that difference compared with control is significant.

seventh month after the end of injection of the carcinogen by 24.2% ($P < 0.05$) but had no significant effect on the latent period of their development.

Administration of thymosin to the intact animals did not result in any statistically significant difference in the frequency of appearance of mammary gland tumors or in the latent period of their development. Administration of thymosin after adrenalectomy potentiated the resistance of the mice to tumors, as shown by absence of appearance of mammary gland neoplasms in the rats between 3 and 5.5 months after the end of DMBA injections. The total number of tumors in this group of animals was 38.2% less than in the control ($P < 0.05$). The mean latent period of their development was 118 ± 14.1 days.

Injections of BSA caused a sharp increase in the rate of tumor development. The carcinogenic action of DMBA on the mammary glands after injection of BSA was effective in the course of 5 months: The mean latent period of development of the tumors under these circumstances was 58.8 ± 6 days ($P < 0.05$).

In 40-50% of cases the animals of the first four groups developed several tumor nodules. The procedures used, except injection of BSA, did not affect the number of tumor nodules formed. The mean number of tumors per tumor-bearing rat in the different groups of animals varied from 1.47 to 1.65. The number of animals with multiple tumors increased after the use of BSA to 64.3%, and the mean number of neoplasms per rat in this case was 2.28 ± 0.4%.

Intensification of chemical carcinogenesis in the mammary glands of rats by BSA may indicate that thymosin increases antitumor resistance of the recipient not because of the injection of foreign protein, but because of activation of cell-mediated immunity reactions.

The results of the study of the effect of the procedures used on the rate of growth of the mammary gland tumors are given in Table 2.

With the exception of BSA injections, all the procedures used delayed growth of mammary gland tumors at different times after their appearance. The most marked slowing of tumor growth was observed in the adrenalectomized rats, especially in those subsequently receiving thymosin. The rate of tumor growth in animals receiving BSA after the end of the DMBA injections was indistinguishable from that in the control.

The results of these experiments thus confirmed the view that the antitumor resistance of animals is enhanced by administration of thymosin in the presence of hypocorticalism. In the writers' view the effect of these therapeutic tactics on the development of induced mammary gland tumors in rats can be attributed to strengthening predominantly of cell-mediated immunity on account of removal of the limiting influence of glucocorticoids on the lymphoid system. Hypocorticalism in rats after adrenalectomy, it will be noted, is temporary in character for these animals have accessory adrenal tissue.

Considering that progressive growth of tumors is frequently accompanied by increased secretion of glucocorticoids [2, 7], the writers suggest that immunotherapy with thymosin against a background of temporary depression of hormone formation in the adrenal cortex (by means of chloditan, for example) will result in the more effective treatment of cancer patients.

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INSULIN AND GLUCOCORTICOID LEVELS IN ANIMALS WITH TRANSPLANTED TUMORS

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In mice with Ehrlich's ascites carcinoma and in rabbits with Brown–Pearce carcinoma, hypoglycemia was shown not to be the result of hyperinsulinemia. In rats with Zajdela's ascites hepatoma normoglycemia was the rule, and their blood insulin level did not change during growth of the tumor. The fall in the blood glucose level of rabbits with tumors was not due to weakening of the glucocorticoid function of the adrenals.

KEY WORDS: tumor; hypoglycemia; gluconeogenesis; insulin; glucocorticoids.

Profound hypoglycemia sometimes develops in cancer patients [8] and in animals with tumors [11, 14]. However, the pathogenetic mechanism of this phenomenon has not yet been explained. The view has been expressed that hypoglycemia in hormone-independent tumors is the result of excessive secretion of insulin of pancreatic or ectopic origin [7, 9]. Meanwhile there is conflicting evidence on the combination of hypogly-

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