LOCALIZATION OF MYOCARDIAL ANTIGENS BY IMMUNOFLUORESCENCE

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The localization of two myocardial antigens, previously discovered by immunodiffusion methods, was studied by the indirect immunofluorescence method with the aid of antibodies isolated from the sera of rabbits immunized with a heterologous heart tissue homogenate. One antigen was found to be an organ-specific antigen of the heart and was localized in the sacrolemma of the myocardium in all species of animals studied and in man. The second antigen was found only in primates and was localized in the sarcolemma and disks of the muscle fiber of the myocardium and skeletal muscles, membranous structures of the liver and kidney, and in interstitial connective tissue.

In some autoimmune diseases (rheumatic fever, myasthenia gravis, the postinfarction and postcommissurotomy syndromes) autoantibodies against the same myocardial structure are found in the patients' sera. In these pathological processes autoantibodies against the sarcolemma of the mycardial muscle fiber have been found [13, 14]. Since the autoantibodies may be directed against different antigens of this structure, it is necessary to investigate the antigens of the sarcolemma.

Myocardial antigens have been studied mainly by the immunofluorescence method with the aid of anti-heart [16] and antistreptococcal sera [5, 6, 17, 10]. This method has shown that the sarcolemma of the myocardium evidently contains several antigens.

Eight antigens have been found by immunodiffusion methods in citrate extracts obtained from washed homogenates of human heart tissues [10-12]. One of them – an organ-specific myocardial antigen – when studied by immunofluorescence was found to be localized in sarcolemmal-subsarcolemmal zones and, to some extent, in the disks of the muscle fiber; six antigens are common to cardiac and skeletal muscles and are localized in the sarcolemma and subsarcolemma; an eighth antigen has been found in the myocardium, skeletal muscles, liver, kidney, spleen, and lungs.

Two antigens have been found by immunodiffusion methods in hydrochloric acid extracts obtained from preparations of the myocardium containing sarcolemma, using sera of rabbits immunized with heart tissue homogenate [2]. One of them is specific for heart tissue only and is found in man and guinea pigs (antigen No. 1). The second antigen is found in tissues of the human myocardium, liver, and kidney but is not found in the guinea pig (antigen No. 2).

The object of the present investigation was to study, by means of immunofluorescence methods, the localization of myocardial antigens previously found by immunodiffussion methods in hydrochloric acid extracts obtained from preparations of the myocardium containing sarcolemma.

EXPERIMENTAL METHOD

Experiments were carried out by the indirect immunofluorescence method using pure antibodies against rabbit γ-globulin labeled with fluorescein isothiocyanate (the Coons' method in Éngel'gardt's

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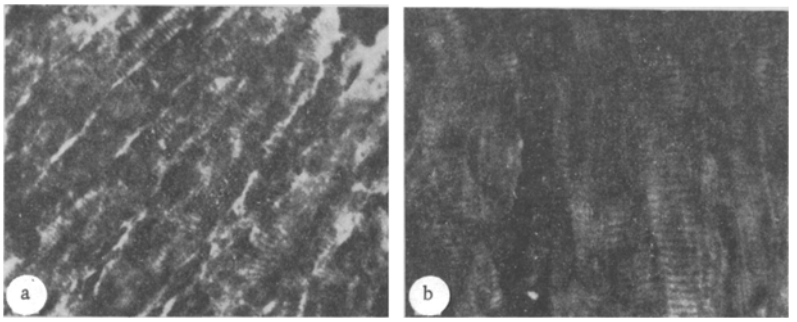

Fig. 1. Tests for antibodies isolated from serum No. 438: a) unadsorbed antibodies. Section through monkey heart tissue – fluorescence of sarcolemma, sarcoplasm, and disks; b) antibodies adsorbed by fraction containing antigen No. 1. Section through monkey heart tissue – fluorescence of sarcoplasm and disks. Fluorescence of sarcolemma absent. Here and in Fig. 2, objective 40×, Homal 5×.

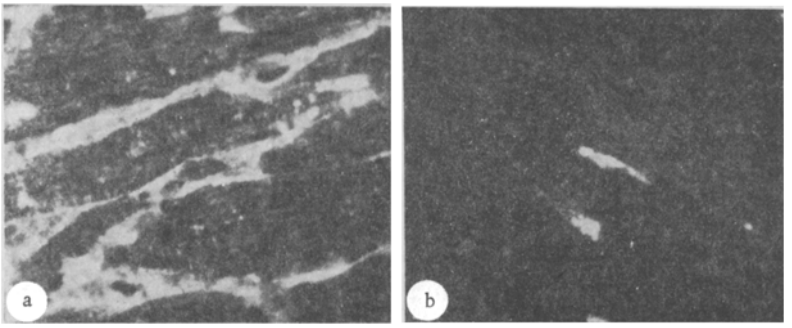

Fig. 2. Test of antibodies isolated from serum No. 117: a) unadsorbed antibodies. Section of human heart tissue – fluorescence of sarcolemma, disks, and interstitial connective tissue; b) antibodies adsorbed by fraction containing antigen No. 2. Section of human heart tissue – no fluorescence of its elements. Deposits of fat are fluorescent.

modification [7]). γ-Globulin was obtained by the method of Baumstark et al. [9]. Pure antibodies were isolated from ass serum against rabbit globulins with the aid of an immunosorbent by the method of Aurameas and Ternynck [8] and conjugated with fluorescein isothiocyanate by the method of Riggs et al. [19] in the modification of Blagoveshchenskii and Kul'berg [1].

The localization of the antigens was studied in unfixed sections of normal tissue of the human heart, skeletal muscles, liver, and kidney (blood group O). The tissues of the organs were taken from persons dying from accidents. Tissues of organs from monkeys (Macaca rhesus) and guinea pigs and bovine tissues also were used. The method of preparing the sections and treating them with antibodies against tissue antigens and with labeled antibodies against γ-globulin is described elsewhere [7]. The specimens were examined with the ML-2 luminescence microscope (objective 40×, Homal 5×).

Antibodies isolated from the sera of rabbits immunized with a homogenate of human or guinea pig heart tissue were used in the work. (The scheme of immunization is given in a paper by Borodiyuk et al. [3].) To isolate the antibodies two sera were chosen, each of which contained antibodies against only one of the antigens studied. Serum No. 438 was obtained as a result of immunization of a rabbit with guinea pig heart tissue homogenate and it contained antibodies against antigen No. 1. Serum No. 117 was obtained after immunization with human heart tissue homogenate, and it contained antibodies against antigen No. 2. This was shown by immunodiffusion methods by Borodiyuk [2]. The sera were first treated with normal human or guinea pig serum. Antibodies against the antigens to be studied were isolated from the sera with the aid of immunosorbent. The immunosorbent was prepared by cross-linking the hydrochloric acid extract obtained from preparations of human myocardium containing sarcolemma with glutaldehyde by the method of Avrameas and Ternynck [8]. The hydrochloric acid extract obtained from preparations of the myocardium

was taken for "cross-linking" in a concentration of 50 mg protein/ml. The preparation containing sarcolemma was obtained by treating human myocardial homogenate by McCollester's method [18].

The antibodies isolated with the immunosorbent were adsorbed by two different fractions containing the antigens studied. The fractions were obtained by preparative electrophoresis. To obtain the fractions hydrochloric acid extracts prepared from samples of myocardium containing sarcolemma were used. Preparative electrophoresis was carried out in the modification of Abelev and Tsvetkov [4]. The presence of antigens Nos. 1 and 2 in the fractions was determined by the precipitation test in agar gel with antiheart sera. One fraction contained antigen No. 1, whose mobility in immunoelectrophoresis is slightly greater than that of serum albumin. The second fraction contained antigen No. 2, migrating at the rate of the serum β-globulins. The antibodies were adsorbed by antigens at the rate of 600 μg antigen protein to 0.5 ml of eluate containing antibodies. The eluate of the antibodies was used in a working dilution of 1:4. The mixture of antibodies and antigen was incubated for 2 h at 37°C.

EXPERIMENTAL RESULTS

Antibodies isolated from serum No. 438 reacted with the sarcoplasm, disks, and sarcolemma of the muscle fiber in sections of guinea pig myocardial and skeletal muscle tissues (Fig. 1a). Fluorescence of bile capillaries was observed in the sections of liver tissue and fluorescence of the basement membranes of the tubules and glomeruli in sections of the kidney. Fluorescence of all these structures also was observed in sections of human, bovine, and monkey heart, skeletal muscle, liver, and kidney tissues. Fluorescence of interstitial connective tissue was not observed in any of these organs.

Antibodies isolated from serum No. 117 reacted with the sarcolemma and disks of the muscle fiber and also with the interstitial connective tissue in sections of human myocardial and skeletal muscle tissue. Fluorescence of the sarcoplasm was not observed (Fig. 2a). In tests of the antibodies in the liver tissue sections fluorescence of the membranes of the bile capillaries was observed, while in sections of kidney tissue there was fluorescence of the basement membranes of the tubules and glomeruli. Fluorescence of elements of the interstitial connective tissue also was observed. Similar results were obtained in tests of antibodies in sections of the monkey organs. No reaction was observed with tissue sections from guinea pig and bovine organs.

As a result of adsorption of the antibodies obtained from serum No. 438 by the fraction containing antigen No. 1, no fluorescence of the sarcolemma was found in tissue sections of the human and animal heart. Fluorescence of the disks and sarcoplasm of the muscle fiber of the myocardium and skeletal muscles, the sarcolemma of the skeletal muscles, and also the membranous structures of the liver and kidney was preserved (Fig. 1b). Adsorption of antibodies obtained from the same serum by the fraction containing antigen No. 2 did not change the character of fluorescence of the elements of the myocardium and other tissues.

Tests of antibodies obtained from serum No. 117 showed that adsorption by the fraction containing antigen No. 1 did not affect the character of fluorescence of the elements in any of the tissues. In a study of the same antibodies after adsorption by the fraction containing antigen No. 2, fluorescence was absent in tissue sections not only of the myocardium, but also of human skeletal muscles, liver, and kidney (Fig. 2b). Similar results were obtained in tests of the antibodies after adsorption by fractions containing antigen Nos. 1 and 2 in monkey tissue sections.

The localization of some myocardial antigens contained in hydrochloric acid extracts of preparations of human myocardium was thus studied by the indirect immunofluorescence method. An organ-specific heart antigen was found with the aid of antibodies isolated from serum No. 438. (This serum contains antibodies against antigen No. 1.) It is localized in the sarcolemma of the myocardium of man and of all the species of animals studied. This antigen is evidently identical with the organ-specific antigen previously found by Borodiyuk [2] by immunodiffusion methods, for the fraction containing this antigen abolished the reaction of the antibodies with the sarcolemma of the muscle fiber of the myocardium only. Antigen No. 1 is similar in some of its properties with the organ-specific antigen described by Espinosa and Kaplan [12]. Both antigens are protein in nature, thermostable, and not extracted by lipid solvents [2, 12]. However, the antigen found in the writers' laboratory is localized in the sarcolemma of the myocardial muscle fiber, and its immunoelectrophoretic mobility is a little higher than that of serum albumin [2]. The antigen described by Espinosa and Kaplan, on the other hand, was found in sarcolemmal-subsarcolemmal zones and also, to some extent, in the disks of the myocardial muscle fiber. Its immunoelectrophoretic mobility is between that of the serum α_1- and β_2-globulins [12].

In the study of antibodies isolated from serum No. 117 fluorescence of such different structures as the sarcolemma and the disks of the myocardial and skeletal muscle fiber, the membranous structures of the liver and kidney, and the interstitial connective tissue was observed. On adsorption of these antibodies with the fraction containing antigen No. 2, complete suppression of the fluorescence of all these structures was observed. Possibly the antigen may be the same and localized in different structures, but found only in primates. The possibility cannot be ruled out that the fraction in which antigen No. 2 is found contains several antigens.

Espinosa and Kaplan [12] also found an antigen common to the human heart, muscles, liver, kidney, and spleen. However, before this antigen can be compared with the common antigen for different tissues found in the authors' investigations, they must receive further study.

Antibodies isolated from serum No. 438 continue to react with some structures after adsorption by the fraction containing antigen No. 1. These reactions also were not abolished after adsorption with the fraction containing antigen No. 2. The hydrochloric acid extract obtained from preparations of the myocardium containing sarcolemma evidently contains not only antigens Nos. 1 and 2, but also other antigens which are not found by immunodiffusion methods.

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