A SURFACE RECEPTOR SPECIFIC FOR HUMAN IgA ON GROUP B STREPTOCOCCI POSSESSING THE Ibc PROTEIN ANTIGEN

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Many strains of group A, C, and G streptococci have been shown to possess surface receptors for human IgG (1-6). Receptors for human IgA have not been found on group C and G streptococci, though a small number of group A streptococci, specifically those of M type 4 and M type 60, do bind IgA (1, 7-9). Schalen (9) and others (6, 7) have shown that the receptors for IgG are different from those for IgA, and that binding is specific for the Fc fragment of the antibody molecules.

Group B streptococci have also been examined for their ability to bind IgG and IgA (2, 7). Christensen and Kronvall (2) found that 47% of the group B strains that they tested were capable of agglutinating IgG-sensitized sheep red blood cells. In another study, Christensen and Oxelius (7) could not demonstrate significant uptake of 125I-labeled IgA by any of 14 group B strains tested.

In this paper we report that a number of group B streptococci are capable of binding IgA and that IgA binding is correlated with the presence of a 130,000 mol wt, detergent-extractable protein, and with a reaction with Ic typing serum. The IgA-binding protein has been purified and we have demonstrated that it binds the Fc region of human serum or secretory IgA, but not any other immunoglobulin.

Materials and Methods

Bacterial Strains. All group A and B streptococcal strains used in this study were obtained from the Lancefield strain collection currently maintained in our laboratory. The group A strains were grown in Todd-Hewitt medium. The group B strains were grown to late log phase in 200 ml of chemically defined medium (10), centrifuged, washed once in Tris-HCl buffer (0.2 M, pH 7.4), and finally suspended in 3.0 ml of the Tris-HCl buffer. Aliquots of 200 µl of this suspension were used to test IgG and IgA binding. All strains were typed as Ia, Ib, Ic, II, or III using the typing sera prepared by Dr. R. C. Lancefield.

Immunoglobulin Preparations. Human IgG was obtained from Sigma Chemical Co., (St. Louis, MO) and human IgA1 and IgA2 were purified from the sera of patients with IgA monoclonal gammopathy. Purified human myeloma proteins of the IgG1, IgG2, IgG3, IgG4, and IgA2 subclasses were donated by Ms. F. Prelli. Secretory IgA was purified from colostrum by the method of Cebra and Robbins (11). Immunoglobulins were labeled with 125I by the chloramine T method (12), or by using Iodo-Beads obtained from Pierce Chemical Co., Rockford, IL. Fc and Fab fragments of IgA1 were prepared by cleaving IgA with gonococcal IgA1-protease (13).
Separation of these two fragments by conventional ion exchange, or molecular sieve chromatography is difficult because the two fragments have very similar isoelectric points and molecular weights. To separate the Fc and Fab fragments of protease-treated IgA, a method using hydrophobic affinity chromatography was developed. The cleaved IgA was mixed with an equal volume of 2 M ammonium sulfate in 0.2 M Tris, pH 7.6, loaded onto a 2.5 × 5 cm column of phenyl-Sepharose previously equilibrated with 1.0 M ammonium sulfate in 0.1 M Tris HCl, pH 7.6, and the Fab fragment eluted. The column was washed with 5 column volumes of 0.8 M ammonium sulfate in 0.1 M Tris HCl, pH 7.6. The Fc fragments were then eluted by washing with 0.1 M Tris HCl, pH 7.6. The fractions were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoglobulin Binding to Streptococci. A 200-μl aliquot of bacteria was mixed with 10 μl of 125I-labeled immunoglobulin (300 ng) and incubated at 22°C for 15 min. The suspension was centrifuged and washed with 1.5 ml of Tris buffer. The percentage of IgA bound to the bacteria was calculated from the number of counts in the bacterial pellet and those in the supernatant and the wash. Binding inhibition experiments were also performed. The various human IgA preparations (60 μg of 7S, 9S, or Fc fragments) or 200 μl of serum from various species of animal were added to the 125I-labeled immunoglobulin before incubation with the bacteria.

Triton Extraction of the Bacteria. Overnight cultures of bacteria were centrifuged, washed once in Tris-HCl buffer (pH 7.4, 0.2 M), and resuspended in 0.2% Triton X-100 in 0.1 M citrate buffer, pH 5.5, 0.1 M NaCl. Triton extraction was allowed to proceed for 48 h at 37°C after which the bacteria were pelleted and the supernatant analyzed by SDS-PAGE (14) using a 10% polyacrylamide gel. Duplicate gels were processed by the Western blot method (15) to transfer the proteins onto nitrocellulose sheets. These were washed once with 0.1 M Tris-HCl, pH 8.0 containing 0.1 M NaCl and 0.05% Brij 35, and incubated with 5% bovine serum albumin before the addition of 125I-IgA. After incubation with 125I-IgA for 2 h at 37°C, the nitrocellulose sheets were washed extensively (0.15 M NaCl containing 0.05% Brij), dried, and exposed to film.

Extraction and Purification of IgA-binding Protein. The Ib strain H86B5 was used to inoculate 60 l of dialyzed Todd-Hewitt broth (Difco Laboratories, Detroit, MI) supplemented with 3 kg of dextrose and harvested by centrifugation in mid-log phase growth. The pelleted bacteria were suspended in 1.5 l of 2% SDS (Sigma Chemical Co.) in distilled water and boiled with stirring for 60 min. The bacteria were removed by centrifugation and the proteins in the supernatant precipitated by the addition of 2.5 vol of cold (4°C) ethanol. After centrifugation, the pellet was resuspended in 500 ml of 10 mM Tris-HCl, pH 8.0 (Tris buffer). Polysaccharides and other contaminants were removed by precipitation of the proteins with trichloroacetic acid (10% wt/vol). The supernatant was discarded and the pellet resuspended in 10 mM Tris buffer, pH 8.0 containing 0.5 M NaCl, 10 mM EDTA, and 2% SDS (Tris-SDS-2%).

The protein was applied to a 5.3 × 185 cm column of Sepharose 6B (Pharmacia Fine Chemicals Piscataway, N.J) previously equilibrated with the 0.1 M Tris buffer containing 0.5 M NaCl, 10 mM EDTA, and 0.1% SDS (Tris-SDS-0.1%), and chromatographed at a flow rate of 60 ml/h at 37°C. Fractions (20 ml) were collected and tested for IgA-binding activity in the following manner: An aliquot (200 μl) from each fraction was precipitated with ethanol, redissolved in 0.1 M Tris-HCl, pH 9.8 (100 μl), and used to sensitize an enzyme-linked immunosorbent assay (ELISA) plate (Immulon II; Dynatech Labs, Inc., Alexandria, VA) overnight at 22°C. The plates were washed six times with a solution of Brij 35 (0.05%) in saline (Brij-NaCl) and IgA (10 μg/ml in phosphate-buffered saline (PBS), 0.05% Brij 35) was added for a period of 4 h. The plates were washed again with Brij-NaCl before the addition of rabbit anti-human light chain serum (Dako Corporation, Santa Barbara, CA) to which alkaline-phosphatase had been conjugated (16). After overnight incubation at 20°C the plates were washed with Brij-NaCl and the alkaline-

\[^1\] Abbreviations used in this paper: SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
phosphatase substrate, p-nitrophenyl phosphate (Sigma Chemical Co.), added. Fractions showing IgA-binding activity were pooled, precipitated with ethanol, dissolved in Tris-SDS-2% and rechromatographed on a Sephacryl S-400 column (2.5 × 185, 15 ml/h in Tris-SDS-0.1%) as previously described. Fractions (10 ml) were collected, tested, and pooled as before.

Results

**Binding of IgG and IgA to Group B Streptococci.** A number of strains of type Ia, Ib, Ic, II, and III group B, and type 4 group A streptococci were tested for their ability to bind human IgG and IgA. None of the strains tested bound >10% of the 125I-labeled IgG. We were able to confirm the results of Schalen et al. (8) that the majority of group A strains of M type 4 bind human IgA (Fig. 1). We found that many of the Ib and Ic strains bound radiolabeled IgA. When significant binding was defined as >15%, then 16 of 23 Ib and 5 of 6 Ic exhibited IgA binding (Fig. 1). None of 16 type Ia, 5 type II, or 4 type III strains bound IgA (results not shown). A number of strains have been found that show positive precipitin reactions with two different typing sera and several of these were tested. The II × Ic type is strains with the type II polysaccharide antigen and the Ibc protein, whereas II × Ib strains express antigenic determinants of the type II and Ib polysaccharides. The II × Ic strains, 22RP279, F393, and 57RP145 bound 70.3, 13, and 3%, respectively, of the IgA (Fig. 1). The II × Ib strains, F325 and F326, bound 3% of the IgA (data not shown).

**Inhibition of IgA Binding by Bacteria.** To further define the nature of the binding of IgA, inhibition studies were performed using the Ib strain H36B-5 (the strain which bound the most IgA among those tested). Binding of 25 ng of

![Figure 1](image_url)
radionabeled human IgA to strain H36B-5 (type Ib) was completely inhibited by premixing the IgA with 200 μl of human serum. Binding of intact IgA was also completely inhibited by premixing the radiolabeled IgA (300 ng) with 60 μg of purified IgA1 or with 60 μg of purified IgA1 Fc fragments. Addition of the same amount of cat, mouse, goat, horse, burro, alligator, or grey nurse shark serum did not inhibit the binding of human IgA to this strain.

**Analysis of Triton-extractable Proteins by SDS-PAGE.** 11 group B strains were extracted with Triton X-100 and the proteins separated on duplicate SDS-PAGE gels. One gel was stained for protein with Coomassie Blue (Fig. 2a). The proteins in the other gel were transferred to nitrocellulose, and, after washing, the sheet was exposed to labeled IgA. The autoradiograph is shown in Fig. 2b. It can be seen in Fig. 2a that a number of strains revealed two major proteins of ~130,000 mol wt. These were released from IgA-binding strains, but not from nonbinding strains (note the two Ia strains in lanes 1 and 2, as well as the 2 II × Ib strains, lanes 8 and 9, which did not bind IgA). Furthermore, there appeared to be a correlation between the intensity of the 130,000 mol wt protein band and the extent of IgA binding listed in the figure legend. The identity of the IgA-binding proteins is shown in Fig. 2b. The major bands labeled were the two proteins of ~130,000 mol wt. In all IgA-binding strains, however, a number of smaller molecular weight proteins also bound IgA. It is likely that these proteins are breakdown products of the larger proteins.

**Extraction and Purification of the IgA-binding Protein.** In preliminary attempts to purify the IgA binding protein it was noted that the material was readily degraded and lost. When, however, the bacterial pellet from a mid-log phase culture of the group B streptococcal strain, H36B5, was boiled in SDS, a major protein of 130,000 mol wt was released without the concomitant release of active proteolytic enzymes. Subsequent molecular sieve chromatography of this extract in the presence of 0.1% SDS and isolation of the IgA-binding fractions resulted in the copurification of this protein with the IgA-binding activity. When SDS polyacrylamide gels of this preparation were blotted onto nitrocellulose and incubated with ^125^I-IgA, it was found that this major protein was indistinguishable from the IgA-binding band (Fig. 3).

**Specificity of the IgA-binding Protein.** To establish the specificity of the purified IgA-binding protein, competition assays with purified immunoglobulins were performed by iodinating pure human myeloma IgA1 and adding equal or greater concentrations of unlabeled immunoglobulin preparations. The results are summarized in Table I and indicate that serum IgA1 and colostral IgA are highly inhibitory. Using purified gonococcal IgA1 protease and hydrophobic affinity chromatography, the Fab and Fc fragments were isolated, and only the Fc fragment was inhibitory. It was found that commercial preparations of IgG did inhibit the binding, but much less effectively than purified IgA (data not shown). To determine whether this was due to trace contamination with IgA or to affinity by one of the IgG subclasses, we tested purified myeloma proteins representing each of the subclasses. None of these inhibited the IgA binding significantly. Human IgM was also noninhibitory. Two preparations of human IgA2 myeloma proteins did cause inhibition of binding, but less effectively than IgA1.
Figure 2. Binding of \(^{125}\text{I}\)-labeled IgA to Western blots of Triton X-100 extracts of strains of group B streptococci. Triton extracts of various strains of group B streptococci were subjected to SDS-PAGE and stained with Coomassie Brilliant R (Fig. 2a). Duplicate gels were blotted and, after appropriate preparation, were incubated with \(^{125}\text{I}\)-labeled IgA (4 \(\mu\)g/ml), washed, and used to expose photographic film (Fig. 2b). Extracts of strains are as follows: (lane 1) 090R, (2) D870 (type Ia), (3) A909, (4) F343-3 (type Ic), (5) A670, (6) H36B-5, (7) A943 (type Ib), (8) F325, (9) F326 (type II \(\times\) Ib), (10) 20RP279, and (11) F393 (type II \(\times\) Ic). These strains bound 3, 3, 73, 43, 35, 91, 45, 3, 3, 70, and 13% of added radioactivity when tested in the manner described for Fig. 1.
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Discussion

The present study describes the presence of a receptor for human IgA on the surface of a number of strains of group B streptococci. This is in contrast to the work of Christensen and Oxelius (7) who found no significant binding of IgA to 14 untyped group B streptococcal strains.

The ability of group B streptococci to bind IgA appears to be restricted to strains that react with the anti-Ibc serum, namely, 5 Ic and 16 lb strain, and one II × 1c strains. Strains of the Ia, II, III, and II × lb serotypes bound <15% of added IgA. Binding of IgA was found to be specific for the Fc fragment of the immunoglobulin molecule, and also appeared to be remarkably specific for human IgA.

Fischetti et al. (17) used Triton to extract M protein from the surface of group
A streptococci. We have used this procedure with group B organisms. SDS-PAGE analysis of Triton-extractable proteins revealed a correlation between the extent of IgA binding and the presence of two major protein bands of ~130,000 mol wt. Blotting of these gels onto nitrocellulose reaffirmed that the 130,000 mol wt proteins were indeed the IgA-binding proteins. It is noteworthy that the IgG-binding protein isolated from group A streptococci by Havlicek (6) had a molecular weight of 100,000, distinctly smaller than the IgA-binding protein. As can be seen from Fig. 2a and b, strain H36B/5, a type 1b strain, which bound 91% of the radiolabeled IgA, had considerably more of the 130,000 mol wt proteins and could bind more 125I-IgA than strains A670 and A943 (also 1b strains), which bound only 35 and 45% of the IgA. Strain A640, an 1b strain that did not bind IgA, completely lacked the high molecular weight protein (results not shown).

Reactivity with the Ibc antiserum has previously been shown (18, 19) to be due to the presence of at least two antigenically distinct proteins, the alpha and the beta determinant. However, only half of the strains reactive with the Ibc antiserum possessed both of these antigens (19). This may explain why 7 of the 23 type 1b strains tested in this study did not bind IgA.

We have been able to purify the IgA-binding protein in an active form. Solid phase radioimmunoassays indicate that the protein binds the Fc region of monomeric or polymeric IgA, and that it does not bind IgM or any subclass of IgG. In competition assays it appears that IgA2 is able to compete with IgA1 for the binding protein, but less effectively, suggesting that the affinity of the binding with IgA2 may be lower. The potential of this protein as an immunochemical reagent analogous to staphylococcal protein A, but specific for human IgA, deserves exploration.

While the results of this study do not address the possible role of the IgA-binding protein in the pathogenesis of group B streptococcal disease, Lancefield and coworkers (20) have previously shown that rabbit antibodies to the Ibc proteins are protective in mice. Baltimore et al. (21) have also demonstrated that similar antibodies (also raised in rabbits) are capable of eliciting phagocytosis and killing of strains carrying the Ibc protein antigen. It is interesting to note that a much higher concentration of antibody was required to kill the 1b or 1c strains (which bear the IgA-binding protein) than that required to kill type Ia, II, or III strains.

Summary

A number of group B streptococcal strains of various serotypes, Ia, Ib, Ic, II, and III were examined for their ability to bind human IgG and IgA. No strains of group B streptococci were found to bind IgG, but many strains possessing the Ibc protein antigen(s) were found to bind a significant amount of IgA. The extent of IgA binding correlated with the amount of a 130,000 mol wt, detergent-extractable protein, and reactivity with the Ic typing sera. Using nitrocellulose blots, it was found that the 130,000 mol wt protein bound human IgA. A method was developed to purify the protein while retaining its ability to bind human IgA. Using solid phase radioimmunoassays, it was determined that the protein
bound to the Fc region of monomeric or polymeric IgA and that it failed to bind IgM or any IgG isotype.

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