THE EFFECT OF ANTITUMOR SERUM ON THE INTENSITY OF CELL DIVISION IN TUMORS

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Several workers [1, 3, 6, 7, 8, 11, 12] have shown that antitumor sera lengthen the latent period of development of cancer, and retard the growth and progress of tumors and formation of metastases.

It has also been shown [2, 9, 10] that antitumor serum, when injected into experimental animals with developing tumors (Ehrlich's adenocarcinoma) has a depressing action of the mitotic activity of tumor cells in comparison with sera of different specificity.

As a rule, however, the antitumor sera used experimentally contained, besides antibodies against the tumor, antibodies against normal tissues.

The aim of the present work was to compare the action of an adsorbed antitumor serum, containing mainly antibodies against the tumor, with that of ordinary unadsorbed antitumor serum on the intensity of division of the tumor cells in a subcutaneous Ehrlich's adenocarcinoma.

As a control we used the serum of a normal, nonimmune rabbit and sera against the liver and spleen of a healthy mouse. The latter appeared desirable in view of the antigenic closeness of the spleen and the tumor [4]. The mitotic activity was studied, besides in the tumor, in the corneal epithelium of the same experimental animals in order to ascertain the effect of sera of different specificity on it.

EXPERIMENTAL METHOD

The sera used in the experiments were obtained by immunization of rabbits of the chinchilla variety with saline extracts of the ascitic cells of an Ehrlich's adenocarcinoma and of the liver and spleen of a healthy mouse.

The rabbits were immunized by means of 6 intravenous injections at intervals of 3 days.

The sera obtained were tested by means of the complement fixation reaction at a temperature of 37° for the height of the titer of the antibodies contained in them and for their specificity. The experiments comprised series I and II.

The following sera were used in the experiments in series I:

1). Adsorbed antitumor serum which reacted with tumor antigen in a dilution of 1:160 + + +, spleen 1:40 + + and liver 1:40 ±.

2). Unadsorbed antitumor serum with a titer of antitumor antibodies of 1:400 + + +, antispleen 1:200 + + and antiliver 1:100 ±.

3). Antispleen serum with a titer of antispleen antibodies of 1:200 + + +. It reacted with tumor antigen in a dilution of 1:100 + + but did not react with liver antigen in dilutions of 1:100 or higher.

4). Serum of a normal, nonimmune rabbit, containing no antibodies against the antigens tested.

TABLE 1

Mitotic Coefficient (MC) in Mouse Tumors in Series I Experiments after Three Injections of Sera (per 1000)

Mouse No.	Sera				Control (nothing injected)
	absorbed antitumor 1:160 + + +	unadsorbed antitumor 1:400 + + +	antispleen 1:200 + + +	normal rabbit's	
	1st group	2nd group	3rd group	4th group	5th group
1	0.70	4.08	7.97	6.60	8.75
2	4.48	4.17	8.82	7.38	10.90
3	7.36	4.65	10.55	9 06	11.41
4	7.71	4.99	10.93	9.47	11.50
5	7.77	5.36	11.14	10.40	11.50
6	8.22	5.66	11.51	11.60	12.13
7	8.36	7.04	12.23	13.87	18,14
8	Necrosis of tumor	7.43	14.56	18.03	
Mean MC	6,37	5,42	10,96	10,78	12,04

Probability of chance variation of mean values of:

1 and 3 groups $P=0,003$ — 2 and 3 groups $P=\infty$
1 and 4 groups $P=0,027$ — 2 and 4 groups $P=0,002$
1 and 5 groups $P=0,003$ — 2 and 5 groups $P=\infty$

It must be pointed out that the height of the titer of antitumor antibodies in the adsorbed antitumor serum and of antispleen antibodies in the antispleen serum were on roughly the same level.

The following sera were used in the experiments in series II.

1). The adsorbed antitumor serum had identical immunological characteristics with the analogous serum used in series I.

TABLE 2

Mitotic Coefficient (MC) in the Corneas of the Mice in Series I Experiments after Three Injections of Sera (per 1000)

Mouse No.	Sera				Control (nothing injected)
	absorbed antitumor 1:160 + + +	unadsorbed antitumor 1:400 + + +	antispleen 1:200 + + +	normal rabbit's	
	1st group	2nd group	3rd group	4th group	5th group
1	2.64	2.02	2.47	1.38	3.75
2	2.64	2.92	2.63	2.34	3.80
3	3.97	3.45	2.92	3.86	3.94
4	4.21	3.90	3.02	3.87	4.08
5	4.67	4.19	3.43	3.87	4.13
6	4.74	4.19	4.23	4.27	6.92
7	4.90	4.62	4.56	4.56	Preparation spoiled
8	5.41	5,70	5.09	6.01	
Mean MC	4.14	4.12	3.54	3.77	4.44

2). The unadsorbed antitumor serum reacted with tumor antigen in dilution of 1:800 + + +, with spleen 1:400 + + + and liver 1:100 +.

3). The antispleen serum had a titer of antispleen antibodies of 1:800 + + +, antitumor antibodies 1:200 + + and antiliver 1:100 ±.

4). The antiliver serum reacted with homologous antigen in a dilution of 1:800 + +, with spleen antigen in a dilution of 1:200 + +, and did not react at all with tumor antigen in dilutions of 1:100 and higher.

5). The serum of the normal normal nonimmune rabbit contained no antibodies against the antigens tested.

Thus in the experiments of series II, the height of the titer of antitumor antibodies in the unadsorbed antitumor serum, of antispleen antibodies in the antispleen serum and of antiliver antibodies in the antiliver serum were roughly the same.

The sera were adsorbed by the method introduced in Prof. P. N. Kosyakov's laboratory [5], with a mixture of formalinized liver and spleen tissue, but we took whole serum and not serum diluted 1:10 for the adsorption.

In the two series of experiments we used altogether 100 male white mice with an Ehrlich's adenocarcinoma implanted under the skin of the dorsal region. Of these mice, 39 were in series I of the experiments and were divided into 5 groups. One group (7 mice), which received no injections, acted as controls, and the remaining 4 groups (with 8 mice in each) were injected with one of the above-mentioned sera. In series II of the experiments 61 mice were used, and these were divided into 6 similar groups.

The sera were injected three times, on alternate days starting on the 8th day after implantation of the tumor material, subcutaneously in doses of 0.5 ml at some distance from the tumor.

All the experimental animals were killed at the same time, i.e., in the morning 20 hours after the last injection of serum.

The tumor was fixed in Carnoy's fluid. The corneas were fixed in a 5% solution of acetic acid in 70° alcohol. Sections, 8 μ in thickness, were cut from the tumor, and total preparations of the cornea were made. In both cases staining was by Carazzi's hematoxylin.

After counting the mitoses, we calculated the mitotic coefficient (MC) for each test object, which expressed the ratio between the dividing cells and the total number of cells examined (per 1000).

We also calculated the coefficient K (the ratio between the total number of early phases of division and the late phases).

The experimental results were treated statistically. The difference between the values compared was regarded as significant if the value of P was not greater than 0.01.

EXPERIMENTAL RESULTS

The results of the counting of mitotic activity in the mouse tumors of all the experimental groups of the series I experiments showed that the mean percentage of the different phases of division was approximately the same for each group (P ≈ 1-2%; M ≈ 78-83%; A ≈ 3-4%; T ≈ 11-17%). The ratio between the total number of early phases of division and the late phases was also on roughly the same level (K ≈ 4-5), from which it could could be concluded that the tempo of cell division was approximately equal in both the control and the experimental animals. We therefore judged the intensity of cell division mainly by the value of the mitotic coeffient.

The results given in Table 1 show that the values of the mitotic coefficient in the 1st and 2nd experimental groups, in which antitumor (adsorbed and unadsorbed) sera were injected, were the least and were roughly equal (6.37 and 5.42 respectively).

In the remaining groups the value of the mitotic coefficient was much higher (3rd group 10.96, 4th group 10.78, 5th group 12.04). The values of the mitotic coefficient in the groups in which adsorbed and unadsorbed anti-tumor sera were injected were evidence of the identical action of these sera on the intensity of division of the tumor cells.

The results of the counting of mitotic activity in the corneal epithelium of the experimental animals of the series I experiments showed that the mean percentage of different phases of division in the various experimental groups showed slight variations (P ≈ 18-30, M ≈ 34-48, A ≈ 3-5 and T ≈ 23-30). Nevertheless the ratio of the total number of early phases of division to that of the late phases was roughly the same (K ≈ 2-2.6).

The values of the mitotic coefficient are shown in Table 2, from which it can be seen that the mitotic coefficient in all 5 groups varied between narrow limits (4.14, 4.12, 3.54, 3.77 and 4.44). Consequently, no essential change in the mitotic activity could be observed in the corneal epithelium under the influence of sera of different specificity.

The results of the series II experiments were in the main analogous with those obtained in series I. An exception was the group of mice which received an injection of antispleen serum, since the majority of the mice of this group (8 of 11) dies after the injection of this serum with signs of cytotoxic shock. This was evidently due to the excessively high titer of antispleen antibodies (1:800 + + +).

Our findings thus showed that an adsorbed antitumor serum, containing antibodies mainly against the tumor, has the same depressing action on the intensity of division of the tumor cells in a subcutaneous Ehrlich's adenocarcinoma as an unadsorbed anti-tumor serum. The experimental results demonstrate that the inhibitory effect of the antitumor serum on the mitotic activity of the tumor can be ascribed to the presence in the serum of antitumor antibodies.

The antitumor serum (adsorbed or unadsorbed) had no depressing effect in our experiments on cell division in the corneal epithelium of mice developing a subcutaneous adenocarcinoma.

SUMMARY

The effect was compared of adsorbed antitumor serum with mainly tumor antibodies and of the usual nonadsorbed antitumor serum on the intensity of the tumor cell division in the subcutaneous Ehrlich's adenocarcinoma.

The results of experiments staged on 100 male white mice demonstrated that the adsorbed and the nonadsorbed sera exercise an equal inhibitory effect on the mitotic activity of the tumor. The rabbits' serum immunized with liver and spleen tissue of healthy mice as well as the blood serum of normal rabbit exerted no such effect on the tumor. The cited sera effected no changes in the mitotic activity of the corneal epithelium in experimental animals.

Thus, the inhibitory effect of the antitumor serum exerted on the mitotic activity of the tumor, should be ascribed to the presence of antitumor antibodies in it.

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