AN ATTEMPT TO DETECT HUMORAL ANTITUMOR ANTIBODIES IN MICE IMMUNE TO ISOGENIC SARCOMAS, INDUCED BY METHYLCHOLANTHRENE

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The state of immunity in the presence of tumors is a manifestation of reactions of incompatibility between the tumor and the host, due to the presence of antigens in the tumor, which are absent in the normal host tissues. The mechanisms of specific antitumoral immunity have practically not been studied. This is explained by the difficulty in the development of tests permitting the elucidation of the role of humoral and cellular antibodies, participating in the resorption of tumoral cells in the immune organism. The scanty literature material on this problem gives evidence that the "cellular" antibodies are "responsible" for the antitumoral immunity, and evidently the same mechanisms participate here as in transplantation immunity. This has been demonstrated on models of carcinogenic sarcomas [5, 6]. As for the humoral antibodies, they appear regularly only in the case of lymph induced by viruses [9, 10].

This work represents an attempt to detect humoral antibodies in mice immune to isogenic sarcomas. The task of the investigation consisted of finding a test for humoral or "cellular" antibodies, under the control of which a study of the antigenic structure of the tumors might be conducted and the specific tumoral antibodies might be detected.

EXPERIMENTAL PROCEDURE

Mice of five highly inbred lines— C57BL10/Sn, C57BL/10 H-2d·(B10D2), C3H-H-2p (C3HNB), CC57W, CC57BR and cultures of isogenic sarcomas, induced by methylcholanthrene, were used in the work. The methods of immunization were described in detail earlier [1]. On the seventh and tenth days after the injection of the mice with live isogenic tumor cells in a dose to which the animals were immune, blood was taken from them from the retroorbital sinus.

Only fresh sera, obtained on the day of the experiment, were used for the cytotoxic reaction accordin to Gorer and O'Gorman [3]. To a test tube containing a suspension of 20,000 or 50,000 tumoral cells in 0.05 ml and an equal volume of serum of immune mice we added 0.05 ml of the complement obtained from a guinea pig, and incubated the mixture at 37° for 1.5-2.5 and 19 h. After incubation, the percent of live and dead cells was determined [8] in test tubes with immune sera, and sera of normal mice. The cytotoxic index [4] was calculated to evaluate the results.

For the reaction of 50% complement fixation, we used the Tarkhanova-Konikov modification [2], where a suspension of cells of the tumor under study was used as the antigen; the number of cells used in the reaction comprised 10,000 and 50,000.

Passive skin anaphylaxis according to Ovary [7] was conducted on guinea pigs 230-250 g in weight. Each pig received an intracutaneous injection of 0.1 ml of serum each from immune and healthy mice. Then at various periods (after 2 h 45 min and 6 h), 1-2 ml of a mixture of equal volumes of 1% Evans blue and tumor extract was

injected intravenously. The guinea pigs were killed 30 min after the intravenous injection. The degree of the reaction was evaluated by an examination of the inside of the skin.

RESULTS OF THE EXPERIMENTS

Using the cytotoxic reaction, we investigated 15 samples of sera of immune mice. According to the literature data, serum may be considered active if the cytotoxic index is equal to or greater than 0.15. In our experiments, the index varied from 0 to 0.11.

Using the complement fixation reaction, we proceeded on the assumption that specific antibodies are fixed on the surface of the cells, and this complex should absorb the complement from the system, in spite of the fact that the cytotoxic effect is not manifested in this case. Using this method, we investigated 6 samples of sera of immune and 12 sera of healthy mice. We noted a nonspecific bonding of the complement by sera both from immune and from healthy animals.

The method of passive skin anaphylaxis was developed on the model of egg albumin and the corresponding rabbit antiserum. In this immune system, distinct positive reactions were observed expressed in the formation of a blue spot on the skin of the test animal at the site of injection of the antibodies. The coloration was absent when normal sera were injected. Analogous results were obtained with serum of a rabbit immunized with mouse sarcoma SA-1. However, in isologous and even in homologous systems, the results were negative. In repeated experiments, we investigated 8 samples of sera of mice immune to an isologous tumor and 6 samples of sera of mice immune to a homologous tumor. In not one case did we observe even weak reactions.

Specific tumoral antigens are detected only under conditions of preimmunization of the host and induce low-intensity immunity. It may be that the mechanisms participating in the reactions of antitumoral immunity are analogous to those observed in the use of weak transplantation antigens of the H-1 and H-3 systems. Hence, methods permitting detection of an antibody to weak antigens of tissue incompatibility may prove more effective in the detection of specific antitumoral antibodies in an isologous system. The possibility also remains that in the case of specific antitumoral immunity, no humoral antibodies are formed.

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