ISOLATION OF HIGH-AFFINITY ANTIBODIES TO GROUP A STREPTOCOCCAL POLYSACCHARIDE REACTING WITH EPITHELIAL CELLS OF THYMUS AND SKIN

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Previous investigations revealed cross reactions between group A streptococcal polysaccharide (polysaccharide A) and epithelial cells of thymus and skin [5, 9, 10]. On the basis of these findings it was suggested that injury to the thymus tissues by a reaction of antibodies against polysaccharide A with epithelial cells can evidently cause a disturbance of the immunodepressor function of that organ and the development of an autoimmune process in rheumatic fever [4]. A further study of cross reactions of antibodies against polysaccharide A with epithelium of thymus and other tissues accordingly appeared interesting.

Not all antigens isolated by the method of decomposition of the precipitate from animal serum and reacting with polysaccharide A in the gel diffusion test (GDT), were found to be active when tested by the immunofluorescence method (IF) on epithelial tissues [11]. Antibodies against polysaccharide A isolated from the sera of animals immunized with group A streptococci also have been shown to differ in their specificity. For instance, antibodies reacting in GDT not only with polysaccharide A, but also with polysaccharide of group L streptococci (polysaccharide L), have been obtained from some sera by affinity chromatography. Antibodies specific only for polysaccharide A also have been isolated [3]. This suggests that differences in the ability of antibodies to react with epithelial cells can be explained by differences in their specificity. Furthermore, in the reaction with epithelial cells, the affinity of the antibodies may play an essential role.

The object of the present investigation was to study the effect of the degree of affinity of antibodies on their reaction with epithelium of thymus and skin, and also to determine the role of antibodies against polysaccharide A, reacting with polysaccharide L, when tested with epithelial tissue of thymus and skin.

EXPERIMENTAL METHOD

Sera with high titers of antibodies against polysaccharide A were obtained by immunization of rabbits with a culture of group A streptococcus, type I, grown on medium with casein hydrolysate and treated with pepsin [12]. The sera were used after two cycles of immunization with increasing doses (from $7.5 \cdot 10^9$ to $30 \cdot 10^9$) or after 2-4 cycles (from $0.5 \cdot 10^9$ to $2 \cdot 10^9$) bacterial cells. Polysaccharides A and L were prepared by formamide extraction from cultures of group A (strain J17A4, No. 6/49) and group L (strain SHC16, No. 43/59) [3, 6].

The immunosorbent was prepared as follows. Sepharose 4B (Pharmacia), in a volume of 10 ml, was activated with CNBr in 2 M Na_2CO_3 [7]. Binding of activated Sepharose with partially deacetylated A-polysaccharide was carried out in 0.5 M $NaHCO_3$ at pH 8.6 [3]. To block groups which remained active, the sorbent was treated with 1 M glycine in 0.1 M $NaHCO_3$, pH 8.6, and washed in 0.85% NaCl solution made up in 0.15 M potassium-sodium phosphate buffer (PBS), pH 7.4. Antibodies were isolated by a modified method of Jonson and Garvey [7]. To 5 ml of immunosorbent, placed on a porous glass filter, 5 ml of serum dissolved in PBS was added, and after mixing the product was washed with PBS to remove nonspecifically bound proteins. Completeness of washing was verified by measurement of the optical absorption of the washings at 280 nm. Low-

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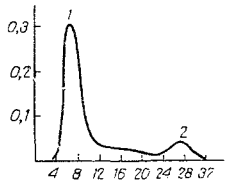

Fig. 1. Chromatographic fractionation of antibodies from immunosorbent with polysaccharide A. Abscissa, Nos. of fractions; ordinate, optical absorption at 280 nm. 1) Low-affinity, 2) high-affinity antibodies.

affinity antibodies were separated after incubation of the immunosorbent with 20 ml of 0.1 M Na-acetate buffer, pH 5.0, for 15 min. The immunosorbent was washed with 50 ml of PBS, mixed with 10 mg of 3 M KCNS, made up in PBS containing 10 mg of bovine serum albumin. The eluate was collected, a second portion of KCNS solution was added, the residual antibodies were removed by washing with 10 ml PBS, and the pooled eluate was desalted by gel-filtration on a column with Sephadex F-25 (2.4× 45 cm).

Antibodies were fractionated on a column with immunosorbent containing polysaccharide A, in a gradient of 0.01 M Na-acetate bufrer, pH 5.0, made up in 0.85% NaCl solution, and in 1 M NaCl in 0.5 M CH_3COOH, pH 2.5, as described previously [3].

Undiluted sera and preparations of isolated antibodies (0.1-1 mg/ml) were tested in the GDT [2] with polysaccharide A (from 1 mg to 6 μg/ml). The antibodies were inhibited by mixing equal volumes of antibodies (2-3 mg/ml) and polysaccharide A (200-300 μg/ml). The absence of antibodies unconnected with antigen was verified in the GDT after incubation at 4°C for 24 h; the precipitate was first removed by centrifugation at 12,000g for 20 min. When antibodies against polysaccharide A were tested on tissue sections in the indirect IF method, antibodies against rabbit IgG, isolated from sera with the aid of immunosorbent and labeled with fluorescein isothiocyanate, were used. The method of preparing the antibodies and tissue sections and their subsequent treatment were described previously [1]. Antibodies against polysaccharide A (0.5-1.5 mg/ml) were tested on sections of human and guinea pig thymus and skin tissue. The tissue sections were incubated with antibodies for 45 min or 2 h at 18-20°C [8]. The sections were then washed, labeled antibodies were applied to them, and incubation was carried out for 35 min.

EXPERIMENTAL RESULTS

Preparations of antibodies against polysaccharide A, designated low-affinity antibodies, were isolated from the majority of sera obtained by immunization of rabbits with low doses of streptococcal culture by elution with acid buffer, pH 5.0 (Fig. 1). The low-affinity antibodies isolated from some sera cross-reacted with polysaccharides A and L. Some of the low-affinity antibodies reacted in the GDT with polysaccharide A only. None of the eight batches of low-affinity antibodies studied reacted with epithelium of skin and thymus. Of the seven sera obtained after a second cycle of immunization of the animals with high doses of streptococcus, no significant quantities of low-affinity antibodies could be isolated by elution with acid buffer.

A small quantity of antibodies with a higher degree of affinity was isolated from some sera by the same method (Fig. 1). These antibodies reacted with epithelium of the stratum basale of the epidermis. Later antibodies were isolated without the use of columns, by means of 3 M KCNS. Low-affinity antibodies were first washed off with acetate buffer, pH 5.0. The antibodies thus obtained, designated high-affinity antibodies, reacted with polysaccharide A in the GDT (Fig. 2). No reactions of high-affinity antibodies with polysaccharide L were found. The addition of bovine serum albumin to the potassium thiocyanate had a marked stabilizing action on the activity of the antibodies in the GDT with polysaccharide A and in the reaction with skin and thymus epithelium. Eleven batches of antibodies obtained from six immune sera reacted with the epithelial cells of these tissues (Fig. 3). The preparations differed in their activity, depending on the serum used for isolation. Four series of high-affinity antibodies obtained from two sera did not react with epithelium of skin and thymus. In control experiments, preparations containing only serum albumin did not give fluorescence of the epithelial structures when tested by the indirect IF method. Preparations of antibodies active when tested by this method on skin and thymus sections, virtually lost their ability to react with epithelial cells after inhibition by polysaccharide A.

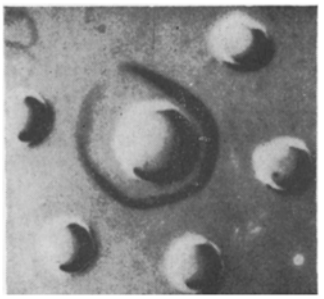

Fig. 2

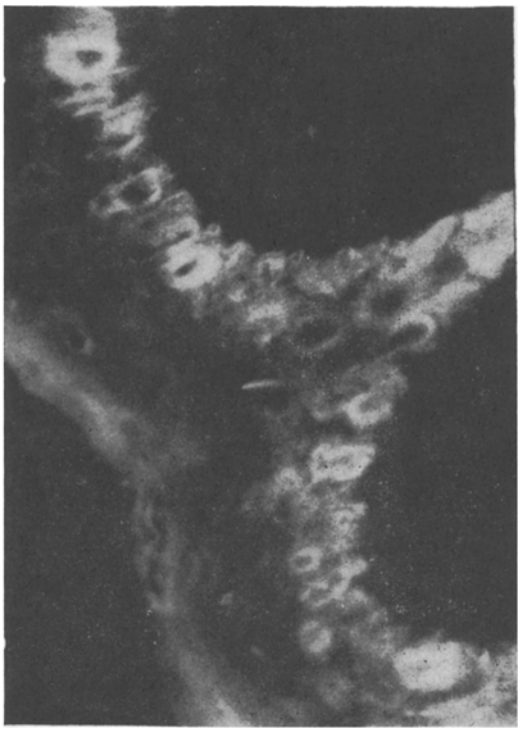

Fig. 3

Fig. 2. Testing antibodies removed from immunosorbent containing polysaccharide A in GDT with polysaccharide A. Central well contains antibodies in a concentration of 1 mg/ml; peripheral wells contain polysaccharide A in concentrations of 6-100 μg/ml.

Fig. 3. Testing high-affinity antibodies to polysaccharide A by the indirect IS method on section of human embryonic skin. Reaction of antibodies with cytoplasm of cells of stratum basale of epidermis.

The majority of preparations of high-affinity antibodies against polysaccharide A, isolated from the sera of rabbits immunized with group A streptococcus, thus reacted with epithelial cells of thymus and skin. The ability of antibodies to react with a tissue antigen evidently depends on their degree of affinity, for none of the preparations of low-affinity antibodies, which were obtained under mild conditions and reacted well with polysaccharide A in the GDT, reacted with epithelium of skin and thymus.

Inhibition of active antibodies by polysaccharide A leads to abolition of the reaction with epithelium, evidence of its specificity. Previous investigations showed that the reaction of antibodies to polysaccharide A with epithelial cells was unconnected with the presence of antibodies against other group A streptococcal antigens and the rhamnose determinant of polysaccharide A as impurities [5, 10]. Ability to react with epithelial cells likewise is evidently not connected with antibodies against the common determinant for polysaccharides A and L, for this reaction was found not to depend on the presence of antibodies against polysaccharide L. Consequently, the difference in the activity of the antibodies in reactions with epithelial cells of thymus and skin was evidently not due to differences in their specificity.

Potassium thiocyanate is an effective agent for removing antibodies from an immunosorbent [7, 13]. However, its use leads to considerable denaturation of antibodies against polysaccharide A [13]. In fact, some batches of antibodies isolated in the absence of serum albumin were inactive or weakly active when tested in the GDT and in the reaction with epithelial cells. Albumin evidently has a stabilizing action, though this does not rule out a possible disturbance of the conformation of some antibody molecules. These antibodies may perhaps react with the more accessible antigenic determinant of polysaccharide A in the GDT, but they are inactive in the reaction with tissue antigens. Antibodies obtained from different animals evidently differ in their resistance to the denaturing action of KCNS. These suggestions help to explain the absence of reactions of certain preparations, containing high-affinity antibodies against polysaccharide A in the GDT with epithelial cells.

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RECOVERY OF SENSITIVITY OF J-41 CELLS TO COXSACKIE B3 VIRUS BY TREATMENT WITH EXOGENOUS ALKALINE PHOSPHATASE

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Investigations in the writers' laboratory have shown that the sensitivity of cells to Coxsackie B virus is largely determined by alkaline phosphatase (Enzyme Nomenclature 3.1.3.1) activity. Activity of this enzyme is sharply reduced in cells resistant to this virus [1-3, 7, 8, 10]. Alkaline phosphatase activity is unchanged in cells resistant to poliovirus; it was shown later on a different object (human–murine hybrid cells) that the sensitivity of cells to poliovirus is linked with glucose phosphate isomerase activity [9].

If loss of sensitivity of cells to particular viruses is based, inter alia, on an insufficiently high activity of certain enzyme systems, it would be logical to attempt to restore the lost sensitivity by treatment with the corresponding enzymes.

In the present study an attempt was made to restore the sensitivity of human J-41 cell cultures, which had become specifically resistant to Coxsackie B3 virus after a period of chronic infection, by introducing exogenous alkaline phosphatase.

EXPERIMENTAL METHOD

Cultures of cells of human origin were used: continuous cell line J-96, highly sensitive to Coxsackie B3 virus, and subline J-41, resistant to this virus and obtained from J-96 cells by exposure to Coxsackie B3 virus [4, 6]. After a short period of chronic infection and removal of the virus the J-41 subline was found to be specifically resistant to homologous virus and to differ from the original cells by its low alkaline phosphatase activity [1]. The cells were cultured in 50-ml flasks with strips of coverslips in medium 199 with 10% bovine

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