AN EXPERIMENTAL STUDY OF THE ROLE OF ANTIBODIES IN IMMUNITY TO TUMORS

R.M. Radzikhovskaia

From the Department of Immunology and Malignant Tumors (Head – Active Member Acad. Med. Sci. USSR L.A. Zil'ber) of the N.F. Gamaleia Institute of Epidemiology and Microbiology (Director – Prof. S.N. Muromtsev) Acad. Med. Sci. USSR, Moscow

(Received June 21, 1957. Presented by Active Member Acad. Med. Sci. USSR L.A. Zil'ber)

Despite the large volume of work devoted to a study of the role of antibodies in the mechanism of protection against tumors, this problem remains unsolved. Of the greatest importance in this connection are investigations in which the role of antibodies is examined by their direct action on the malignant cell, i.e., by their effect on the vital functions of tumor tissue.

It has been shown in numerous reports [4, 5, 10, 15, 17, 20] that in the serum of animals immunized with heterologous tumor tissue antibodies appear which inactivate tumor cells in vitro and inhibit growth of this tumor in vivo. The antineoplastic action of the sera of animals immunized with heterologous tumor tissue is not, however, sufficiently direct proof of the role of antibodies in defense against tumors, for according to the findings of Bogomolets [1], Kavetskii and Fedushin [3], Vinogradova [2] and others, normal animal sera also exhibit a cytotoxic action on tumors of animals of another species.

This action is intensified not only by immunization of the donors with the corresponding tumor tissue but also by administration to them of extracts of spleen, thymus and other normal organs rich in reticuloendothelial tissue.

Of rather greater interest are investigations directed to the discovery of protective antibodies in animals susceptible to a particular tumor. Reports of these are few in number and contradictory in their conclusions [6, 7, 9, 14, 18].

We may dwell in some detail on certain of these investigations, made in recent years. Falls [11] performed experiments with Gardner lymphosarcoma, arising in mice of the C_3H strain and inoculated into mice of the CBA strain. Irradiation of this strain of mice with x-rays caused absorption of the tumor and subsequent immunity to further transplantation. On pairing immune mice parabiotically with nonimmune mice of the same strain (CBA), the latter also became immune to transplantation of the lymphosarcoma. Schreck and Preston [19] reported that transplantation and subsequent absorption of Bagg lymphosarcoma in rats of the Sprege-Daul'* strain is accompanied by the appearance in the serum of these animals of antibodies which inactivate the lymphosarcoma cells in vitro and cause severe cytological changes in the tumor cells; the sera have no action on cells of other tumors nor on normal rat tissues. Normal rat serum does not cause cytological changes and does not inactivate lymphosarcoma cells.

Gorer and Amos [12, 13] immunized mice of strains A, BALB/C and C_3H with tumor cells from mice of strain $C_{57}BL$ suffering from EL_4 leukemia. Injection of immune sera into mice of all the strains mentioned produced in them complete or partial inhibition of tumor growth after subsequent implantation of leukemic cells. Similar properties were acquired by the serum of BALB/C mice immunized with ascitic sarcoma BP_8: injection

*Transliterated from Russian.

of this serum into mice of strain C_3H, susceptible to sarcoma, was accompanied by inhibition of growth of the tumor in the mice. In contrast to the above experiment, Todd and Kidd [21] could detect no depressing action on tumor cells by sera of mice with a developing lymphoma or after absorption of this tumor. Negative results were obtained also in experiments by Miller [16]. It may be thought that the cause of failure was an insufficient intensity of immunization of the animals, with consequent feeble development of the antineoplastic properties of the test sera.

The purpose of this investigation was to ascertain the possibility of passive transmission of immunity against tumors to susceptible animals from immunized animals of the same species.

EXPERIMENTAL METHOD

The investigation was carried out on Brown-Pearce rabbit carcinoma. Rabbits of an impure strain, weighing 3-3.5 kg, were inoculated with a suspension of Brown-Pearce tumor intradermally. From five to seven days later at the site of injection intradermal tumors appeared and, in the majority of cases were reabsorbed. Afterwards the rabbits were reinjected 10-12 times with a suspension of the carcinoma. The intervals between the injections were 21-30 days. In this way the rabbits became highly resistant to inoculation with even high doses of tumor tissue. Blood was taken from the rabbits before the beginning of the vaccinations and on the 7th, 9th, 14th and 20th days after its conclusion. In all the experiments fresh serum was used, which had been stored in a refrigerator for not more than five days.

In the first series of experiments a fresh rabbit was injected intramuscularly with 10 ml of serum from an immune rabbit. After 24 hours this rabbit was inoculated in the muscle of the same or another limb with 1 ml of tumor suspension in a dilution of 1:5-1:10. In some experimental animals the serum was injected once only, in others up to ten times from the time of inoculation of the tumor material. The volume of serum injected varied from 10 to 60 ml per rabbit. As controls healthy rabbits were used, not subjected to any form of interference, or injected with normal rabbit serum only. The control rabbits were inoculated at the same time as the experimental rabbits. The animals were kept under observation for 1-1½ months, after which they were killed and examined post mortem.

EXPERIMENTAL RESULTS

The results obtained are shown in Table 1.

Altogether 17 immune sera were tested. A protective action was shown by four sera, three of which completely inhibited tumor growth and one partially. This was shown by the fact that the weight of the tumor in the experimental rabbit was 1.6 g while the weight of the tumor in the control animals was 15 and 20.5 g. It must

TABLE 1

Action of Immune Serum in Vivo

Serum	Number of rabbits in experiment	Number of immune rabbits	Number of rabbits with partial inhibition of tumor growth
Immune	51	5	1
Normal	10	0	0
Not injected	10	0	0

be pointed out that the tumor in the experimental rabbit resembled a continuous necrotic mass. The protective properties of each serum were tested in several experiments. Identical results were obtained. The effect of the serum depended on the time it was taken. For example, serum No. 188, obtained from blood taken on the 16th and 27th day after vaccination, completely inhibited growth of the tumor in fresh rabbits; the same serum No. 188 obtained on the 47th and 60th days after the conclusion of vaccination had no protective action. The volume of serum injected did not affect the results of the experiment. A negative action on the protective proper-

ties of the serum was shown by excessive hyperimmunization and frequent taking of blood from a rabbit. For example, serum No. 375 gave good results after the fourth and sixth vaccinations but had no action after the tenth vaccination.

Besides intramuscular injection of serum and tumor suspension, in three experiments we injected the serum and suspension intraperitoneally, using the method described above. The tumor suspension was injected in a dilution of 1:5 and a volume of 1 ml. In two experiments no difference was observed between the course of the tumor process in the animals of the experimental and control groups, and in one experiment the immune serum caused partial inhibition of tumor growth: spread of the tumor in the experimental animals was considerably more limited than in control animals, and the average weight of the tumors in the experimental animals was correspondingly 26 times less than in the controls injected with normal serum.

The results obtained are evidence of the possibility of passive transmission of immunity to Brown-Pearce tumors by means of antibodies. However, the fact that out of 17 sera obtained from highly immune rabbits, only four showed any protective properties, suggests that the antibody concentration of the sera is low. In some cases we were unable to detect such a low concentration of antibodies because in order to test the immunity very large doses of tumor material were injected (suspension in dilutions of 1:5-1:10). We used large doses because we were working with rabbits obtained from the market whose sensitivity to Brown-Pearce carcinoma varies widely. If we had used smaller doses of the suspension, tumors would not have developed in all the control animals, which would have made it impossible to evaluate the results obtained. In this case, in order to obtain statistically significant data it would have been necessary to include in the experiment a very large number of rabbits, which we could not have done. In a second series of experiments we produced transmission of immunity by means of transplantation of the spleen of immune rabbits into fresh animals.

The spleen of an immune rabbit was finely shredded with scissors into small pieces and transplanted intraperitoneally into two fresh rabbits in the region of the greater omentum. After 24 hours an intraperitoneal injection of 1 ml of tumor suspension diluted 1:5 was given to these rabbits and also to controls into which normal rabbit spleen had been transplanted. The rabbits were kept under observation for 1-1½ months, after which they were killed and examined post mortem. The results of these experiments are shown in Table 2.

TABLE 2

Transplantation of the Spleen of Immune Rabbits into Animals not Exposed to Any Form of Treatment

Material transplanted	Number of rabbits in experiment	Number of rabbits with complete inhibition of tumor growth	Number of rabbits with partial inhibition
Spleen of immune rabbits	17	3	2
Spleen of normal rabbits	18	0	0

Altogether nine spleens from immune and nine from normal rabbits were transplanted. Two immune spleens produced complete inhibition of tumor growth, and one partial inhibition, as shown by the fact that tumor growth in the experimental rabbits was considerably less intensive and widespread than in controls. The experiments which were carried out showed the possibility of transmission of immunity by means of transplantation into fresh rabbits of the spleen of resistant animals. However, in this case, the results obtained were feebly expressed, partly on account of the fact that in order to test the immunity we had to use very large doses of the tumor suspension.

In the third series of experiments we attempted to produce immunity by transplanting the lymphatic glands of immune animals into fresh rabbits. Rabbits resistant to Brown-Pearce tumors were immunized intradermally

in the posterior surface of the shank of the hind limbs. On the ninth day after immunization their popliteal lymphatic glands were removed. After being cut up with scissors they were transplanted into the peritoneal cavity of two fresh rabbits. Into control rabbits were transplanted lymphatic glands from normal animals. After 24 hours the experimental and control animals were injected intraperitoneally with tumor suspension. During the month after inoculation the control animals died. At necropsy multiple tumor nodules were found in all the internal organs, together with ascites. The weight of the tumors was about 200-220 g. The experimental rabbits were killed 1½ months after inoculation.

At necropsy of one rabbit a uniformly necrotic tumor nodule weighing 1.5 g was discovered in the parietal peritoneum at the site of injection. In a second rabbit solitary tumor nodules were found in the mesentery, weighing 10 g. In this experiment an obvious difference was found in the intensity of tumor growth in the experimental and control animals, but the experiment must be repeated using a larger number of animals.

The spleen and lymphatic glands are organs which produce antibodies, and transmission of immunity is effected in this case by means of antibodies. It must be pointed out that it is not only these antibodies which are found in the spleen and lymphatic glands of immune donors which play an important part, but also antibodies which continue to be produced by the transplanted tissues in the body of the recipient. Thus, the transmission of immunity from rabbits resistant to Brown-Pearce carcinoma to normal animals may be effected by means of antibodies present in the serum, spleen and lymphatic glands. So that the question of the nature of these antibodies – true tumor antibodies or isoantibodies – can be answered, the whole of this work must be reproduced in pure-bred animals in which a tumor has arisen and is kept going by implantation in other animals. This would allow the doses of tumor material in testing immunity to be reduced, and also would shed light on the nature of the antibodies formed.

SUMMARY

This work is devoted to the study of the role of the antibodies in induction of immunity to Brown-Pearce carcinoma. Serum or splenic tissue and lymph node tissue, obtained from rabbits immune to the tumor was introduced to rabbits. The subsequent inoculation of the tumor tissue to experimental animals was connected in a number of cases with complete or partial inhibition of the tumor growth. These data demonstrate the significance of the antibodies in the mechanism of antitumor protection of the organism.

LITERATURE CITED

[1] A.A. Bogomolets, Vrachebnoe Delo 8, 562-566 (1938).

[2] V.D. Vinogradov, Experimental Study of Specific and Nonspecific Resistance to Malignant Tumors (Experimental Investigation Using Artificial Immunization of White Mice), Candidate's Dissertation* (Moscow, 1948).

[3] R.E. Kavetskii and M.P. Fediushin, Transactions of the 1st Congress of Oncologists of the Ukrainian SSR, 1940, pp. 374-377.

[4] M.S. Lomakin, Biull. Eksptl. Biol. i Med. 9, 40-44 (1956).**

[5] P.P. Filatov, Biull. Eksptl. Biol. i Med. 11, 56 (1955).

[6] A. Besredka and L. Gross, Wien. med. Wschr. 86, 509-514 (1936).

[7] J. Bichel and I. Holm-Jensen, Acta path. a. microbiol. Scand. 26, 319-322 (1949).

[8] J.S. Colter, H.H. Bird, H. Koprowski et al., Nature 177, 4517, 993-994 (1956).

[9] G. Damagk, Ztschr. f. Krebsforsch.56, 247-252 (1949).

[10] A.D. Dulaney and K. Arnesen, Proc. Soc. Exper. Biol. and Med. 72, 665-668 (1949).

[11] N.G. Falls and A. Kirschbaum, Proc. Am. Assoc. Cancer Research 1, 15 (1953).

[12] P.A. Gorer, Ann. New York Acad. Sci. 1, 15 (1956).

*In Russian.

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

[13] P.A. Gorer and D.B. Amos, Cancer Research 16, 338-343 (1956).

[14] J.G. Kidd, J. Exper. Med. 83, 227-240 (1946).

[15] T. Lumsden, Lancet. 1, 116-122 (1927); Am. J. Cancer 15, 563-640 (1931); 31, 430-440 (1937).

[16] D.G. Miller, J. Nat. Cancer Inst. 16, 1473-1484 (1956).

[17] A. Nettleship, Am. J. of Pathol. 21, 527-541 (1945).

[18] H.J. Phelps, Am. J. Cancer 31, 441-445 (1937).

[19] R. Schreck and F.W. Preston, J. Nat. Cancer Inst. 16, 1021-1033 (1956).

[20] A.C. Snell and B.V. Favata, Cancer Research 11, 335-340 (1951).

[21] J.E. Todd and J.G. Kidd, Proc. Soc. Exper. Biol. and Med. 86, 865-868 (1954).