ANTIBODIES AGAINST HETEROLOGOUS CONNECTIVE TISSUE ISOLATED FROM SERUM OF PATIENTS WITH RHEUMATIC FEVER

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Antibodies against connective-tissue structures of various bovine organs were isolated from the sera of rheumatic fever patients by means of immunosorbents containing antigens of bovine connective tissue and erythrocyte stroma. The antibodies thus obtained were not identical and contained antibodies against different bovine connective tissue antigens. These antibodies did not react with the components of human connective tissue.

KEY WORDS: rheumatic fever; connective tissue; antibodies.

Previous investigations [2-4] showed that the sera of patients with rheumatic fever, when tested by immunofluorescence, react with interstitial connective tissue cells of the bovine heart but do not react with connective tissue of the human heart. Adsorption experiments failed to show that the antigen (or antigens) against which these antibodies are formed are constantly present in bovine connective tissue and erythrocytes.

The object of this investigation was to isolate antibodies against these antigens from the sera of patients with rheumatic fever and then to study them.

EXPERIMENTAL METHOD

Tests were carried out by the indirect immunofluorescence method, using pure antibodies against human IgG prepared by the method of Avrameas and Ternynck [7] with the aid of an immunosorbent and labeled with fluorescein isothiocyanate. The conditions of preparation and labeling of the antibodies were described previously [2]. In some experiments fluorescein-labeled immunoglobulin fractions containing antibodies against particular classes of human immunoglobulins (anti-IgG, anti-IgM, anti-IgA), prepared in the Laboratory of Luminescent Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology, were used.

The connective tissue extract was prepared from bovine pericardium. The pericardium was carefully minced in a tissue microblender at 4°C. The resulting homogenate was sonicated in the MSE-20 ultrasonic disintegrator (12 cycles, 15-20 min). The residue was treated with 0.85% NaCl solution or 0.2 M phosphate buffer, pH 7.2, for 24 h at 4°C. The supernatant was separated by centrifugation at 8000 rpm. The extracts were dialyzed against 0.085% NaCl solution, freeze dried, concentrated, and converted into an immunosorbent with the aid of glutaraldehyde. Subsequent treatment of the immunosorbent was by the method of Avrameas and Ternynck [7].

Bovine erythrocyte stroma was obtained by Dausset's method [5]. Washed erythrocytes were lysed with distilled water, frozen and thawed three times, and the stroma was precipitated at pH 5.8. The resulting stroma was washed twice with 0.1 M glycine–HCl buffer, pH 2.8.

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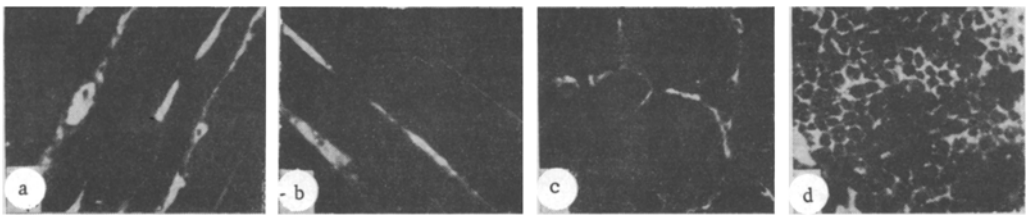

Fig. 1. Tissue sections of bovine organs treated with antibody preparations isolated from the sera of rheumatic fever patients with the aid of an immunosorbent prepared from bovine connective tissue antigens: a) section through heart tissue; fluorescence of fibroblasts, capillary cells, and sarcolemma; b) section through skeletal muscle; fluorescence in zone of sarcolemma and connective tissue cells; c) section through kidney tissue; fluorescence of cells in fibrous structures of interstitial connective tissue; fluorescence of cells in fibrous structures of interstitial connective tissue; d) section through thymus; fluorescence on surface of thymocytes.

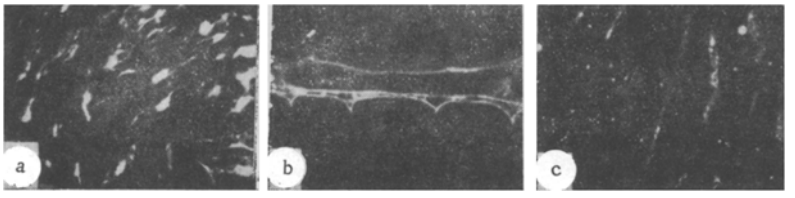

Fig. 2. Tissue sections of bovine organs treated with antibody preparations isolated from sera of rheumatic fever patients with the aid of bovine erythrocyte stroma: a) section through heart valve; fluorescence of connective tissue cells; b) section through skeletal muscle; fluorescence in region of sarcolemma; c) section through liver tissue.

The antibodies were isolated with the aid of serum from patients with rheumatic fever in the active phase, which reacted (++++) with interstitial connective tissue cells of bovine heart. A mixture of active sera was incubated with immunosorbents for 30 min, with vigorous mixing, at room temperature and for 1 h at 4°C. The antibodies were eluted with 0.1 M glycine–HCl buffer, pH 2.8, then dialyzed against 0.085% NaCl solution, and concentrated by evaporation under a fan. The final samples of antibodies contained 300-400 μg/ml protein. The antibodies were preserved frozen at −20°C.

The antibodies were tested on fresh tissues of the bovine heart, heart valves, liver, kidney, thymus, and lymph glands, and also on the human heart, heart valves, and thymus from persons of blood group O (the material was obtained at autopsy or from fetuses). Sections 4 μ in thickness were cut in a cryostat at −20°C from frozen, unfixed tissue and they were used unfixed. Sections of bovine tissues were treated with antibodies at room temperature for 45 min, whereas sections of human tissues were kept with antibodies for 30 min at room temperature and for 18 h at 4°C. The incubation time of the sections with luminescent antibodies was the same in both cases: 45 min at room temperature.

The preparations containing antibodies were adsorbed with bovine and human erythrocyte stroma and with sheep's erythrocytes. The erythrocytes or stroma were added at the rate of 0.05 ml to 0.2 ml of antibodies. The sugars determining the specificity of human blood group antigens (D-galactose and N-acetylgalactosamine) were added at the rate of 10 mg sugar to 0.2 ml antibodies. The antibodies also were adsorbed by preparations of streptococcal origin (A-polysaccharide, and also a polysaccharide and mucopeptide isolated from streptococci of the A variant) by Fuller's method [8] in Krause's modification [9]. The preparations of polysaccharides were used in sufficient amount to remove specific antibodies from the antistreptococcal sera, namely 200 μg polysaccharide to 1 ml serum [1], and the mucopeptide in a dose of 2 mg/0.2 ml antibodies. Incubation continued for 2 h at 37°C and for 18 h at 4°C.

EXPERIMENTAL RESULTS

Tests of the preparation containing antibodies against connective tissue antigens (CTA) on bovine heart tissue sections showed a strong reaction with interstitial connective tissue cells: fibroblasts and capillary cells. In addition, reactions were observed with fibrous structures of connective tissues and also with the sarcolemma (Fig. 1a). CTA did not react with sections of bovine heart valves. On skeletal muscle sections fluorescence was observed in a narrow zone, corresponding in localization to the sarcolemma, and also in the endomysium and cytoplasm of the connective tissue cells (Fig. 1b). In the liver tissues fluorescence of Kupffer cells and the endothelial cells of the larger vessels was observed, and in kidney cells there was fluorescence of the cellular and fibrous structures of the interstitial connective tissue (Fig. 1c); in the tissues of the thymus fluorescence was observed in structures of the connective-tissue stroma, and weaker fluorescence also was present on the surface of the thymocytes (Fig. 1d). A similar reaction was found when the localization of CTA was studied in sections of a lymph gland.

Antibodies isolated with the aid of bovine erythrocyte stroma (BEA) reacted with the same structures of heart tissue as CTA, but the reaction was weaker. In addition, BEA reacted with myocardial cell nuclei. In tissue sections of heart valves BEA reacted with connective tissue cells of different shapes and sizes, including with fibroblasts (Fig. 2a). Clear fluorescence of the outlines of a fiber, evidently of the sarcolemma, was observed in sections of muscle (Fig. 2b). The reactions of BEA and CTA in sections of the liver (Fig. 2c) and kidney agreed in all respects. However, in sections through the thymus tissue BEA reacted weakly with the connective-tissue stroma and punctate fluorescence was observed on the territory of the thymocyte (2-3 points per cell). Similar punctate fluorescence of lymphocytes was observed in sections of the lymph gland.

The control to these experiments was a preparation of normal human γ-globulin in a concentration of 400 μg/ml, which did not react or reacted only very weakly (+ or −) with bovine tissue sections.

Reactions of CTA and BEA with the bovine tissues were shown to take place on account of IgG. When anti-IgM and anti-IgA were used, fluorescence was completely absent.

In tests of CTA and BEA on tissue sections of human heart, heart valves, and thymus, no specific fluorescence of the connective tissue structures was found.

Adsorption experiments showed that the reactions of CTA and BEA with bovine heart tissue were completely abolished by adsorption with bovine erythrocyte stroma. Sheep's erythrocytes did not abolish these reactions. The negative reactions of CTA and BEA with bovine heart tissue, just as in the experiments with whole sera, were obtained on inhibition with D-galactose. Although D-galactose is the terminal determinant of human erythrocytes of blood group B, in experiments in which the CTA preparation was adsorbed by group B erythrocytes only very slight inhibition of the reaction with bovine heart tissue was obtained. The reactions of the sera and isolated antibodies with bovine heart connective tissue thus evidently had nothing to do with the presence of β isoantibodies in the sera, for the positive reactions were independent of blood group: the number of positive reactions with sera of blood group I (α and β isoantibodies) was 42%, but with sera of group IV (not containing isoantibodies) it was 50%.

Whereas D-galactose completely abolished the reaction of BEA with bovine heart tissue, the reaction of BEA with bovine heart valve tissue still remained. N-Acetylgalactosamine was virtually inactive in the adsorption experiments.

On inhibition of CTA and BEA by streptococcal polysaccharides (A and A variant) fluorescence of the connective tissue structures of the heart and valves was preserved intact. Partial inhibition of fluorescence of these structures was observed after adsorption of the antibodies by streptococcal mucopeptide.

Antibodies against connective-tissue structures of various bovine organs could thus be extracted from the sera of rheumatic fever patients with the aid of immunosorbents. These antibodies were directed toward components of the cytoplasm and cytoplasmic membrane of the connective-tissue cells and against certain fibrous structures. The results indicate that the CTA and BEA preparations were not identical and that, consequently, extracts of bovine connective tissue and bovine erythrocyte stroma contain both common and different antigens. The BEA preparation also is heterogeneous and evidently contains antibodies directed against different antigens; some antibodies (those against heart connective tissue) are inhibited by D-galactose; antibodies reacting with valve tissue still remain after this treatment. The sera of rheumatic fever patients thus contain antibodies against several bovine connective tissue antigens, but they do not all react with the components of human connective tissue.

Two problems that still require explanation are whether the corresponding antigens are present in human connective tissue and whether the antibodies which react with antigens of bovine connective tissue are autoantibodies. It was suggested previously [2, 3] that human connective tissue contains antigens identical with those of bovine connective tissue, but that the corresponding determinants are hidden [6]. The present investigation provides some confirmation for this view, for D-galactose, which is not only the terminal determinant of group B erythrocytes but also occurs in various components of human tissues, inhibited the reaction of the isolated antibodies with bovine heart connective tissue. The discovery of partial inhibition of reactions with bovine connective tissue structures by streptococcal mucopeptide also is interesting. These results point to a possible role of cross-reacting microbial antigens in the induction of antibodies against bovine connective tissue antigens in rheumatic fever patients.

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