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Identification of an Immunoglobulin A Binding Motif Located in the β-Antigen of the c Protein Complex of Group B Streptococci

PIERRE G. JERLSTRÖM,† SUSANNE R. TALAY, PETER VALENTIN-WEIGAND, KENNETH N. TIMMIS, AND GURSHARAN S. CHHATWAL*

Department of Microbiology, Technical University/GBF–National Research Centre for Biotechnology, Braunschweig, Germany

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The β-antigen of the c protein complex of group B streptococci contains two immunoglobulin A (IgA)-binding domains called A and B. A 73-amino-acid segment in domain A is responsible for most of the IgA-binding activity. To identify the IgA binding motif, the 73-amino-acid domain was divided into 60 14-amino-acid overlapping peptides spot synthesized onto a cellulose membrane. A 20-residue putative antigenic epitope was identified and expressed as a fusion protein. The fusion protein was purified by fast protein liquid chromatography and used to raise rabbit antiserum. By use of a membrane with spot-synthesized peptide amino acids of decreasing length (from 14 to 6 amino acids), the major antigenic epitope recognized by the anti-fusion protein antibodies was mapped to motif MLKKIE. Anti-fusion protein antibodies inhibited the binding of IgA to group B streptococci. This inhibition could be blocked by the peptide containing the motif MLKKIE. These results indicate that the motif MLKKIE is located in the IgA-binding site. The IgA-binding domain of β-antigen from three group B streptococcal strains reacted with the anti-fusion protein antibodies, and their coding sequences gave positive signals in Southern hybridization. The sequences of β-antigen from these strains were amplified by PCR, and sequence analysis showed them to be identical. The results indicate that the motif MLKKIE is required for IgA binding and is present in different group B streptococcal strains.

The Group B streptococcal species *Streptococcus agalactiae* is one of the most frequent etiologic agents isolated from the blood or cerebrospinal fluid of neonates (3). Group B streptococci (GBS) cause a variety of diseases in neonates and are associated with very high neonatal mortality rates (29, 33). The determinants involved in pathogenicity as well as the mechanism of GBS infection remain unresolved. Immunity to GBS is associated with the six well-described type-specific capsular polysaccharides (18, 35). There is evidence that protein components of GBS are also important in conferring protective immunity (21, 41). In particular, the antibodies to c protein complex, consisting of four antigenic components (α, β, γ, and δ), have been associated with the development of protective immunity (4, 8, 25). About 60% of GBS isolates contain the c protein complex, and there is increasing evidence that this complex may have virulence properties (12). Part of the c protein complex consists of two independently expressed antigens, a trypsin-sensitive β-antigen and a trypsin-resistant α-antigen (45). It has been suggested that α- and β-antigen-positive strains are more resistant to opsonization and intracellular killing (32). Immunity to each of these antigens is protective (5, 28, 32), and recently, a GBS polysaccharide–β-antigen conjugate was found to elicit protective antibodies to multiple GBS serotypes (31). Of interest is that β-antigen specifically binds the Fc region of human immunoglobulin A (IgA) (14, 30, 36). Since IgA is the predominant immunoglobulin defense against microbial infection of human mucosal surfaces (15, 43), nonproductive sequestering of IgA may be a mechanism for avoiding the first line of host defense. As suggested for immunoglobulin receptors of group A streptococci, the β-antigen may alternatively act as an adhesin for bacterial attachment of surfaces coated with antibody and/or a sensory system to inform the organism of its location in the host (13).

Both the α- and β-antigens have been cloned as two distinct fragments (14, 32). We previously expressed subfragments of the β-antigen as fusion proteins and showed that the IgA-binding activity was located in two distinct regions of the protein. In this study, we have further subcloned the main binding domain and have attempted to identify peptides carrying IgA-binding activity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *Escherichia coli* K-12 strains 537 and W6, GBS strain LA239, the expression vector pEx31, and plasmid pPGJ6, expressing the main IgA-binding region, have been described previously (24). The GBS strains H36B, 70339, A909, 15626, 126539, and 335 belong to serogroups Ib/cβ, Ia/cβ/R, Ia/cβ, IV/cβ, Ib/cβ, and Ia/c, respectively, and were supplied by L. Bevanger. *E. coli* 537 was grown in AM3 (BBL Microbiology Systems) containing 20 μg of kanamycin per ml and 100 μg of ampicillin per ml when harboring plasmids. GBS strains were grown in Todd-Hewitt broth (Oxoid, Basingstoke, England).

Standard techniques. Transformation of *E. coli*, preparation of plasmid DNA and chromosomal DNA, and agarose gel electrophoresis were performed basically as described by Sambrook et al. (38). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs and used as described in the instructions of the manufacturer. Extraction of β-antigen from GBS was basically as described by Lindahl et al. (30). Serum IgA was obtained from Dianova, Hamburg, Germany. Fc fragment was prepared by cleaving serum IgA with gonococcal IgA protease (Boehringer GmbH, Mannheim, Germany), followed by purification with hydrophobic affinity chromatography by the method of Russell-Jones et al. (36). Serum IgA and IgA Fc fragments were radiolabelled with ^{125}I by the method of Hunter and Greenwood (23). Rabbit antiserum to purified fusion proteins was raised in our laboratory by a standard method.

Subcloning of the IgA-binding domain. Plasmid pPGJ3, which contains a 1.2-kb *Cla*I-*Hap*I fragment coding for the IgA-binding region, and pEx31b were digested with *Bgl*II and *Acc*I. The fragments were separated on agarose gels, and

* Corresponding author. Mailing address: Biozentrum der Technischen Universität, Spielmann Strasse 7, 38106 Braunschweig, Germany. Phone: 49 531 391 5860. Fax: 49 531 391 5858. Electronic mail address: Chhatwal@Gbf-Braunschweig.de.

† Present address: Biochemistry Department, The University of Queensland, Brisbane, Queensland 4072 Australia.

the 0.92-kb fragment from pPGJ3 coding for the IgA-binding region and the 3.1-kb vector fragment from pEx31b were isolated with a DNA extraction kit (Diagen, Hilden, Germany). The 0.92-kb fragment was further digested with *Mbo*I, and the two resulting 0.71-kb *Mbo*I-*Acc*I and 0.216-kb *Bgl*II-*Mbo*I fragments were ligated to pEx31b and to *Bgl*II-cut pEx31, respectively. Putative clones were grown on selective plates overnight at 28°C, and fusion proteins were expressed at 42°C for 3 to 4 h and analyzed for IgA binding in colony blots or tested with rabbit polyclonal antiserum in Western blots (immunoblots).

Protein extraction, electrophoresis, and immunoblotting. Fusion proteins were expressed as described previously (24). Proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 16% gels (27) and either stained with Coomassie brilliant blue or electroblotted onto nylon membranes (Immobilon; Millipore, Bedford, Mass.). Membranes were blocked in phosphate-buffered saline (PBS) containing 10% skim milk for 30 min. After washing in PBS for 10 min, the membranes were incubated for 1 h in PBS containing 1.6 μg of peroxidase-conjugated serum IgA (Dianova) per ml. Finally, membranes were washed three times with PBS, and the bands were visualized with the chromogen 4-chloro-1-naphthol. In one experiment, the membranes were incubated with ^{125}I-labelled IgA Fc fragment and, after washing, exposed to X-ray film.

For colony blotting, putative clones were grown overnight on selective plates at 28°C. Fusion proteins were expressed at 42°C for 3 h, and the colonies were allowed to adsorb onto nitrocellulose membranes (Bio-Rad, Munich, Germany) and then lysed and blocked as described by Talay et al. (42). Membranes were incubated with IgA as described above.

Extraction of inclusion bodies and purification of β-antigen fusion protein. Cultures of *E. coli* 537 harboring the plasmid pPGJ8 were grown overnight and induced as described previously (24). Fusion proteins were purified by the following mechanical disruption method. Induced cells were harvested by centrifugation and washed twice with buffer (50 mM Tris-HCl [pH 8.0]). The pellets were resuspended in 30 ml of buffer and disrupted in a French press (Sopra, Büttelborn, Germany) three times, keeping the suspension at 4°C to inhibit proteases. After centrifugation for 15 min, the supernatant was discarded and the pellet containing cell debris and inclusion bodies was resuspended in buffered 8 M urea. After centrifugation, the particle-free supernatant containing the fusion protein was then purified by fast protein liquid chromatography (FPLC) with a Mono Q ion-exchange column (HR 10; Pharmacia LKB, Uppsala, Sweden) eluted with a linear salt gradient. The fractions under each peak were pooled, lyophilized, and dialyzed against PBS prior to use in inhibition experiments.

Preparation of spot membranes and analysis of epitopes in the IgA-binding domain. The 73-amino-acid IgA-binding domain coded for by pPGJ8 was divided into 60 14-amino-acid overlapping peptides with an offset of 1 amino acid. The peptides were synthesized onto cellulose paper (1Chr; 3MM, Whatman, Maidstone, England) with an array of 10 rows of six spots as described by Frank (19). On another membrane, a 25-amino-acid region from the IgA-binding domain (amino acids 25 to 49) was divided into 80 overlapping peptides, consisting of five series with lengths of 14, 12, 10, 8, and 6 amino acid residues, respectively. Each series spanned the entire 25-amino-acid region, with an offset of 1 amino acid. For the IgA-binding assay, the membrane was washed three times with TBS (8 g of NaCl, 0.2 g of KCl, 6.1 g of Tris base per liter; adjusted to pH 7) for 10 min and incubated overnight in blocking buffer (Super Block; Pierce, Rockford, Ill.). After washing with T-TBS (TBS plus 0.05% Tween 20), the membrane was incubated in blocking buffer containing 4 μg of serum IgA per ml for 2 to 4 h. Following two washes with T-TBS, the membrane was incubated for 2 h in blocking buffer containing a 1/1,000 dilution of goat-anti-IgA antiserum coupled to biotin (Dianova). The membrane was washed twice with T-TBS and incubated with a 1/500 dilution of β-galactosidase-conjugated avidin in blocking buffer for 2 h. After two washes with T-TBS followed by two washes with PBS, the membrane was treated with chromogen solution [3 mg of 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (Sigma), 3 mM potassium ferricyanide(III), and 5 mM $MgCl_2$ in 10 ml of PBS] for 10 to 30 min. Positive spots developed a blue color. Staining was stopped by washing with PBS. In assays with rabbit polyclonal antiserum, the incubation times were identical except that the first two 2-h incubations were carried out with a 1/200 dilution of rabbit polyclonal antiserum and a 1/1,000 dilution of swine anti-rabbit antiserum coupled to biotin (Dianova), respectively.

Binding experiments. Cultures of GBS were harvested by centrifugation, washed twice with PBS, and suspended in PBS containing 0.1% Tween 20 to give 10% transmission at 600 nm (10, 11). Twenty-microliter aliquots of increasing concentrations of rabbit polyclonal anti-fusion protein antiserum (0 to 50 μg of IgG) were incubated with 200 μl of streptococcal suspension for 5 min at room temperature. Ten nanograms of ^{125}I-labelled IgA (specific activity, 1 mCi/mg) was then added, and a further incubation for 40 min at room temperature followed. Nonbound IgA was separated by centrifugation, and the amount of cell-bound IgA was determined in a gamma counter. Preimmune serum served as a negative control. In some experiments, the polyclonal antiserum was preincubated for 5 min at room temperature with 20-μl aliquots of increasing concentration of a peptide consisting of a 20-amino-acid putative antigenic epitope (0.1 to 1 mg), before it was added to the cells. Identical amounts of an unrelated peptide were used as a negative control.

Southern blot analysis. Chromosomal DNA was prepared from GBS strains A909, LA239, H36B, 70339, 126539, 15626, and 335. Purified DNA was digested with *Hin*dIII, separated on agarose gels, and blotted onto positively charged nylon membrane (Boehringer) under alkaline conditions as described by Sambrook et al. (38). A Southern hybridization probe containing the 216-bp region coding for IgA binding activity was designed and amplified via PCR with pPGJ8 plasmid DNA as a template and the set of primers described below. Labelling of the hybridization probe, hybridization of the filters, and detection of reactive bands were performed with a digoxigenin labelling and detection kit (Boehringer) as described in the manufacturer's protocol.

PCR. Primers 1 and 2 were synthesized for amplification of the 216-bp insert in pPGJ8. Primer 1 (5′-GCCCGCTGCAGTAGATCTAA-GCAATATTGA CAAA-3′) was used as the upstream primer, and primer 2 (5′-GCCCGTCTA GAGATCCAGACCAGCTTTAGTTGA-3′) represented the downstream primer. Chromosomal DNA from each of the GBS strains used for Southern blot analysis served as template DNA for amplification. The reaction mixture contained 1 μg of template, 60 pmol of each primer, 200 ng of each deoxynucleoside, 25 U of *Taq* polymerase, and 1× buffer (Boehringer). The PCR was run for 25 cycles, each consisting of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 120 s. For DNA sequencing, the same reaction mixture was used except with 60 pmol and 0.6 pmol of primers 1 and 2, respectively. Each of the 25 PCR cycles consisted of 97°C for 30 s, 45°C for 30 s, and 63°C for 240 s.

DNA sequencing. The 5′ and 3′ extremes of the insert in pPGJ8 were sequenced with synthetic oligonucleotides as described by Chen and Seeburg (9). Sequencing reactions were performed with Sequenase (U.S. Biochemicals, Cleveland, Ohio) by the dideoxy chain termination method (39) with 6% acrylamide gels. PCR fragments were purified by use of quick-spin columns (Qiagen), dialyzed against water, and used in cycle sequencing reactions that were analyzed by an auto sequencer (Applied Biosystems).

RESULTS

Subcloning of the sequence coding for IgA-binding region A. In our previous study, the IgA-binding activity was approximately localized by expressing subfragments of the β-antigen as fusion proteins with the expression vector pEx31. Two distinct but adjacent DNA segments, region A and region B, specified peptides that bound IgA (24). In the present study, the sequence coding for region A was further subcloned since the fusion peptide from this region exhibited a ladder of smaller IgA-binding subfragments in Western blots, indicating that a shorter sequence codes for IgA binding (data not shown).

To subclone the 746-bp *Bgl*II-*Hpa*I fragment coding for region A, a 1.38-kb *Cla*I-*Acc*I fragment was isolated from pPGJ3; this fragment consisted of a 1.2-kb *Cla*I-*Hpa*I fragment from the β-antigen coding sequence and a 180-bp *Hpa*I-*Acc*I fragment from the pEx31 vector. After digestion with *Mbo*I, the two resulting fragments, a 216-bp *Bgl*II-*Mbo*I fragment encoding a peptide of 8.5 kDa and a 530-kb *Mbo*I-*Hpa*I fragment encoding a peptide of 20 kDa, were cloned into plasmids pPGJ8 and pPGJ9, respectively (Fig. 1). Complete sequence analysis of the inserts confirmed that the cloned sequences were correct. The pPGJ8 fusion peptide encoded the 73 N-terminal amino acids of region A. Transformants harboring pPGJ8 expressed a fusion peptide that bound IgA in colony blots, and Western blot analysis with IgA verified that the 8.5-kDa peptide specified IgA-binding activity. This fusion peptide also interacted with IgA Fc fragment, indicating that the nonimmune portion of the IgA molecule is involved in this binding. The fusion expressed by pPGJ8 was named IABF, for IgA-binding fusion. The 20-kDa peptide encoded by the pPGJ9 fusion showed no binding activity. A 60-bp subclone of PGJ8 expressing a smaller part of the IgA-binding region was also constructed. The resulting fusion peptide, however, did not bind IgA.

Mapping of the IgA-binding activity. Three avenues were taken to identify the shortest peptide that could still bind IgA. First, the 8.5-kDa region coding for 73 amino acids mentioned above was divided into 60 14-amino-acid peptides with an offset of 1 amino acid. The peptides were synthesized onto a cellulose sheet with an array of 10 rows of six spots and were

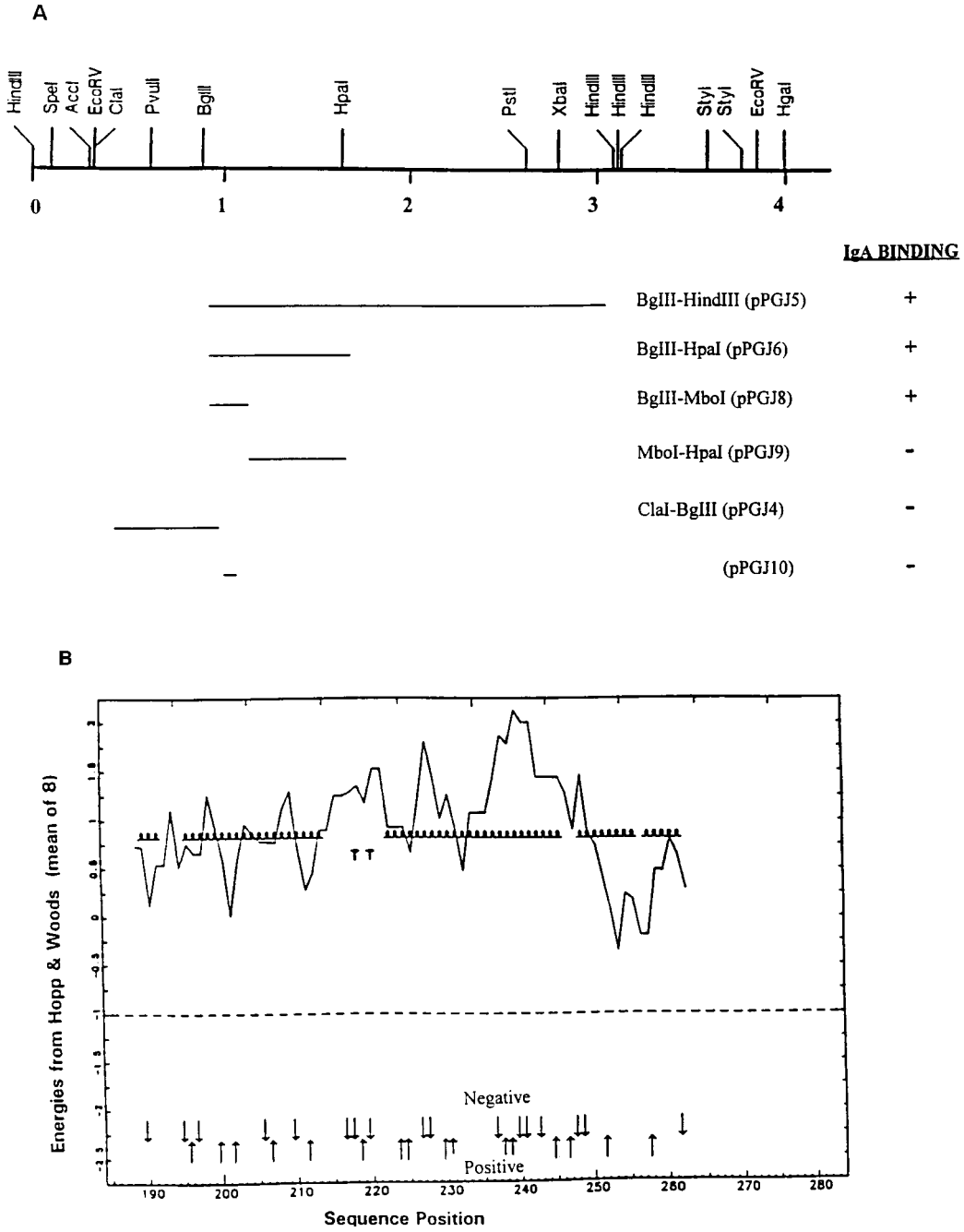

FIG. 1. Structural features of the β-antigen and its gene. (A) Restriction map of β-antigen gene region and deletion derivatives of β-antigen gene region showing corresponding IgA-binding activity; (B) Hopp and Woods hydropathy plot of serum IgA-binding domain.

tested for IgA binding. No clear binding could be detected (data not shown). Second, from a hydropathy plot of the 73-amino-acid binding sequence, one putative antigenic epitope was located around amino acid position 218 in the β-antigen (Fig. 1B); it contained the only two β-turns in the sequence, and it was situated at a hydrophilic maxima with four charged residues. A 20-amino-acid peptide spanning this region (peptide AI; amino acids 212 to 231) was synthesized and tested for IgA binding after spotting onto an Immobilon membrane and in a competition experiment for binding of radiolabelled IgA to streptococci. No binding activity was detected, however. Third, since protein structure may be important for binding, two synthetic oligonucleotides (5′ and 3′ strands) coding for peptide AI were cloned into pEx31 to give plasmid pPGJ10, and a fusion peptide to MS2 polymerase was expressed. No IgA-binding activity was evident by the peptide in Western blots.

Purification of fusion peptide IABF coding for the 73-amino-acid IgA-binding sequence and preparation of antiserum. Cells expressing IABF were disrupted in a French press, and

A

DLSNIDKELNHQKSQVEKMAEQ**KGITNEDKDSMLKKIEDIRK**QAQQADKKEDAEVKV
REELGKLFSSTKAGLD

B

Size-scan: overlapping peptides, start position=1, offset=1

length	spot No	positives	**ITNEDKDSMLKKIEDIRKQAQQADK**
14	1-12	1-9	**ITNEDKDSMLKKIEDIRKQAQQ**
12	13-26	15-21	**NEDKDSMLKKIEDIRKQA**
10	27-42	32-35	**KDSMLKKIEDIRK**
8	43-60	49-51	**DSMLKKIEDI**
6	61-80	69	**MLKKIE**

epitope sequence underlined

C

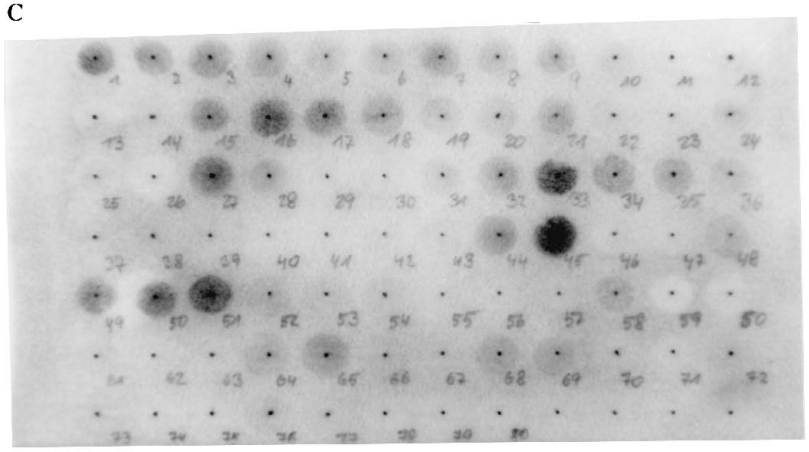

FIG. 2. Mapping of antigenic epitopes recognized by rabbit anti-IABF. (A) Amino acid sequence of IABF coded by pPGJ8. Bold, underlined amino acids represent synthetic peptide AI. (B) Strategy for the synthesis of overlapping peptides, showing peptides recognized by the antibody. Epitope sequence is underlined. (C) Membrane treated with anti-IABF antiserum.

IgA-binding activity was found in the cytoplasm of *E. coli* harboring pPGJ8, localized in inclusion bodies. Initially, the inclusion bodies were extracted with detergents, but this procedure impeded the purification of the protein by FPLC. Cells were therefore disrupted mechanically, and the inclusion bodies were solubilized with 8 M urea. IABF was purified on a FPLC Mono Q ion-exchange column. The peak containing active fusion was eluted at 0.13 M NaCl. Purified IABF gave one band in SDS-PAGE which reacted with IgA in Western blots. Antiserum was raised in rabbits against purified IABF and was shown to be specific for the IABF peptide and native β-antigen in Western blots (data not shown). This antiserum was used in the inhibition studies described below.

Antigenic epitopes of the IgA-binding region. The membrane consisting of 60 offset peptides spanning the IgA-binding domain was screened with the anti-IABF polyclonal antiserum. One series of peptides reacted strongly with the antiserum, suggesting one dominant epitope in the IgA-binding region (data not shown).

The region reactive with the antiserum was then analyzed in more detail by dividing it into 80 peptides consisting of five series of peptides of different length (from 14 to 6 amino acid residues), each series spanning the entire region, with an offset of 1 amino acid residue (Fig. 2). The 14-amino-acid peptides (numbers 1 to 12) were identical to those of the previous membrane. Every peptide reactive with anti-IABF antiserum contained the common sequence motif MLKKIE (Fig. 2C). The positive reactions observed for spots 48, 58, 68, and 76 were nonspecific and also gave a color reaction in the control blot with only secondary antibody. Although the reactive peptides of spots 27, 28, 44, 45, 64, and 65 contained the sequence DKDSM, other peptides also containing this sequence did not give a positive signal and were therefore regarded as false positives.

The MLKKIE motif is contained in the putative antigenic epitope determined from the hydropathy plot and was thus included in peptide AI and its respective fusion protein expressed from pPGJ10 (Fig. 2). The antiserum also reacted with peptide AI spot blotted onto an Immunodyne membrane (data not shown).

Inhibition of IgA binding to GBS by rabbit anti-IABF antiserum and the effect of peptide AI on inhibition. Increasing concentrations of rabbit anti-IABF IgG decreased the amounts of IgA bound to GBS cells. With 50 μg of calculated IgG in the

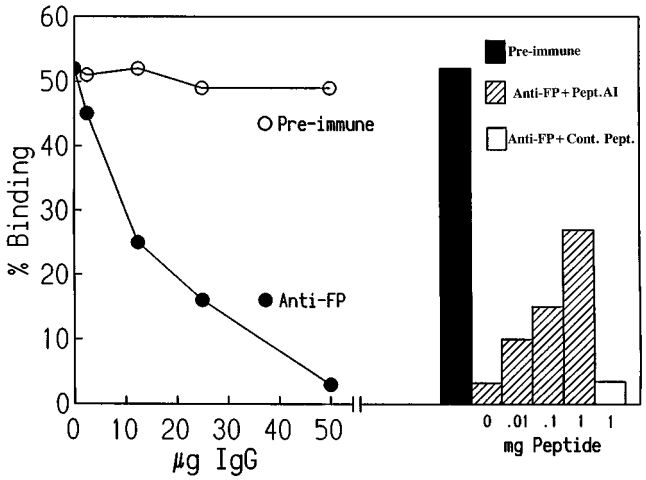

FIG. 3. Inhibition of binding of GBS to IgA by rabbit anti-IABF (anti-FP) and the effect of peptide AI (Pept.A1) on inhibition. The amino acid sequence of peptide AI and its structural features are shown in Fig. 2A and Fig. 1B, respectively. Results are expressed as mean percent binding of two independent experiments performed in duplicate. A peptide from β-antigen lacking the motif MLKKIE served as the control peptide (Cont. Pept.).

antiserum, an almost 100% inhibition of binding was reached. In control experiments with the same concentrations of preimmune serum, there was no significant inhibition of IgA binding (Fig. 3). Preabsorption of antiserum with increasing amounts of peptide AI caused a concentration-dependent decrease in the inhibition by the rabbit anti-IABF. The addition of an unrelated peptide had no effect on the inhibition of binding (Fig. 3).

Sequence homology in the IgA-binding domain of β-antigen from various GBS strains. The IgA-binding domains of β-antigen isolated from several GBS strains was first compared by testing these with anti-IABF antiserum and IgA in Western blots. The strains used in this series of experiments included LA239, A909, H36B, 126539, 70339, 14626, and 335. All β-antigens except those from strains 70339 and 15626 reacted with both the anti-IABF serum and IgA. A blot with antiserum against whole β-antigen revealed that these two proteins were considerably smaller in size than the others. Chromosomal DNA from these strains was digested with *Hin*dIII and analyzed by Southern blotting by using the IABF coding sequence from pPGJ8 as a probe. A band of approximately 3.1 kb hybridized with the four strains that reacted with anti-IABF antiserum.

The DNA sequence coding for the 73-amino-acid IgA-binding region was amplified from the chromosomes by PCR with oligonucleotide primers homologous to the 5′ and 3′ ends of the corresponding sequence from strain LA239. The expected fragment was obtained from all of the strains except strains 70339 and 15626 as well as the negative control strain 335. The fragments were sequenced with the PCR primers. All of the sequences were identical to that from LA239.

DISCUSSION

In this work, we attempted to further characterize the main IgA-binding activity of the β-antigen. By subcloning into the expression vector pEx31, the sequence coding for the main binding activity has been mapped to a 73-amino-acid region. This peptide interacted with the IgA Fc fragment, indicating the involvement of nonimmune portion of IgA in this binding. In an attempt to obtain an even shorter peptide with binding activity, peptides of 14 amino acids covering this whole region were synthesized. None of them showed any clear binding. We also synthesized a 20-amino-acid peptide, which represents a putative surface-exposed sequence of the 73-amino-acid region, and a respective chimeric protein fused to the MS2 polymerase N terminus, but neither of them bound IgA. Our results showed that the peptides synthesized here were of insufficient length and that a longer sequence is required for a complete active site or that additional structural residues are needed for IgA binding. This is supported by the fact that the binding sequences of IgG receptor protein G from group G streptococcus and protein A from *Staphylococcus aureus* are 55 and 58 amino acids, respectively (16, 34, 40, 44).

Further attempts to obtain an IgA-binding peptide could be accomplished by subcloning the IABF coding sequence by PCR and by the synthesis of corresponding peptides as already described here. From the analyses of the spot-synthesized peptides, the motif MLKKIE appears to be the main antigenic epitope in the IgA-binding region which is recognized by the polyclonal anti-IABF antiserum. It is not yet resolved whether the motif MLKKIE is actually involved in binding IgA or provides the necessary structure to permit the folding of the IgA domain. This motif, however, correlates with the IgA binding. This is supported by the binding inhibition of anti-IABF antiserum to cell-surface-located β-antigen by peptide AI, which contains this motif, and leads to increased inhibition of antibody activity. The sequence homology search revealed that other IgA-binding proteins, such as IgA receptor from *Streptococcus pyogenes* (20), do not contain this motif. The binding domain of β-antigen containing the motif MLKKIE has a much higher affinity for human serum IgA than other IgA-binding proteins (unpublished observation). It is likely that this motif attributes this property to β-antigen. Two other proteins, yeast RNA polymerase (1) and CENP-C protein, an autoantigen in scleroderma (37), contain the motif MLKKIE. Nothing is known about the interaction of these two proteins with IgA.

Although complete β-antigen has been isolated previously from strain LA239, the β-antigen isolated here from strain LA239 appears to be expressed in a truncated form as revealed by the blots with antisera and IgA. This may be due to the low levels of β-antigen expressed from this strain since, from our experience, it appears to be a general characteristic of β-antigen when stored at dilute concentrations. Our sequencing results show that the IgA-binding domains in β-antigen of strains LA239, H36B, A909, and 70339 are identical, which suggests a high conservation of this region in β-antigen from strains of polysaccharide types Ia and Ib. The binding domain of the β-antigen from the Ib/c strain SB35 reported by Heden et al. (22) is also identical and further reinforces this notion. In fact, the entire sequence of the gene is identical to our previously published sequence except for a shorter proline-rich region. More β-antigen genes need to be sequenced to determine whether this is the only sequence variability among β-antigens from strains that express the entire antigen. In light of this, the size difference of the *Hin*dIII fragments observed in the Southern blot in this work may be due to variations in the proline-rich region.

Brady and Boyle (7) have reported the secretion by some GBS strains of low-molecular-weight β-antigen lacking IgA Fc binding. In our studies, β-antigens from the Ia/cβ/R strain 70339 and from the IV/c strain 15626 were much smaller in size in immunoblots, and unlike the truncated antigen from strain LA239, they did not bind IgA nor react with anti-IABF antiserum. There was also no signal in the Southern blot with chromosomal DNAs from the two strains described above.

Since the coding sequence of the binding domain is totally conserved among the GBS strains expressing IgA-binding activity, the sequence appears to be absent in these β-antigen genes; frameshift mutations or point mutations giving rise to premature stop codons and truncated binding domains would still allow hybridization of the remaining homologous coding sequence. The expression by some GBS of only truncated forms of the β-antigen lacking the IgA Fc-binding sequence suggests that this protein may have additional functions. More β-antigens have to be screened with antisera raised from specific parts of the β-antigen, and the respective coding sequences must be studied with DNA probes, followed by DNA sequence analysis. This will provide a better understanding of the heterogeneity of the β-antigen and help to unravel its function in GBS pathogenicity.

Identification of the IgA binding motif might have some analytical applications. Compared with the lectin jacalin, which binds IgA1 but also interacts nonspecifically with serum proteins, the β-antigen specifically binds both IgA1 and IgA2 (2, 6, 26). Faulmann et al. (17) have shown that β-antigen can be useful in immunochemical procedures involving IgA. The synthesis of an active synthetic peptide would be an inexpensive reagent useful for the detection and purification of both IgA1 and IgA2.

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REFERENCES

1. **Allison, L. H., M. Moyle, M. Shales, and C. J. Ingles.** 1985. Extensive homology among the largest subunits of eukaryotic and prokaryotic RNA polymerases. Cell **42:**599–610.
2. **Aucoutier, P., E. Mihaesco, and J. L. Preud'homme.** 1987. Characterization of jacalin, the human IgA and IgD binding lectin from jackfruit. Mol. Immunol. **24:**503–511.
3. **Baker, C. J.** 1993. Vaccine prevention of group B streptococcal disease. Pediatr. Ann. **22:**711–714.
4. **Bevanger, L.** 1983. Ibc proteins as serotype markers of group B streptococci. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **91:**231–234.
5. **Bevanger, L., and A. I. Naess.** 1985. Mouse protective antibodies against the Ibc proteins of group B streptococci. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **93:**121–124.
6. **Biewenga, J., I. Stenker, and D. M. H. Hameleers.** 1988. Effect of serum albumin on the recovery of human IgA^1 from immobilized jacalin. J. Immunol. Methods **115:**199–207.
7. **Brady, L. J., and M. D. P. Boyle.** 1989. Identification of non-immunoglobulin A-Fc-binding forms and low-molecular-weight secreted forms of the group B streptococcal β antigen. Infect. Immun. **57:**1573–1581.
8. **Brady, L. J., U. Daphtary, E. M. Ayoub, and M. D. P. Boyle.** 1988. Two novel antigens associated with group B streptococci identified by a rapid two stage radioimmunoassay. J. Infect. Dis. **158:**965–972.
9. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoiling sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4:**165–170.
10. **Chhatwal, G. S., G. Albohn, and H. Blobel.** 1987. Novel complex formed between a nonproteolytic cell wall protein of group A streptococci and α_2-macroglobulin. J. Bacteriol. **169:**3691–3695.
11. **Chhatwal, G. S., K. T. Preissner, G. Muller-Berghaus, and H. Blobel.** 1987. Specific binding of the human S protein (vitronectin) to streptococci, *Staphylococcus aureus*, and *Escherichia coli*. Infect. Immun. **55:**1878–1883.
12. **Chun, C. S. Y., L. J. Brady, M. D. P. Boyle, H. C. Dillon, and E. M. Ayout.** 1991. Group B streptococcal C-protein associated antigens: association with neonatal sepsis. J. Infect. Dis. **163:**786–791.
13. **Cleary, P., and D. Retnonigrum.** 1994. Group A streptococcal immunoglobulin-binding proteins: adhesins, molecular mimicry or sensory proteins? Trends Microbiol. Sci. **2:**131–136.
14. **Cleat, P. H., and K. N. Timmis.** 1987. Cloning and expression in *Escherichia coli* of the Ibc protein genes of group B streptococci: binding of human immunoglobulin A to the beta antigen. Infect. Immun. **55:**1151–1155.
15. **Delacroix, D. L., G. N. Malburny, and J. P. Vaerman.** 1985. Hepatobilary transport of plasma IgA in the mouse: contribution to clearance of intravascular IgA. Eur. J. Immunol. **15:**893–899.
16. **Fahnestock, S. R., P. Alexander, J. Nagle, and D. Filpula.** 1986. Gene for an immunoglobulin-binding protein from group G streptococcus. J. Bacteriol. **167:**870–880.
17. **Faulmann, E. L., J. L. Duvall, and M. D. P. Boyle.** 1991. Protein B: a versatile bacterial Fc-binding protein selective for human IgA. BioTechniques **10:**748–755.
18. **Feldman, R. G., and A. Fleer.** 1992. The immune response to the group B streptococcus. Rev. Med. Microbiol. **3:**52–58.
19. **Frank, R.** 1992. Spot-synthesis: an easy technique for the positionally addressable, parallel chemical synthesis on a membrane support. Tetrahedron **48:**9217–9232.
20. **Frithz, E., L.-O. Heden, and G. Lindahl.** 1989. Extensive sequence homology between IgA receptor and M protein in *Streptococcus pyogenes*. Mol. Microbiol. **3:**1111–1119.
21. **Heard, S. R., and J. A. Mawn.** 1993. New phenotyping scheme for group B streptococci. J. Clin. Pathol. **46:**145–148.
22. **Heden, L.-O., E. Frithz, and G. Lindahl.** 1991. Molecular characterisation of an IgA receptor from group B streptococci: sequence of the gene, identification of a proline-rich region with unique structure and isolation of N-terminal fragments with IgA-binding capacity. Eur. J. Immunol. **21:**1481–1490.
23. **Hunter, W. J., and F. C. Greenwood.** 1962. Preparation of iodine 131 labelled growth hormone of high specific activity. Nature (London) **194:**495–496.
24. **Jerlström, P. G., G. S. Chhatwal, and K. N. Timmis.** 1991. The IgA-binding beta antigen of the c protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions. Mol. Microbiol. **5:**843–849.
25. **Johnson, D. R., and P. Ferrieri.** 1984. Group B streptococcal Ibc antigens: distribution of two determinants in wild type strains of common serotypes. J. Clin. Microbiol. **19:**506–510.
26. **Kondoh, H., K. Kobayashi, K. Hagiwara, and T. Kaji.** 1986. Jacalin, a jackfruit lectin, precipitates IgA_1 but not IgA_2 subclass on gel diffusion reaction. J. Immunol. Methods **88:**171–173.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London) **227:**680–685.
28. **Lancefield, R. C., M. McCarty, and W. N. Everly.** 1975. Multiple mouse-protective antibodies directed against group B streptococci: special reference to antibodies effective against protein antigens. J. Exp. Med. **142:**165–179.
29. **Lim, D. L., W. J. Morales, A. F. Walsh, and D. Kazaris.** 1986. Reduction in morbidity and mortality rates for neonatal group B streptococcal disease through early diagnosis and prophylaxis. J. Clin. Microbiol. **23:**489–492.
30. **Lindahl, G., B. Åkeström, J.-P. Vaerman, and L. Stenberg.** 1990. Characterization of an IgA receptor from group B streptococci: specificity for serum IgA. Eur. J. Immunol. **20:**2241–2247.
31. **Madoff, L. C., L. C. Paoletti, J. Y. Tai, and D. L. Kasper.** 1994. Maternal immunization of mice with group B streptococcal type III polysaccharide-beta c protein conjugate elicits protective antibody to multiple serotypes. J. Clin. Invest. **94:**286–292.
32. **Michel, J. L., L. C. Madoff, D. E. Kling, D. L. Kasper, and F. M. Ausubel.** 1991. Cloned alpha and beta c-protein antigens of group B streptococci elicit protective immunity. Infect. Immun. **59:**2023–2028.
33. **Musser, J. M., S. J. Mattingly, R. Quentin, A. Gondeau, and R. K. Selander.** 1989. Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B streptococcus) causing invasive neonatal disease. Proc. Natl. Acad. Sci. USA **86:**4731–4735.
34. **Olsson, A., M. Eliasson, B. Guss, B. Nilsson, U. Hellman, M. Lindberg, and M. Uhlèn.** 1987. Structure and evolution of the repetitive gene encoding streptococcal protein G. Eur. J. Biochem. **168:**319–324.
35. **Rubens, C. E., M. R. Wessels, L. M. Heggen, and D. L. Kasper.** 1987. Transposon mutagenesis of type III group B *Streptococcus*: correlation of capsule expression with virulence. Proc. Natl. Acad. Sci. USA **84:**7208–7212.
36. **Russell-Jones, G. J., E. C. Gotschlich, and M. S. Blake.** 1984. A surface receptor specific for human IgA on group B streptococci possessing the Ibc protein antigen. J. Exp. Med. **160:**1467–1475.
37. **Saitoh, H., J. Tomkiel, C. A. Cooke, H. Ratrie III, M. Maurer, N. F. Rothfield, and W. C. Earnshaw.** 1992. CENP-C, an autoantigen in scleroderma, is a component of the human inner kinetochore plate. Cell **70:**115–125.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**4767–4771.
40. **Sjöbring, U., L. Björck, and W. Kastern.** 1991. Streptococcal protein G: gene structure and protein binding properties. J. Biol. Chem. **266:**399–405.
41. **Stålhammar-Carlemalm, M., L. Stenberg, and G. Lindahl.** 1993. Protein Rib: a novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. J. Exp. Med. **177:**1593–1603.

42. **Talay, S. R., E. Ehrenfeld, G. S. Chhatwal, and K. N. Timmis.** 1991. Expression of fibronectin-binding components of *Streptococcus pyogenes* in *Escherichia coli* demonstrates that they are proteins. Mol. Microbiol. **5:**1727–1734.
43. **Tomasi, T. B., and A. G. Plaut.** 1985. Humoral aspects of mucosal immunity, p. 31–61. *In* J. Gallin and A. S. Fauci (ed.), Advances in host defense mechanisms, vol. IV. Raven Press, New York.
44. **Uhlen, M., B. Guss, B. Nilsson, S. Gatenbeck, L. Philipson, and M. Lindberg.** 1984. Complete sequence of the staphylococcal gene encoding protein A. A gene evolved through multiple duplications. J. Biol. Chem. **259:**1695–1702.
45. **Wilkinson, H. W., and R. G. Eagon.** 1971. Type-specific antigens of group B type Ic streptococci. Infect. Immun. **4:**596–634.

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