# THE USE OF THERMOSTABLE ANTIGENS OF MALIGNANT RAT TUMORS IN SEROLOGIC AND CELLULAR TESTS

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High specificity of thermostable antigens of tumors of rat muscle tissue induced by dimethylbenzanthracene was demonstrated in the gel precipitation test. The use of thermostable tumor antigens increases the sensitivity and specificity of the macrophage adhesion inhibition test.

KEY WORDS: thermostable antigens; induced tumors; macrophage adhesion inhibition test.

The reaction of sensitized peritoneal macrophages and peripheral blood leukocytes with malignant tumor antigens is nowadays becoming increasingly used in clinical and experimental work for the immunodiagnosis of malignant disease [8-10] and to study the state of cell-dependent immunity to tumors [11, 13]. However, as the writers showed previously [4], these reactions are not strictly specific in character in all cases.

According to reports in the literature, during temperature fractionation of certain biologically active substances it is possible to obtain more active [3, 4, 14] and homogeneous preparations [1, 2].

In the present investigation, in order to increase the specificity of these tests, attempts were made to use thermostable antigens of malignant tumors of animals, the existence of which was described previously [5].

## EXPERIMENTAL METHODS

Female Wistar rats weighing 120-200 g were used; malignant tumors were induced in the animals by injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) in sterile mineral oil into the thigh muscles in a dose of 4.5-5 mg per animal.

Normal female rats of the same breed were used for the immunologic investigations; these animals had primary induced tumors implanted subcutaneously in a dose of $10^7$ cells at the 6th-7th month of carcinogenesis.

To determine thermostable antigens in the malignant tumors and also to detect immune antitumor antibodies in the sera of tumor-bearing rats, the gel precipitation test was used. Protein in the antigens was determined by Lowry's method on a spectrophotometer at a wavelength of 750 $\mu$. To detect immune antibodies, blood was taken from the experimental animals on the 12th-15th day after implantation of the tumor. To obtain heterologous immune sera, chinchilla rabbits were immunized intravenously five times, at intervals of 3-5 days, with extracts from native tumor tissues and with the same extracts heated to 100°C for 10 min. Fuller details of the thermostable antigens of malignant tumors and normal rat organs and tissues will be found in the writers' previous paper [5]. Immune sera of rabbits and rats were used either untreated or concentrated by McErlean's method [15].

The test of inhibition of adhesion of sensitized peritoneal macrophages to a glass surface in the presence of specific antigens (the macrophage adhesion inhibition test – MAIT) also was used, in the method described in [12]. The test samples were: 1) macrophages + Hanks' solution; 2) macrophages + extracts from tissues of primary induced tumors, centrifuged at 20,000 and 105,000g; 3) macrophages + tumor extracts centrifuged as described above and heated to 100°C for 10 min. Macrophages of tumor-bearing rats and extracts of the

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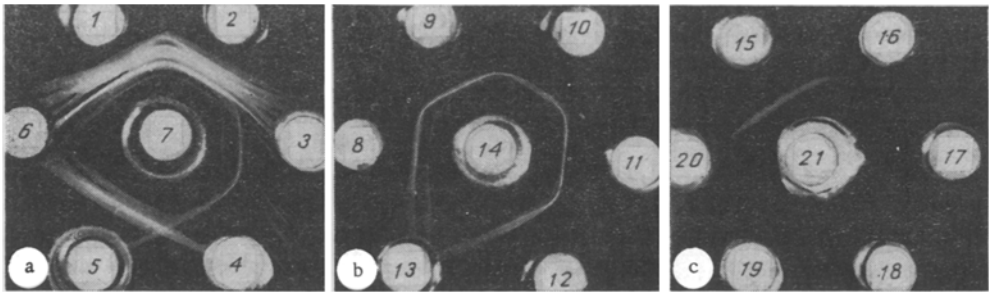


Fig. 1. Gel precipitation test between sera of immune rabbits and rats and antigens from normal and tumor tissues of rats. 1) Native tumor No. 18; 2) native tumor No. 21; 3) heated tumor No. 18; 4) heated tumor No. 21; 5) native muscle; 6) heated muscle; 7) fraction IV of concentrated serum of rabbit immunized with native tumor; 8, 9, 10, 11, 12) heated tumors Nos. 5, 9, 13, 18, and 21 respectively; 13) heated spleen; 14) fraction IV of concentrated serum of rabbit immunized with heated tumor; 15) heated tumor No. 21; 16) native tumor No. 21; 17) native muscle; 18) heated muscle; 19) native spleen; 20) heated spleen; 21) fraction III of concentrated serum of tumor-bearing rat No. 21. a, b, c) See text.

TABLE 1. Results of MAIT (% of nonadherent cells) in the Presence of Native and Heated Antigens of Tumor Tissues and Normal Tissues of Rats

| Rat No. | Antigen | Antigen from tumor | Antigen from muscle | P |
|---|---|---|---|---|
| 1 | Native | 93,2±0,5 | 71,6±4,4 | <0,05 |
| | Heated | 81,9±2,6 | 29,3±1,7 | <0,01 |
| 2 | Native | 58,4±2,9 | 63,4±0,6 | Not significant |
| | Heated | 80,7±3,2 | 36,7±1,1 | <0,01 |
| 3 | Native | 93,9±4,1 | 49,0±2,9 | <0,05 |
| | Heated | 85,8±3,6 | 10,2±2,0 | <0,01 |
| 4 | Native | 82,1±6,0 | 20,3±1,2 | <0,01 |
| | Heated | 91,7±6,5 | 11,9±2,4 | <0,01 |
| 5 | Native | 93,1±4,7 | 14,0±1,1 | <0,01 |
| | Heated | 86,1±6,9 | 12,8±1,2 | <0,01 |
| 6 | Native | 87,8±1,0 | 38,7±2,9 | <0,01 |
| | Heated | 42,5±1,9 | 4,7±1,4 | <0,01 |
| 7 | Native | 67,1±3,5 | 76,5±1,2 | Not significant |
| | Heated | 70,6±4,7 | 11,8±2,4 | <0,01 |
| 8 | Native | 83,9±7,6 | 93,6±0,8 | Not significant |
| | Heated | 92,9±3,8 | 50,9±2,6 | <0,05 |
| 9 | Native | 89,3±4,8 | 60,7±1,2 | Not significant |
| | Heated | 58,9±2,3 | 36,9±3,6 | <0,05 |
| 10 | Native | 98,5±1,1 | 83,2±3,2 | Not significant |
| | Heated | 96,4±3,1 | 28,5±5,3 | <0,01 |
| 11 | Native* | 58,2±3,7 | 67,5±2,6 | Not significant |
| | Heated* | 54,5±3,2 | 25,2±1,1 | <0,01 |
| 12 | Native* | 15,8±2,2 | 43,2±3,2 | Not significant |
| | Heated* | 73,7±6,3 | 28,5±5,3 | <0,01 |
| 13 | Native* | 15,9±1,2 | 48,3±1,1 | Not significant |
| | Heated* | 18,9±2,3 | 7,1±2,3 | <0,05 |
| | | Macrophages of normal rats | | |
| 14 | Native | 62,9±2,9 | 52,9±5,7 | Not significant |
| | Heated | 16,4±2,3 | 7,4±1,1 | » |
| 15 | Native | 40,4±1,1 | 37,8±0,6 | Not significant |
| | Heated | 28,4±1,1 | 26,8±1,7 | » |

*Centrifugation carried out at 105,000g, in all other cases at 20,000g.

normal tissue homologous with the tumor (thigh muscles), and also macrophages of normal rats served as controls. The reactions were read after incubation of the tubes at 37°C for 2 h. The numerical results were subjected to statistical analysis by Student's t-test.

Experiments using the gel precipitation test showed that the sera of rabbits immunized with extract of the native tumor gave six precipitation bands with homologous antigens and three bands with antigens from normal muscle tissue. The same sera with heated tumor antigens formed two or three precipitation lines and gave negative results with heated muscle antigens (Fig. 1a). A similar picture also was observed when the sera of rabbits immunized with heated tumor extracts were tested with the above-mentioned antigens. The results of one such experiment are illustrated in Fig. 1b. These sera likewise did not react with heated extracts of other normal organs and rat tissues. These results indicate that specific antigens, not found in normal organs and tissues, can be detected by the temperature fractionation method in primary induced tumors. As previous observations [5] showed, these specific thermostable tumor antigens have electrophoretic mobility in the zones of $\beta$-, $\alpha$-, and $\alpha_1$-globulins.

In the next experiment thermostable antigens of malignant tumors were used to seek immune antibodies in the sera of rats with developing primary neoplasms. Altogether 16 experimental animals were used. These experiments showed that nine sera of tumor-bearing rats formed one band each with tumor extracts heated to 100°C for 10 min in the gel precipitation test, but did not react with native extracts of the same tumors or with native and heated extracts of normal organs and tissues (Fig. 1c). To detect immune antibodies in this experiment, fractions III and IV of concentrated sera of tumor-bearing rats were used.

The results show that in this experiment also thermostable tumor antigens were the most specific components of the immunologic test.

The results of the MAIT, using native and heated antigens from tumor and normal tissues are given in Table 1. They show that statistically significant positive results of the MAIT were obtained in all cases when heated antigens were used compared with native tumor antigens. Moreover, when the MAIT gave a significantly positive result with the use of both native and heated tumor antigens (rats Nos. 1, 3, 5, and 6) the percentage of nonadherent macrophages during the reaction with heated tumor antigens was considerably higher than with heated muscle antigens. Ultracentrifugation (105,000g) was found to have no advantage over the use of heated extracts.

The two immunologic tests thus showed that highly thermostable antigens of primary induced tumors of rat muscle tissue exhibit greater specificity than native tumor antigens. During heating it is evident that mainly the nonspecific tumor antigens are inactivated, whereas the most specific components remain in the active form. Data obtained by other workers indirectly confirm these results [6, 7].

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