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SERUM ANTIBODIES AGAINST HEART VALVE FIBROBLASTS IN PATIENTS WITH RHEUMATIC FEVER

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Antibodies reacting in immunofluorescence tests with human and bovine heart valve fibroblasts were isolated from the serum of patients with rheumatic fever by means of an immunosorbent prepared from human heart valve tissues. The antibodies did not react with fibroblasts of the interstitial connective tissue of the myocardium. Fibroblasts of the myocardium and valves are evidently antigenically different, whereas fibroblasts of human and bovine heart valves have common antigens.

KEY WORDS: *rheumatic fever; fibroblasts; antibodies.*

Deposits of bound immunoglobulins have been found [7, 8] in connective-tissue structures of the myocardium and heart valves of patients with rheumatic fever. However, when the sera were studied by the immunofluorescence method, no circulating antibodies against connective tissue antigens were found [9, 13]. Previous investigations showed that the sera of patients with rheumatic fever react with cells of the interstitial connective tissue of bovine heart and from the heart of other animals [1]. Antibodies reacting with fibroblasts of various bovine organs, including heart valve fibroblasts, have been isolated by means of immunosorbents containing bovine connective tissue antigens from the sera of patients with rheumatic fever, but the antibodies thus obtained did not react with the connective tissue components of human heart [2].

The object of this investigation was to isolate antibodies from the sera of patients with rheumatic fever with the aid of an immunosorbent prepared from human heart valve tissue and to investigate them by the immunofluorescence method on sections from bovine and human heart valve and myocardial tissues.

EXPERIMENTAL METHOD

In order to isolate antibodies against human heart valve connective tissue antigens an immunosorbent was prepared from the valve tissues of healthy persons who had died from injury. The material was carefully homogenized in a tissue microblender at 4°C. The resulting homogenate was washed 4 or 5 times with 0.85% NaCl solution. Mechanical mincing of the homogenate and subsequent washing were repeated 4 or 5 times. The homogenate was then treated with 0.1 M glycine—HCl buffer, pH 2.8, and washed with 0.2 M phosphate buffer, pH 7.2, until protein disappeared from the supernatant.

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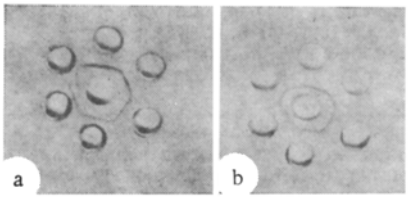

Fig. 1

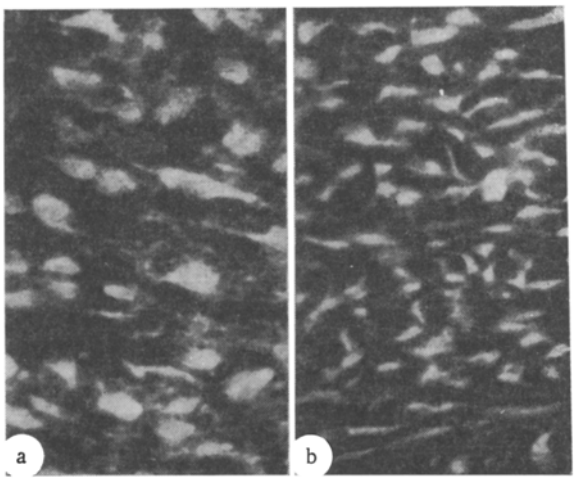

Fig. 2

Fig. 1. Reaction of preparations of antibodies isolated from sera of rheumatic fever patients (a) and sera of blood bonors (b) with serum against human IgG. Central well contains preparations of antibodies concentrated fivefold (2 mg protein to 1 ml); peripheral wells contain serum against human IgG (top well is undiluted, thereafter successive dilutions to 1:32).

Fig. 2. Reactions of antibodies isolated from sera of rheumatic fever patients with sections of tissues of human fetal valves (a) and joints (b). Luminescence of fibroblasts and other connective tissue cell components can be seen.

Sera of patients with rheumatic fever in the active stage of the disease were used for isolation of the antibodies. A mixture of sera (5 ml) was incubated with the immunosorbent for 30 min at room temperature and for 18 h at 4°C. The antibodies were eluted with 0.1 M glycine—HCl buffer, pH 2.8. The antibody preparations thus obtained, containing 300-400 µg protein in 1 ml, were kept at —20°C. Similar preparations were obtained as the control from donors' sera. Antibodies obtained from the sera of patients with rheumatic fever and blood donors were tested with serum against IgG and against human serum in the precipitation test.

Immunofluorescence tests were carried out by the indirect method, using pure antibodies against human IgG prepared with the aid of immunosorbents by the method of Avrameas and Ternynck [4] and with antibodies labeled with fluorescein isothiocyanate as described earlier [1]. In some experiments immunoglobulin fractions against individual classes of human immunoglobulins (anti-IgG, -IgM, -IgA), prepared in the laboratory of luminescent sera of the N. F. Gamaleya Institute of Epidemiology and Microbiology, labeled with fluorescein were used. Sections used in the experiments were obtained from heart valve, myocardium, liver, and kidney of persons of blood group O (the material was obtained at autopsy from previously healthy subjects dying from injury), the valves and joints of a human fetus aged 16-20 weeks, and bovine valves and myocardium. Sections were cut to a thickness of 4 µ in a cryostat from tissue frozen to —70°C and were used unfixed. The sections were washed for 10 min with 0.85% NaCl solution buffered with phosphate to pH 7.5 and treated with antibodies for 30 min at room temperature and for 18 h at 4°C. The sections were washed for 10-15 min, the labeled antibodies were applied to them for 45 min, after which they were again washed for 15 min and mounted in glycerol at pH 7.0. As a first step, 54 sera from patients with rheumatic fever and 25 sera from blood donors, as the control, were studied on sections of human heart valves. The sera, twice absorbed with mouse liver powder, were tested in a dilution of 1:4.

Preparations containing antibodies were absorbed with human red cells of all blood groups and with bovine and sheep's red cells (0.2 ml antigen to 0.05 ml of red cells) and also with sugars determining the specificity of the human blood group antigens, namely D-galactose and N-acetylgalactosamine (0.2 ml antigen to 10 mg sugar). In addition, the various preparations from group A streptococci were used for absorption: cell walls, membranes obtained by mechanical disintegration (5 mg to 0.2 ml antibodies), polysaccharide (40 µg to 0.2 ml antibodies), and peptidoglycan (2 mg to 0.2 ml), obtained by Fuller's method [5] in Krause's modification [12].

The stained sections were examined in the ML-2 luminescence microscope with 40× and 90× objectives. The Homal 3× ocular was used for photography.

EXPERIMENTAL RESULTS

As a rule when the sera of rheumatic fever patients were tested on sections of human heart valve tissues strong reactions were found with the cell component (from ++ to +++). However, the donors' sera also reacted with the cell components of the valve, although less strongly (+, ++). In both cases, moreover, luminescence of the ground substance of the tissues was observed, and this was evidently nonspecific and due to the presence of acid mucopolysaccharides in the tissue. This fluorescence could not be abolished either by absorption of the sera twice with liver powder or by prolonged rinsing of the sections at different stages of their treatment.

When the sera were tested on sections of bovine valves the luminescence of the ground substance was much weaker. Luminescence of the cell components also was observed with sera both from patients with rheumatic fever and from blood donors. The subsequent tests were carried out only with preparations of isolated antibodies.

Antibodies obtained from the sera of rheumatic fever patients gave a clear precipitation line in the precipitation tests with serum against human IgG. The preparation of antibodies from the donors' sera reacted with the same serum much less strongly (Fig. 1). Both preparations gave several precipitation bands with serum against human serum, i.e., besides IgG they also contained other human serum proteins which were not identified.

When antibodies isolated from the sera of rheumatic fever patients were tested on sections of heart valves from persons of blood group O a strong (+++) reaction was observed with fibroblasts of dense and loose connective tissue. The fluorescence was localized in the region of the cytoplasm, cell processes, and cell nucleus. In experiments with luminescent monospecific immonoglobulins, the reaction was shown to take place on account of IgG. Three batches of antibodies were obtained from the sera of rheumatic fever patients and they actively reacted with fibroblasts of human heart valves.

When the antibodies were tested on sections of human fetal heart valves, bright fluorescence of the fibroblasts was observed (Fig. 2a). On sections from the joints a strong reaction was observed with fibroblasts of loose and dense connective tissue, osteocytes, and chrondrocytes (Fig. 2b). Similar bright luminescence of the cells also was found when the antibodies were tested on sections of bovine valves.

On sections of human myocardium weak luminescence (+, ++) of the cell nuclei of the muscle fiber and connective tissue was observed. Luminescence of the nuclei was observed also on sections of human liver and kidney and bovine myocardium. On sections of both human and bovine myocardium there was no reaction with fibroblasts of the interstitial connective tissue.

Two series of corresponding preparations were obtained from sera of blood donors. One of them gave weak (+) reactions with human valve and joint fibroblasts and with bovine valve fibroblasts. The other series proved to be virtually inactive.

In the experiments in which antibodies were absorbed by human red cells of different blood groups and by bovine and sheep's red cells and also by the monosaccharides which determine the specificity of human blood group antigens, the luminescence was completely preserved.

Consequently, reactions with valve fibroblasts are not attributable to human and bovine isoantigens or to Forssman antigen. Preliminary experiments showed that luminescence of valve fibroblasts can be removed by absorption of the antibodies with a culture of normal fibroblasts from human skin (the cell residue washed free from culture medium). Absorption of the antibodies by preparations obtained from group A streptococci had no effect on the results of the test.

Antibodies reacting with human valve fibroblasts were thus isolated from the sera of patients with rheumatic fever with the aid of an immunosorbent prepared from human valve tissues. A reaction with fibroblasts also was observed on sections of the valve and joint of a human 16-20-week-old fetus. The antibodies, incidentally, did not react with fibroblasts from the human myocardium or other organs. Fibroblasts of the heart valve evidently differ antigenically from the fibroblasts of other organs, including from fibroblasts of the myocardial interstitial connective tissue. The antigen against which these antibodies are directed is

common to bovine and human valve fibroblasts. Antibodies against valve fibroblasts are evidently present in very small quantities in healthy human serum.

Reactions of the sera of rheumatic fever patients with human heart valve fibroblasts also were found by Kaplan and Clemente [7]. According to their observations, antibodies against fibroblasts belong to the IgG class, they are found more often in the active stage of the disease, and they do not react with fibroblasts of the myocardium and epicardium. The same workers showed that deposits of immunoglobulins and fixed complement in the valves of rheumatic fever patients are distributed mainly in the fibroblasts and adjacent collagen fibers and they can evidently exert a harmful action on the tissue structures.

There is evidence that group A streptococci contain antigens cross-reacting with fibroblasts [11]. Goldstein et al. [6] found cross reactions between the polysaccharide of group A streptococci and valve glycoprotein; admittedly, these findings were not confirmed by other workers [3, 10]. Although luminescence of the fibroblasts in the present experiments was not abolished by absorption with streptococcal components, the problem of the connection between the reactions observed and cross-reacting streptococcal antigens cannot be regarded as having been solved.

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