EFFECT OF IMMUNIZATION OF RATS WITH A HETEROGENEOUS COMPLEX ON GROWTH AND METASTASIZATION OF INDUCED TUMORS

M. S. Lomakin and E. V. Sokolova

UDC 616-006.04-092.9-092-02 : 615.366.006

Immunization of rats by cells of autologous induced tumors combined with heterogeneous protein (human serum γ globulins) leads to marked inhibition of growth of primary tumors, to their regression in some animals, and to a reduction in the intensity of metastasization.

Man and animals, when affected by a malignant tumor, can react against the development of metastasis [1, 2, 6, 11, 13]. According to data in the literature, the response of the organism to tumors depends on the one hand on the antigenicity of the tumor [9, 12, 13], and on the other hand, on the intensity of the defensive reactions of the organism affected by the tumor [2, 3, 10].

As a rule, metastasization of tumors takes place most intensively when the defensive reactions of the organism are weakened. However, according to some reports, reactivity of the host against a tumor can be increased by certain procedures [4-6]. For this reason the development of experimental methods of influencing metastasization is of great interest to oncology.

The object of the investigation described below was to study the character of growth and metastasization of malignant tumors in rats following immunization of the animals with cells of an autologous tumor bound with heterogeneous protein by means of diazobenzidine.

The basis for this investigation was the work of Landsteiner [8], who obtained antibodies against nonantigenic substances by binding them to heterogeneous proteins.

EXPERIMENTAL METHOD

Tumors of the muscle tissue of the thigh were induced in Wistar rats aged 6-8 months by injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) dissolved in mineral oil, in a dose of 5 mg per animal. By surgical operation 4 months after injection of the carcinogen, the greater part of the tumor was removed from all animals of the experimental group and 3 control groups, and used for immunization, for the preparation of histological sections, and for immunological studies. Since tumors induced in muscle tissue as a rule grow by infiltration, small areas of tumor tissue were left behind in the rats after the operation.

Cells of an autologous tumor, bound by means of diazobenzidine with human serum γ globulin by the method of Cabot and Mayer [7], were used for immunization of the rats, by 2 injections at an interval of 12 days. A mixture of tumor cells (0.5 ml) with Freund's complete adjuvant (1 ml) was used so that the total dose per animal was 1.5 ml.

For the histological study of the tumors before and after immunization, material was fixed in 10% formalin. Sections were stained with hematoxylin and eosin.

Laboratory of Immunology of Growth and Development, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 9, pp. 69-72, September, 1970. Original article submitted August 18, 1969.

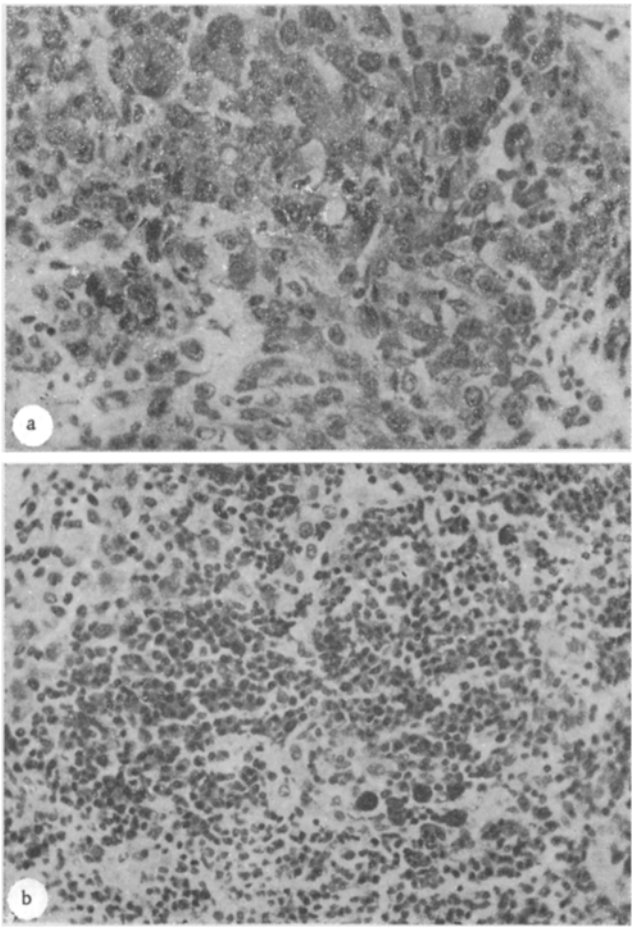

Fig. 1. Primary induced polymorphocellular sarcoma in a rat (200 ×): a) before immunization; b) after immunization of rat with heterogeneous complex.

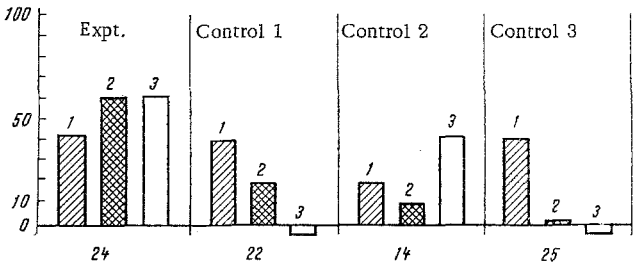

Fig. 2. Results of agar precipitation test with sera of rats immunized with heterogeneous complex. Abscissa, number of sera; ordinate, percentage of reacting sera; 1) antigen from normal rat muscle tissue; 2) antigen from autologous tumor tissue; 3) human serum γ globulin.

In parallel tests before and after each immunization of the experimental animals, the level of antibodies reacting with antigens from tumor tissues and normal tissues was determined by Ouchterlony's agar diffusion reaction.

The animals were divided into 5 groups: 1) experimental group of 24 rats immunized with cells of an autologous tumor bound with human serum γ globulin; 2) control group 1 consisting of 22 rats immunized with cells of an autologous tumor (native); 3) control group 2 consisting of 14 rats immunized with a mixture of

TABLE 1. Effect of Immunization of Rats with a Heterogeneous Complex on Growth and Metastasization of Induced Tumors

Group of rats	No. of rats with tumors		No. of rats with metastases	Development of metastases	
	before Immunization	after Immunization		lungs	lymph glands
Immunized with cells of autologous tumor + HSG + FA (experiment)	24	14	4	3	1
Immunized with cells of autologous tumor + FA (control 1)	22	19	12	9	3
Immunized with HSG + DB + FA (control 2)	14	12	8	7	1
Removal of tumor (control 3)	25		14	10	4
No treatment (control 4)	25		13	9	4

Notation: HSG – human serum γ globulin; FA – Freund's adjuvant; DB – diazobenzidine.

diazobenzidine and human serum γ globulin; 4) control group 3 made up of 25 rats from which the tumor was removed and no other treatment given; 5) control group 4 consisting of 25 rats receiving no treatment but possessing induced tumors.

All the experimental animals and rats of the control groups were sacrificed on the 40th day after the last immunization or after the end of the experiment.

EXPERIMENTAL RESULTS

The experimental results are summarized in Table 1.

The results showed marked inhibition of growth and metastasization of primary induced tumors in animals immunized with cells of the autologous tumor bound with heterogeneous protein ($P = 0.0003$; when calculating P, the percentage of metastasization in the experimental and control groups was compared).

Morphological investigation of tumors taken from the rats before immunization showed that they consisted of clearly defined polymorphocellular and spindle-cell sarcomas and rhabdomyosarcomas (Fig. 1a). Intensive lymphoid infiltration and necrosis of tumor cells were observed in tumors taken from the experimental animals after immunization (Fig. 1b).

In the control groups, diffuse collections of lymphocytes and also single lymphocytes were observed in the field of vision in material taken from individual rats.

Immunological investigations using the agar precipitation test showed (Fig. 2) that the sera of the experimental animals each form one precipitation band with antigens from tissues of the autologous tumor in 62.5% of cases. These same sera reacted with antigens from normal muscle tissue, but in a smaller percentage of cases (41).

Sera from rats of the control groups reacted positively in the agar precipitation test with antigen from autologous tumor tissue in 27% of cases (control 1) and 15% of cases (control 2), and with antigens from normal muscle tissue in 40 and 21% of cases, respectively.

The sera of unimmunized rats (controls 3 and 4) formed precipitation lines in only 0.8% of cases with antigen from autologous tumor tissue and in 40% of cases with antigen from normal muscle tissue.

Postmortem examination of the experimental animals revealed a marked increase in size of the spleen compared with control animals of both the immunized and unimmunized groups.

The results indicate that following immunization of rats with autologous tumor cells bound with heterogeneous protein, marked activation of cellular and humoral factors of immunity is observed. These factors evidently play a role in the inhibition of growth and metastasization of the primary induced tumors.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov, I. N. Maiskii, and V. S. Gostev, Abstracts of Proceedings of the 10th Session of the General Assembly of the Academy of Medical Sciences of the USSR [in Russian], Moscow (1956), p. 38.

2. N. N. Petrov (editor), Malignant Tumors [in Russian], Vol. 1, Part 1, Leningrad (1947).
3. E. V. Sokolova, Abstracts of Proceedings of a Conference of Junior Scientific Workers of the Institute of Experimental Biology [in Russian], Moscow (1967), p. 116.
4. N. Czajkowski, M. Rosenblatt, F. Cushing, et al., Cancer (Philadelphia), 19, 739 (1966).
5. N. P. Czajkowski,M. Rosenblatt, P. L. Wolf, et al., Lancet, 2, 905 (1967).
6. R. Gershon, R. Carter, and K. Karinari, Science, 159, 640 (1968).
7. E. Cabot and M. Mayer, Experimental Immunochemistry [Russian translation], Moscow (1968).
8. K. Landsteiner, The Specificity of Serological Reactions, Springfield (1936), p. 3.
9. L. Old, E. Boyse, D. Clarke, et al., Ann. New York Acad. Sci., 101, 89 (1962).
10. H. Sanford, Transplantation, 5, 557 (1967).
11. C. Southam, A. Brunschwig, A. Levin, et al., Cancer (Philadelphia), 19, 1743 (1967).
12. T. Takeda, M. Aizawa, J. Kikuchi, et al., Gann, 57, 221 (1966).
13. K. Takeda, Y. Kikuchi, S. Yamawaki, et al., Cancer Res., 28, 2149 (1968).