ANTIBODY FORMATION AGAINST CONNECTIVE TISSUE COMPONENTS IN ANIMALS IMMUNIZED WITH HETEROLOGOUS HEART TISSUE

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In rabbits immunized with human and guinea pig heart tissue homogenate antibodies are formed against the components of the muscle fiber and connective tissue of the heart and other organs (kidneys, liver).

In animals immunized with heterologous heart tissue, antibodies reacting with components of heterologous, homologous, and autologous heart tissue are produced [6, 8, 10]. Under these conditions antibodies against organ-specific antigens of the heart tissue are formed [8-10]. Meanwhile, some investigators have found crossed reactions of the sera with tissues of other organs [2, 8].

The localization of the components of heart tissue against which antibodies are formed by immunization with heterologous heart tissue has been studied by the immunofluorescence method. According to Kaplan and Meyerserian [10], the sera of rabbits immunized with bovine heart homogenate contained two types of antibodies: against the subsarcolemmal and intermyofibrillary components of the muscle fiber. Intermyofibrillary fluorescence corresponded to the localization of the organ-specific antigen of heart tissue. In more recent investigations [7] after immunization of rabbits with citrate extracts of human heart tissue, antibodies reacting with the sarcolemma and subsarcolemma of the muscle fiber were obtained. The sera of these rabbits also reacted with elements of other organs, namely the basement membranes reticulin, and intima of the blood vessels. Hence, in these investigations no antibodies against the connective tissue of the heart were found.

In the investigation described below, the possibility of antibody formation against the connective tissue of the heart after immunization of animals with homogenate of the heart tissue of another species was studied. In a parallel investigation, antibodies against components of the muscle fiber were studied. To investigate the manner in which antibodies against particular tissue structures penetrate in the intact organism, bound γ globulin was studied in the heart tissue of the immunized rabbits.

EXPERIMENTAL METHOD

The immunofluorescence method of investigation with pure antibodies against rabbit γ globulin was used. The rabbits were immunized with human or guinea pig heart tissue homogenate. Fresh or frozen (−20°C) heart tissue was washed with 0.85% NaCl solution to remove blood and then homogenized with the addition of 4 ml 0.85% NaCl solution, made up in 0.01 M phosphate buffer, pH 7.2, per gram tissue. The homogenate was mixed with an equal volume of alumina and injected into the animals once a week in a dose of 4 ml of the mixture subcutaneously into the scapula and femoral regions. Altogether 9 cycles of immunization were carried out (each cycle consisted of four injections). Eight rabbits were used for the

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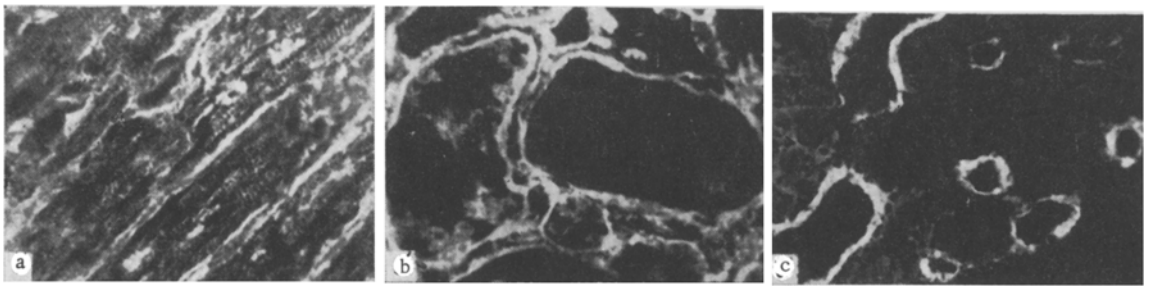

Fig. 1. Test of sera of rabbits immunized with human heart tissue homogenate on sections of human tissues: a) section of heart tissue, fluorescence of sarcolemma, sarcoplasm, disks, and interstitial connective tissue; b) section through kidney tissue, fluorescence of basement membranes of tubules and interstitial connective tissue; c) section of liver tissue, fluorescence of basement membranes of biliary capillaries. Here and in Fig. 2: Magnification: objective 40 ×, ocular 3 × (Homal).

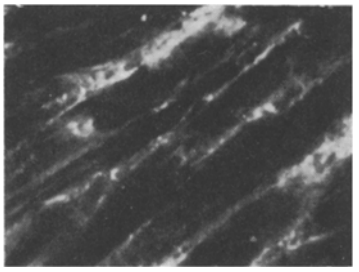

Fig. 2. Bound γ globulin in heart tissue of a rabbit immunized with human heart tissue homogenate.

experiment; four were immunized with human heart tissue homogenate and four with guinea pig heart homogenate. To remove the antibodies against serum components, the sera of the immunized rabbits were absorbed with human or guinea pig serum (0.1 ml/ml antiserum).

Pure antibodies against rabbit γ globulin were obtained from an anti-rabbit ass serum by the method of Avrameas and Ternynck [3] by binding the γ globulin with glutaraldehyde. The rabbit γ globulin, obtained by the method of Baumstark et al. [4], was dissolved in 0.1 M phosphate buffer, pH 7.0, in a concentration of 50 mg/ml, and treated with 2.5% aqueous solution of glutaraldehyde in the proportion of 10 mg/100 mg protein. The gel thus formed was kept for 3 h at room temperature and washed with 0.2 M phosphate buffer, pH 7.2, to remove unbound protein. Antiserum against rabbit globulins was added to the washed immunosorbent, the mixture incubated for 30 min, and the gel then washed with buffered physiological saline, pH 7.0, and the antibodies eluted with 0.1 M glycine-HCl buffer, pH 2.8. The pure antibodies were conjugated with fluorescein isothiocyanate by the method of Riggs et al. [12] in Blagoveshchenskii and Kul'berg's modification [1]. The unbound dye was removed by filtration through Sephadex G-25. Circulating antibodies were studied in sections of normal human (tissue taken from persons dying accidentally), rabbit, and guinea pig heart, kidney, and liver tissue. The pieces of tissue were frozen at −70°C. Sections 4 μ in thickness were cut in a cryostat from frozen, unfixed tissue. Unfixed tissue sections were used in the experiments.

The circulating antibodies were studied by the indirect immunofluorescence method. The sections were treated with antiserum for 40 min at room temperature washed for 15 min with buffered physiological saline, pH 7.0, and then stained for 30 min with labeled antibodies. To remove nonspecific fluorescence, the sera and labeled antibodies were twice absorbed with mouse liver powder. The bound γ globulin was studied by the direct immunofluorescence method. Sections of the organs of immunized rabbits (heart, kidney, liver, muscle) were washed for 30 min with buffered physiological saline, pH 7.3, and stained for 45 min with labeled antibodies against rabbit γ globulin.

The sections were examined with the ML-2 luminescence microscope using a 40 × objective and Homal 3 × ocular.

EXPERIMENTAL RESULTS

Antisera against human heart, when tested on human tissue sections, reacted strongly with the sarcolemma and sarcoplasm of the muscle fiber. In some cases fluorescence of the discs was observed, and rarely fluorescence of the intercalated disks of the muscle fiber. In addition, fluorescence of elements of the interstitial connective tissue could be seen (Fig. 1a). When the same sera were tested on sections of other human organs, the following results were obtained. On kidney tissue sections fluorescence of the basement membranes of the glomeruli and tubules was observed. Fluorescence of elements

of the interstitial connective tissue also was seen (Fig. 1b). In liver tissue, the membranes of the biliary capillaries and connective tissue of the triads fluoresced brightly (Fig. 1c).

To determine whether the fluorescence observed in the human tissue sections was associated with the presence of isoantibodies in the rabbit sera, the sera were absorbed with group IV (AB) human erythrocytes (undiluted sera were mixed with an equal volume of erythrocytes for absorption and left overnight at 4°C). The absorbed sera were still able to react with the above-mentioned tissue elements.

In tests of human heart antiserum on sections of guinea pig heart tissue, fluorescence of the same elements of the muscle fiber (sarcolemma, sarcoplasm, disks) was observed. The fluorescence of the interstitial connective tissue also remained, although it was less bright than in the human heart sections. In sections of guinea pig, kidney, and liver tissue, fluorescence of the connective tissue was observed. Fluorescence of the basement membranes was less distinct.

In sections of rabbit heart tissue, only elements of the muscle fiber showed a reaction. Fluorescence of the connective tissue was absent. The intensity of the reactions was weaker than in human and guinea pig tissue sections. When the sera were tested on sections of other rabbit organs, no reaction took place. Fluorescence of elements of the muscle fiber also remained when the sera were tested on sections of the heart of an immunized animal, i.e., autoantibodies were found in the sera.

The sera of rabbits immunized with guinea pig heart tissue homogenate, when tested on sections of guinea pig heart tissue, reacted strongly with components of the muscle fiber and, in particular, with the sarcolemma and sarcoplasm, the transverse disks and, less frequently, the intercalated disks. Fluorescence of elements of the interstitial connective tissue also was observed. In sections of kidney and liver tissue, fluorescence of the parenchymatous elements was not found. As in sections of human tissue, the reaction was localized to the basement membranes and interstitial connective tissue, but was weaker in its intensity. When tested on sections of human organs the sera reacted with the same tissue elements as on sections of guinea pig organs.

γ-Globulin bound with the tissues was found in the heart tissues of all eight immunized rabbits between the 14th and 30th day after immunization. No later tests were carried out. The γ globulin was localized mainly in the interstitial connective tissue, but in some cases fluorescence was found on the territory of the muscle fibers, as individual spots or as diffuse staining. Bound γ globulin also was found in the kidney tissue of immunized rabbits, in the interstitial connective tissue, and also in the region of the basement membranes of the glomeruli and tubules. Virtually no bound γ globulin was found in the liver and skeletal muscle tissue.

After immunization of animals with homogenate of heterologous heart tissue, antibodies are thus formed against the connective tissue components of the heart and other organs. Fluorescence of the interstitial tissue and basement membranes was observed in sections of human and guinea pig tissues but was absent in sections of rabbit tissues. However, bound γ globulin was found in the tissues of immunized rabbits, mainly in connective-tissue structures. On this basis it can be postulated that antibodies are formed against various connective-tissue antigens. Antibodies against a common antigen to human, guinea pig, and rabbit connective tissue could be bound with the corresponding components of the rabbit tissue and be absent from the blood. Another possibility is that the same connective-tissue antigen is located differently in different species of animals, and in the rabbit it is inaccessible to circulating antibodies because of its localization in the depth of the tissue.

The results also indicate that common antigenic determinants exist in the connective tissue of different organs: heart, kidney, and liver. Similar results were obtained previously by Cruickshank and Hill [5] who described a common antigen in the sarcolemma, basement membranes, reticulin, and neurilemma of the rat. Other evidence of the existence of common antigens in connective tissue has also been obtained [11].

The antibodies detected in these experiments against muscle components of heart tissue were autoantibodies, for they reacted with the heart tissue of the immunized animal itself.

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