IMMUNOFLUORESCENCE STUDY OF ANTIBODIES AGAINST GROUP A STREPTOCOCCAL POLYSACCHARIDE IN CONNECTIVE TISSUE SECTIONS

N. A. Borodiyuk, I. I. Rassokhina, T. A. Danilova, and I. M. Lyampert

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Pure antibodies were obtained against the specific determinant of the group A streptococcal polysaccharide. These antibodies were studied by the immunofluorescence method in tissue sections of the heart and heart valves. Cross reactions were not found between the group A streptococcal polysaccharide and mammalian connective tissue.

KEY WORDS: streptococcal polysaccharide; connective tissue; cross-reacting antigens; antibodies.

Some workers consider that autoantibodies against the connective tissue of the heart in rheumatic heart disease are caused because the group A streptococcal polysaccharide (A-polysaccharide) and the connective tissue glycoprotein (CTGl) of the heart valves and other organs are cross-reacting (CR) antigens [6, 8]. The specific antigenic determinant for the polysaccharide is known to be the terminal determinant of the side chains in which there is a β-linked N-acetylglucosamine group. The second determinant of A-polysaccharide contains rhamnose oligosaccharides and it is common to streptococci of group A and certain other groups. Streptococcal polysaccharide of the A type has no terminal determinant and it contains only rhamnose oligosaccharides [12]. Goldstein et al. [8] found that cross reactions with CTGl depend on the common antigenic determinant, which is the specific determinant of the A-polysaccharide containing N-acetylglucosamine.

The question of the presence of a CR-determinant in A-polysaccharide and CTGl has not yet been settled, for other workers have obtained negative results [10, 11].

The study of CR-determinant of A-polysaccharide and CTGl has been carried out mainly with sera obtained against the whole microbial cell, which usually contain a large assortment of antibodies against various antigens of the group A streptococcus.

The object of this investigation was to obtain pure antibodies against the specific determinant of the group A streptococcal polysaccharide and to test these antibodies by the indirect immunofluorescence method on tissue sections of the human and animal heart and heart valves.

EXPERIMENTAL METHOD

Sera with a high titer of antibodies against A-polysaccharide were obtained by immunization of rabbits with increasing doses (from 0.5 to $2 \cdot 10^9$) of group A type I streptococcal cells killed by heating and treated with pepsin to remove cell wall proteins [13]. The culture was grown on meat medium or on broth

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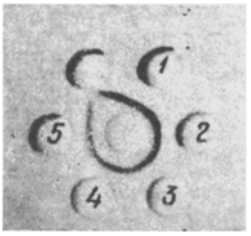

Fig. 1. Reaction of pure antibodies against A-polysaccharide with the A-polysaccharide preparation. Central well contains pure antibodies (9 mg/ml); peripheral wells (1-5) contain A-polysaccharide (50-12 μg/ml).

containing casein hydrolysate. Antibodies against A-polysaccharide were isolated by the method of Osterland et al. [14] from sera obtained after two or three cycles of immunization. To remove antibodies against other streptococcal antigens the sera were first absorbed with an A-variant culture. The precipitate formed on mixing serum with polysaccharide (optimal dose from 50 to 12.5 μg/ml serum) was washed off in neutral salt solution, dissociated at pH 2.65, and applied to a Sephadex G-100 column. The eluted fractions were neutralized and their protein content was determined by Lowry's method and their polysaccharide content was estimated as rhamnose. Fractions containing protein but not rhamnose were lyophilically dried and tested for antibodies against A-polysaccharide. Sera concentrated fivefold and preparations of pure antibodies (5-10 mg protein/ml) were tested in the precipitation test (PT) and by immunoelectrophoresis (IEP) in gel (modification of the methods of Zil'ber and Abelev [3]) with the various antigens of the group A and A-variant streptococci. Streptococcal polysaccharides of group A and A-variant and a mucopeptide isolated from the A-variant culture were obtained from the cell walls by treatment with formamide after preliminary degradation of the proteins by a proteolytic enzyme [13]. Fractions containing cell wall proteins and nonprotein antigens, e.g., polyglycerophosphate (PGP) and antigen E_4 [15], were isolated by preparative electrophoresis from saline extracts (HCl extracts) obtained from group A type type I streptococcus. To determine the specificity of the reactions with A-polysaccharide, the sera and preparations of pure antibodies were absorbed with mucopeptide (10-50 mg/ml), and also with preparations of A or A-variant polysaccharides (0.2-2 mg/ml). The reaction of antibodies with A-polysaccharide was inhibited with synthetic N-acetylglucosamine (β-rotation, 80 mg/ml). To test antibodies against A-polysaccharide on tissue sections in the indirect immunofluorescence method, antibodies against rabbit IgG labeled with fluorescein isothiocyanate were used. The antibodies were isolated with the aid of an immunosorbent [7]. The method of preparation and labeling of the antibodies and also the method of preparation of the tissue sections were described previously [2].

Pure antibodies against A-polysaccharide (1.5 mg protein/ml) were tested on tissue sections of guinea pig, bovine, and human heart and on tissue sections of bovine and human heart valves. A preparation of normal rabbit immunoglobulin and antibodies against DNP, in the same concentration as antibodies against A-polysaccharide, were used as the control. The tissue sections were treated for between 45 min and 2 h at 18-20°C or for 30 min at 18-20°C followed by 18 h at 4°C [9]. Labeled antibodies were applied to the sections for 35 min.

EXPERIMENTAL RESULTS

Sera obtained after two or three cycles of immunization of rabbits with the pepsin-treated group A streptococcal culture formed one precipitation band in agar gel when tested with A-polysaccharide (10.0-0.03 mg/ml).

Absorption of the sera with the A-variant culture abolished the reaction with PGP and with antigen E_4, antibodies against which were present in some sera, but did not remove antibodies against A-polysaccharide. Adsorption of the sera with mucopeptide likewise did not reduce the titer of the antibodies against A-polysaccharide, indicating the absence of reactions in this particular system on account of mucopeptide. Fractions containing antibodies against A-polysaccharide were tested by immunoelectrophoresis with serum against rabbit serum proteins. In this case one precipitation band located in the zone of IgG mobility was obtained. Altogether six series of preparations of pure antibodies were obtained. All these preparations reacted with A-polysaccharide (Fig. 1) but did not react with other antigens contained in HCl-extracts of group A streptococcus with the A-variant polysaccharide. The reaction of the antibodies with polysaccharide could be inhibited by synthetic N-acetylglucosamine or by the A-polysaccharide preparation.

In the tests of the antibodies against A-polysaccharide on tissue sections of the bovine, guinea pig, and human heart and heart valves, fluorescence was completely absent. Meanwhile, during tests of the same preparations of antibodies in the immunofluorescence test on skin and thymus tissues from man and various species of animals, positive results were obtained [5].

Cross reactions between the group A streptococcal polysaccharide and the connective tissue of the heart were thus not obtained in studies by the immunofluorescence method using pure antibodies against the specific determinant of the A-polysaccharide.

These results agree with those of investigations by the immunofluorescence method of whole antistreptococcal sera [11]. Cross reactions found by some workers could thus have depended on the presence of antibodies in their sera against other streptococcal antigens. Meanwhile the possibility cannot be ruled out that the positive results described by certain workers [6, 8] could be attributed to the fact that to study cross-reactions with polysaccharide they used preparations of glycoprotein obtained by extraction with urea from connective tissue previously treated with TCA at a high temperature. Treatment of the tissues in this way could evidently cause the CR determinant of connective tissue to become accessible to antibodies.

In previous investigations the writers found a reaction of antistreptococcal sera with interstitial connective tissue after treatment of the sections with streptococcal proteinase. On this basis it was postulated that antigens capable of reacting with antistreptococcal sera are "concealed" antigens [1, 4]. The production of pure antibodies against the specific determinant of A-polysaccharide presents opportunities for the further study of this question.

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