THERMOSTABLE TUMOR-ASSOCIATED ANTIGENS IN THE BLOOD SERUM OF TUMOR-BEARING ANIMALS AND IN TISSUE CULTURES OF MALIGNANT TUMORS

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UDC 616-006-092.9-097.2

KEY WORDS: thermostable tumor-associated antigens; induced tumors; supernatants of culture fluids.

Investigations have shown that the blood serum of an animal with a growing tumor contains tumor-associated antigens, to which an important biological role is ascribed in the development of malignant neoplasms [2, 5-8, 9, 10, 12]. Indirect confirmation of these data has been obtained in attempts to detect these antigens in supernatants of cultures of malignant cells after explantation on a particular medium [10, 14].

The view has been expressed that the blood serum of an animal with a growing tumor contains a complex consisting of tumor-associated antigen and antitumor antibody, predominantly with an excess of this antigen [13]. The present writers showed previously that malignant rat tumors contain highly thermostable antigens which cannot be detected in most normal organs and tissues [3]. Humoral antibodies are known to be inactivated at a high temperature.

With these data in mind it was decided to study the presence of thermostable tumor-associated antigens (TTAA) in the blood serum of tumor-bearing rats and in the supernatant of culture fluids after explantation of malignant cells into them.

EXPERIMENTAL METHOD

Wistar rats of both sexes aged 6-10 months in which tumors had been induced in the thigh muscle by 9,10-dimethyl-1,2-benzanthracene (DMBA) were used. TTAA were determined in the blood serum of rats with both primary and transplanted induced tumors, and also in the supernatants of culture fluids after 8-12-day explantation of malignant cells of these tumors in 20% normal rabbit blood serum, inactivated at 80°C for 30 min, and diluted with medium No. 199 with the addition of penicillin (200 units/ml). Malignant tumor cells were cultured in the blood serum of the normal rabbits which were subsequently immunized with extracts of the tumors taken for explantation [4]. The gel-diffusion test and immunoelectrophoresis were used to study the sera of Chinchilla rabbits immunized with saline extracts from tissues of primary induced tumors, both native and heated to 100°C for 10 min, by the scheme described previously [3], and also the sera of rats hyperimmune to primary induced tumors, in which these tumors grew temporarily in the first passage, but then regressed, and which were reimmunized 1 month after resolution of the tumors by three intraperitoneal injections, at 3-day intervals, of extracts from primary induced tumors, both native and heated to the temperature indicated above.

The writers showed previously [1] that the sera of rats repeatedly immunized with antigens from homologous primary induced muscle tissue tumors contained, in a certain percentage of cases, antibodies which on immunoelectrophoresis were located in the γ-globulin zone. The complete identity of antigens of this tumor in the gel diffusion test has also been demonstrated when rabbit and rat antitumor sera were used. In the present investigation similar tests also were carried out beforehand.

Research Laboratory of Experimental Immunobiology, 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. 89, No. 4, pp. 452-454, April, 1980. Original article submitted March 13, 1979.

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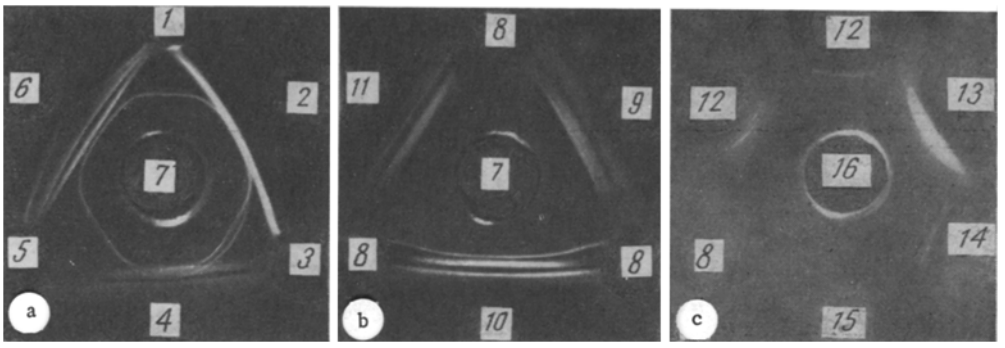

Fig. 1. Gel-diffusion test of rabbit and rat antitumor sera with rat antigens native and heated to 100°C for 5-10 min. 1, 3, 5) Different samples of heated tumor antigens; 2, 4, 6, 9-11) different samples of blood sera of tumor-bearing rats; 7) fraction IV of concentrated rabbit antitumor serum; 8) heated normal rat serum; 12) native supernatant of 12-day culture fluid; 13) the same supernatant, concentrated by Lifogel; 14) the same supernatant concentrated by Lifogel and heated; 15) heated extract of thigh muscle; 16) fraction IV of concentrated rat antitumor serum. Remainder of explanation in text.

Sera of tumor-bearing rats and of normal animals, both native and heated to 100°C for 10 min, native and heated saline extracts from tissues of the thigh muscle, lung, spleen, and supernatants of culture fluids obtained after explantation of malignant tumor cells on the above-mentioned nutrient medium, were used as the sources of antigens. The protein content in the antigens was determined by Lowry's method. Both native immune sera of rabbits and rats and also sera concentrated by the method described in [11] were investigated in immunologic tests. When this method is used five fractions of concentrated serum are obtained, of which fractions IV and V contain the highest concentration of antibodies. Supernatants of culture fluids of malignant cells were concentrated as described previously [3] with Lifogel (polyacrylamide gel for macromolecular concentration), which absorbs substances with a molecular weight of under 20,000.

EXPERIMENTAL RESULTS

An attempt was made in this investigation to determine TTAA which, according to our observations [3], are not found in homologous normal tissue, in the sera of tumor-bearing rats. For this purpose four fractions of concentrated rabbit antitumor sera, the sera of rats with developing primary induced tumors and of animals with transplanted tumors, and also saline extracts from tissues of primary induced tumors were tested. All antigens were heated to 100°C for 10 min. The experimental results showed that sera of tumor-bearing rats formed 2-4 bands in the gel-diffusion test, of which two lines were completely identical with thermostable antigens contained in tumor extracts (Fig. 1a, b). Antitumor rabbit serum gave only one very weak line with heated normal rat serum (Fig. 1b).

During immunoelectrophoresis the rabbit antitumor serum formed 2-4 precipitation bands with heated sera of tumor-bearing rats and with tumor extracts, which were located in the zones of β-, α-, and α_1-globulins. When heated normal rat serum was used only one line was obtained, in the α_1-globulin zone (Fig. 2). These results indicate that TTAA can be discovered in the sera of rats both with primary induced tumors and with transplanted tumors, by the use of the above-mentioned immunologic tests.

We also found TTAA in 12-day supernatants of culture fluids of malignant cells after their explantation on the above-mentioned nutrient medium. Rabbit antitumor serum gave one precipitation band with the supernatant of 12-day culture fluid, which was completely identical with one of the TTAA of the primary induced tumor, and one band which was not identical with heated normal rat serum. Hyperimmune rat antitumor serum formed one band with the native supernatant of the culture fluid and one band with the supernatant concentrated on Lifogel and heated to 100°C for 5 min, but did not react under these circumstances with heated extracts from normal organs (lung, spleen, thigh muscle) or with heated normal rat serum (Fig. 1c). During immunoelectrophoresis, TTAA of the supernatant of culture fluid was located in the α-globulin zone.

By the method of temperature fractionation TTAA could thus be detected in the sera of tumor-bearing rats and in supernatants of 12-day culture fluids of malignant cells after explantation to inactivated normal

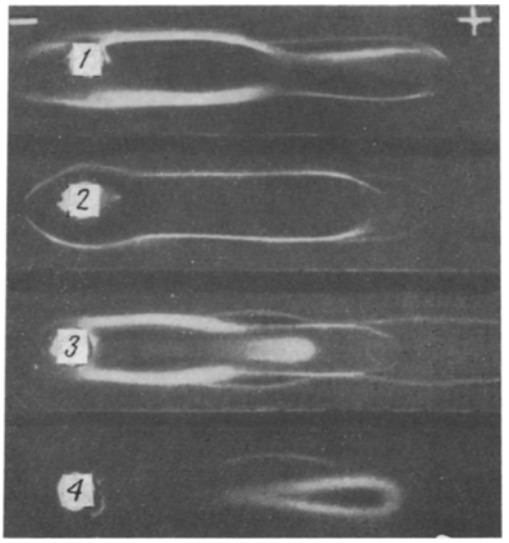

Fig. 2. Immunoelectrophoresis of fraction IV of concentrated rabbit antitumor serum with rat antigens heated to 100°C for 10 min. 1) Serum of rat with primary induced tumor; 2) extract of primary induced tumor; 3) serum of rat with progressively growing transplanted tumor; 4) normal rat serum. Gutters contain rabbit antitumor serum.

rabbit serum. It can be postulated that TTAA are synthesized by the malignant cells themselves and are secreted by them into the general blood stream.

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