

THE EFFECT OF ANTISERA AGAINST IRRADIATED MALIGNANT
TISSUES ON THE GROWTH OF EXPERIMENTAL TUMORS
IN IRRADIATED ANIMALS

REPORT II. EFFECT OF THE SERUM AGAINST IRRADIATED ASCITIC CELLS ON THE GROWTH
OF SUBCUTANEOUS AND ASCITIC FORMS OF EHRLICH'S ADENOCARCINOMA IN MICE

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Several authors [7-12] succeeded in obtaining an antigrowth effect by employing cancer antisera on tumor cells in vitro and in vivo. On the basis of the fact that ionizing radiation induces a change in the antigenic properties of tumor [3,4] as well as normal tissues [1,2,5], it seemed interesting to follow up experimentally the antitumor effect of antisera, obtained in relation to cancer tissues which had been subjected in vitro to the effect of x-rays. The method of obtaining antisera to the irradiated cancer tissues and their serological characteristics were described in our Report I. In the present report are presented the results of the effect on the growth of Ehrlich's mouse adenocarcinoma of antisera obtained via immunization of rabbits irradiated with Ehrlich's ascitic cells.

METHOD

The objects of experimentation were male mice, weighing 20-25 gm, inoculated with Ehrlich's adenocarcinoma. A total of 240 mice were used in the experiments; of these, 160 were divided into eight groups (20 mice in each) and used for experimental series with subcutaneous inoculation of tumor cells, and 80 mice (eight groups, ten in each) with intraperitoneal inoculation.

TABLE 1. Effect of Sera against Irradiated Tumor Cells on the Development of a Subcutaneous Ehrlich Carcinoma in Irradiated Mice

Group of mice	Serum injected	Quantity of injected serum (in ml)	No. of animals in the group	No. of animals	
				with tumor	without tumor
First	Against irradiated tumor cells	0.9	20	4	16
Second	The same	0.6	20	9	11
Third	Against nonirradiated tumor cells	0.6	20	13	7
Fourth	The same	0.6	20	12	8
Fifth	Nonimmunized rabbit serum	0.9	20	14	6
Sixth	The same	0.6	20	14	6
Seventh	Control I (nonirradiated mice, serum was not injected)	-	20	14	6
Eighth	Control II (irradiated mice, serum not injected)	-	20	15	5

On the day following the inoculation of tumor material, all mice with the exception of controls were subjected to x-ray irradiation with a 100 r dose. The irradiation was carried out in plexiglass boxes at 10 ma current strength, 160 kv voltage, 3 mm Al filter, 48 cm focal distance, 5 min 15 sec exposure, 19 r/min dose strength. Within 24 hr after irradiation, the injection of sera was started; in subcutaneous inoculation, the serum was injected subcutaneously in the area of the dorsum; in intraperitoneal inoculation - into the peritoneum. Antisera were used which had been obtained via immunization of rabbits with extracts from irradiated ascitic Ehrlich's cells, and with extracts from native, nonirradiated tumor cells, and sera of healthy, nonimmunized rabbits. Each of these sera was injected into two groups of mice in a 0.3 and 0.2 ml dose, thrice, at two-day intervals.

In the series with subcutaneous inoculation of Ehrlich adenocarcinoma cells to mice of the first group, the serum against irradiated ascitic Ehrlich cells was injected in a 0.3 ml dose; mice of the second group were given the same serum in the amount of 0.2 ml; Mice of the third group were given the serum against tumor cells which had not been exposed to x-ray irradiation, 0.3 ml per injection; the 4th group of mice received 0.2 ml of the same antiserum; the 5th and 6th group - 0.2-0.3 ml of the native serum. The 7th and 8th group of mice received no antiserum; these were control animals: mice of the 7th group, as control of the inoculability of tumor cells in nonirradiated animals; mice of the 8th group, as control of the effect of irradiation on the percentage of tumor inoculability. The experimental series on the effect of antisera in intraperitoneal inoculation of tumor cells were staged identically with those described above, differing only in the number of mice (10 in each group).

TABLE 2. Effect of Sera Against Irradiated Tumor Cells on the Development of the Ascitic form of Ehrlich Carcinoma in the Irradiated Mice

Group of mice	Serum injected	Quantity of injected serum (in ml)	No. of animals in the group	Average quantity of ascitic fluid (in ml) on the 8th day
First	Against irradiated tumor cells	0.9	10	2.1
Second	The same	0.6	10	3.1
Third	Against nonirradiated tumor cells	0.9	10	2.6
Fourth	The same	0.6	10	4.0
Fifth	Nonimmunized rabbit serum	0.9	10	2.9
Sixth	The same	0.6	10	0.3
Seventh	Control I (nonirradiated mice, serum was not injected)	-	10	4.7
Eighth	Control II (irradiated mice, serum not injected)	-	10	4.3

RESULTS

Results of our experiments on the effect of sera against irradiated tumor cells on the subcutaneous form of Ehrlich adenocarcinoma following inoculation are shown in Table 1.

As seen in Table 1, on the 8th day of inoculation, in the group of animals which had been injected, the serum against irradiated cells of Ehrlich carcinoma in the amount of 0.9 ml, in 16 mice, i.e., in 80% of cases no tumors appeared, and in four mice (20%) the tumors developed. Of 20 mice which received this antiserum in the amount of 0.6 ml, tumors were absent in 11, i.e., in 55%

Less effective was injection of the serum against nonirradiated Ehrlich tumor cells. In a group of mice which received this serum thrice at 0.3 ml per injection the tumor did not develop in 8 mice, i.e., in 40% of the 20 experimental animals.

Approximately similar results were observed in the group of mice which had received the same serum in the total amount of 0.6 ml.

Upon injection of the serum of a nonimmunized rabbit, the tumors developed at the same rate as in the control groups.

In the control groups of animals inoculated with Ehrlich adenocarcinoma the presence of the tumor was observed in 70-75% of cases.

The intensity of the tumor growth was judged according to its size. The size was largest in all control and experimental animals which had received immune sera against nonirradiated tumor cells, or native (crude) rabbit's sera. Thus, whereas in control groups a tumor the size of a pea and larger was observed in 12 mice (60%) in animals, injected with the serum against malignant cells, only in two mice (10%) the tumors scarcely reached the size of a pea.

Sera against irradiated ascitic cells had no effect on the growth of Ehrlich tumor in animals which had not been subjected to the action of x-rays.

Upon intraperitoneal inoculation of tumor cells we evaluated the effect of the sera, according to the accumulation of ascitic fluid on the 8th day following inoculation. The results are shown in Table 2.

It is seen in Table 2 that the smallest amount of ascites (2.1 ml) was found in the group of mice which had been injected with the serum against irradiated tumor cells thrice, at 0.3 ml each time, whereas in the control groups the amount of ascitic fluid exceeded more than 2-fold the above quantity (4.7 ml).

The difference between the two figures proved reliable upon statistical treatment ($P = 0.01$).

In mice which had been injected with the serum against nonirradiated tumor cells, or with normal series in the same doses, the amount of ascitic fluid was larger.

Thus, upon observation of the development course of the ascitic as well as the subcutaneous form of the tumor in x-ray irradiated animals, it was noted that in the groups of experimental animals which had received the serum against irradiated malignant tumors, the experimental tumors developed slower and, in a large percentage of cases, they could not be diagnosed on the 8th day following inoculation.

We staged another series of experiments, analogous to the one described above, differing only in that the irradiation of mice was performed prior to the inoculation of the tumor material. We employed 230 animals which were divided into analogous groups. The inoculation of tumor cells was carried out subcutaneously and intraperitoneally.

Analogous results were obtained in these series. Thus, in the group of mice with a subcutaneous Ehrlich carcinoma; which had been injected with the serum against irradiated tumor cells, a still longer latent period was noted (13 days instead of $6\frac{1}{2}$ days in the control animals), also a slower growth of the emerged tumors and a considerable increase of the life span of the animals (57.1 days in the experiment and 41.1 days in control). In mice with an intraperitoneal inoculation of tumor cells, upon injection of the serum against irradiated tumor tissues, the accumulation of ascitic fluid was also less noted than in the other groups.

Thus, the results of the experiments attest to the fact that sera, obtained against an irradiated cancer antigen, possess a more pronounced property of inhibiting the growth of Ehrlich's carcinoma (subcutaneous and ascitic form) in the irradiated animals as compared to the specific immune or normal sera.

It is difficult to interpret this phenomenon in precise terms. Apparently, the serum against irradiated malignant tissues removes to a certain extent the harmful effect of x-rays on the protective reactions of the organism.

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