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Identification of Non-Immunoglobulin A-Fc-Binding Forms and Low-Molecular-Weight Secreted Forms of the Group B Streptococcal β Antigen

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The β antigen expressed on the surfaces of certain strains of group B streptococci has been reported to bind to the Fc region of human immunoglobulin A (IgA). In this study, we screened 100 isolates of group B streptococci for expression of both β antigen and IgA-Fc-binding activity. We identified two isolates which expressed the β antigen but could not bind human IgA Fc fragments and also observed variability in IgA-Fc-binding activity among other β-antigen-expressing strains. Novel low-molecular-weight forms of β antigen were secreted by four β-antigen surface-negative isolates and included IgA-Fc-binding (M_rs, 55,000 and 53,000) and non-IgA-Fc-binding (M_r, 38,000) molecules. These results suggest that the IgA-Fc-binding site represents a unique domain of the β antigen. The 55,000- and 53,000-M_r forms of secreted β antigen were functionally and antigenically representative of the size-heterogeneous (M_r, up to 145,000) β-antigen molecules expressed by surface-positive strains. The cell surface-localized IgA-Fc-binding molecules could bind only human serum IgA efficiently; however, once solubilized, these molecules could bind both human serum and secretory IgAs.

Recently, a binding protein specific for the Fc region of human immunoglobulin A (IgA) was reported to be expressed on the surfaces of certain isolates of group B streptococci (GBS) (34). Expression of this IgA-Fc-binding protein was reported to correlate with the presence of the β-antigen component of the c-protein-typing marker of GBS. The nomenclature of type-specific antigens present on GBS has recently been reviewed (16). Briefly, GBS are typed serologically on the basis of the presence of one of six acid-extractable carbohydrate antigens (16, 17). In addition, a protein marker called the c protein, previously designated Ibc or Ic, has been identified (39). This marker is almost always found on strains which express the Ib carbohydrate but can be found in association with other carbohydrate antigens as well (19). The c protein has been reported to consist of at least two acid-extractable antigens called α (trypsin resistant) and β (trypsin sensitive) (2–6) which are expressed independently of one another (19) and reside on the bacterial surface as nonlinked molecules (3). More recently two additional antigens, γ and δ, have also been described (7).

The GBS IgA-Fc-binding protein reported by Russell-Jones et al. was always found on c-protein-positive strains, but not all such strains exhibited IgA-Fc-binding activity (34). Their experiments using anti-α and anti-β monoclonal antibodies revealed that IgA-Fc-binding activity was associated with β antigen. Western (immuno-) blot analysis of detergent-extracted proteins indicated that it was β antigen which conferred the ability to bind human IgA (33). The α and β antigens have been cloned (9). The protein product of the cloned β gene was able to bind human secretory IgA (sIgA) but not IgG or IgM, while the product of the cloned α gene was unable to bind any isotope of human immunoglobulin.

The results presented in this report indicate that although GBS IgA-Fc-binding ability is closely associated with the expression of β antigen, that correlation is not absolute. We have identified two isolates which express β antigen yet are unable to bind human IgA. Variation in the ratio of surface β-antigen expression relative to IgA-Fc-binding capacity among GBS strains positive for both of these markers was also observed. It has been reported that β antigen can be secreted by some GBS strains (12), and we provide evidence that IgA-Fc-binding proteins and β antigen are secreted by all GBS tested in our laboratory which express these markers on their surfaces. In addition, several GBS isolates were identified which secrete IgA-Fc-binding activity or β antigen or both in the absence of surface expression. We found that cell surface forms of IgA-Fc-binding proteins can bind only serum IgA efficiently, while solubilized molecules have the ability to bind both human serum and sIgAs.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. One hundred laboratory isolates of GBS were used in this study. All isolates were confirmed as GBS by screening with the Phadebact streptococcus test (Pharmacia Diagnostics, Piscataway, N.J.). Bacteria were grown to late log phase in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) or chemically defined medium for streptococci (Hazelton Research Products, Inc., St. Lenexa, Kans.) for 18 to 24 h at 37°C. The bacteria were harvested by centrifugation and were washed in 0.15 M phosphate-buffered saline (PBS; pH 7.4). Light scatter at 550 nm was used to standardize the concentration of organisms used in subsequent tests. Stock cultures were stored in glycerol at −70°C.

Detection of CAMP factor. GBS were streaked perpendicular to, but not touching, a streaked inoculum of *Staphylococcus aureus* on 5% sheep blood agar plates. The plates were incubated for 18 h at 37°C under anaerobic conditions. A positive CAMP reaction was detected as a flame-shaped zone of synergistic complete hemolysis at the interface

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between the two bacterial species as described elsewhere (10). All GBS isolates tested were found to be CAMP factor positive.

Source of anti-β antiserum. Rabbit anti-c-protein typing serum was kindly provided for this study by R. Facklam, Centers for Disease Control, Atlanta, Ga. This antiserum was rendered monospecific for reactivity against β antigen by selective adsorption with strains expressing α, γ, and δ antigens (7). Bacterial strains were chosen as appropriate adsorbents after characterization by two-stage radioimmunoassay as described previously (7). The bacteria from 5 ml of a Todd-Hewitt broth overnight culture were pelleted by centrifugation and were washed once with 2 ml of PBS (pH 7.4). One hundred microliters of anti-c-protein antiserum was added to the washed bacterial pellet and rotated at 4°C for 1 h. The bacteria were removed from the antiserum by centrifugation. The adsorption was repeated at least twice or until all reactivity against the adsorbing strain was eliminated as detected by the two-stage radioimmunoassay. The specificity of the adsorbed antiserum was confirmed by immunoelectrophoresis.

Source of human serum IgA and preparation of IgA Fc fragments. Human IgA1 was isolated from the serum of a myeloma patient by DEAE-cellulose chromatography as described elsewhere (26). Fc and $F(ab')_2$ fragments were generated by cleavage with *Haemophilus influenzae* IgA1 protease (29). Fc and $F(ab')_2$ fragments were separated by using hydrophobic affinity chromatography as described previously (34). The cleaved IgA1 was mixed with an equal volume of 2 M ammonium sulfate in 0.2 M Tris hydrochloride (pH 7.6) and was chromatographed on a column of phenyl-Sepharose which had been preequilibrated in 0.1 M Tris hydrochloride (pH 7.6) containing 0.8 M ammonium sulfate. $F(ab')_2$ fragments were eluted with 0.8 M ammonium sulfate, and Fc fragments were eluted with 0.1 M Tris hydrochloride (pH 7.6). The Fc and $F(ab')_2$ fragments were characterized by immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Iodination of proteins. Protein A (Pharmacia), IgA Fc fragments, IgG Fc fragments (Jackson Immuno Research Labs, West Grove, Pa.), and sIgA (Sigma Chemical Co., St. Louis, Mo.) were radioiodinated by the mild lactoperoxidase method, using Enzymobeads (Bio-Rad Laboratories, Richmond, Calif.) (30). The labeled protein was separated from free iodine by passage over a G25 column (PD 10, Pharmacia) and was collected in 0.15 M Veronal-buffered saline (pH 7.4) containing 0.001 M Mg^{2+}, 0.00015 M Ca^{2+}, and 0.1% gelatin (VBS-gel). Proteins labeled by this method routinely have a specific activity of approximately 0.3 mCi/mg (30).

Preparation of hot-acid extracts of GBS. Cultures (150 ml) of laboratory isolates of GBS were grown to late log phase at 37°C in Todd-Hewitt broth (approximately 18 h). Bacteria were harvested by centrifugation and were extracted as described previously (39). Bacterial pellets were suspended in 0.2 N HCl in 0.85% NaCl to adjust the pH to 2.0 (approximately 1 ml). These suspensions were boiled for 10 min, adjusted to neutral pH with 0.2 N NaOH, and centrifuged at 1,000 × *g* to remove bacterial debris, and the supernatants were decanted. Hot-acid extracts were concentrated 10-fold, using Minicon macrosolute concentrators (Amicon Corp., Danvers, Mass.).

Preparation of culture supernatants. Bacteria were grown to late log phase in Todd-Hewitt broth (BBL) or chemically defined medium for streptococci (Hazelton Research Products) for approximately 18 h at 37°C. Bacterial cells were pelleted by centrifugation, and the supernatants were decanted. Residual bacteria were removed by filtration through 0.2-μm Acrodiscs (Gelman Sciences, Inc., Ann Arbor, Mich.) or 0.22-μm Nalgene disposable filterware (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.). The filtered Todd-Hewitt broth supernatants were concentrated 15- to 50-fold, using Minicon macrosolute concentrators (Amicon). The filtered chemically defined medium supernatants were concentrated 300- to 500-fold, using PM10 Diaflo ultrafiltration membranes (Amicon). Sodium azide was added to a final concentration of 0.02% to stored culture supernatants.

Direct binding of ^{125}I-radiolabeled proteins to GBS. The presence of Fc-reactive proteins on bacteria was detected by their ability to bind ^{125}I-labeled human IgA Fc fragments, ^{125}I-human IgG Fc fragments, or ^{125}I-human sIgA directly to their surfaces. Approximately 1×10^9 bacteria were incubated with ^{125}I-labeled protein (approximately 3×10^4 cpm) in 100 μl of VBS-gel for 1 h at 37°C. Bacteria were pelleted by centrifugation and were washed twice with 2 ml of VBS-gel containing 0.01 M EDTA (EDTA-gel). Radioactivity associated with the bacteria was determined in a 5500 auto-gamma counter (Beckman Instruments, Inc., Fullerton, Calif.). Background radioactivity was determined in control tubes containing radiolabeled proteins only. Assays were performed in duplicate with less than 5% variation observed between duplicate samples. None of the 100 GBS isolates tested bound human IgG Fc fragments.

Two-stage radioimmunoassay for determination of the GBS β antigen. The two-stage radioimmunoassay described previously (7) was used to detect β antigen on the surfaces of GBS. Bacteria were grown to late log phase in Todd-Hewitt broth, were pelleted by centrifugation, and were suspended in PBS (pH 7.4). The bacteria were incubated with rabbit monospecific anti-β antiserum and were washed, and anti-β antibody bound to the GBS was quantified by using ^{125}I-radiolabeled protein A as the tracer. All isolates were also incubated with normal rabbit serum as a negative control. None of the 100 GBS isolates studied demonstrated reactivity with nonimmune rabbit IgG. Bacteria-associated radioactivity was quantitated in a 5500 auto-gamma counter (Beckman). Background radioactivity was measured in control tubes containing bacteria and ^{125}I-protein A only. Assays were performed in duplicate with less than 5% variation observed between duplicate samples.

Dot blot autoradiographic procedure to detect cell surface and secreted IgA-Fc-binding proteins, IgG-Fc-binding proteins, and β antigen. Dot blots were performed by using the Bio-Dot microfiltration apparatus (Bio-Rad) and a modification of the Bio-Rad procedure. A piece of nitrocellulose previously soaked in 25 mM Tris–192 mM glycine (pH 8.3)–20% (wt/vol) methanol (wash buffer) was placed in the apparatus. Fifty microliters of 15-fold-concentrated Todd-Hewitt broth culture supernatants or bacterial suspensions in PBS (pH 7.4) (approximately 5×10^7 organisms) was pipetted into the wells. The wells were washed twice with 200 μl of PBS, and the nitrocellulose was removed from the apparatus. The nitrocellulose filters were blocked and probed for IgA-Fc- or IgG-Fc-binding proteins or β antigen as described below for the Western blotting procedure. A normal rabbit serum control was included in each sandwich-type assay used to detect β antigen.

Polyacrylamide gel electrophoresis and Western blotting procedure to detect IgA-Fc-binding proteins and β antigen. Protein samples (100 μl of 10-fold-concentrated hot-acid extracts or 100 μl of 50-fold-concentrated Todd-Hewitt broth culture supernatants) were denatured by being boiled for 5

min in 2% (wt/vol) sodium dodecyl sulfate–5% (wt/vol) β-mercaptoethanol–10% (wt/vol) glycerol–0.01% (wt/vol) bromphenol blue–0.5 M Tris hydrochloride (pH 6.8). Denatured proteins were electrophoresed on 7.5% polyacrylamide slab gels at 5 mA per slab for 16 h by the method of Laemmli (23). Prestained molecular weight markers (Sigma) were run in one lane of each gel for the determination of estimated molecular weights. The proteins on the gels were transferred electrophoretically to nitrocellulose by a modification of the method described by Towbin et al. (35). The gels were presoaked for 30 min in 25 mM Tris–192 mM glycine–20% methanol (pH 8.3), were assembled into the high-field-intensity Trans Blot system (Bio-Rad), and were electrophoresed in that buffer at 70 V for 3 h.

The nitrocellulose filters were blocked by being washed four times (15 min per wash) with 250 ml of 0.15 M Veronal-buffered saline (pH 7.4) containing 0.25% gelatin and 0.25% Tween 20 (VBS-gel-Tween). The blocked filters were probed with 10 ml of VBS-gel-Tween containing 3×10^5 cpm of ^{125}I-human IgA Fc fragments or ^{125}I-human sIgA per ml for 3 h at an ambient temperature. The filters were then washed four times (15 min per wash) with 250 ml of VBS-gel-Tween containing 0.01 M EDTA and 1 M NaCl, were dried, and were autoradiographed by using XAR-5 film and X-Omatic intensifying screens (Eastman Kodak Co., Rochester, N.Y.) at −70°C for 1 day.

For detection of β antigen, the blocked nitrocellulose filters were incubated for 3 h at an ambient temperature with 10 ml of a 1:400 dilution of the rabbit monospecific anti-β antiserum or normal rabbit serum (diluted in VBS-gel-Tween), were washed four times with 250 ml of VBS-gel-Tween, and were incubated with 10 ml of VBS-gel-Tween containing approximately 3×10^5 cpm of ^{125}I-protein A per ml for 3 h at an ambient temperature. The filters were then washed four times with 250 ml of VBS-gel-Tween containing 0.01 M EDTA and 1 M NaCl, were dried, and were autoradiographed by using XAR-5 film and X-Omatic intensifying screens (Eastman Kodak) at −70°C for 6 h.

Colony blot procedure to detect IgA-Fc-binding proteins and β antigen. The colony blot procedure was carried out by the method of Yarnall et al. (40). An overnight suspension of bacterial strain ss618 was diluted in Todd-Hewitt broth to yield 10 to 100 colonies when 100 μl was plated on Todd-Hewitt agar (Todd-Hewitt broth containing 1.5% agar). The plate was incubated at 37°C for 16 h and was replica plated onto Todd-Hewitt agar plates, using a RepliPlate colony transfer pad (FMC Corp., Marine Colloids Div., Rockland, Maine). The agar was removed from each petri dish, and a circular piece of nitrocellulose, previously soaked in 25 mM Tris–192 M glycine–20% (vol/vol) methanol (pH 8.3), followed by a circular piece of buffer-soaked 3MM paper (Whatman, Inc., Clifton, N.J.) were placed on top of the colonies on each of the plates. Another circular piece of buffer-soaked Whatman 3MM paper was placed against the agar on the side away from the colonies. The bacterial colonies and diffusible bacterial proteins within the agar were transferred to the nitrocellulose by electrophoresis at 70 V for 3 h in the buffer described above, using the high-field-intensity transblot system (Bio-Rad). After electrophoresis, the nitrocellulose filters were blocked and probed with ^{125}I-IgA Fc fragments or rabbit anti-β antiserum followed by ^{125}I-protein A as described above for the Western blotting procedure.

Competitive inhibition of ^{125}I-IgA Fc fragment and anti-β antiserum binding by GBS culture supernatants. GBS were grown to late log phase in chemically defined medium for streptococci (Hazelton Research Products), and concentrated supernatants were prepared as described above. Serial twofold dilutions of concentrated culture supernatants were prepared in PBS (pH 7.4). Samples (100 μl) of the serial dilutions were added concomitantly with bacteria and ^{125}I-IgA Fc fragments as described above for the direct binding assay, or with anti-β antiserum and bacteria as described above for the first stage of the two-stage radioimmunoassay. The percent inhibition of specific binding was calculated by comparison of the counts per minute associated with bacterial pellets in test samples with the counts per minute associated with bacterial pellets in control samples containing 100 μl of PBS in place of the dilution of culture supernatant. Assays were performed in duplicate.

TABLE 1. Summary of IgA-Fc-binding proteins (IgAFcBP) and β antigen (β Ag) on the surface or secreted by GBS isolates[a]

Strain	Surface expression of:		Secretion of:	
	IgAFcBP	β Ag	IgAFcBP	β Ag
HG381	+	+	+	+
A909	+	+	+	+
HG806	−	−	−	+
2AR	−	−	+	+
TC795	+	+	+	+
PF549AR-B	+	+	+	+
HG805	+	+	+	+
HG769	+	+	+	+
TC137	(+)[b]	(+)	(+)	(+)
H-36	−	+	−	+
ss618	+	+	+	+
DL469M	−	−	+	+
DL471B	−	−	+	+
HD501L	+	+	+	+
HD652E-B	+	+	+	+
HD653E-M	+	+	+	+
HD1822E	+	+	+	+
HD1988E	+	+	+	+

[a] Characteristics of the 18 β-antigen-positive isolates (of 100 screened) are shown. The 82 isolates that neither expressed nor secreted IgAFcBP or β antigen were N86K, NP$_1$AR, HG824, ss617, HG346, PF534AR, J46, HG783, HG784, HG812, HG811, PF58AV, PF536AR, PF549AR-NB1, PF541AR, J44, PF65BV, PF25AR, PF610AR, NPF$_1$AV, HG818, HG819, 9B200, HG768, HG804, HG774, HG782, HG820, VC75, HG780, HG757, HG814, HG754, HG802, HG738, J48, J52, Peh, J51, HG786, HG795, HG828, HG770, HG771, DL263M, DL265B, DL413B, DL414M, DL506M, DL507B, DL587B, DL588M, DL235B, DL245B, DL324B, DL361B, DL369B, DL394B, DL400B, HD612, HD1023E, HD1073L, HD1150E, HD1228L, HD1575L, HD1604L, HD1471, HD1630E-M, HD1631E-B, HD1676E, HD1702L, HD1710, HD1719L, HD1723E, HD1728E, HD1734E, HD1735E, HD1818E, HD1976E, HD3055L, 10W817-M, and 10B1657-B.

[b] Strain TC137 bound lower quantities of IgA Fc fragments and rabbit anti-β antibodies than did other positive strains.

RESULTS

Distribution of β antigen and IgA-Fc-binding activity associated with GBS. One hundred laboratory isolates of GBS were screened for IgA-Fc-binding activity and expression of β antigen both on their surfaces and in culture supernatants (Table 1). Thirteen isolates exhibited surface binding of IgA Fc fragments. Fourteen isolates (including the thirteen IgA-Fc-binding isolates) demonstrated surface expression of β antigen. All isolates which demonstrated surface expression of IgA-Fc-binding proteins or the β antigen or both also secreted these markers into culture supernatants. In addition, four strains secreted β antigen in the absence of surface expression, but only three of these secreted IgA-Fc-binding proteins into culture supernatants. A total of 18 of the 100

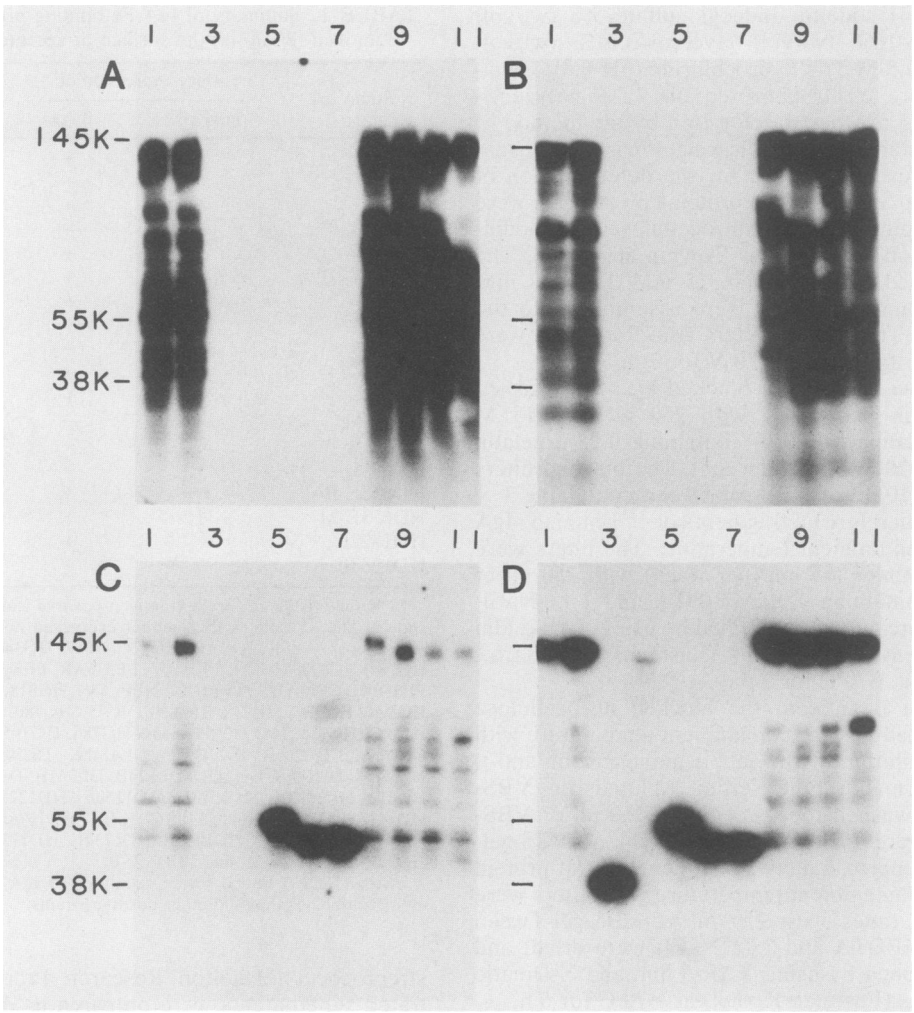

FIG. 1. Analysis of IgA-Fc-binding activity and β antigen in extracts and culture fluids of GBS. Parallel Western blots (7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of hot-acid extracts and concentrated supernatants of representative streptococcal isolates were performed as follows. Hot-acid extracts (10-fold concentrated) were probed directly with ^{125}I-IgA Fc fragments (A) or were reacted with anti-β antiserum, washed, and then probed with ^{125}I-protein A (B), and Todd-Hewitt broth culture supernatants (50-fold concentrated) were probed directly with ^{125}I-IgA Fc fragments (C) or were probed by using the anti-β antiserum-^{125}I-protein A sandwich procedure (D). Lanes 1 through 11 contain concentrated hot-acid extracts or concentrated culture supernatants from strains HG381, ss700, HG806, TC137, 2AR, DL469-M, DL471-B, PF549AR-B, HG805, HG769, and TC795, respectively. Autoradiography was carried out for 24 h at −70°C. Strain TC137 demonstrated β-antigen and IgA-Fc-binding activity in both hot-acid extracts and concentrated culture supernatants when the autoradiographs were exposed for longer periods of time.

GBS isolates studied secreted β antigen, with only 16 of the corresponding culture supernatants demonstrating IgA-Fc-binding activity.

Comparison of β antigen and IgA-Fc-binding proteins. The relationship between GBS IgA-Fc-binding proteins and β antigen was studied further by examining 11 GBS isolates by Western blot analysis. Concentrated hot-acid extracts were used to determine surface characteristics, and concentrated culture supernatants were used to study secreted molecules. Comparison of the autoradiographs shown in Fig. 1 revealed several distinct reactivity profiles. The hot-acid extracts of the β-antigen surface-positive isolates bound both IgA Fc fragments and anti-β antibody (Fig. 1A and B, lanes 1, 2, and 8 through 11). All bands which were reactive with anti-β antiserum also bound IgA Fc fragments. A range of molecular-weight forms of β antigen/IgA-Fc-binding proteins, the highest of which had an M_r of approximately 145,000, was observed for surface-positive isolates. Isolates with β-antigen/IgA-Fc-binding activity in their hot-acid extracts demonstrated corresponding reactivity in their culture supernatants (Fig. 1C and D). Secreted molecules were less size heterogeneous than acid-extracted molecules, but again there was a similar pattern of bands reacting with anti-β antiserum and IgA Fc fragments (Fig. 1C and D; compare lanes 1, 2, and 8 through 11). None of the surface-positive GBS secreted β antigen/IgA-Fc-binding proteins of M_r less than 53,000.

A second group of four GBS isolates that failed to bind either anti-β antiserum or ^{125}I-labeled IgA Fc fragments to their surfaces was also studied. As expected, the hot-acid extracts of these isolates showed no β-antigen or IgA-Fc-binding activity (Fig. 1A and B, lanes 3 through 7). However, each of these GBS isolates secreted a homogeneous low-molecular-weight form of β antigen (M_rs, 55,000, two of 53,000, and 38,000) (Fig. 1D, lanes 3 through 7). A comparison of the IgA-Fc-binding properties of these low-

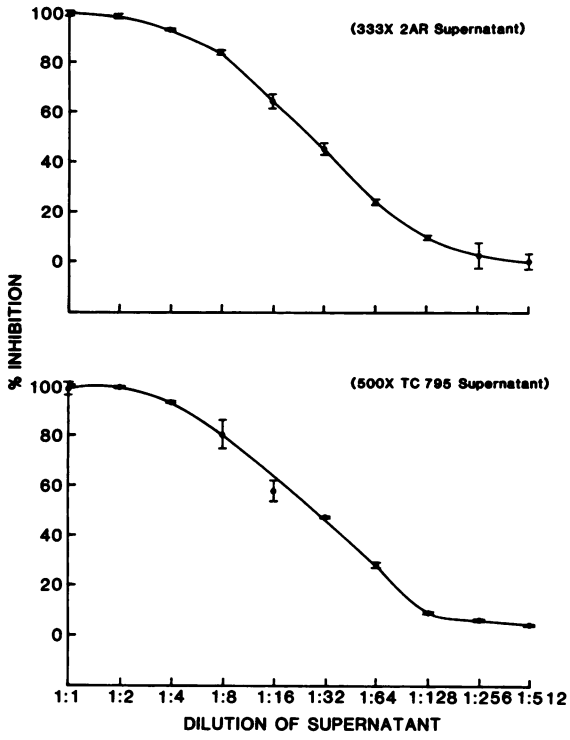

FIG. 2. Inhibition of binding of IgA Fc fragments to strain TC795 by culture supernatants from selected GBS isolates. Competitive binding experiments were carried out with twofold serial dilutions of concentrated culture supernatant from strain 2AR (concentrated 333-fold) (top) or from strain TC795 (concentrated 500-fold) (bottom) added concomitantly with ^{125}I-IgA Fc fragments to a standardized bacterial suspension of the β-antigen surface-positive strain TC795. Inhibition was determined by comparing the quantity of radioactivity associated with the bacterial pellet in the presence of added culture supernatant with binding observed in the presence of buffer alone. Assays were performed in duplicate.

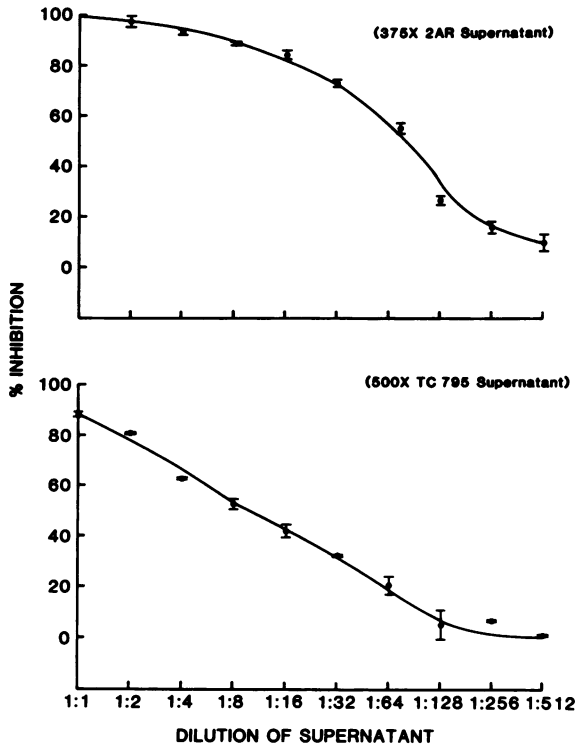

FIG. 3. Inhibition of binding to strain TC795 of rabbit anti-β antiserum by culture supernatants from selected GBS isolates. Competitive binding experiments were carried out with twofold serial dilutions of concentrated culture supernatant from strain 2AR (concentrated 375-fold) (top) or from strain TC795 (concentrated 500-fold) (bottom) added concomitantly with monospecific rabbit anti-β antiserum added to a standardized bacteria suspension of strain TC795 in the first stage of the two-stage radioimmunoassay for detection of cell surface β antigen. Rabbit anti-β antibodies bound to the bacteria were quantified, using ^{125}I-labeled protein A. All assays were performed in duplicate. Inhibition was determined by comparing the quantity of ^{125}I-protein A associated with the bacterial pellet in the presence of added supernatant with that bound in the presence of buffer alone.

molecular-weight forms of secreted β antigen revealed that only three of the four β-antigen-positive supernatants bound IgA Fc fragments (Fig. 1C and D; compare lanes 3 through 7). The 38,000-M_r form of β antigen secreted in the absence of surface expression did not bind ^{125}I-radiolabeled IgA Fc fragments.

Relationship of low-molecular-weight secreted β antigen to other forms of the molecule. To characterize further β antigen which had IgA-Fc-binding activity, a series of competitive binding experiments was performed to test whether the single-molecular-weight secreted forms of β antigen/IgA-Fc-binding protein were functionally and antigenically representative of those molecules expressed by surface-positive GBS. Concentrated culture supernatant from strain 2AR, which demonstrated a 55,000-M_r band on Western blot analysis, was able to completely inhibit binding of both IgA Fc fragments and anti-β antibodies to the representative surface-positive strain TC795 (Fig. 2 and 3). Despite differences in the anti-β antibody/IgA Fc fragment-binding profiles of supernatants from strains TC795 and 2AR when analyzed by Western blotting, supernatants from both strains were equally effective inhibitors of IgA Fc binding (Fig. 2). Inhibition of binding of anti-β antibodies was also observed when concentrated culture supernatant from either strain 2AR or TC795 was added to the TC795 suspension concomitantly with anti-β antiserum (Fig. 3). The presence of β antigen in 2AR and TC795 culture supernatants was also confirmed by immunoelectrophoresis (data not shown).

Similar inhibition curves were generated when concentrated culture supernatants from other β-antigen surface-positive strains were substituted for TC795 or when concentrated culture supernatant from strain DL471B (which demonstrated a 53,000-M_r band on Western blot analysis) was substituted for 2AR in these competitive binding experiments. Concentrated culture supernatant from strain HG806 (which contained the 38,000-M_r form of secreted β antigen) had no effect on ^{125}I-IgA Fc fragment binding but could partially inhibit binding of rabbit anti-β antiserum to surface-positive strains (data not shown).

Expression of β antigen/IgA-Fc-binding proteins by individual colonies of GBS. Variation in surface expression of IgG-Fc-binding proteins has been reported for many group A streptococcal isolates (8, 32, 40); consequently, experiments were performed to determine whether there was colony-to-colony variation in expression of β antigen or IgA-Fc-binding proteins by a surface-positive strain. The GBS strain ss618 was replica plated, colony blotted, and probed

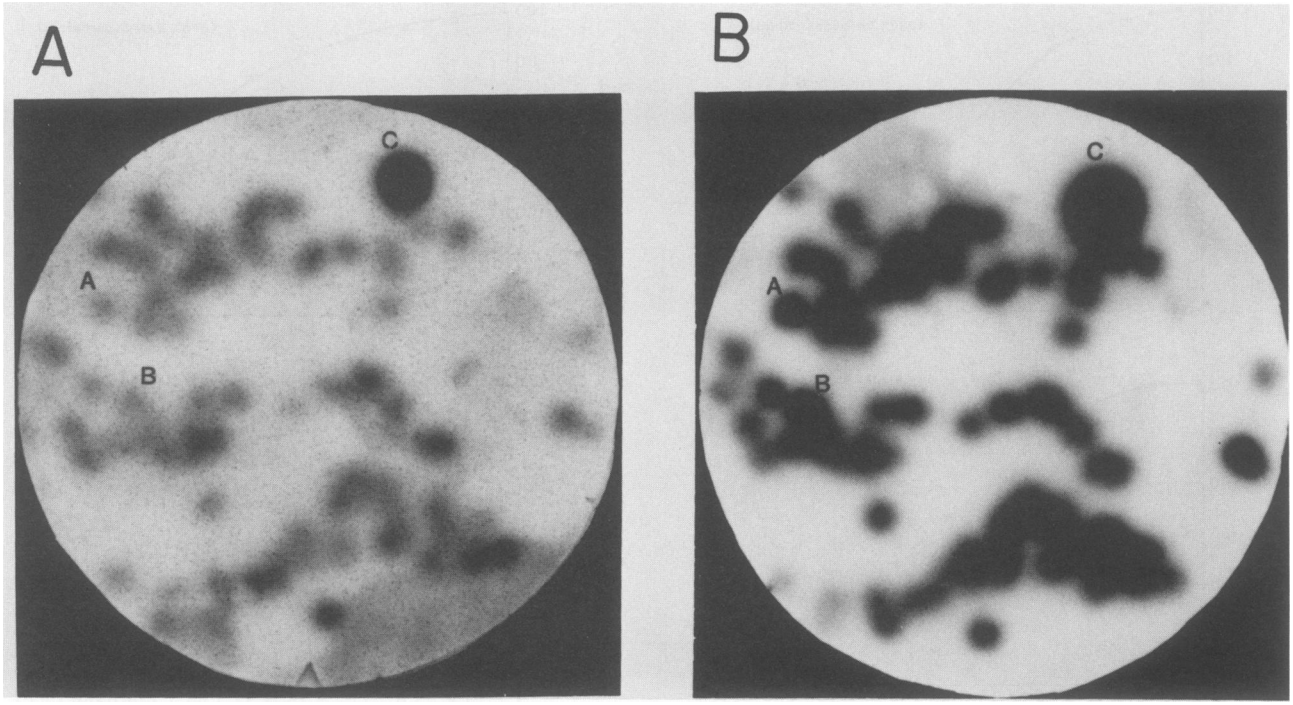

FIG. 4. Colony blots of replica plates of GBS strain ss618. (A) Colonies were transferred to nitrocellulose, and the filter was probed directly with ^{125}I-IgA Fc fragments. (B) Colonies were transferred to nitrocellulose, and the filter was probed indirectly by using a sandwich procedure, i.e., first with monospecific rabbit anti-β antiserum and then with ^{125}I-protein A.

directly with ^{125}I-radiolabeled IgA Fc fragments (Fig. 4A) and indirectly with rabbit anti-β antiserum followed by ^{125}I-radiolabeled protein A (Fig. 4B). Variation in intensities of the colonies detected by autoradiography after these two probing procedures led to the isolation and subculturing of three colonies. Colonies A and B appeared to bind less of the IgA Fc fragment probe relative to the amount of β antigen detected, while colony C bound high levels of both probes.

To analyze these observed differences quantitatively, subcultures of colonies A, B, and C, the parent ss618 strain, and six additional β antigen/IgA-Fc-binding surface-positive strains were grown and assayed for their degree of β-antigen surface expression and capacity for binding ^{125}I-IgA Fc fragments to their surfaces. ^{125}I-IgA Fc fragments, anti-β antiserum, and ^{125}I-protein A were added in excess to ensure that maximum binding occurred. The ratio of radioactivity associated with the bacterial pellets following each of the two assays was calculated, and these data are presented in Fig. 5. A ratio of approximately 10:1 was observed except for the ss618 parent strain, ss618 colonies A and B, and strain TC137. These bacteria demonstrated substantially increased ratios, indicating a lowered ability to bind IgA Fc fragments relative to the amount of β antigen present on their surfaces.

Reactivity of cell-bound and secreted β antigen with sIgA. All of the experiments reported thus far have used ^{125}I-labeled IgA Fc fragments as the probe for IgA-Fc-binding activity. Since GBS tend to colonize mucosal surfaces (37), we extended our studies to assess the interaction of cell-bound and secreted β antigen with sIgA. Figure 6 shows the results of a representative experiment. Seven of these fourteen strains demonstrated a significant IgA-Fc-binding capacity (at least five times the background), while none of the strains were able to bind significant quantities of sIgA, i.e., greater than approximately twice the background level. In contrast, serum IgA molecules of both IgA1 and IgA2 subclasses were bound efficiently to the surfaces of IgA-Fc-binding strains (data not shown). None of the 100 GBS isolates were able to bind ^{125}I-sIgA efficiently to their surfaces, including the 13 strains which bound ^{125}I-radiolabeled IgA Fc fragments (listed in Table 1).

Culture supernatants were tested for secretion of sIgA-binding activity by dot blot assay. In contrast to the results for surface binding ability, all 16 strains which secreted IgA-Fc-binding activity (Table 1) also secreted sIg-A-binding activity (data not shown). No isolate secreted a molecule

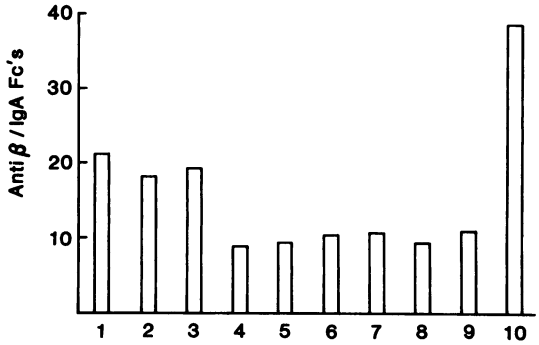

FIG. 5. Comparison of IgA Fc binding with β-antigen expression on various GBS isolates. The radioactivity associated with bacterial pellets after the two-stage radioimmunoassay for detection of cell surface β antigen (minus background) was divided by the radioactivity associated with duplicate bacterial pellets after a direct binding assay using ^{125}I-IgA Fc fragments as the probe (minus background). Bars 1 through 10 correspond to GBS isolates ss618p, ss618A, ss618B, ss618C, HG381, ss700, HG805, HG769, TC795, and TC137, respectively. All assays were performed in duplicate, and less than 5% variation was observed between replicate samples.

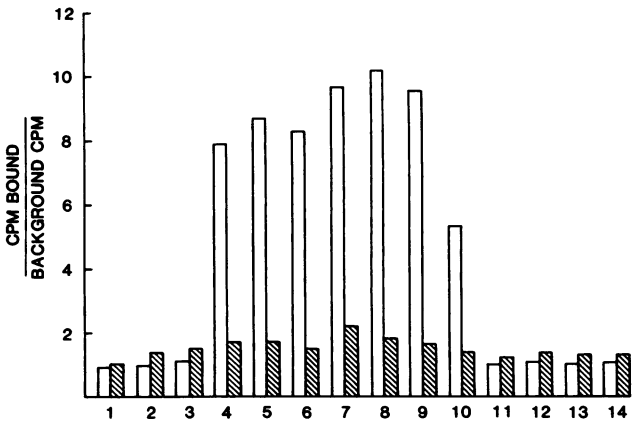

FIG. 6. Comparison of the reactivity of various GBS isolates with serum and sIgA. The ratios of radioactivity associated with bacterial pellets over background radioactivity after direct binding assays using ^{125}I-IgA Fc fragments (□) or ^{125}I-sIgA (▨) as a probe are plotted on the *y* axis. A ratio of 1 indicates no specific binding over background levels. Bars 1 through 14 correspond to GBS isolates N86K, 9B200, HG770, HB381, ss700, PF549AR-B, HB805, HG769, TC795, ss618p, 2AR, DL469-M, DL471-B, and HB806 respectively. All assays were performed in duplicate, and less than 5% variation was observed between replicate samples.

that would bind sIgA which did not also bind serum IgA. The ability of solubilized IgA-Fc-binding proteins to bind sIgA was next examined by Western blot analysis. Figure 7 shows the Western blot of hot-acid extracts of six GBS isolates probed with ^{125}I-sIgA. The isolates shown in lanes 2, 3, and 5 demonstrated the same pattern of bands observed when ^{125}I-IgA Fc fragments were used as a probe (data not shown), indicating that the same molecules bind both IgA Fc fragments and sIgA when they are solubilized. Strains which did not demonstrate surface binding of IgA Fc fragments did not contain sIgA-binding material in their hot-acid extracts (lanes 1, 4, and 6). Western blot analysis was also performed with concentrated culture supernatants (data not shown), and identical reactive bands were observed with both ^{125}I-IgA Fc fragment and ^{125}I-sIgA probes.

DISCUSSION

The results presented in this report indicate that the correlation between expression of β antigen by GBS and the ability to bind to the Fc region of human IgA is not absolute. Two of the eighteen β-antigen-positive isolates identified were totally lacking in their ability to bind IgA Fc fragments (Table 1). Variation in the capacity to bind IgA Fc fragments with respect to the amount of β antigen detected on their surfaces was demonstrated as well for several GBS isolates (Fig. 5). These data are consistent with the hypothesis that the β antigen contains an IgA-Fc-binding site that is altered or absent from some forms of the molecule.

Size heterogeneity and lower-molecular-weight forms of β antigen extracted from GBS have been observed in the past (4, 9, 33, 34, 36, 38). These have been assumed to be breakdown products resulting from acid hydrolysis during the extraction procedure (4) or proteolysis resulting from exogenous enzymes produced by the bacteria themselves (33). The large degree of size heterogeneity observed for β antigen/IgA-Fc-binding proteins secreted by surface-positive isolates (Fig. 1C and D, lanes 1, 2, and 8 through 11) indicates that the harshness of the extraction procedure alone cannot account for all of the reactive bands seen in hot-acid extracts, although it does contribute to some degree (compare Fig. 1B and D).

It is not clear why there are such striking differences in the Western blot profiles of secreted β antigen/IgA-Fc-binding proteins between surface-positive and -negative strains (Fig. 1). These may reflect variations within the β antigen such as the presence or absence of membrane anchor sequences. The results of competitive binding assays (Fig. 2 and 3) indicate that the 55,000- and 53,000-M_r molecules secreted by surface-negative strains are functionally and antigenically representative of the multiple-molecular-weight forms of β antigens/IgA-Fc-binding proteins expressed by surface-positive GBS. The ability of supernatants containing the 38,000-M_r form of secreted β antigen to partially inhibit binding of anti-β antibodies to surface-positive strains yet not interfere with IgA Fc binding again suggests that the IgA-Fc-binding site represents a unique domain of the β antigen.

Cleat and Timmis have cloned a GBS gene which encodes β antigen (9). The have identified lower-molecular-weight forms of recombinant β antigen produced in *Escherichia coli* and suggest that similar processing and degradation of β antigen from high- to low-molecular-weight forms occurs in *E. coli* as in GBS. Although these authors comment that the estimated molecular weight of their recombinant β antigen slightly exceeds the coding capacity for the 3.3-kilobase insert of streptococcal DNA, the information to code for the entire β antigen does appear to be present on this fragment. The finding that some surface-negative strains of GBS produce homogeneous lower-molecular-weight forms of β antigen that are representative of higher-molecular-weight forms would suggest that lower-molecular-weight forms represent core β antigen and that processing can occur from low- to high-molecular-weight forms of the molecule.

Binding proteins specific for the Fc region of IgG molecules have been isolated from a variety of staphylococcal and streptococcal species (13, 15, 22, 24, 27, 30, 31); however, we have found no evidence for the existence of the unspecific antibody-binding activity that has recently been suggested to be associated with CAMP factor, a secreted product of GBS (21). Since the expression of a bacterial protein that would bind IgG antibody molecules nonspecifically would complicate our assays, we have included normal rabbit serum controls in our experiments. We have also screened the GBS test isolates for secretion and surface expression of human IgG-Fc-binding proteins with negative results, despite secretion of CAMP factor by these strains.

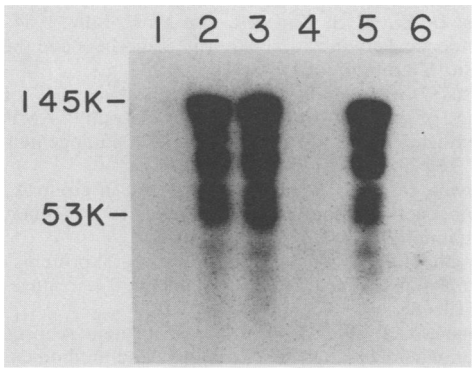

FIG. 7. Western blot analysis on (7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of 10-fold-concentrated hot-acid extracts from six GBS isolates probe with ^{125}I-sIgA. Lanes 1 through 6 contain extracts from GBS isolates HG806, HG805, HG769, HG346, HG381, and HG819, respectively.

None of the isolates of GBS which were able to bind ^{125}I-IgA Fc fragments to their surfaces could bind ^{125}I-sIgA efficiently, although solubilized molecules present in hot-acid extracts and culture supernatants bound both ^{125}I-IgA Fc fragments and ^{125}I-sIgA. The importance to GBS strains of secreting sIgA-binding material into their environment while not binding that molecule efficiently to their surfaces is unknown. Two explanations may account for this observation. Either IgA-Fc-binding proteins are unable to interact efficiently with sIgA until they are released from the bacterial cell surface, or IgA-Fc-binding protein-sIgA complexes are shed rapidly from the bacteria after binding occurs. The unusual interaction of the β antigen with sIgA is intriguing since one would expect sIgA to be the first line of defense against GBS infections at mucosal surfaces and in mothers' milk.

The observations presented in this report could have important implications for studies of GBS sepsis, in particular neonatal sepsis, which results in over 12,000 infant deaths annually in the United States (25). Urogenital isolates are more likely than other GBS isolates to express c-protein marker antigens (2, 18), and most infants who develop early-onset sepsis are exposed to the disease-causing organism during passage through the birth canal (1, 11, 14, 20, 28). We have recently identified two novel antigens, γ and δ, which react, in addition to α and β, with the rabbit c-protein typing antiserum of the Centers for Disease Control, Atlanta, Ga. (7). This suggests that the concept of a c-protein marker may be overly simplistic and that many GBS strains exhibit a more complicated antigenic structure than recognized in the past. This situation becomes more complex when considering our results which indicate that, although clearly related, expression of β antigen does not always predict the ability of a GBS to bind to the Fc region to human IgA. In addition, identification of GBS isolates which secrete IgA-Fc-binding protein or β antigen or both in the absence of surface expression indicates that studies designed to evaluate these markers with respect to pathogenesis should examine secreted molecules as well as surface expression.

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