DETERMINATION OF CIRCULATING ANTIBODIES AND TISSUE-BOUND γ-GLOBULIN IN RABBITS WITH ALLERGIC MYOCARDITIS BY THE IMMUNOFLUORESCENCE METHOD

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Antibodies reacting with components of the I-disks of myocardial muscle fibers in the region of the subsarcolemma and with the cytoplasm of smooth-muscle cells of the blood-vessel walls were found in the blood serum of rabbits immunized with a homogenate of homologous heart tissue mixed with a streptotoccal culture. A γ-globulin was found in the heart tissues of immunized rabbits, localized in the connective-tissue structures of the organ.

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Investigations have shown that repeated injection of a homogenate of homologous heart tissue mixed with a streptococcal culture into animals is followed by the development of allergic myocarditis [11, 12]. The severest changes in the tissues and changes in the heart were obtained practically in all animals when a living culture was used [2, 6]. Antibodies reacting with the tissue components of the heart [7, 10, 11] were found by various serologic methods in the blood serum of the immunized animals [7, 10, 11].

Serum injected into recipient animals has a cardiotoxic action, manifested by death of myocardial muscle fibers [1, 2]. However, despite the available data, the pathogenesis of experimental allergic myocarditis has not yet been fully explained. For example, the precise components of myocardial tissue against which antibodies are formed have not been established.

In the present investigation an attempt was made, by means of the immunofluorescence method, to determine the structures of heart tissue with which the antibodies react and to detect bound γ-globulin in the tissue of the affected organ.

EXPERIMENTAL METHOD

Experiments were carried out on 75 rabbits with a mean weight of 3 kg. The animals were divided into four groups: group 1 (30) were injected intraperitoneally with homogenate of homologous heart tissue mixed with a living streptococcal culture (Group A, Type 1). The animals of groups 2 and 3 (15 in each) were injected with tissue homogenate only and streptococcal culture only, respectively, and those of group 4 (15 animals) acted as the control. The method of immunization (a modified Cavelti's method) was described previously [2].

Circulating antibodies in the serum of the immunized animals were determined by the indirect immunofluorescence method [8, 18].

To exclude the possibility of a reaction between the components of myocardial tissues and serum proteins, such as albumin [9, 16, 17], pure antibodies against γ-globulin obtained by Gurvich's method [4] with the aid of immunosorbent, were used. A layer of serum was poured on to unfixed sections, cut in a freezing microtome (-20°) from frozen heart tissue of rabbits, guinea pigs, or rats and subsequently treated with pure antibodies against rabbit γ-globulin labeled with fluorescein isothiocyanate. Tissue-

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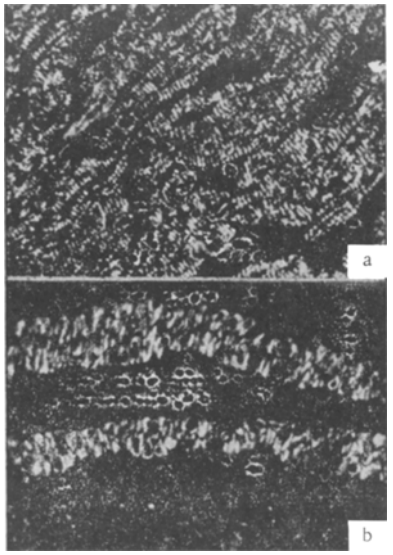

Fig. 1. Sections through guinea pig heart tissue treated with serum of rabbit immunized with homogenate of homologous heart tissue mixed with a streptococcal culture: a) fluorescence of I-disks of myocardial muscle fiber in region of subsarcolemma; b) fluorescence of cytoplasm of smooth-muscle cells of vessel wall. Indirect immunofluorescence method. Objective 40, ocular, homal 3.

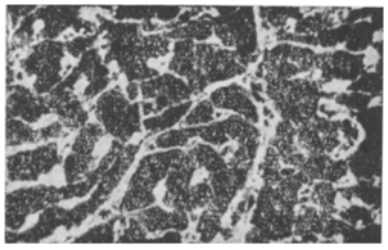

Fig. 2. Section through heart tissue of rabbit immunized with homogenate of homologous heart tissue mixed with streptococcal culture. Fluorescence in interstitial tissue of myocardium due to bound γ-globulin. Direct immunofluorescence method, objective 40, ocular, homal 3.

bound γ-globulin was detected by the direct immunofluorescence method [15], by covering sections of heart tissues from immunized rabbits with pure labeled antibodies against rabbit γ-globulin. Sections stained with hematoxylin-eosin after fixation in alcohol were used as the histological control.

EXPERIMENTAL RESULTS

Morphological investigation revealed manifestations of focal myocarditis in the heart tissue of all rabbits of group 1. Antibodies reacting with components of the I-disks of the myocardial muscle fiber in the region of the subsarcolemma, and also with cytoplasmic components of smooth-muscle cells of the myocardial vessel walls were found in the blood serum of these (13 of 16) animals (Fig. 1). No circulating antibodies reacting with connective-tissue components were found in the serum. The investigated sera did not react with parenchymatous elements of other organs (liver, kidney). The reaction with myocardial muscle fibers and smooth-muscle cells disappeared after preliminary adsorption of the serum with heart tissue homogenate.

Antibodies reacting with components of the I-disks of the muscle fiber were found in the serum of rabbits immunized with a streptotoccal culture in 3 of 7 cases, although this reaction was much weaker than that in sera of the animals of group 1. Production of these antibodies was due either to liberation of components of myocardial muscle fibers into the blood stream as a result of streptococcal action [3, 12], or to adsorption of components of muscle fibers from the meat nutrient medium on the cell surface of the streptococci [14].

Sera of rabbits immunized with heart tissue homogenate only caused a weak, diffuse fluorescence of the whole muscle fiber in 5 of 7 cases. Sera of unimmunized (control) rabbits gave no fluorescence in 4 of 10 cases, a weak diffuse fluorescence of the cytoplasm of the myocardial muscle fibers in 2 cases, and fluorescence of the intercalary disks in 4 cases. Similar results have been obtained by other investigators studying sera of apparently healthy animals [5, 13].

In tests to detect γ-globulin bound with heart tissues, application of pure labeled antibodies against rabbit γ-globulin to heart-tissue sections from rabbits of group 1, sacrificed after the 3rd cycle of immunization, was followed by bright fluorescence of the connective-tissue structures of the heart, the interstitial tissue, the adventitia of the blood vessels, the endocardium, epicardium, and areas of scar tissue (Fig. 2). In no case was fluorescence observed in the structures of the myocardial muscle fibers or the smooth-muscle cells of the vessel walls. Preliminary adsorption of antibodies by pure rabbit γ-globulin completely prevented the fluorescence. No fixed γ-globulin was found in heart-tissue sections of animals receiving one cycle of immunization, just as in the controls. Bound γ-globulin was found in small amounts in heart-tissue sections from rabbits sacrificed after 2 cycles of immunization.

The bound γ-globulin began to disappear 40-50 days after the end of the 3rd cycle of immunization, and a further increase in its content took place only after reimmunization.

There are good grounds for believing that the γ-globulin found in the tissues consists of antibodies against components of connective-tissue structures. It is also possible that bound γ-globulin is a component of antigen – antibody complexes located within the connective tissue. Further investigations are being carried out to determine the nature of this γ-globulin. The observed discrepancy between the localization of components against which antibodies circulating in the blood stream are directed and the localization of antibodies bound with the tissues must evidently be due to the fact that muscle fibers, constituting a well-isolated structure, are inaccessible in the living body to circulating antibodies, whereas a constant transfusion of serum proteins, including γ-globulin, takes place through the connective tissue, as a result of which, antibodies directed against connective-tissue components may be bound by the corresponding antigens. This phenomenon is also observed during the study of clinical material. According to published data [15, 16], the blood serum of patients with rheumatic fever contains antibodies against components of myocardial muscle fibers, while sections of heart tissues from these patients reveal bound γ-globulin in the connective-tissue structures and sarcolemma, and fluorescence of small foci in muscle cells is observed only in occasional cases.

On the basis of these observations a hypothesis can be put forward to explain the reason for selective involvement of connective-tissue structures in rheumatic fever in man and, perhaps, allergic myocarditis in animals.

The results obtained confirm the view that tissue antibodies play a pathogenetic role in the development of experimental allergic myocarditis.

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