THE ACTION OF HOMOLOGOUS IMMUNE SERA ON THE CELLS OF IMPLANTED TUMORS

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(Received September 7, 1958. Presented by Active Member AMN SSSR L. A. Zil'ber)

We know of many investigations as a result of which it has been possible to obtain sera possessing a cytotoxic action on tumor cells. For this purpose in the majority of cases heterologous sera were used. In order to explain the mechanisms of antitumor immunity, however, it is much more important to study homologous sera, i.e., sera obtained from animals of the same species as the one bearing the tumor. There are only few investigations of this type (see the detailed survey by R. M. Radzikhovskaya [2]) and fresh information on this problem would be of undoubted interest.

EXPERIMENTAL METHOD

Test sera were prepared by means of immunization of rats with tissue from transplanted rat sarcomas. We tried out several methods of immunization of animals.

Goldfeder [4] irradiated tumor transplantates with definite doses of x-rays which injured the cells but did not cause their death. Being implanted into animals, these transplantates were absorbed at the conclusion of the period of progressive growth, after which the animal developed immunity to implantation of the tumor. We were unable to select the dose of irradiation necessary for obtaining this immunizing effect. After irradiation, the transplantates of M-1 rat sarcoma either would not take on implantation and the animals did not develop immunity, or they took on implantation which grew progressively, leading to death of the animals.

We attempted to create immunity by absorption of an implanted and growing tumor. For this purpose we injected Gordeev's fluid into the substance and beneath the base of an M-1 sarcoma. Despite the fact that in several cases we used doses of the fluid bordering on those lethal for experimental animals (0.15-0.2 ml), the tumors continued to grow progressively and the animals died.

In the next series of experiments necrosis of the tumor was caused by the application of a ligature to its base. F. M. Brikker's recommendation [1] — to tie the ligature not very tightly, in order to embarrass the blood supply of the tumor but not to stop it completely — was not applicable to the tumors investigated. When the M-1 sarcoma was not ligated completely at its base, it rapidly spread under the ligature and continued to grow progressively. We obtained the best results, as in the experiments of Foley [3] and of Keilova and Sorm [5], by ligating the base of the tumor as tightly as possible. The tumor became necrotic and sloughed, whereupon another tumor was implanted into the animal, when it developed, the tumor was again ligated, and this operation was repeated until the animal became completely insusceptible to further implantation of this strain of tumor. Usually the operation had to be repeated 4 or 5 times.

The immune rats were killed by exsanguination and serum was prepared from their blood. At the same time we prepared control sera from the blood of healthy rats which had never received implantation of tumors.

The sera were mixed with equal volumes of a suspension of tumor cells, containing in different experiments from 400,000 to 1 million cells in 0.1 ml of physiological saline. The cell suspensions were prepared as follows: tumor tissue was cut up finely with scissors, physiological saline was added and the suspension was filtered through sterile silk material to remove large aggregates of cells. The cells were counted in a counting chamber and the required concentration of cells was obtained by the addition of physiological saline. The mixtures of cells and sera were injected into rats, after a definite period of contact, in doses of 0.1 ml intradermally

TABLE 1

The Action of an Immune Homologous Serum on Cells of an M-1 Rat Sarcoma

Material implanted	Number of implantations			Mean weight of tumors, in mg
	total	successful	unsuccessful	
Cells of M-1 sarcoma + serum of rats immune to this tumor	25	18	7	1,139
Cells of M-1 sarcoma + control serum	25	23	2	3,782

(experimental and control suspensions into the same animal at symmetrical points on the body). After 2 weeks the rats were killed, and the tumors developing in the animals were excised and weighed.

EXPERIMENTAL RESULTS

It was found that for the sera to display a cytotoxic action, the conditions of their contact with the tumor cells were of great importance. When, before inoculating the rats, we kept the mixture of cells and sera for 30 minutes at 37°, we were unable to find any appreciable difference in the action of the experimental and control sera.

TABLE 2

The Action of an Immune Homologous Serum on Cells of a Sarcoma 465 of Rats

Material implanted	Number of implantations			Mean weight of tumors, in mg
	total	successful	unsuccessful	
Cells of sarcoma 465 + serum of rats immune to this tumor	32	18	14	750
Cells of sarcoma 465 + control serum	32	30	2	2,600

The following experiments were performed differently: the mixtures of cells and sera were kept overnight in the refrigerator and then for 30 minutes at 37°; the rest was done as described above. The results of three similar experiments are summarized in Table 1. It can be seen from the table that the addition of the immune serum to the transplanted cells depressed the chances of a successful take of the tumor and the subsequent growth of the transplantes. Of 25 implantations, 18 experimental ones were successful, by comparison with 23 controls. The mean weight of the experimental tumors was significantly less than that of the controls:

1139 mg compared with 3782 mg. This difference is statistically significant. The probability of chance discrepancy of the mean values (P) is less than 0.001.

In a similar manner we carried out experiments with another rat tumor, sarcoma 465. The results of this experiment were the same (Table 2).

Table 2 is compiled from the combined results of two similar experiments. As may be seen from these findings, the immune sera depressed the chance of successful implantation and growth of sarcoma 465. Of 32 implantations only 18 experimental ones were successful, compared with 30 controls. The mean weight of the experimental tumors was significantly less than that of the controls: 750 mg compared with 2600 mg. This difference was statistically significant, $P < 0.001$.

We were thus able to obtain homologous sera which depressed the growth in vitro of two implanted tumors. In order to judge the role of antibodies in immunity against tumors, however, further experiments are essential, in conditions which exclude the action of isoantibodies.

SUMMARY

The bases of M-1 or 465 sarcomas transplanted to rats were tightly ligated, resulting in necrosis of the tumors. The operation was repeated many times until complete immunity to the inoculation of the above tumors was obtained. The serums of immune rats acquired the property of rendering a definite cytotoxic effect in vitro on the cells of the corresponding tumors, depressing their inoculation ability and growth.

LITERATURE CITED

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