

REACTION OF ANTIBODIES TO ANTIGENS OF GROUP A STREPTOCOCCUS WITH FIBROBLASTS OF INTERSTITIAL CONNECTIVE TISSUE OF THE HUMAN HEART

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The sera of rabbits immunized by fractions containing nontype-specific group A streptococcal antigens, belonging to cell wall proteins, were studied by the indirect immunofluorescence method. Antibodies reacting with fibroblasts of the interstitial connective tissue of the human heart were found in the sera. On the basis of the results of absorption experiments it is postulated that a cross-reacting antigen common with an antigen of the fibroblasts is present in some hydrochloric acid extracts obtained from streptococci of groups A and C.

KEY WORDS: cross-reacting antigens; antibodies; streptococcus; fibroblasts; myocardium

Cross reactions between antigens of group A streptococcus and connective tissue components, including the structural glycoprotein of the heart valves and other organs, the protein-polysaccharide complex of the synovial fluid and cartilage, have been described [4, 10]. The presence of cross reactions between streptococcal polysaccharide and the glycoprotein of connective tissue was not subsequently confirmed [2, 5]. Some antistreptococcal sera have been shown to react with fibroblasts of human heart valves [11]. No antibodies against antigens of the interstitial connective tissue (ICT) of human heart have been found in sera against whole bacterial cells. Meanwhile, in rheumatic fever, deposition of bound immunoglobulin is observed in the ICT, and this suggests the presence of autoantibodies against the structures present [10]. In this connection it is interesting to continue the search for cross-reacting antigens (CRA) of group A streptococcus common with the elements of the ICT of the heart.

During immunization with whole bacterial cells, antibodies do not arise to all the antigens contained in the various structures. For that reason, the most rational solution is to study sera obtained as a result of immunization by fractions containing different sets of antigens.

The object of this investigation was to study, by the immunofluorescence method in sections of human heart tissues, the sera of rabbits immunized with fractions containing nontype-specific antigens (NTSA) of group A streptococcus. Previous investigations have shown that these antigens belong to the cell wall proteins of group A streptococcus [1, 7].

EXPERIMENTAL METHOD

The indirect immunofluorescence method with pure antibodies against rabbit IgG, labeled with fluorescein isothiocyanate, was used in the experiments. The methods of obtaining the pure antibodies, preparation of the sections, and their treatment with antibodies were described previously [6]. Unfixed sections of the heart tissues of six healthy persons dying from injury (blood group O) were used. The sections were examined with the ML-2 luminescence microscope (objective 40 ×, homal 3 ×).

Altogether 25 sera from rabbits immunized with fractions containing NTSA of group A streptococcus were investigated. The fractions were isolated by preparative electrophoresis from HCl extracts prepared from cultures of group A streptococcus of types 1 and 29, grown on broth with casein hydrolysate [7]. As the control, 36 sera not containing antibodies against streptococcal cell wall proteins were tested: 4 sera were used from animals immunized with peptidoglycan, isolated from group A streptococcus [12] and 32 sera

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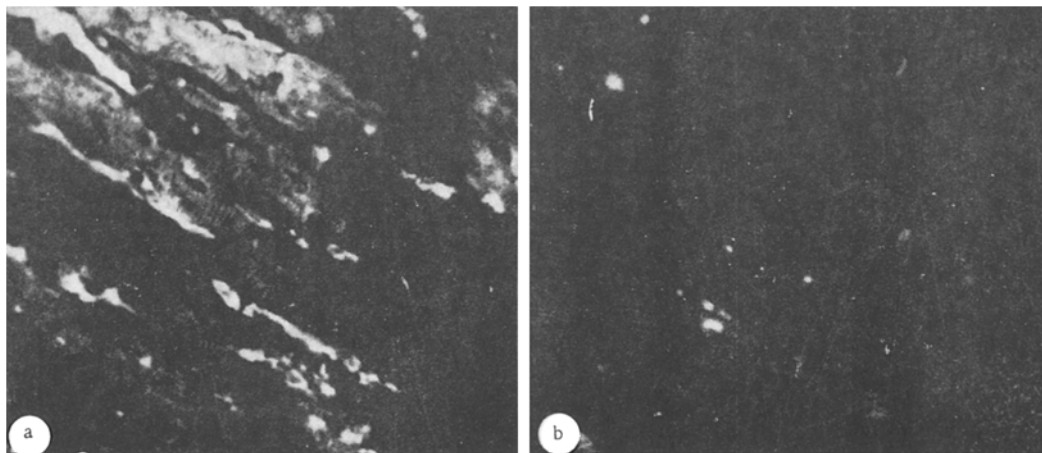


Fig. 1. Test of serum from rabbit immunized with NTSA of group A streptococcus: a) unabsorbed serum; tissue section from human heart - fluorescence of fibroblasts of ICT and sarcoplasm of muscle fiber; b) serum absorbed with HCl extract containing streptococcal NTSA; tissue section of human heart - fluorescence of sarcoplasm. No fluorescence of fibroblasts. Luminous dots indicate deposits of fat. Objective 40 \times , homal 3 \times .

were used from rabbits immunized with cultures of group A or A-variant streptococcus, treated with pepsin [14]. Besides these, 97 sera from unimmunized rabbits, 7 sera from rabbits injected with Freund's complete adjuvant only, and 4 sera of animals immunized with nutrient medium and adjuvant, were tested. Immunization with fractions containing NTSA, peptidoglycan, Freund's adjuvant, and nutrient medium with adjuvant was carried out by the method of Goudie et al. [9].

The sera were absorbed with: whole bacterial cells of group A or C streptococcus (36×10^{10} bacterial cells/ml serum); preparations of cell walls, membranes, and cytoplasm obtained by mechanical disintegration of streptococci [8, 15] (12-20 mg dry weight/ml serum in a working dilution of 1/16); HCl extracts at the rate of 80 mg protein/ml serum. A culture of human embryonic fibroblasts, washed with 0.85% NaCl solution (1 ml of fibroblast residue to 1 ml serum),* also was used for absorption. Absorption was carried out for 2 h at 37°C and for 18 h at 4°C.

EXPERIMENTAL RESULTS

When the sera of rabbits immunized with fractions containing NTSA were tested on sections of human heart in 7 of 25 cases positive reactions were found with the sarcolemma of the myocardial muscle fiber. Some sera also reacted with the sarcoplasm and the intercalated and other disks. The sera of rabbits immunized with pepsin-treated cultures of group A (15 of 24 sera) or A-variant (3 of 8 sera) streptococcus reacted more often with the sarcolemma. Besides reactions with the structures mentioned above, in tests of 25 sera from animals immunized with NTSA, intensive reactions were observed in 11 cases (45%) with cells of the ICT, namely fibroblasts (Fig. 1a). A reaction was observed much less frequently with the fibroblasts when sera were tested from rabbits immunized with a culture of group A streptococcus treated with pepsin (in 3 of 24 cases, 12.5%). No positive reactions were found with fibroblasts when sera were tested from rabbits immunized with the peptoglycan preparation, with a culture of the A-variant of streptococcus treated with pepsin, with Freund's complete adjuvant, or with broth and adjuvant (23 sera altogether). In the study of 97 sera from immunized rabbits, reactions with fibroblasts of the ICT were observed in 11 cases (11.3%). The differences between the results obtained in the experimental and control series were statistically significant ($P < 0.01$).

In the experiments to study absorption of the sera of rabbits immunized with fractions containing streptococcal NTSA and reacting with fibroblasts, the following results were obtained. Absorption by preparations of membranes and cytoplasm did not affect the intensity of fluorescence of the fibroblasts. Partial inhibition of the reactions was obtained by absorption of the sera with whole bacterial cells of group A streptococcus (types 1 and 29) and group C streptococcus. Similar results were obtained by absorption of the sera with cell walls prepared from group A streptococci of the same types. After absorption with HCl extracts prepared from

*The culture of fibroblasts was generously provided by E. P. Ugryumov of the D. I. Ivanovskii Institute of Virology.

streptococci of groups A and C more marked inhibition of fluorescence of the fibroblasts was observed. Some HCl extracts (types 1 or 29) caused total inhibition of the reactions with fibroblasts (Fig. 1b). In some of these experiments the reactions with other structures of the myocardium were not abolished. Abolition of the reactions with fibroblasts was observed after absorption by a culture of embryonic fibroblasts. Under these circumstances fluorescence of other structures remained (intercalated disks, sarcoplasm).

Thus in nearly half of the animals immunized with fractions containing streptococcal NTSA, antibodies reacting with fibroblasts of ICT of the heart were found. As was shown previously, NTSA belonged to the cell wall proteins of group A streptococcus and one of them is also found in groups C and G [1, 7]. Sera taken as the control and not containing antibodies against cell wall proteins of group A streptococcus did not react at all or reacted in a small percentage of cases with fibroblasts just like sera from nonimmunized rabbits.

Besides antibodies reacting with fibroblasts, tests of the sera against NTSA also revealed antibodies reacting with the sarcolemma and with other structures of the myocardium. Reactions with the sarcolemma were observed, in addition, during tests on sera not containing antibodies against cell wall proteins. These reactions were evidently connected with antibodies against various CRA described previously [4, 10].

Immunization with the fraction containing NTSA can be used to obtain antibodies which evidently do not arise as a result of immunization with the whole bacterial cell, for no reactions with fibroblasts of ICT could be found when sera against whole bacterial cells were tested [3, 4]. Inhibition of reactions with fibroblasts during absorption of the sera by HCl extracts suggests that some of the HCl extracts obtained from cultures of groups A and C streptococcus contain CRA common with the antigen of the fibroblasts. Experiments involving absorption by a culture of fibroblasts confirmed that sera against NTSA do in fact react with fibroblasts of ICT of the human heart.

Some antigens of streptococcus of groups A and C are known to react nonspecifically with the Fc-segments of immunoglobulins [13]. In some experiments to study absorption of sera obtained against NTSA by HCl extracts, besides inhibition of reactions with fibroblasts, antibodies reacting with other structures of the myocardium were not removed. These findings are evidence of the specificity of the results.

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