THE ACTION OF A SPECIFIC IMMUNE SERUM ON THE DEVELOPMENT OF M-1 SARCOMA IN RATS

Z. I. Rovnova

From the D. I. Ivanovskii Institute of Virology (Director — Prof. P. N. Kosyakov) of the AMN SSSR, Moscow

(Received February 27, 1958. Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)

A large volume of experimental research has shown that tumor antisera may modify the growth of a tumor and may injure tumor cells in experiments in vitro [3, 9, 10, 11, 13 and others]. These findings suggested that tumor antisera might also be effective in vivo and restrain the growth of tumors.

Observations showed that tumor antisera in some cases may depress cell division in malignant tumors [1, 8], and delay growth of certain tumors and the formation of metastases [5, 8, 9, 11, 14]. In other cases these sera have no effect whatsoever on the growth of tumors [16, 17].

In experimental research into the localization of tumor antibodies labeled with radioactive isotopes, it was shown [2, 13, 16, 18 and others] that these antibodies are present in both tumor tissue and the normal organs of the animal, for the immune sera used contained both tumor antibodies and antibodies common to normal and tumor tissues.

After P. N. Kosyakov and his co-workers [4] had devised a method of obtaining specific tumor antisera, we studied the action of a specific antiserum to Ehrlich's adenocarcinoma of mice on the development of an ascitic form of this tumor. The results of this study showed that the specific tumor antiserum has an effective action on the growth and development of the ascitic form of Ehrlich's adenocarcinoma of mice; large doses of the concentrated serum inhibit the growth of the tumor and, on the other hand, small doses stimulate it [6, 7].

The ascitic form of Ehrlich's adenocarcinoma of mice was a very convenient model for studying the action of this specific serum, for with experiments both in vitro and in vivo it was possible to create the best conditions for direct contact between specific antibodies and "pure" tumor cells.

It appeared to be of interest to study the action of a specific serum on the growth and development of another type of tumor — the M-1 sarcoma of rats. This was the purpose of the present experimental investigation.

EXPERIMENTAL METHOD

An M-1 sarcoma was cultivated in adult rats (140-180 g) by subcutaneous implantation (with a syringe) of a suspension of tumor tissue, chopped up finely with scissors in physiological saline (dose 0.2 cm^3). The tumor was implanted after 14-16 days.

Specific immune antisera to M-1 sarcoma and rat spleen were obtained by immunization of rabbits. The spleen antiserum had an antibody titer in the complement fixation reaction of 1:400. We used this as an antireticulocytotoxic serum and designated it ACS. The titer of specific antibodies in the adsorbed tumor antiserum was 1:160 to 1:80.

Experiments to study the action of the tumor antiserum on tumor tissue in vitro. Under the most sterile conditions possible the tumors in the rats were excised, freed from necrotic areas and cut up finely with ophthalmic scissors or a high-speed mincer. Samples of equal weight of the minced tumor tissue were placed in

TABLE 1

The Action of Various Dilutions of Specific Tumor Antiserum on Tumor Transplantates of M-1 Sarcoma in Rats (in vitro)

Group of rats	Treatment of tumor transplantate before inoculation	Experiments									
		1		2		3		4		5	
		number of rats included in experiment	average weight of tumor (g) on the 19th day	number of rats included in experiment	average weight of tumor (g) on the 20th day	number of rats included in experiment	average weight of tumor (g) on the 21st day	number of rats included in experiment	average weight of tumor (g) on the 14th day	number of rats included in experiment	average weight of tumor (g) on the 21st day
1 experimental	Tumor antiserum in a dilution of 1 : 10	30	2,1 ($P=0,0278$)	32	2,5 ($P=0,0574$)	26	11,1 ($P=0,2713$)	23	3,8 ($P=0,0019$)	28	0,48 ($P=0,0124$)
2 experimental	The same 1 : 60	29	5,7 ($P=0,2301$)	33	6,8 ($P=0,4237$)	26	12,2 ($P=0,0799$)	23	7,2 ($P=0,000$)	15	2,8
3 control	Physiological saline	31	4,1	31	5,2	25	8,0	23	1,7	28	2,1

flasks. To one flask was added specific tumor antiserum in a dilution of 1:10, to the second tumor antiserum in a dilution of 1:60, and to the third physiological saline (3-4 cm^3 of serum or physiological saline to 1 g of tumor tissue). The suspensions obtained were kept for 2 hours in the incubator and then they were injected subcutaneously into rats in a dose of 0.2 cm^3.

On the 14th - 21st day after inoculation the rats were killed and the tumors weighed. The mean weight of the tumor was determined for all the rats of each group. The values obtained for the mean weight of the tumor were treated statistically. The results were regarded as significant if the value of P was less than 0.05.

Experiments to study the action of the serum in vivo. Tumor antiserum was injected subcutaneously or intravenously a few hours before or from the first day after inoculation of the tumor in a dose of 0.1 to 2 cm^3, diluted 1:10, 1:20 or 1:40, from 4 to 10 injections being given either daily or at intervals of two days. In some experiments injection of tumor antiserum was combined with small doses of ACS (1 cm^3 of a 1:20,000 dilution) or with guinea pig serum.

Control rats were injected with physiological saline or normal rabbit serum, or were left quite untreated.

On the 25th - 44th day after inoculation of the tumor the rats were killed.

The tumors were weighed and the mean weight in grams calculated. In some experiments in the course of development of the tumor its length and breadth were measured through the skin and the mean area of the tumor in cm^2 was calculated.

EXPERIMENTAL RESULTS

Effect of the serum on M-1 sarcoma in vitro. In Table 1 are shown the results of experiments in which the tumor tissue was treated before inoculation with tumor antiserum (1:10 or 1:60) and with physiological saline (control).

Comparison of the mean weight of the tumor in different groups of animals shows that tumor antiserum in a dilution of 1:10 has a depressing action on the viability of tumor tissue. The average weight of the tumor in the rats of this group in experiments Nos. 1, 2 and 5 was considerably less than the average weight of the tumors in the animals of the control groups. Depression of the growth of the tumor by the action of the serum was statistically significant

TABLE 2

The Action of Specific Tumor Antiserum on the Development of M-1 Sarcoma in Rats in Experiments in Vivo

Experiment	Group of rats	Treatment of rats	No. of rats incl. in expt.	No. of rats with tumors on day killed	average weight of tumor, g
1	1	10 subcutaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	8	6	18.1
	2	Without treatment	13	8	7.45
2	1	10 subcultaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	12	4	1.37
	2	10 subcutaneous injections each of 1 cm^3 of ACS (1:20,000)	10	0	0
	3	Without treatment	10	0*	0
3	1	10 subcutaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	29	23	7.2
	2	10 subcutaneous injections each of 1 cm^3 of ACS (1:20,000)	27	18	4.2
	3	4 subcutaneous injections of 1 cm^3 of normal rabbit serum (1:100)	15	11	9.1
	4	Without treatment	13	7	15.5
5	1	5 intravenous injections of 2 cm^3 of tumor antiserum (1:10)	20*	6	0.41
	2	5 intravenous injections of 0,1 cm^3 of tumor antiserum (1:10)	19	12	0.64
	3	5 intravenous injections of 2 cm^3 of normal rabbit serum (1:10)	20	12	0.99
	4	5 intravenous injections of 0,1 cm^3 of normal rabbit serum (1:10)	13	8	1.0
	5	Without treatment	22	12	0.55
6	1	5 injections of 2 cm^3 of tumor antiserum (1:10) subcutaneously and intravenously	30**	12	20.9
	2	5 injections of 2 cm^3 of tumor antiserum	31	26	17.4
	3	Without treatment	28	25	16.1

* Four rats in this group died from injections of serum.

** Eighteen rats in this group died after 3 injections of serum.

in these experiments [in experiment No. 1 — P = 0.0278, experiment No. 2 — P = 0.0574 (on the borderline of significance) and experiment No. 5 — P = 0.0124].

In the groups of rats which were inoculated with tumor tissue treated with tumor antiserum diluted 1:60 growth of the tumor was stronger than in the controls.

In all the experiments the average weight of the tumor in the rats of these groups exceeded the average weight of the tumor in the rats of the control groups, although only in experiment No. 4 was this stimulation statistically significant (P = 0.000).

In two experiments (Nos. 3 and 4) stimulation of the growth of the M-1 sarcoma was also observed in the groups of rats inoculated with tumor tissue that had been treated with tumor antiserum diluted 1:10.

TABLE 3

Stimulation of Growth of an M-1 Sarcoma in Rats by Specific Tumor Antiserum

Group of rats	Treatment of rats	No. of rats incl. in expt.	Average area of tumor (cm^2) on the 15 day	Average area of tumor (cm^2) on the 20 day	Average weight of tumor (g) on the 21 day
1 experimental	10 injections of 1 cm^3 of specific serum (1:10)	10	4.8 (P=0.041)	10.4 (P=0.089)	16.8 P=0.151)
2 experimental	10 injections of 0.5 cm^2 of specific serum (1:10) and 0.5 cm^2 of guinea pig serum	10	4.3 (P=0.041)	9.2	16.8
3 control	Without treatment	10	2.0	5.3	8.1

Stimulation of the growth of the tumor transplantates by the action of the serum in dilution of 1:10 may evidently be due to the different degree of mincing of the tumor tissue in the various experiments. Poorly minced tissue fragments elicit a weaker action from the specific antibodies, i.e. in these experiments a dilution of 1:10 was the low, stimulating dose of the serum.

The experiments carried out show that concentrated specific tumor antiserum lowers the viability of tumor cells; a smaller concentration of serum on the other hand may stimulate the viability of the tumor tissue and encourage growth of the tumor.

Experiments with M-1 sarcoma in vivo. In order to study the action of the specific serum on the growth of M 1 sarcoma we undertook a series of experiments, the results of which are shown in Table 2. In experiments Nos. 1 and 2 the rats of the experimental groups received daily subcutaneous injections of 1 cm^3 of specific serum diluted 1:10 and of 1 cm^3 of ACS diluted 1:20,000 for 10 days, starting on the 1st day after inoculation of the tumor.

Comparison of the mean weight of the tumor in rats treated with the sera and in the controls showed that injection of the sera is accompanied not by inhibition but, on the contrary, by some degree of stimulation of growth of the tumor. However, the differences between the average weight of the tumors in the treated and control rats were not statistically significant (experiment No. 1 — P = 0.0888; experiment No. 2 — P = 0.105). In experiment No. 3 (see Table 2) some retardation of growth of the tumor was observed after injection of specific tumor antiserum and a small dose of ACS, but the differences between the average weights of the tumors of the experimental and control groups were not statistically significant.

In view of the fact that the dose of specific serum injected (1 cm^3 daily for 10 days) did not materially affect the growth of the M-1 sarcoma, in subsequent experiments we increased the dose of each injection of serum to 2 cm^3.

These experiments (Nos. 5 and 6) showed that the injection of large doses of specific serum had no action whatsoever on the development of the tumor. Intravenous injections of large doses of the foreign serum led to death of the animals.

In experiment No. 7 (the results of which are given in Table 3) the serum was injected intravenously the first time, 3 hours before inoculation of the tumor. Subsequent injections were given subcutaneously, starting on the first day after inoculation of the tumor, with intervals of 1-2 days (dose 1 cm^3). In this experiment stimulation of growth of the tumor by the injections of serum was observed.

Measurement of the mean area of the tumor on the 15th day after inoculation showed that its value in the control group was 2 cm^2, whereas in groups Nos. 1 and 2, which received tumor antiserum, the mean area of the tumor was 4.8 and 4.3 cm^2 (the difference between these groups and the control is statistically significant — P = 0.041).

On the 20th day the average area of the tumor in the treated rats also exceeded the average area of the tumor in the control animals but the difference was not significant (P = 0.089). On the 21st day the rats were killed. The average weight of the tumor in groups Nos. 1 and 2 was 16.8 g and in the control group — 8.1 g (difference not statistically significant: P = 0.151).

Comparison of the experimental research which we carried out on the effect of specific sera on the growth and development of the ascitic form of Ehrlich's adenocarcinoma of mice and of M-1 sarcoma in rats shows that the cells of Ehrlich's adenocarcinoma were more amenable to the action of specific antibodies in experiments both in vitro and in vivo. Both in vitro and in the peritoneal cavity of mice, the latter had a direct destructive action on "pure" tumor cells of Ehrlich's adenocarcinoma.

When studying the action of the specific serum on M-1 sarcoma transplantates we did not possess "pure" tumor cells. The serum acted on the whole fragment of tumor tissue, exerting its damaging influence on the superficial layers only. Evidently for this reason the depressing influence of the tumor antiserum was shown less clearly.

Tumors developing in animals were even less amenable to the action of the specific antibodies. The experiments showed that injection of rats subcutaneously or intravenously with up to 10 cm^3 of specific serum had no material influence on the development of an M1 sarcoma. In no experiment were we able to observe diminished growth of the tumor among the group of rats in which tumor antiserum was given alone or in conjunction with a small dose of ACS and normal guinea pig serum.

Wissler [18] explained the absence of inhibiting action of tumor antiserum on the growth of the Flexner-Jobling sarcoma and the accumulation of a higher concentration of tumor antibodies in normal organs than in tumors by the impermeability of the capillary walls to antibodies.

The experimental investigations which we carried out with the two different tumors may assist in the explanation of the failure of many research workers to influence the actively growing tumors of man and experimental animals by intravenous and subcutaneous injections of antisera.

Experiments with Ehrlich's adenocarcinoma of mice and M-1 sarcoma of rats have shown that an antiserum is only effective when conditions are present for the direct contact of the antibodies with the cells.

In some experiments in vitro and vivo stimulation of growth of an M-1 sarcoma by the use of tumor antiserum was observed. These findings of the stimulating action of sera which we obtained in the case of two different tumors suggest that in the clinical trial of tumor antisera it is essential to select the doses of the serum very carefully and to pay attention to their titer, since in place of depression of growth of the tumor stimulation may result.

SUMMARY

The author studied the effect of specific antitumor serum on the transplants of M-1 rat's sarcoma in experiments in vivo and in vitro. It was demonstrated that the treatment of these transplants with the specific serum (1:10 solution) decreases their vital capacity. On the contrary, a more diluted solution of this specific serum may have a stimulating effect on the M-1 sarcoma transplants. Administration of up to 10 cc of the specific antitumor serum (1:10 solution) had no significant effect on the development of M-1 sarcoma. No inhibition of the tumor growth could be noted in any of the experiments. On the contrary, in certain experiments administration of the serum was associated with the stimulation of the tumor growth.

LITERATURE CITED

[1] V. N. Dobrokhotov, Byull. Éksptl. Biol. i Med. 3, 59-62 (1955).

[2] R. E. Kavetskii, A. I. Danilenko and Yu. A. Umanskii, Vrachebnoe Delo. 7, 581-586 (1955).

[3] M. M. Kapichinkov, The Immunology of Normal and Malignant Tissues, 41-53, Moscow (1956).*

[4] P. N. Kosyakov, V. S. Korosteleva and N. I. Kuznetsova, Byull. Éksptl. Biol. i Med. 9, 63-65 (1955).

[5] R. P. Martynova, Voprosy Onkol. 3, 295-302 (1956).

*In Russian.

[6] Z. I. Rovnova, Voprosy Onkol. 4, 4, 391-398 (1958).

[7] Z. I. Rovnova, Byull. Éksptl. Biol. i Med. 5,54-58 (1956). * *

[8] S. V. Sukhorukikh, The Immunology of Normal and Malignant Tissues, 71-86, Moscow (1956). *

[9] P. P. Filatov, Byull. Éksptl. Biol. i Med. 11, 56-60 (1955).

[10] M. Flax, Cancer Res. 16, 774-783 (1956).

[11] B. Kalfayan and J. G. Kidd, Exper. Med. 97, 145-162 (1953).

[12] J. G. Kidd, Exper. Med. 83, 227-250 (1946).

[13] L. Korngold and D. Pressman, Cancer Res. 14, 96-102 (1954).

[14] T. Lumsden, J. Path. a. Bact. 34, 349-355 (1931).

[15] W. J. Nungester and H. Fischer, Cancer Res. 14, 284-288 (1954).

[16] D. Pressman and L. Korngold, Cancer 6, 619-623 (1953).

[17] J. S. Thompson, Blood 10, 228-235 (1955).

[18] R. W. Wissler, et al. Cancer Res. 16, 761-773 (1956).

* In Russian.

* * Original Russian pagination. See C. B. Translation.