Streptococcal β Protein Has Separate Binding Sites for Human Factor H and IgA-Fc

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The group B streptococcus (GBS) is the most important cause of life-threatening bacterial infections in newborn infants. Protective immunity to GBS infection is elicited by several surface proteins, one of which, the β protein, is known to bind human IgA-Fc. Here, we show that the β protein also binds human factor H (FH), a negative regulator of complement activation. Absorption experiments with whole human plasma demonstrated binding of FH to a GBS strain expressing β protein but not to an isogenic β-negative mutant. This binding was due to a direct interaction between β and FH, as shown by experiments with purified proteins. Inhibition tests and studies with β fragments demonstrated that FH and IgA-Fc bind to separate and non-overlapping regions in β. Heparin, a known ligand for FH, specifically inhibited the binding between β and FH, suggesting that FH has overlapping binding sites for β and heparin. Bacteria-bound FH retained its complement regulatory activity, implying that β-expressing GBS may use bound FH to evade complement attack. The finding that β protein binds FH adds to a growing list of interactions between human pathogens and complement regulatory proteins, supporting the notion that these interactions are of general importance in bacterial pathogenesis.

Among pathogenic bacteria, the group B streptococcus (GBS) is the most common cause of life-threatening septicemia, pneumonia, and meningitis in the neonatal period. In recent years, GBS has also attracted attention as a significant cause of disease in adults with underlying conditions (1, 2). Despite its importance as a human pathogen, relatively little is known about the molecular mechanisms by which this bacterium causes disease and evades attack from the human immune system, and a vaccine against GBS disease is not yet available.

Attempts to develop a GBS vaccine have mainly focused on the polysaccharide capsule, which elicits antibodies that protect against experimental infection (1, 2). However, strains of GBS also express surface proteins that elicit protective immunity and these proteins have attracted increasing interest for studies of pathogenic mechanisms and for vaccine development (3–9). One of these proteins is the β protein (also known as Bac or β C), which is of interest not only because it elicits protective immunity but also because it binds to the Fc part of human IgA (10–14). The IgA-binding region is situated in the N-terminal part of the β protein (13) and has been localized to a sequence comprising 73 amino acid residues (15). The role of β in infection is not known, but a recent study suggests that the ability of β to bind IgA-Fc may allow it to interfere with IgA effector function (16). Here, we show that β also binds another component of the human immune system, the complement regulator factor H (FH).

Human FH is a 150-kDa single chain plasma glycoprotein belonging to the regulators of complement activation (RCA) family. Like other members of the RCA family, FH is composed of domains designated short consensus repeat (SCR) or complement control protein (CCP) modules, and there are 20 such modules in FH (17–20). FH plays an important role in the regulation of the alternative pathway of complement activation by down-regulating the formation of the alternative pathway C3 convertase, resulting in decreased production of C3b, and by acting as a cofactor for factor I (FI) in the degradation of C3b (20).

Our data show that the β protein has separate binding sites for FH and IgA-Fc and that bacteria-bound FH retains its ability to down-regulate complement activation. These findings focus interest on the role of β in the pathogenesis of GBS infections and suggest that GBS may exploit FH to evade complement attack.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Media—The GBS type Ia strain A909 expressing the β protein (10, 21) was obtained from Dr. J. L. Michel (Channing Laboratory, Boston, MA). A series of β-expressing GBS isolates used for analysis of FH binding (Fig. 4C) was available in our collections. Plasmid pJR5233 is a shuttle vector in which replication is temperature-sensitive in Streptococcus pyogenes (22) and also in GBS. Plasmid pLZ12Spec is a shuttle vector in which replication is not temperature-sensitive (23). Plasmid pBAC601 is a pUC18 derivative containing an insert that includes the entire bac gene, the structural gene for the β protein, and the chromosomal regions upstream (~1.3 kb) and downstream (~2.9 kb) of bac (13). For construction of a derivative of pLZ12Spec carrying bac, the entire bac gene was amplified from plasmid pBAC601, using synthetic oligonucleotides 5′-AAATTTGGAT-TCTGCGAGGAAATTTATCCCCAGTG-3′ and 5′-AAATTTGGATCC-GTATTTTACGCTTACAC-3′ as primers. The resulting PCR fragment was digested with EcoRI and BamHI, recognition sequences for which had been introduced through the primers. This fragment was ligated into pLZ12Spec, generating plasmid pLZbac. All GBS strains were grown in Todd-Hewitt broth (Oxoid, Basingstoke, Hampshire, UK)

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at 37°C without shaking. GBS transformed with the pLZ12Spec derivative pLZbac was grown in presence of spectinomycin (70 μg/ml).

**Purified Proteins and Antiserum—**The group B streptococcal β, Rib, and α proteins were purified from extracts of strains SB35, BM110, and A909, respectively, as previously described (5, 24). The N-terminal B6 fragment of the α protein was prepared from GBS strain ATCC 12643, the C-terminal Bac fragment from GBS strain 411/83, and the entire proline-rich XPZ region has been deleted, will be described elsewhere. This β-derivative was purified from extracts of whole GBS bacteria expressing the protein (12). Purified human FH, F1, and C3b were purchased from Calbiochem. One preparation of purified human FH was from the kind gift of Dr. D. L. Kasper (University of Lund, Sweden). Monoclonal human IgA was kindly provided by Dr. J. M. Woof (University of Dundee Medical School, Dundee, UK). Polyclonal human IgA was purchased from Cappel-Origan Teknika (Turnhout, Belgium). Protein G was from Amersham Biosciences. Rabbit antiserum to the β protein was raised as described (5). Antiserum to human FH was purchased from Calbiochem or The Binding Site (Birmingham, UK). Rabbit antiserum to human IgA was from Dakopatts (Copenhagen, Denmark). Rabbit antiserum directed against the GBS type 1a capsular polysaccharide was kindly provided by Dr. D. L. Kasper (Channing Laboratory, Boston, MA).

**Construction of a β-Negative Mutant of GBS Strain A909—**A mutant of strain A909 was constructed in which bac, the structural gene for β, had been replaced by the kanamycin resistance cassette. The procedure used to construct this mutant employed a derivative of pRS233, a shuttle vector in which replicon is temperature-sensitive in Gram-positive bacteria, allowing efficient selection of recombinants through homologous recombination (22). The pRS233 derivative, designated pRSΔbac, carried the hkm2 kanamycin resistance cassette surrounded by chromosomal regions located upstream and downstream of the bac gene, respectively (see below). Plasmid pRSΔbac was transformed into strain A909 as described (26), and a mutant was recovered after homologous recombination between the plasmid and the bacterial chromosome (22). The structure of the mutant was verified by PCR (data not shown). This β-negative mutant of strain A909 will be referred to as Δbac. Transformation of strain Δbac with plasmid pLZbac generated the trans-complemented strain Δbac/ pLZbac.

The insert in plasmid pRSΔbac was constructed as follows. The bac gene in pBAC601 was deleted using two EcoRV sites located 6 bp upstream of the start codon and 30 bp downstream of the stop codon, respectively. This pBAC601 derivative was blunt ligated to SmalI-cleaved fkm2 cassette. The resulting plasmid had an insert in which the fkm2 was surrounded by the chromosomal regions located upstream and downstream of the bac gene, respectively. This insert was isolated after SacI digestion and ligated into SalI-cleaved pRS233, resulting in plasmid pRSΔbac (Fig. 1A).

**Purification of a 75-kDa C-terminal Fragment of β—**A 75-kDa C-terminal fragment of β was prepared by alkaline hydrolysis. A solution (22 ml) of β (35 μg/ml in 10 mM NaCl) was mixed with the same volume of 20 mM glycine-NaOH, pH 11.0, and incubated at 60°C for 20 h. After neutralization and concentration by ultrafiltration, the preparation was analyzed by Western blotting. A distinct 75-kDa polypeptide in the hydrolysate lacked reactivity with antiserum to the N-terminal B6 residue Pro441, and the size of the fragment suggests that it corresponds to the entire C-terminal part of the β protein. The 75-kDa fragment contains the XPZ region, as shown by analysis with XPZ-specific antibodies (data not shown).

**Plasma Absorption Experiments—**Plasma proteins binding to whole bacteria were identified in absorption experiments. Bacteria in an over-night culture were washed twice in PBS and suspended to 10 mg/ml. Samples (2 ml) of this suspension were added to 7 ml of fresh human plasma, supplemented with EDTA (9 m mM) to avoid complement activation, and the mixture was incubated for 2 h at room temperature with gentle shaking. The bacteria were washed three times in PBS, and bound plasma proteins were eluted with 1 ml of glycine-HCl buffer, pH 2.0, and the eluates were neutralized with 1 ml Tris, pH 8.0, and concentrated ~7-fold in a Centricon YM-10 centrifugal filter device (Millipore Corp., Bedford, MA).

Plasma proteins binding to pure β were identified by passing plasma through a column containing immobilized β. Pure β (1 mg) was immobilized in a 1-ml Hitrap column (Amersham Biosciences), following the instructions provided by the supplier. Fresh human EDTA-plasma (2 ml) was centrifuged and filtered through a 0.45-μm filter to remove particulate material, diluted 4-fold in PBS, and passed through the column. After washing the column with 15 ml of PBS, bound proteins were eluted with 5 ml of 0.1 M glycine-HCl, pH 2.0, and the eluates were immediately neutralized with 1 ml Tris, pH 8.0.

**Binding Assays With Whole Bacteria—**To analyze binding of pure FH to the β-expressing strain A909 and its β-negative mutant Δbac, over-night cultures of GBS strain 411/83 were washed twice in PBS (PBSAT) containing 0.02% NaCl and 0.05% Tween 20 and suspended to 10 mg/ml (final concentrations indicated). Identical samples (200 μl) of bacterial suspension were added to each of a series of tubes and resuspended with PBSAT (200 μl) containing 125I-labeled protein G (~15,000 cpm). After incubation for 2 h, washing with PBSAT, and centrifugation, the radioactivity associated with each bacterial pellet was determined in a gamma-counter. All incubations were performed at room temperature. Binding of anti-β antibodies and 125I-labeled IgA to whole bacteria was analyzed as described in (27).

**Competitive Inhibition Tests—**For competitive binding tests with proteins, the wells of microtiter plates were coated overnight with β protein (50 μl; 1 μg/ml in PBS). This coating step was performed at 4°C, while all subsequent steps were performed at room temperature. The wells were washed three times with PBSAT and blocked by incubation for 1 h with the same buffer. To analyze the ability of FH or IgA to inhibit the binding of IgA to the immobilized β, unlabeled FH or polyclonal IgA was mixed with 125I-labeled polyclonal IgA in a total volume of 100 μl of PBSAT to the final concentrations indicated, and these mixtures were added to the wells coated with β. After a 2-h incubation and three washes with PBSAT, bound 125I-labeled IgA was detected in a gamma-counter. Maximal binding (i.e. binding of 125I-labeled IgA in the absence of inhibitor) was ~7%. Non specific binding to wells (<0.3%) was determined by analyzing binding of the 125I-labeled IgA to uncoated wells. To analyze the ability of heparin to inhibit the binding of FH to β, unlabeled FH or IgA (negative control) was mixed with different amounts of heparin (final concentrations indicated) in a total volume of 115 μl of PBSAT. FH or IgA was used at a final concentration of 4.3 μg/ml. After incubation, the radioactivity associated with each well was determined in a gamma-counter. Bound IgA was detected with anti-IgA antibodies (diluted 1,000-fold in PBSAT) and 125I-labeled protein G, as described above. Maximal binding (i.e. binding in the absence of inhibitor) was about 7% for FH and 47% for IgA, respectively. Each of these experiments was performed at least twice, with similar results.

**Cofactor Activity of FH Bound to Bacteria—**Overnight cultures of GBS bacteria were washed twice in TBS-T (50 mM Tris, 100 mM NaCl, 0.05% Tween 20, pH 7.3) and resuspended to 2 × 106 cells/ml. Aliquots (20 μl) were centrifuged, and the bacterial pellets (4 × 106 cells) were resuspended in 19 μl of TBS-T containing pure FH (3.15 μg). After incubation for 2 h at room temperature, unbound FH was removed by three washes with TBS-T (450 μl). To determine the cofactor activity of the bacteria-bound FH, 125I-labeled C3b (~320,000 cpm, corresponding to ~8 ng) and F1 (44 ng) were added in TBS-T (total volume 20 μl). After incubation for 2 h at 37°C, the tubes were centrifuged, and the supernatants were collected. To analyze whether FH that had dissociated from bacteria during the experiment was responsible for cofactor activity, the β-expressing strain A909 with bound FH was immobilized in TBS-T alone for 2 h at 37°C and then incubated. The reaction was stopped after another 2 h with F1 and 125I-labeled C3b, as described above. As a positive control, pure FH was mixed with F1 and 125I-labeled C3b and incubated as described above. The amount of FH used was the same as that bound to the bacteria. To analyze whether the β-expressing strain A909 can degrade C3b in the absence of FH, a sample of this strain (19 μl) was incubated with F1 and 125I-labeled C3b for 2 h at 37°C. For analysis of C3b degradation, samples corresponding to

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RESULTS

Bacteria Expressing β Selectively Bind FH and IgA in Whole Human Plasma—Several studies have shown that β binds to the Fc part of human serum IgA (10–14). To analyze whether surface-expressed β can bind human plasma proteins other than IgA, we compared a β-expressing GBS strain and an isogenic β-negative mutant for ability to bind plasma proteins.

The β-negative mutant was derived from the β-expressing strain A909, a strain of capsular type Ia that expresses both β and IgA uncomplexed α protein (10, 21, 29). The construction of this mutant is described under “Experimental Procedures” and in Fig. 1A. In this mutant, which is designated Δbac, the entire structural gene for β, the bac gene, is replaced with the kanamycin resistance cassette ΔKm2. Surface expression of β was completely abolished in Δbac, as shown by analysis with rabbit anti-β serum (Fig. 1B). Moreover, the wild type strain A909 bound IgA, while Δbac failed to bind IgA, confirming that expression of β is abolished in Δbac (Fig. 1C). In contrast, surface expression of the type Iα polysaccharide capsule and the α protein were not affected in Δbac, and the in vitro growth rate of Δbac was not different from that of the parental strain (data not shown). When the Δbac mutant was trans-complemented with the bac gene on a plasmid, the resulting strain, designated Δbac/pLZbac, expressed β at a level very similar to that of the parental strain (Fig. 1, B and C).

To compare wild type bacteria and the β-negative mutant Δbac for ability to bind plasma proteins, bacteria were incubated in human EDTA-plasma, and bound proteins were eluted with 0.1 M glycine, pH 2.0 (see “Experimental Procedures”), and analyzed by SDS-PAGE. The eluate from the wild type strain A909 contained three protein species of ~60 kDa that were not present in the eluate from Δbac (Fig. 2A). The absence of the polypeptides in the eluate from the β-negative mutant Δbac was not due to a polar effect of the ΔKm2 cassette on a gene located downstream, because the same three plasma proteins were also found in eluates from the trans-complemented and β-expressing strain Δbac/pLZbac. Thus, absorption of the three polypeptides from plasma was mediated by surface-located β protein. Determination of N-terminal sequences showed that the sequence of the ~150-kDa protein was EDXNELPP, which is identical to that of human FH. The identity of this ~150-kDa protein was confirmed by Western blot analysis with specific anti-human FH antibodies (Fig. 2A). For unknown reasons, the eluted FH migrated as a doublet band, with a minor component of lower molecular weight. A similar observation has been made in another bacterial system (30). The N-terminal sequence analysis revealed that the ~130-kDa protein was identical to the β protein itself, indicating that this protein is partially released from the bacterial cell wall during the elution step. This observation was not surprising, because previous studies have shown that β is selectively released from whole bacteria when incubated at nonneutral pH (12). Finally, the ~60-kDa protein was identified as the heavy chain (αH) of human IgA (data not shown). Together, these data indicate

\[ \text{expression of } \beta \text{ is abolished in } \Delta \text{bac (Fig. 1C). In contrast, surface expression of the type Iα polysaccharide capsule and the } \alpha \text{ protein were not affected in } \Delta \text{bac, and the in vitro growth rate of } \Delta \text{bac was not different from that of the parental strain (data not shown). When the } \Delta \text{bac mutant was trans-complemented with the } \text{bac gene on a plasmid, the resulting strain, designated } \Delta \text{bac/pLZbac, expressed } \beta \text{ at a level very similar to that of the parental strain (Fig. 1, B and C).} \]

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that β-expressing GBS not only bind IgA-Fc but also bind human FH.

**Pure β Protein Binds FH and IgA in Human Plasma**—To analyze whether pure β protein has the same properties as β expressed on the bacterial cell surface, human EDTA-plasma was passed through a column containing highly purified β. Elution of bound proteins and analysis by SDS-PAGE demonstrated protein species of ~150 and ~60 kDa, and the ~150-kDa protein was identified as FH using specific antibodies (Fig. 2B). The ~60-kDa protein was similarly identified as IgA heavy chain (data not shown). The eluates also contained protein species of ~75, ~55, and ~30 kDa. Amino-terminal sequencing identified the ~55-kDa protein as a variant of the IgA heavy chain that for unknown reasons migrated faster than the major species. The ~30-kDa proteins were identified as Ig light chains (L) (data not shown). The identity of the minor ~75-kDa protein species is unknown. Absorption of these plasma proteins was not seen when plasma was passed through a column containing the GBS surface α protein, which is unrelated to β (29). These results show that pure immobilized β specifically binds FH and IgA present in human plasma.

**Pure FH Binds to the Surface of β-Expressing GBS**—The interaction between FH and protein β was further characterized by analyzing whether pure FH binds to whole β-expressing streptococci. The β-expressing wild type strain A909 was indeed able to bind pure FH (Fig. 3). No binding of FH was observed to the β-negative strain Δbac, but binding of FH was restored in the trans-complemented strain Δbac::plZbac. Thus, GBS bacteria were able to bind pure FH, and the binding was mediated by β.

**Interaction between Pure β and Pure FH**—To investigate the ability of pure β to bind pure FH, the β protein was subjected to Western blot analysis using unlabeled FH as probe and anti-FH for detection. As shown in Fig. 4A, pure β bound FH in this analysis, while no binding was observed for two control proteins, the GBS surface proteins Rib and α, which are unrelated to β (5, 29, 31). Similar results were obtained when the three GBS proteins were analyzed for FH-binding ability after immobilization in the wells of microtiter plates (data not shown).

Binding of pure β to FH could also be demonstrated in a Western blot, in which FH was present on the blotting membrane and unlabeled β was used as the probe (Fig. 4B). In this analysis, the β protein also bound its other known ligand, human serum IgA, while no binding was detected for the control protein bovine serum albumin (Fig. 4B). Similar results were obtained when β in solution was analyzed for its ability to bind FH, IgA, and bovine serum albumin immobilized in the wells of microtiter plates (data not shown). Together, these data show that β binds directly to FH and that no other components in plasma or on the bacterial cell surface are required for the interaction.

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**FIG. 3. Binding of pure FH to whole bacteria.** Different amounts of FH (final concentrations indicated) were incubated with 2 × 10⁹ bacteria. Strains used were the β-expressing strain A909, its β-negative mutant Δbac, and the trans-complemented strain Δbac::plZbac. Surface-bound FH was detected with anti-FH serum and [125I]-labeled protein G. Nonspecific binding (≤7%) has been subtracted. This experiment was performed three times with similar results.

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To analyze whether β proteins expressed by different GBS strains have similar properties with regard to FH binding, β was extracted from five different GBS strains of serotypes Ia and Ib. These extracts were prepared by incubating whole bacteria at elevated pH, which causes selective release of the β protein in almost pure form (12). Western blot analysis showed binding of FH to all β proteins studied (Fig. 4C). The β protein expressed by these strains showed slight variation in size, due to size variation in a proline-rich region with periodic structure (13, 14).⁴

**FH and IgA Have Separate Binding Sites in the β Protein**—To analyze whether FH and IgA bind to different sites in β, competitive inhibition analysis was performed with purified proteins. The binding of radiolabeled IgA to β immobilized in microtiter wells was not inhibited by FH, but as expected, the binding was completely inhibited by unlabeled IgA, indicating that FH and IgA have separate binding sites in protein β (Fig. 5A). The reciprocal experiment could not be performed, because radiolabeling eliminated the ability of FH to bind β.

The FH-binding region in β was further characterized by using fragments corresponding to different parts of β (Fig. 5B). The recombinant B6 fragment corresponds to the N-terminal part of β and includes the IgA-binding region (13, 15). The 75-kDa fragment, which corresponds to the C-terminal part of the molecule, was derived from β by alkaline hydrolysis, as described under “Experimental Procedures.” These two fragments were subjected to Western blotting and analyzed for

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*Footnote:* L.-O. Hedén, T. Areschoug, and G. Lindahl, manuscript in preparation.
mains have been reported to be localized in SCRs 7, 13, and 20 (32–34). To analyze whether β binds to a site in FH that overlaps with one of the heparin binding sites, heparin was used in various concentrations to inhibit binding of FH to immobilized β protein. Interestingly, heparin inhibited binding of FH to β (Fig. 6). This inhibition was not nonspecific, because heparin was not able to block the binding of IgA to β. These data suggest that β and heparin may have overlapping binding sites in FH.

**FH Bound to the Surface of GBS Retains Its Cofactor Activity**—The cofactor activity of FH bound to the surface of β-expressing GBS was analyzed in a C3b degradation assay (Fig. 7). The C3b protein is composed of α’- and β-chains (lane A). The enzymatic activity of FI in the degradation of C3b is dependent on FH, which acts as a cofactor for FI (20). Thus, when C3b is incubated with FI only, no degradation of C3b is observed (lane A). However, the α’-chain is cleaved when C3b is incubated with pure FH and FI, generating the 43-kDa fragment ααα (lane B). When FH was replaced with the β-expressing strain A909 that had been preincubated with FH, degradation of the α’-chain was also observed (lane C). The degradation of the α’-chain was not due to protease activity of the bacteria, as shown by incubation of C3b and the β-expressing strain A909 in the absence of FH (lane D). When the β-negative A909 mutant Δbac was preincubated with FH, followed by incubation with C3b and FI, very little degradation of the α’-chain was seen (lane E). This slight degradation of the α’-chain may be due to low levels of nonspecific binding of FH to the Δbac strain. As expected, degradation of the α’-chain was observed with the trans-complemented and β-expressing strain Δbac/pLZbac, which had been preincubated with FH (lane F). To analyze whether the observed cofactor activity of bacteria-bound FH was due to FH that had dissociated from the bacteria during the incubation, bacteria with bound FH were incubated as in the other assays, and after centrifugation, the supernatant was used as a source of cofactor activity. Only weak degradation of C3b was seen in this case (lane G). Together, these results show that most of the observed cofactor activity was due to FH bound to the β protein on the surface of GBS.

**DISCUSSION**

Like other Gram-positive bacteria, GBS is not sensitive to complement-mediated lysis because it lacks an outer membrane and has a thick cell wall. Therefore, elimination of GBS from an infected individual depends on phagocytosis and killing by leukocytes. Several lines of evidence indicate that both specific antibodies and complement have important roles in the opsonization of GBS for phagocytosis (35–37). A possible way for the bacterium to avoid such opsonization would be to reduce surface complement deposition. In this study, we have demonstrated that the surface protein β of GBS binds human FH and that bacteria-bound FH retains its ability to down-regulate complement activation. Thus, binding of FH to β may lead to
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Previous studies have shown that a binding site for human IgA-Fc is located in the N-terminal part of the β protein (13, 15). The data presented here indicate that the binding site for FH is located in the C-terminal half of β. Thus, β has separate and nonoverlapping binding sites for two important components in the human humoral immune system. Although the exact function of these ligands is not known, one may speculate that they both contribute to phagocytosis resistance. As mentioned above, FH may exert this function by down-regulating complement deposition on the bacterial surface. With regard to IgA, the β protein might interfere with IgA-mediated phagocytosis, due to its ability to block the binding of IgA-Fc to the human IgA receptor CD89 on phagocytes (16).

Interestingly, the ability to bind both IgA-Fc and a human complement regulator is not only a property of the β protein but is also a common property among M proteins, which are major virulence factors of S. pyogenes (48). Many M proteins have nonoverlapping ligand-binding domains that allow them to simultaneously bind with high specificity to IgA-Fc and the complement regulator C4BP (48, 49, 60, 61). Because M proteins and the β protein are expressed by different bacterial species and are structurally unrelated, the ability to simultaneously bind IgA-Fc and an RCA protein has apparently arisen independently during the evolution of surface proteins of S. pyogenes and GBS. These data suggest that the ability to simultaneously bind the two ligands may confer a selective advantage. In this context, it is of interest to note that the PspC/SpsA-like proteins of S. pneumoniae have been reported to bind both the secretory component of secretory IgA and FH, providing yet another example of a bacterium binding both an RCA protein and a polypeptide found in immunoglobulins (44, 45, 62). In this case, there is evidence that the bacteria may use free secretory component as a receptor on the surface of epithelial cells (63).

The location of the binding site for β in FH is not known, but the observation that binding of β to FH is inhibited by heparin suggests that β and heparin may have overlapping binding sites in FH. These data focus interest on SCRs 7, 13, and 20, which have been implicated in the binding of heparin to FH (32–34). Previous studies have shown that some M proteins of S. pyogenes bind to SCR 7 in FH, a binding that is inhibited by heparin (30, 64). Because M proteins bind to SCR 7, they not only bind FH but also bind the naturally occurring splice variant FHL-1, a ~42-kDa plasma protein that corresponds to the first seven SCRs of FH (65, 66). There is even evidence that M proteins selectively bind FHL-1, rather than FH, in human plasma (65). However, we did not detect any binding of FHL-1 to the β protein in plasma absorption experiments, suggesting that β does not bind to SCR 7 but may bind to SCR 13 or 20 in FH. It is noteworthy that the C-terminal part of FH has previously been implicated in the binding of FH to two bacterial surface structures, the sialylated lipo-oligosaccharide of N. gonorrhoeae and the OspE protein of B. burgdorferi, which bind in the regions corresponding to SCRs 16–20 and 15–20, respectively (41, 46).

In summary, we have shown that the β protein of GBS has separate binding sites for human FH and IgA-Fc and that bacteria-bound FH retains its ability to down-regulate complement activation. These data focus interest on FH as a target for pathogenic microorganisms and identify a novel ligand for this human plasma protein. Further studies of this interaction are of interest for analysis of the function of FH and for analysis of pathogenetic mechanisms in infections caused by β-expressing GBS. Moreover, studies of the interaction between β and FH are of interest for vaccine development, since the β protein elicits protective immunity and has been used for the prepara-
tion of a protein-polysaccharide conjugate intended for use as a human vaccine (6).

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