ANTIBODIES TO CONNECTIVE TISSUE IN MONOSPECIFIC ANTITISSUE SERA

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In the immunology of normal and tumor growth, increasing importance comes to be attached to the monospecific sera, i.e., sera which react only with antigens specific to the particular tissue under study.

Immune antitissue sera contain antibodies to various antigens, and only a small proportion are directed against antigens specific to the organ used for the immunization. The great majority of the antibodies give crossed reactions with antibodies of other organs. The crossed reactions may be excluded by exhausting the immune serum with various antigen preparations from hetorologous organs [5-10]. For a comparative study of the antigens of normal and tumorous tissues by precipitation in agar, immune antitissue sera neutralized with extracts of hetarologous organs are often used [1, 2, 4, 12].

In the present work we have shown that such sera are monospecific only with respect to the soluble antigens, and that they contain a large number of antibodies to the insoluble antigens of connective tissue. Antibodies to insoluble antigens are not revealed by the precipitation reaction, but may easily be demonstrated in tissue sections by means of Coons' fluorescent antibodies [13, 14].

METHOD

We used serum from rabbits repeatedly immunized by a homogenate of a digested hepatoma of mice of line SZNA, by the mitochondrial and microsomal fraction of the same hepatoma, or by a homogenate of mouse liver. The sera were obtained by immunization with Z. A. Postnikova's lanoline hand cream. The scheme for the immunization has been described previously [4].

The activity of the sera, the completeness of the neutralization, and the specificity were tested by a micromethod [3] of precipitation in agar. Extracts of mouse organs were used as antigens for precipitation in agar and for neutralization of the sera. The organs were homogenized in a mill for $1\frac{1}{2}$-2 minutes with three times their volume of distilled water. The homogenate was centrifuged for 10 minutes at 1500 revs/minute, the supernatant fluid was made alkaline to pH 8.6-8.9, it was cleared by centrifugation at 8500 revs/minute for 20 minutes, and preserved in 1 : 10,000 merthiolate. The sera and antigens were kept at + 4°.

The sera were neutralized by antigens from heterologous organs with control by precipitation in agar [1].

The distribution of the antigens in the sections of the mouse organs were determined by Coon's indirect method [14], and donkey anti-rabbit serum labeled with fluorescein isocyanate was used. To reduce the nonspecific binding of the sera by the sections, they were adsorbed with acetone liver powder twice per hour at room temperature; 70 mg per ml of serum was used. Donkey serum was adsorbed by mouse liver powder, and rabbit serum by rabbit liver powder. After adsorption, the pH of the sera was measured and brought to 7.2-7.6. The mice were killed by decapitation, large portions of the organs were transferred to test tubes, frozen in a mixture of solid CO_2 and acetone, and sections 3 μ thick were cut in a cryostat at − 10°. The sections were transferred to clean cooled slides, melted, and dried in a stream of air at 4-6° for about one hour, after which some of the sections were fixed for 20 minutes in acetone at room temperature, and the remainder for 15 minutes in 95% alcohol at 37°.

The sections were incubated with the adsorbed sera for 30 minutes at room temperature under Petri dishes containing moist cottonwool. The unbound sera were washed in physiological saline buffered at pH 7.0 with 0.01 M

phosfate buffer. The treated sections were placed in 10% glycerine made up with the same buffered physiological saline, cover slips were placed over them, and held in position by paraffin, and the sections were kept at 4°. They were examined in ultraviolet light under a ML-1 microscope, and photographed on to RF-3 film. They were also examined by phase-contrast. Some of the sections were stained with hematoxylin, or by Hotchkiss' Schiff-iodic acid [16].

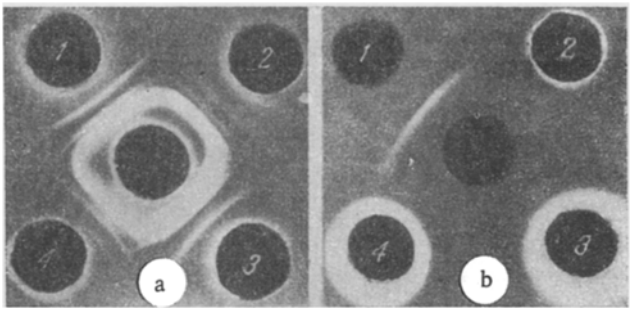

Fig. 1. Precipitation in agar of the serum of rabbits immunized with a homogenate of a hepatoma from a mouse of line SZNA with extracts of mouse organs. a) Initial antihepatoma serum; b) globulin fraction of the same serum after neutralization with extracts of normal mouse organs ("monospecific" antihepatoma serum); 1) extract of hepatoma; 2) extract of spleen; 3) extract of liver; 4) extract of kidney.

RESULTS

With both extracts of normal mouse organs and with hepatoma extracts the original anti-hepatoma serum formed a spectrum of bands in the reaction of precipitation in agar (Fig. 1a). It was neutralized with extracts of normal mouse organs and of mouse serum, and then the β- and γ-globulins were separated from it by electrophoresis. The remaining fraction reacted by precipitation in agar only with the hepatoma extract (Fig. 1b).

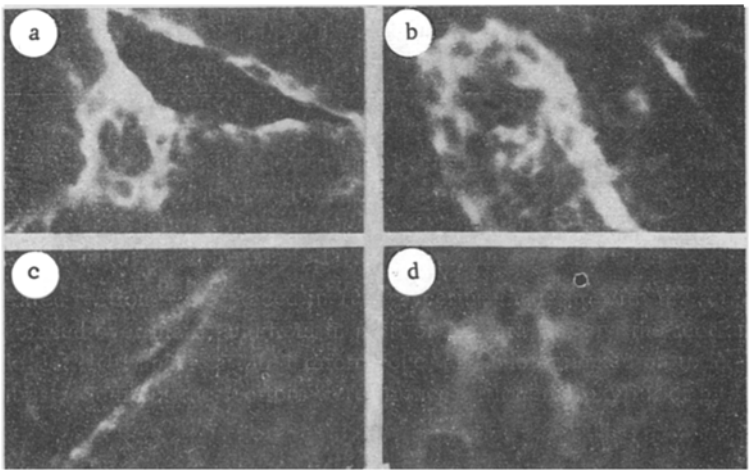

Fig. 2. Sections of (a) a liver and (b) a hepatoma of mice treated with a "monospecific" antihepatoma serum and then with donkey antirabbit serum labeled with fluoresceine isocyanate. In both cases the connective tissue fluoresces. Control: (c) sections of liver and (d) sections of a hepatoma from mice treated with normal 1:10 rabbit serum and then with labeled donkey antirabbit serum. Magnification 900 times.

However, when examined in ultraviolet light, on sections of the hepatoma treated with "monospecific" antihepatoma serum and then with labeled donkey serum, the connective tissue fluoresced very strongly, but the cancerous cells themselves did not (Fig. 2b). The connective tissue also fluoresced brightly in liver sections against a background of completely unstained cells of liver parenchyma, although the serum did not react with liver extract in the agar precipitation test (Fig. 2a). After complete neutralization of the "monospecific" antihepatoma serum with an extract of the hepatoma, it then reacted as before with connective tissue on sections of the hepatoma and of normal mouse organs.

On control sections treated only with labeled donkey serum, and also on sections incubated with normal rabbit serum and then with labeled donkey serum there was no selective staining of the connective tissue (see Fig. 2c, d).

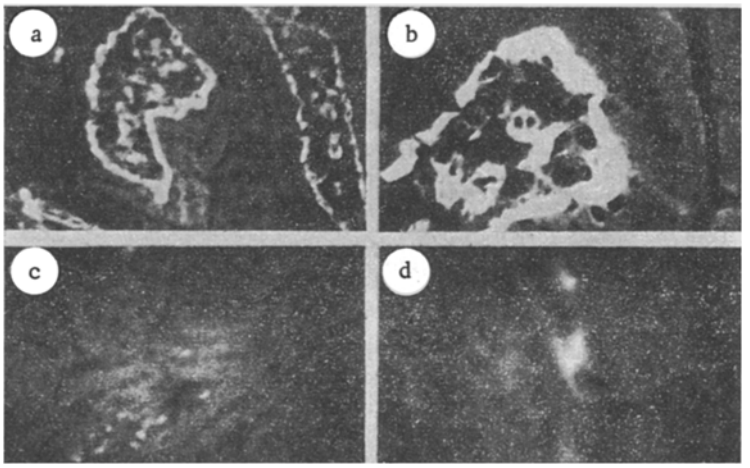

Fig. 3. Sections of a mouse intestine treated with a fraction of the antibodies against connective tissue from serum of rabbits immunized with a homogenate of a hepatoma from mice of line SZNA, and then with labeled donkey antirabbit serum (a, b). Control: sections of a mouse intestine treated with normal 1:10 rabbit serum (c) and with the fraction of normal rabbit serum corresponding to the fraction of the antibodies against connective tissue (d), and then with labeled donkey antirabbit serum. a, c) 400 times; b, d) 900 times.

Staining of the connective tissue in sections of mouse organs was related to the presence in the neutralized antihepatoma serum of antibodies which were not revealed in the agar precipitation test made with extracts of hepatoma and of normal mouse organs and directed against the insoluble antigens of the connective tissue.

Subsequently, these antibodies were purified and separated from all the tested sera of rabbits immunized with mouse liver homogenate (one serum), with a homogenate of a hepatoma of mice of line SZNA (mixture of two sera), and also with the mitochondrial and microsomal fraction of this hepatoma (mixture two sera).

The separated antibodies did not react at all with extracts of the hepatoma, liver, kidneys, spleen, or lungs, but stained strongly and specifically the connective tissue on sections of various mouse organs (Fig. 3).

The antibodies to connective tissue which we found in the "monospecific" serum were apparently identical with those from nephrotoxic sera studied by Coons' direct method by Hill and Cruickshank [15, 17].

It has long been known that sera of animals immunized by the basement membrane of renal glomeruli or by a homogenate of kidneys of another species produce a nephrotoxic effect when injected in vivo. Nephrotoxic antibodies are also formed when the animals are immunized by pulmonary tissue, and to a lesser extent by the tissue of many other organs [11].

Hill and Cruickshank showed that nephrotoxic antibodies are directed against reticulin, the antigen of connective tissue, which is common both to the basement membranes and to the reticular fibers of all the rat organs they studied.

The nephrotoxic activity of the sera did not depend on their activity in the precipitation reaction with renal extracts.

We did not test in vivo the effect of the antibodies revealed in our experiments; however, the type of staining which they produced in sections agreed entirely with the results of Hill and Cruickshank (see Fig. 3).

When neutralized antitissue sera are used it is essential to remember that antibodies against insoluble antigens may be present in them and may show up in various immunological reactions, or when in vivo injections are made.

Even in the sera of rabbits immunized by the mitochondrial and microsomal fractions of a mouse hepatoma we found antibodies against the insoluble mouse connective tissue antigens.

The neutralized antitissue sera which are monospecific in reactions with soluble antigens must be considered as monospecific only with respect to the reactions studied and the antigens used, but not in relation to all tissue antigens in general.

To conclude we would like to express our sincere thanks to A. Ya. Fridenshtein and M. Ya. Korn for their continual help and for advice during the whole of the investigation.

SUMMARY

After neutralization with mouse organ extracts, antibodies against insoluble connective tissue antigens were retained in the sera of rabbits immunized with mouse hepatic tissue or with hepatoma tissue. These antigens were not detectable by precipitation in agar, but were distinctly shown in sections treated by Coons' method. They are evidently identical with nephrotoxic antibodies.

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