IMMUNOLOGIC CROSS-REACTIONS BETWEEN POLYSACCHARIDES OF GROUP A AND L STREPTOCOCCI

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By affinity chromatography antibodies were isolated from the sera of rabbits immunized with group A streptococcus on Sepharose immunosorbents containing A-polysaccharide or synthetic β-N-acetylglucosamine. Antibodies obtained from some sera by means of both immunosorbents reacted with polysaccharides of groups A and L. Tests by the immunodiffusion method revealed partial identity of these polysaccharides. It was shown by adsorption of antibodies by polysaccharides of groups A and L that they differ in specificity. These antibodies probably may be aimed at terminal sites of different sizes of the polysaccharide molecule of the group A streptococcus.

KEY WORDS: streptococcus; polysaccharide; antibodies; affinity chromatography.

The study of antibodies against antigenic determinants of the polysaccharide of the group A streptococcus (A polysaccharide) is important for the development of the best methods of diagnosis. Investigation of these antibodies is also interesting in connection with the discovery of cross reactions between A polysaccharides and connective tissue, and also the epithelium of the skin and thymus [7, 10]. This problem requires further study, for the presence of cross reactions with connective tissue has not been confirmed by other authors [10]. One cause of the contradictory results obtained by determination of cross reactions with tissue antigens could be the presence of antibodies in antistreptoccal sera against different antigenic determinants of A polysaccharide.

We know that A polysaccharide has a branched structure and consists of rhamnose oligosaccharides connected by β-N-acetylglucosamine residues. The specific determinant of the polysaccharide is the terminal region of the side chains, containing β-N-acetylglucosamine [11]. Rhamnose is a component of the polysaccharides of several groups of streptococci.

Antibodies against polysaccharide of group L (L polysaccharide) have been found in sera against group A streptococcus and partial identity between A and L polysaccharides has been demonstrated as the result of tests by the immunodiffusion method with whole sera [1, 8]. The reaction of HCl extracts obtained from group L streptococci and containing L polysaccharide is evidently not connected with antibodies against the "rhamnose" determinants of A polysaccharide, for antibodies against this determinant are not found by this method in sera against group A streptococcus [1]. The structure of L polysaccharide has not been finally established, but it is known to contain N-acetylglucosamine [9].

The object of this investigation was to study cross reactions between A and L polysaccharides. For this purpose, by affinity chromatography on columns with immunosorbents, antibodies against A polysaccharides were isolated from the sera of animals immunized with group A streptococcus. To prepare the immunosorbents, group A polysaccharide and synthetic β-N-acetylglucosamine were used.

EXPERIMENTAL METHOD

A serum (No. 33), obtained by four cycles of immunization with a culture of group A type 1 streptococcus (No. 2/49),* treated with pepsin, and a serum (No. 439), obtained by two cy-

*Strain numbers of the Prague Collection.

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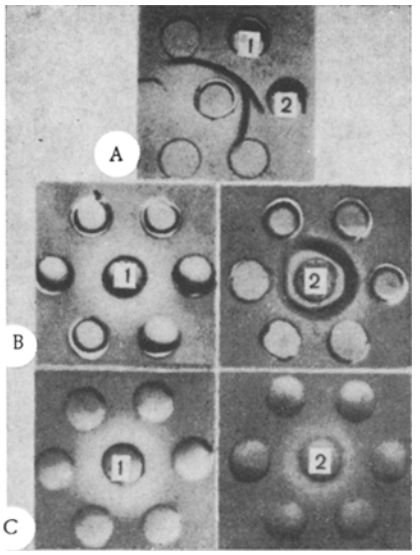

Fig. 1. Testing of A polysaccharide and HCl-L with unadsorbed and adsorbed sera. A: central well contains unadsorbed serum No. 33; peripheral wells contain: 1) A polysaccharide in concentration of 500 μg/ml; 2) HCl-L, undiluted. B and C: 1) serum No. 33 adsorbed with A-polysaccharide; 2) serum No. 33 adsorbed with HCl-L. B: peripheral wells contain A polysaccharide in concentrations of 12-500 μg/ml. C: peripheral wells contain HCl-L undiluted and in dilutions of 1 : 2-1 : 32.

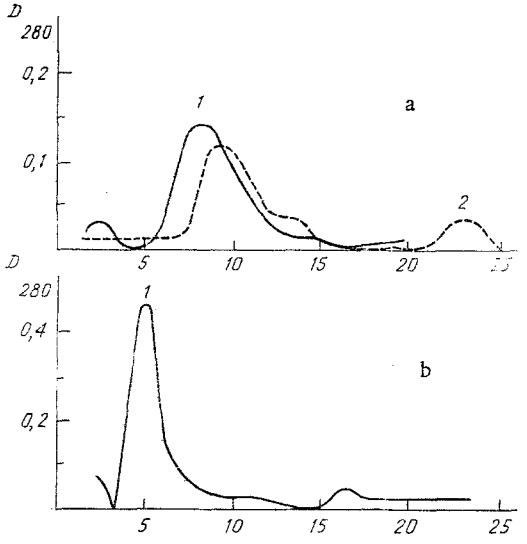

Fig. 2. Chromatographic fractionation of antibodies on immunosorbents containing A polysaccharide (a) or β-N-acetylglucosamine (b). 1) Elution of antibodies from serum No. 33) 2) elution of antibodies from serum No. 439. Abscissa, Nos. of fractions; ordinate, optical absorption at 280 nm.

cles of immunization with whole bacterial cells of group A type 29 streptococcus (No. 15/55), were used [12]. The A polysaccharide was prepared by formamide extraction from a culture of group A/SHT J 17A4 (No. 6/49) [4]. The HCl extract containing L polysaccharide (HCl-L) was prepared from a group L strain (No. 43/59) [2]. The immunosorbents were prepared as follows: 2 g of Sepharose 4B activated with BrCN (Pharmacia) was conjugated with 4 mg A polysaccharide, partially deacetylated with 20% p-toluenesulfonic acid in 0.5 M $NaHCO_3$ solution, pH 8.5. Next, 1 g of BrCN-activated Sepharose 4B (Pharmacia) was conjugated with 10 mg p-aminophenyl-β-N-acetylglucosaminide in 0.5 M carbonate buffer, pH 9.0 [6, 13].

Chromatographic fractionation of the antibodies was carried out on a 1 × 20 cm column containing 5 ml of immunosorbent equilibrated with 0.1 M Na-phosphate buffer (pH 7.2), containing 0.14 M NaCl. From 0.5 to 1 ml of serum was applied to the column. Serum protein bound nonspecifically with the immunosorbent was eluted by 50 ml of the same buffer. The antibodies were fractionated in a gradient of 25 ml 0.01 M Na-acetate buffer, pH 5.0, made up in 0.14 M NaCl solution and 25 ml of a 0.5 M solution of CH_3COOH (pH 2.4), containing 1 M NaCl. Fractions of 2 ml were collected at a rate of elution of 1.5 ml/min. Antibodies against β-N-acetylglucosamine were isolated on a 0.5 × 7 cm column packed with 1.5 ml immunosorbent, in the same gradient and with an elution speed of 2 ml/min. Elution of the antibodies was monitored by measuring their optical absorption at 280 nm. The protein concentration in the fractions was determined spectrophotometrically, on the basis of an extinction

Fig. 3. Comparison of A polysaccharide and HCl-L with antibodies isolated from immunosorbent with β-N-acetylglucosamine. The central well contained antibodies in concentrations of 3 mg/ml; 1) A polysaccharide in concentration of 50 μg/ml; 2) HCl-L in dilution of 1 : 4.

coefficient of 14.6 for rabbit immunoglobulins. Fractions containing protein were dialyzed against 0.01 M Na-phosphate buffer and lyophilically dried.

During adsorption of the sera 500 μg of A polysaccharide or 1 ml HCl was added per milliliter of serum. The gel precipitation test was carried out in the micromodification [3] with sera concentration fivefold in volume or with antibodies in a concentration of 3 mg/ml. A polysaccharide was tested in concentrations of 12-500 μg/ml, and HCl was used undiluted or in dilutions of 1 : 2-1 : 32. The rhamnose content in the A polysaccharide was determined by Dishe's method.

EXPERIMENTAL RESULTS

The preparation of A polysaccharide obtained by the formamide method contained 60% rhamnose and under 5% of protein impurities, in agreement with results obtained by analysis of purified preparations of A polysaccharide. Comparison of the A polysaccharide obtained by the formamide method with the polysaccharide contained in the HCl extract of group A streptococcus, in the gel precipitation test, showed them to be completely identical.

As a result of comparison of A polysaccharide and HCl-L with serum No. 33, a reaction of partial identity was observed (Fig. 1). Serum No. 439 reacted only with A polysaccharide. Adsorption of serum No. 33 with A polysaccharide abolished the reaction with both A polysaccharide and HCl-L. Adsorption of the sera with HCl-L did not abolish the reaction with A polysaccharide but removed antibodies reacting with HCl-L (Fig. 1).

As a result of fractionation of the antibodies on immunosorbent containing A polysaccharide elution curves illustrated in Fig. 2 were obtained. Antibodies both from serum No. 33 and from serum No. 439 were isolated on this adsorbent as a single protein peak. Similar results were obtained on isolation of antibodies from serum No. 33 on immunosorbent prepared from β-N-acetylglucosamine (Fig. 2). Antibodies isolated from serum No. 33 on both adsorbents reacted with A polysaccharide and HCl-L when tested by the immunodiffusion method. A and L polysaccharides in this case gave a reaction of partial identity (Fig. 3).

Consequently, when whole serum obtained against a culture of group A streptococcus and also the antibodies isolated from this serum were tested, partial identity of the A polysaccharide and a substance contained in the HCl extract from a group L streptococcus were demonstrated. These results are in agreement with those obtained previously during tests of A and L polysaccharides with whole sera [8]. Experiments with adsorption of serum by A polysaccharide and HCl-L showed that HCl-L does not adsorb all antibodies reacting with A polysaccharide. Furthermore, antibodies against A polysaccharide, isolated from serum No. 439, did not react with HCl-L.

The results are evidence of the existence of antibodies against A polysaccharide, differing in their specificity, in antistreptococcal sera. Since these antibodies were isolated on an immunosorbent with synthetic β-N-acetylglucosamine, it can tentatively be suggested that they are directed against terminal regions, differing in size, of the side chains of the A polysaccharide. As investigations by other workers who studied antibodies against the polysaccharide of group C streptococcus showed, antibodies directed not only against the monosaccharide itself, but also against larger antigenic determinants, can be isolated on an immunosorbent containing a monosaccharide [5].

It has recently been shown that antibodies against A polysaccharide differ in their affinity [13, 15]. During chromatography of the antibodies on Sepharose immunosorbents in a gradient with decreasing pH and increasing NaCl concentration, antibodies of different specificity could not be separated, evidently because of their close affinity.

Antibodies against A polysaccharide have been used in several investigations to study the idiotypical determinants and structure of the combining site of antibodies. To isolate antibodies immunosorbents containing A polysaccharide or β-N-acetylglucosamine are used. The specificity of the reactions is determined on the basis of inhibition of the antibodies by

synthetic β-N-acetylglucosamine [14]. The data described above are evidence that antibodies differing in their specificity can be isolated on such immunosorbents.

The isolation of antibodies against A polysaccharide which differ in their affinity and specificity, and their subsequent investigation by the direct immunofluorescence method, opens up new prospects for the study of cross reactions between antigenic determinants of A polysaccharide and the tissue antigen of man and animals.

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EFFECT OF METHOTREXATE ON ALLOANTIGEN EXPRESSION IN A POPULATION OF MOUSE LYMPH NODE CELLS

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The effect of intraperitoneal injection of methotrexate on alloantigen expression was studied in a population of lymph node cells from (CBA × C57BL/6j)F_1 mice. For this purpose, cells of F_1 mice, intact or receiving methotrexate, were injected into the foot of CBA mice and the increase in size of the regional popliteal lymph node compared with the contralateral (intact) side was determined. Methotrexate in doses of 50 and 75 mg/kg significantly increased the alloantigenicity of the lymph node cell population of the F_1 mice.

KEY WORDS: lymphocytes; alloantigen expression; methotrexate.

The effects of certain chemical substances and, in particular, of antitumor agents on the expression of transplantation antigens in populations of lymphocytes have been published. For instance, Haspel et al. [4], who studied human lymphoblastoid cell lines *in vitro*, observed a decrease in the number of HLA-antigens on the surface of the cells under the influence of puromycin. Neither actinomycin D nor cycloheximide had any such action. Guttman [3] showed that the lymphocytes of rats receiving cyclophosphamide largely lose their immunogenicity for animals of a different line. In his opinion, cyclophosphamide can depress more or less selectively the synthesis of histocompatibility antigens. Lindahl et al. [5] investigated the effect of interferon on expression of H-2 antigens in mouse lymphoid tissue. Both in experiments *in vitro* and after administration of interferon *in vivo*, expression of H-2 antigens on

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