A STUDY OF THE SIMPLIFIED ANTIGENIC STRUCTURE OF THE MOUSE LIVER DURING EARLY STAGES OF CARCINOGENESIS CARRIED OUT WITH THE AID OF ANALYTICAL IMMUNOELECTROPHORESIS

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During the last decade it has been established that tissues of induced and transplanted liver tumors differ in the antigenic composition from the liver of normal adult animals [2, 4, 6, 8, 9, 10, 11, 14, 16, 18, 19, 21]. Of special interest has been the observation dealing with the antigenic simplification by a hepatoma [7, 6, 21], considered to be an important argument for the "elimination theory" proposed by E. Miller and G. Miller [15].

It has been demonstrated that the antigenic simplification occurs in lymphoid tumors in mice [17]. Data have been obtained of simplification of antigenic structure of human tumors [5].

Different immunological procedures have been used in the above-indicated studies: complement fixation test (CFT), anaphylaxis and desensitization, agar precipitation reaction and fluorescent antibody reaction. The discovery of the antigenic simplification in carcinogenesis has been made by Weiler with the help of CFT [18-21] and by V. I. Gel'shtein, using anaphylaxis reaction [7].

The impossibility of separation of individual antigenic components in such complex mixtures as tissue extracts can be regarded as an inadequacy of the above-indicated methods. In this connection, new possibilities are suggested by the methods combining a separation of protein components according to their electrophoretic migrations with their immunologic characterization [3, 4, 9]. G. I. Abelev and associates studied the antigenic structure of the normal liver and a number of strains of transmissible hepatomas with the help of procedures involving immunofiltration and immunoelectrophoresis, developed and perfected by them. It has been demonstrated that the liver contains at least five individual organospecific antigens [9], and that the extent of loss of these antigens in the transmitted hepatomas varies in different strains and even in substrains.

The purpose of the present study has been the investigation of the phenomenon of antigenic simplification at different stages of chemical liver carcinogenesis, using analytical electrophoresis.

EXPERIMENTAL METHOD

L_3HA mice of both sexes, 2-3 months of age, were divided into 3 groups. A solution of orthoaminoazotoluene (OAAT) in benzol has been used to paint the skin of mice in group I three times a week. Mice belonging to group II received subcutaneous injections of 0.02 ml of pure carbon tetrachloride (CCl_4) twice a week.

The control mice belonged to group III; they were either entirely untreated or their skin has been painted with pure benzol (similar to the first group).

Extracts of livers of mice receiving different quantities of OAAT or CCl_4 served as antigens. For immunization of rabbits the extracts have been prepared in a physiological solution (pH was adjusted to 8.2 by means of 1% solution of NaOH). For adsorption of antisera and setting up of the precipitation reaction in agar, the antigens were prepared in distilled water in the ratio of 1 : 3 at pH 8.2. For immunoelectrophoresis the antigens were prepared in veronal-medinal buffer (pH 8.6). In all, 70 different samples of antigens have been used: 22 from mouse livers,

treated with OAAT; 28 from mouse livers treated with CCl_4; 10 from mouse livers treated with benzol; and 10 from livers of normal mice.

Two-three kilogram rabbits were immunized by subcutaneous and intraperitoneal injections of antigens from livers of normal, clean, mice and mice treated with OAAT and CCl_4, containing 15-20 mg of protein per ml. The first injection was made with Freund's adjuvants subcutaneously. After two weeks, six intraperitoneal injections of successively increasing doses were administered (total amount of protein 200-300 mg). Every 2-3 months the immunization cycles were repeated (3 intraperitoneal injections of increasing doses of antigen, one day apart). The blood used in the experiments has been obtained from marginal ear vein (at times from the heart) on 7-9th day after completion of immunization cycles. The sera were adsorbed by addition to them of mixtures of mouse serum and extracts of mouse kidneys, lungs and spleen. The degree of adsorption was regulated by agar precipitation reaction. The sera were tested by means of microprecipitation reaction in agar, as modified by Abelev [1] and by analytical immunoelectrophoresis [9]. Eighteen antisera against livers of mice with 20 treatments of OAAT, 21 antisera against livers of mice with 20 injections of CCl_4 and 17 against livers of normal mice were tested by the precipitation reaction. Reaction of different antigens with each antiserum has been repeated 3-4 times. Electrophoresis has been carried out in 1% agar, prepared in veronal-medinal buffer of ionic strength of 0.025 with pH 8.6. The potential gradient was 7 in/cm, the duration of reaction 90-120 min, at 12-14 ma. For immunoelectrophoretic reaction organospecific antisera, obtained from different rabbits, immunized with the same antigens, were pooled and concentrated according to Cohn's alcohol precipitation method [12], as modified by Gusev. Three of the sera used were prepared against the livers of mice treated with OAAT, three against livers of mice treated with CCl_4 and four against livers of normal mice.

EXPERIMENTAL RESULTS

The following systems have been compared by the precipitation reaction in agar with complete sera: normal liver against normal liver antiserum, precancerous mouse liver against antiserum to "precancerous" liver. No difference has been demonstrated between these systems.

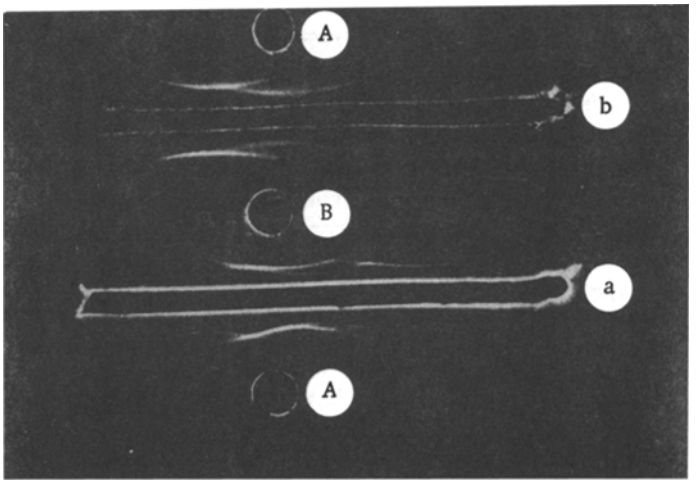
Adsorption of antiliver sera by a mixture of mouse antiserum and a mixture of extracts of normal lung, kidney, spleen and liver at different precancerous stages brought about by OAAT and CCl_4, as a rule led to complete exhaustion of this serum.

Because of the failure of these experiments, we decided to investigate the antisera against "precancerous" liver for the presence of organospecific antiliver antibodies. Adsorption of antiliver antisera with a mixture of serum and an extract of normal organs (except liver), allowed to uncover in this serum antibodies reacting only with the liver extracts. In the immunoelectrophoretic chamber, the reaction of the liver antigen with such a depleted serum showed five precipitation arcs (reaction between A and a, see figure), which fully confirmed the data reported by G. I. Abelev and his associates [9].

The indicated antiserum reacted in an analogous manner with extracts of "precancerous" liver, regardless of the stage of cancerogenesis of the liver used as the source of antigen. This indicated that all characteristic liver organospecific antigens are present in mouse livers at different precancerous stages, induced by OAAT and CCl_4.

At the same time, it became evident that if "precancerous" antisera are adsorbed with the same concentration of extracts of the normal organs, used for adsorption of antisera against normal livers, then the organospecific antiliver antibodies were not observed. Considerably smaller amount of the extracts was required for demonstration of these antibodies. Thus, if in the course of adsorption of antisera against normal liver, equal amounts of serum were added to a mixture of normal components, then 0.7 volume of a mixture of the same normal components was required for adsorption of one volume of antiserum against "precancerous" liver. However, even in these antisera antibodies have been found only against 3 and not all 5 of the antigens. In addition, the antisera reacted similarly with the antigens from normal and "precancerous" liver (reactions between B and b, A and b, see figure). Some sera against "precancerous" liver gave 1 or 2 arcs. None of the six tested antisera showed all the precipitating liver. The precipitation reaction in agar demonstrated that the unadsorbed sera against normal and "precancerous" liver had equal reacting capacity. Therefore, although at the precancerous stage all the organospecific liver antigens are present in the liver tissue, antisera against the normal and "precancerous" liver react in a different manner.

Apparently it is possible to explain this phenomenon by the difference in concentration of organospecific antigens in the normal and "precancerous" liver. It is probable, that with this there is also associated clearly and regularly the observation that in the anaphylaxis with desensitization there is a simplification of antigenic structure [6].



Immunoelectrophoretic reaction between the purified sera against the normal and "precancerous" liver and the antigens from normal mouse liver and from mice treated with the carcinogen. A) Antigen from normal mouse liver; B) antigen from livers of mice treated with 20 applications of OAAT; a) rabbit serum against normal mouse liver, adsorbed with a mixture of mouse sera and extracts of liver, lung, and spleen of normal mice; b) rabbit serum against liver of mice heated 20 times with OAAT, adsorbed similarly to serum a.

It is also possible, that upon addition to antisera of extracts of heterogenous organs, there takes place a more intensive nonspecific adsorption of antiorganospecific antibodies from the sera against "precancerous" liver than from sera against normal liver. It is possible that this phenomenon occurs in Weiler's experiments [18].

The difference in concentration of antigens may, in its way, be related to the homogeneity of cellular population. As it is demonstrated by the cytological experiments, even the parenchymatous cells are quite heterogeneous at the "precancerous" stage. Here one finds "dystrophic" cells, proliferating cells of adenomal nodules and normal cells. In addition, it is known that in the course of carcinogenesis there is an alteration in relationship between parenchymatous and connective tissue elements and epithelium of the bile duct [12]. It is entirely possible that there is also a difference in the antigenic characterization of these cells.

One could differentiate individual cells with the help of immunomorphologic method. However, in the studies reported to date with the marked antisera usually impure antibodies have been used, and therefore the results have been controversial [13, 15, 19].

Apparently, the utilization of antibodies in the immunomorphologic investigations of specific antigens is of greater potential importance, particularly for studies of changes in the antigenic structure during carcinogenesis.

SUMMARY

Analytical immunoelectrophoresis was used to investigate the antigenic structure of the liver during early stages of carcinogenesis induced in C_3HA mice with orthoaminoazotoluene (OAAT) and CCl_4.

The rabbit organospecific antiliver sera exhibit the presence of all the 5 organospecific liver antigens in the reaction with antigens from normal liver and the liver of mice at various stages of carcinogenesis.

But the sera against precancerous liver showed antibodies active against but three organospecific antigens.

In this instance to deplete the above sera a smaller quantity of heterogenous antigens was needed for exhaustion of the antinormal ones. A possible explanation of these findings is discussed.

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