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Experimental Vaccination against Group B Streptococcus, an Encapsulated Bacterium, with Highly Purified Preparations of Cell Surface Proteins Rib and α

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Encapsulated bacteria cause some of the most common diseases in humans. Although the polysaccharide capsules of these pathogens have attracted the most attention with regard to vaccine development, recent evidence suggests that bacterial surface proteins may also be used to confer protective immunity. We have analyzed this possibility in group B streptococcus (GBS), an encapsulated bacterium that is the major cause of invasive bacterial disease in the neonatal period. Previous work has shown that the majority of GBS strains causing invasive infections express the Rib protein, and that most strains lacking Rib express a protein designated α. Here we report that active immunization with highly purified preparations of Rib or α protected mice against lethal infection with strains expressing the corresponding protein. Vaccination with the Rib protein protected against two strains of capsular type III and two strains of type II, and vaccination with the α protein protected against one strain of type II and one strain of type Ib. The mice vaccinated with Rib or α showed a good immunoglobulin G response to the immunogen. These data suggest that a vaccine against GBS disease may be based on cell surface proteins and support the notion that proteins may be used for immunization against encapsulated bacteria.

Infections caused by encapsulated bacteria are among the most common causes of serious disease in humans. Major efforts to develop vaccines against such infections by using the ability of capsular polysaccharides to elicit protective immunity have therefore been made (1). Although this approach has been highly successful in preventing certain infections, e.g., those caused by encapsulated strains of *Haemophilus influenzae* (1), attempts to develop polysaccharide vaccines against other encapsulated bacteria have been complicated by antigenic variation, poor immunogenicity, and cross-reactivity with human tissues (1, 4, 10, 11). Efforts are therefore under way to analyze whether vaccines against encapsulated bacteria, such as *Streptococcus pneumoniae* and *Neisseria meningitidis*, may be based on bacterial proteins, used alone or conjugated to capsular polysaccharides (1–3, 16).

Group B streptococcus (GBS) (*Streptococcus agalactiae*) is an encapsulated bacterium that causes most cases of septicemia and meningitis in the neonatal period (6) and may also cause an increasing number of serious infections in the adult population (9, 34). Various strategies have been devised for preventing GBS disease, and work is in progress to develop a vaccine based on the four classical types of polysaccharide capsule: types Ia, Ib, II, and III (5, 6, 26). The capsule of type III strains has been studied in particular detail, since such strains are responsible for the large majority of serious infections and cause ~90% of cases of neonatal meningitis (6).

Cell surface proteins of GBS have also attracted attention as possible virulence factors and vaccine components. More than 20 years ago, it was shown that some strains of GBS express a protein antigen designated the c protein, and passive immunization experiments with rabbit antiserum indicated that this antigen confers protective immunity (15). The c antigen was later shown to be composed of at least two unrelated proteins, the α and β proteins, which are expressed by many strains of capsular types Ia, Ib, and II (7, 12, 13, 22, 23). For each of these two proteins, it has been demonstrated that rabbit antibodies confer passive immunity against GBS strains expressing the corresponding protein (8, 21, 28). However, the α and β proteins are almost never expressed by type III strains, which cause most cases of invasive infections, and would therefore not be sufficient for the development of a protein vaccine against GBS disease.

In contrast to the α and β proteins, the recently identified protein Rib was found to be expressed by almost all GBS strains of serotype III, and rabbit antiserum against Rib was shown to protect mice against lethal infection with Rib-expressing strains (28). Moreover, most strains that did not express Rib were found to express α. Interestingly, the Rib and α proteins are structurally related and define a novel family of streptococcal surface proteins with extremely repetitive structures (23, 28, 31).

Since ~90% of all GBS strains causing invasive infections were found to express either Rib or α, it seemed possible that these proteins could be used to develop a protein vaccine against GBS disease (28). The β protein may be of more limited interest in this context, since β is less common than α and most strains that express β also express α (7, 28). To analyze whether Rib and α can be used for active immunization against GBS disease, we developed a method for isolation of these proteins in a highly purified form, free of polysaccharides. The results reported here show that vaccination with each of these purified proteins protects mice against lethal infection with GBS strains expressing the corresponding protein.

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MATERIALS AND METHODS

Bacterial strains and media. Six GBS strains, all of them mouse virulent, were used for protection studies and/or purification of proteins. The capsular types

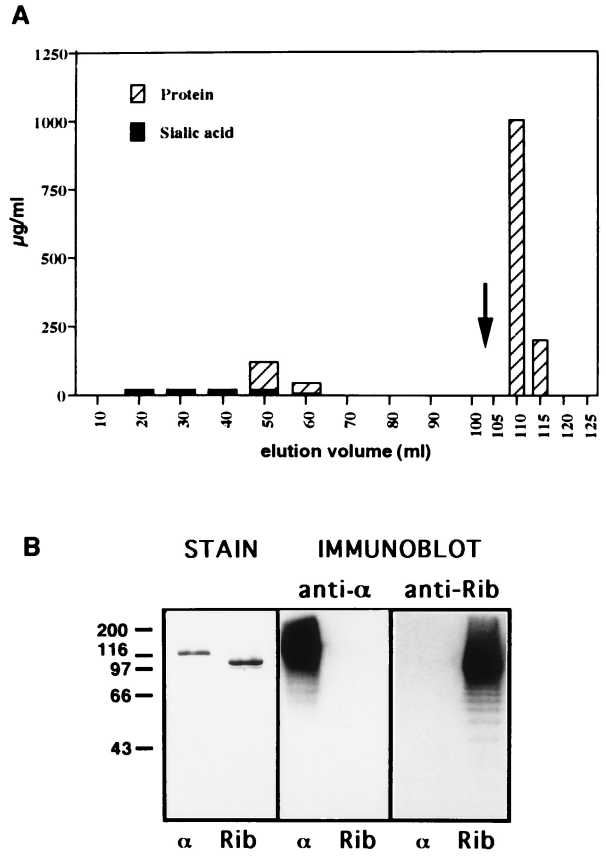

FIG. 1. Purification and Western blot analysis of GBS proteins. (A) Hydroxylapatite chromatography, showing the removal of sialic acid-containing type-specific polysaccharide remaining in Rib previously purified by ion-exchange chromatography and gel filtration (28). Rib was eluted by the addition of 20 ml of 150 mM KP (arrow). The difference in size of the fractions is indicated by the difference in width of the columns. Fractions were analyzed for protein and sialic acid content. The fractions at 110 and 115 ml, containing pure Rib, were pooled. (B) Western blot analysis of the Rib protein and the α protein, both purified by hydroxylapatite chromatography (from strains BM110 and SB35, respectively). The separated proteins were transferred to membranes by electroblotting, and the membranes were incubated with rabbit antisera to the purified Rib and α proteins. Bound antibodies were visualized by incubation of the membranes with ^{125}I-protein G, followed by autoradiography. The autoradiograms were deliberately overexposed to demonstrate the lack of cross-reactivity between the two proteins. Numbers in the left margin are molecular masses in kilodaltons.

and relevant cell surface proteins (Rib or α) of these strains are listed in Tables 1 and 2. The type III strain BM110, a member of a putative high-virulence clone (25), was obtained from S. Mattingly (University of Texas, San Antonio). The type III strain BS30, isolated in Sweden, and the type Ib strain SB35*sed1* have been described (28). Of type II strains, 1954/92 was provided by R. Facklam (Centers for Disease Control and Prevention, Atlanta, Ga.), 118/158 was obtained from J. Jelínková (National Institute of Public Health, Prague, Czech Republic), and BS29, recovered from a Swedish boy with a neonatal infection, was isolated in our laboratory. A collection of strains of serotypes Ia, Ib, and II was kindly provided by L. Burman (Swedish Institute for Infectious Disease Control, Stockholm), R. Facklam, J. Henrichsen (State Serum Institute, Copenhagen, Denmark), and J. Jelínková. The type III strains COH1 and M781 were obtained from J. Michel (Channing Laboratory, Boston, Mass.). Bacterial strains were grown in Todd-Hewitt broth (28).

Purification of the Rib and α proteins. The Rib protein, solubilized from the type III strain BM110 by mutanolysin treatment, was purified by ion-exchange chromatography and gel filtration (28). Such preparations were devoid of contaminating proteins, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), but still contained some contaminating polysaccharide, which was removed by hydroxylapatite chromatography. About 10 mg of Rib in 50 ml of 5 mM potassium phosphate (KP), pH 6.8, was applied to a column (7 ml) of hydroxylapatite (high resolution; Calbiochem, San Diego, Calif.) equilibrated with the same buffer. After washing of the column with 50 ml of 5 mM KP, Rib was eluted by the addition of 20 ml of 150 mM KP. The collection of fractions was started when the protein sample was applied to the column. The fraction size was 10 ml up to the addition of 150 mM KP and was 5 ml afterwards. The chromatographic fractions were analyzed for total protein concentration with the MicroBCA kit (Pierce, Rockford, Ill.). The presence of type-specific polysaccharide was determined by analysis of the content of sialic acid by the periodate-resorcinol method (14). To analyze for the presence of group B polysaccharide, samples (50 μl) were heated in 50 μl of 8 M HCl for 4 h at 100°C, evaporated to dryness, heated in 50 μl of bis-(trimethylsilyl)-trifluoroacetamide (Acros Organics, Geel, Belgium) and 5 μl of pyridine for 30 min at 80°C, and analyzed by gas chromatography-mass spectrometry for the presence of rhamnose. The α protein was isolated from the type Ib strain SB35 (28) by procedures similar to those used for the Rib protein.

Vaccination experiments. Male C3H/HeN mice (Bomholtgaard, Ry, Denmark), 7 to 13 weeks old, were immunized subcutaneously with 25 μg of pure protein (Rib, α, or bovine serum albumin [BSA]) in 0.1 ml of phosphate-buffered saline (PBS) mixed with 0.1 ml of complete Freund's adjuvant. Four weeks later the mice were given boosters of the same amount of protein with incomplete Freund's adjuvant. Two weeks after the booster, the mice were injected intraperitoneally (i.p.) with a 90% lethal dose (LD_{90}) of log-phase bacteria, diluted in 0.5 ml of Todd-Hewitt broth. The LD_{90} for the different strains varied between 10^6 and 10^7 bacteria, as determined in preliminary experiments. Deaths were recorded daily for 7 days.

Analysis of mouse antisera. Mice were immunized with Rib, α, or BSA, as described above, and exsanguinated 2 weeks after the booster injection. The sera were analyzed for immunoglobulin G (IgG) reacting with whole GBS (expressing Rib or α) and for IgG reacting with the purified Rib and α proteins. For experiments with whole bacterial cells, an overnight culture of BM110 or SB35 was washed twice with PBSAT (PBS containing 0.02% azide and 0.05% Tween 20) and suspended in PBSAT at a concentration of about 10^9 bacteria per ml. A sample (180 μl) of this suspension was mixed with 20 μl of mouse serum (diluted in PBSAT), and the mixture was incubated for 1 h. After the addition of PBSAT (2 ml) and centrifugation, the bacterial pellet was washed once with PBSAT and resuspended in 180 μl of PBSAT supplemented with 20 μl of a 1:100 dilution (in PBSAT) of sheep anti-mouse γ chains (The Binding Site, Birmingham, U.K.). Following incubation for 1 h, the bacteria were washed with PBSAT as described above and resuspended in 180 μl of PBSAT. Bound sheep antibodies were detected by the addition of 20 μl (about 10^4 cpm) of ^{125}I-labelled protein G (Pharmacia, Uppsala, Sweden) in PBSAT. After incubation for 1 h, the bacteria were centrifuged and washed once with PBSAT, and the radioactivity in the pellet was determined. All steps were performed at room temperature.

The reactivity of mouse antisera with purified proteins was determined by enzyme-linked immunosorbent assay (ELISA). The wells of microtiter plates (Falcon Microtest III; Becton Dickinson, Oxnard, Calif.) were coated with protein by incubation overnight at room temperature with 100 μl of a solution of Rib or α in PBS (0.6 μg/ml). Control wells were incubated with PBS. After removal of the protein solution, 150 μl of a blocking solution (10 mM Veronal buffer, 0.15 M NaCl [pH 7.4], 0.25% gelatin, 0.25% Tween 20) was added to each well and the plate was incubated for 90 min at room temperature. After four washes with PBSAT, 100 μl of mouse antiserum, diluted in PBSAT, was added to each well and the plate was incubated for 90 min at 37°C. To detect bound mouse IgG, the plates were washed as described above, 100 μl of alkaline phosphatase-conjugated goat anti-mouse γ chains (Sigma, St. Louis, Mo.) diluted 1:2,000 in PBSAT was added to each well, and the plate was incubated for 90 min at 37°C. The wells were washed as described above, and 100 μl of a substrate solution containing 1 mg of *p*-nitrophenyl phosphate (Sigma) per ml of 10% diethanolamine buffer (pH 9.8) was added to each well. After 25 min at 37°C, the plate was read in a microplate reader at 405 nm.

Other methods. Western blot (immunoblot) analysis, radiolabelling of proteins with ^{125}I, and determination of N-terminal sequences were performed as described previously (29). Cell surface expression of the Rib, α, and β proteins by GBS strains was analyzed with polyclonal rabbit antisera (28). Hyperimmune rabbit antiserum to Rib from strain BM110 was prepared as described previously (28). Passive immunization of mice with rabbit antiserum was performed as described previously (28). The Fisher exact test was used for statistical analysis.

RESULTS

Purification of the Rib and α proteins. For immunochemical analysis, the Rib and α proteins were previously purified by ion-exchange chromatography and gel filtration, followed by SDS-PAGE (28). For experiments involving active immunizations, it was necessary to replace SDS-PAGE with a different procedure, allowing recovery of the pure proteins in large amounts, in a native form and devoid of contaminating polysaccharides. Hydroxylapatite chromatography was found to efficiently separate Rib and α from the polysaccharide that remained after the gel filtration step. The data in Fig. 1A show the removal of contaminating type-specific polysaccharide

from a preparation of protein Rib. The proteins purified by hydroxylapatite chromatography contained less than 0.002% sialic acid, a key component of the type-specific polysaccharide capsule (33), and less than 0.01% rhamnose, the major component of the group-specific polysaccharide (24).

Rib was previously isolated from the Swedish type III strain BS30 (28), but for the work described here it was purified from the type III strain BM110, isolated in the United States. Several lines of evidence indicate that the Rib molecule expressed by strain BM110 is similar to that expressed by strain BS30. Both of these proteins were shown to lack cross-reactivity with the α protein when analyzed with specific rabbit antisera in Western blots (28) (Fig. 1B), and the N-terminal sequence of Rib from BM110 was found to be identical to that of Rib from strain BS30 (28) at 11 of 12 positions (amino acid residue 7 was Ser, not Asp). Most importantly, rabbit antiserum to Rib from BM110 was found to passively protect mice against lethal infection with two Rib-expressing strains but not against an α-expressing strain (Table 1). These data indicate that Rib molecules expressed by GBS strains of different geographic origin have similar properties.

The α protein was purified by methods similar to those used for Rib. The α proteins expressed by two different clinical isolates have already been shown to have similar properties and identical N-terminal sequences (17, 23, 28).

Expression of the Rib and α proteins by group B streptococcal strains of different serotypes. Rib was previously shown to be expressed by almost all type III strains isolated from patients with invasive infections (28). This finding made it of interest to analyse whether Rib is expressed by strains COH1 and M781, two type III strains that have been extensively studied by other investigators (26, 30). Using methods described previously (28), we found that both of these strains express Rib, but not α, on the cell surface (data not shown).

Characterization of strains of capsular types Ia, Ib, and II previously indicated that such strains rarely express Rib (28). In agreement with this result, analysis of 24 additional strains of types Ia or Ib showed that none of these strains expressed Rib, while 23 of the 24 strains expressed α. However, among 25 additional type II strains analyzed, 7 expressed Rib and 16 expressed α. Taken together, these data indicate that Rib is expressed not only by almost all type III strains but also by many type II strains. Among strains that do not express Rib, the majority express α.

Vaccination of mice. A mouse model was used to analyze whether active immunization with the purified Rib and α proteins protects against lethal GBS infection. Control mice were immunized with BSA. The immunized mice were challenged i.p. with an LD_{90} of bacteria; four strains expressing the Rib protein and two strains expressing the α protein were used. The results (Fig. 2 and Table 2) show that immunization with Rib, but not with BSA, protected mice against lethal infection with all four Rib-expressing strains (two of type III and two of type II). Most of the nonvaccinated mice died within 24 h, while most of the vaccinated mice survived and showed no signs of infection during the 7-day period of observation. The protection observed was highly significant for all four strains studied. Interestingly, immunization with Rib also appeared to confer partial protection against the two α-expressing strains (one of type II and one of type Ib). However, this cross-protection was not significant in the case of the type Ib strain and was significant only at a P value of 0.036 for the type II strain. On the other hand, the significance of this possible cross-protection is emphasized by the fact that pooled data from all mice immunized with Rib and challenged with an α-expressing strain differed from pooled data from controls at a P value of 0.004.

TABLE 1. Protection of mice against lethal GBS infection by passive immunization with antiserum against Rib purified from strain BM110[a]

Strain	Relevant cell surface protein[b]	Capsular type	No. of mice surviving[c]/no. injected after pretreatment with:		
			Anti-Rib serum	Anti-α serum	Normal serum
BM110	Rib	III	8/10[d]	1/6	0/15
118/158	Rib	II	7/8[e]	ND[f]	0/6
SB35*sed1*	α	Ib	2/10	6/7[g]	ND

[a] C3H/HeN mice were injected i.p. with 0.1 ml of rabbit antiserum (diluted to 0.5 ml with PBS) and challenged i.p. 4 h later with an LD_{90} of log-phase bacteria, diluted in Todd-Hewitt broth. The survival data were analyzed by the Fisher exact test.
[b] Only the surface protein relevant to this study is indicated for each strain. The α-expressing strain also expresses the β protein.
[c] For 4 days.
[d] $P < 0.001$ compared with controls receiving normal serum, and $P < 0.05$ compared with controls receiving anti-α serum.
[e] $P < 0.01$ compared with controls receiving normal serum.
[f] ND, not done.
[g] $P < 0.05$ compared with controls receiving anti-Rib serum.

Immunization with purified α protein conferred protection against lethal infection with the two α-expressing strains but did not protect against a type III strain expressing Rib. The protection that immunization with α conferred against the α-expressing strains was highly significant.

Antibody response in mice immunized with the Rib and α proteins. Mouse antiserum to Rib contained IgG that reacted with the Rib-expressing strain BM110 and also reacted very weakly with the α-expressing strain SB35 (Fig. 3A). In agreement with these data, analysis by ELISA showed that IgG in the anti-Rib serum reacted with purified Rib and also reacted

TABLE 2. Protection against lethal GBS infection by vaccination with the purified Rib and α proteins[a]

Strain	Relevant cell surface protein; molecular mass (kDa)[b]	Capsular type	No. of mice surviving challenge[c]/no. challenged after immunization with:		
			Rib	α	BSA
BM110	Rib; 95	III	22/22[d]		7/22
			14/14[d]		3/15
			10/10[e]	4/14	3/10
BS30	Rib; 95	III	12/15[d]		0/10
1954/92	Rib; 95	II	13/15[d]		2/15
118/158	Rib; 80	II	11/14[d]		1/15
BS29	α; 180	II	10/19[f]	18/20[d]	4/20[g]
SB35*sed1*	α; 110	Ib	7/15[h]	13/15[d]	2/15

[a] C3H/HeN mice were vaccinated with purified Rib or α. Control mice received BSA. The vaccinated mice were challenged i.p. with an LD_{90} of log-phase bacteria. The survival data were analyzed by the Fisher exact test. Each row contains data obtained in one experiment.
[b] Only the surface protein relevant to this study is indicated for each strain. The two α-expressing strains also express the β protein. The molecular masses were determined by Western blot analysis of bacterial extracts, as described previously (28).
[c] For 7 days.
[d] $P < 0.001$ compared with the control.
[e] $P < 0.01$ compared with the control.
[f] $P < 0.05$ compared with the control.
[g] These control mice received PBS, not BSA.
[h] $P > 0.05$ compared with the control.

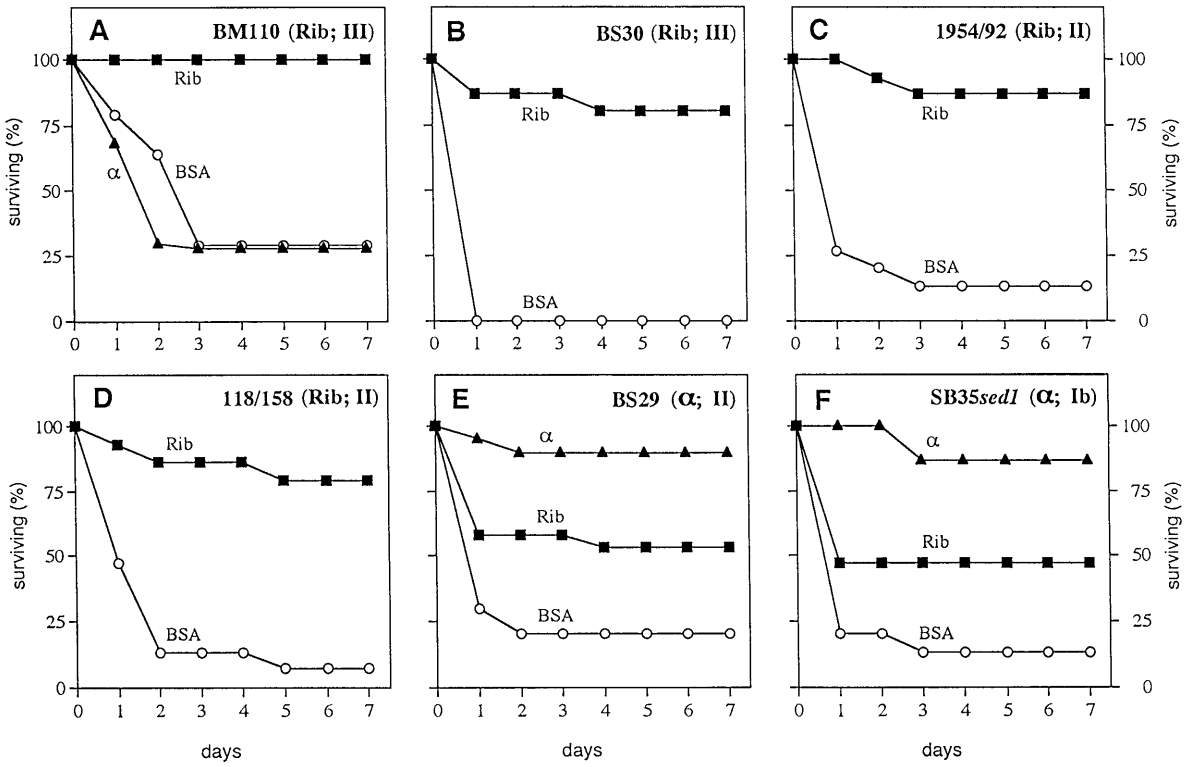

FIG. 2. Vaccination experiments with the purified Rib and α proteins. C3H/HeN mice were immunized with Rib, α, or BSA. Rib had been purified from strain BM110, and α had been purified from strain SB35. The immunized mice were challenged i.p. with an LD_{90} of a GBS strain, and mice surviving the infection were counted daily for 7 days. Each panel represents results obtained with one bacterial strain. For each strain the name and relevant properties (cell surface protein and capsular type) are indicated in the upper right corner of the panel. The data used for this figure are summarized in Table 2, where the statistical analysis of the data is presented. For strain BM110, the data shown here correspond to the experiment reported on line 3 in Table 2.

very weakly with purified α (Fig. 3B). Similar results were obtained with mouse anti-α sera, i.e., such sera reacted with the α-expressing strain and with purified α and also reacted very weakly with the Rib-expressing strain and with purified Rib (data not shown). These data show that the Rib and α proteins elicited an IgG antibody response in the mouse and also suggest that the two native proteins might cross-react weakly by ELISA. However, in agreement with the results obtained with rabbit antisera (Fig. 1B), no cross-reactivity was detected when the mouse sera were used for immunoblot analysis of proteins separated electrophoretically in the presence of denaturing amounts of SDS (data not shown).

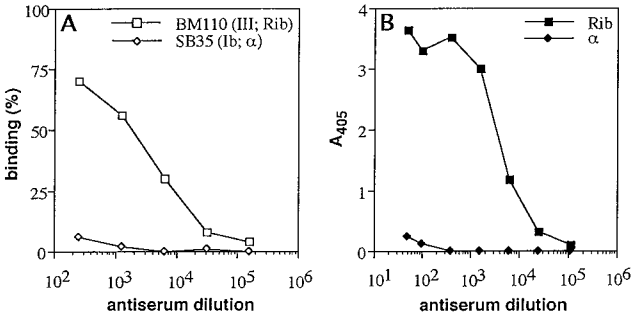

FIG. 3. Analysis of mouse anti-Rib serum. Serum from a mouse immunized with purified Rib was analyzed for the presence of IgG antibodies to the antigens indicated. (A) Reactivity with whole bacteria expressing Rib (strain BM110, type III) or α (strain SB35, type Ib). (B) Reactivity with the purified Rib and α proteins, analyzed by ELISA. The results shown here were obtained with a single mouse serum, but similar results were obtained in experiments with sera from three different mice. Background values, obtained with serum from a mouse immunized with BSA, were subtracted.

DISCUSSION

Development of a protein vaccine against GBS disease is of interest not only with regard to infections caused by this pathogen but also with regard to the more general question of whether protein vaccines against encapsulated bacteria can be developed. In both of these respects, the results reported here are encouraging, since they indicate that lethal GBS infections can be prevented by immunization with highly purified preparations of the Rib and α proteins. Immunization with Rib conferred protection against four Rib-expressing strains of serotype II or III, and immunization with α protected against infection with two α-expressing strains of serotype Ib or II. Since either the Rib or the α protein is expressed by ~90% of strains of the four classical serotypes, and also by many strains of other serotypes (21, 27a, 28), our data indicate that immunization with these two proteins may protect against the large majority of GBS strains causing invasive infections. The different GBS proteins may also be useful as carrier molecules in protein-polysaccharide conjugates, in which both the polysaccharide and the protein component might elicit protective immunity (19). However, the use of a vaccine based only on proteins might avoid possible problems due to cross-reactivity

between bacterial polysaccharides and sialic acid-containing human glycoproteins (10, 27).

The proteins used here were purified from streptococcal cells, and it was therefore essential to ensure that they were free of contaminating polysaccharides, which might affect the outcome of the immunizations. We found that a single step of hydroxylapatite chromatography efficiently removed polysaccharides that remained in protein preparations that had been previously purified by other procedures. With regard to the type-specific polysaccharide, the proteins purified by hydroxylapatite chromatography contained less than 0.002% sialic acid, a key component of this polysaccharide. It can therefore be calculated that the vaccinated mice cannot have received more than about 5 ng of contaminating unconjugated type-specific polysaccharide. However, the unconjugated polysaccharide is very poorly immunogenic, and even 10,000-fold-higher doses of such polysaccharide did not elicit protective antibodies in an animal model (32). Moreover, the possibility that the protection observed in the vaccination experiments was due to contaminating type-specific polysaccharide can be excluded, since the protection was independent of capsular type. With regard to the group-specific polysaccharide, it is also highly unlikely that the results described here could be explained by contamination with this polysaccharide, which has been reported not to confer immunity (20). Our analysis of the purified protein preparations showed that they contained less than 0.01% rhamnose, the major component of the group-specific polysaccharide (24). Therefore, the vaccinated mice cannot have received more than 5 ng of this polysaccharide, which would have been unconjugated. However, even 1,000-fold-larger amounts of conjugated group-specific polysaccharide lacked protective effect in a mouse model (20). In addition, protection that was due to trace amounts of contaminating group-specific polysaccharide, and not to the protein used for immunization, would be independent of the surface protein expressed by the strain used for challenge. However, vaccination with α protected against α-expressing strains, but not against a Rib-expressing strain, and vaccination with Rib protected against Rib-expressing strains, and only weakly against α-expressing strains. Taken together, these data clearly show that the protection observed after vaccination with Rib or α must have been elicited by the proteins used for immunization.

It has recently been shown that immunization with the GBS β protein, either alone or conjugated to type III polysaccharide, confers protection against GBS infection in a mouse maternal immunization-neonatal challenge model (18, 19). The β protein was not included in the experiments reported here, since expression of β alone is uncommon among group B streptococcal strains, while many strains express only the α protein or the Rib protein (7, 21, 28). Expression of β in combination with α is not uncommon, but antibodies to α alone are sufficient to protect against lethal infection with such strains (22, 28) (Tables 1 and 2). A combination of the Rib and α proteins may therefore protect against the large majority of group B streptococcal strains. Future work might even allow the construction of a single chimeric protein including protective epitopes from each of the Rib and α proteins.

Characterization of the Rib and α proteins with rabbit antisera previously showed that these two proteins do not cross-react (28, 31) (Fig. 1B), but the experiments with mouse sera reported here indicate that there may be a very weak cross-reactivity between the two proteins (Fig. 3). This result was not surprising, since there is considerable amino acid residue identity between the Rib and α proteins, which are members of a novel family of streptococcal proteins (31). This structural similarity between the Rib and α proteins may also explain why immunization with Rib appeared to confer partial protection against infections with α-expressing strains (Table 2). With regard to vaccine development, such cross-protection should only be an advantage.

Serum antibodies are apparently sufficient to confer protection in the mouse model used here, since mice can be passively protected against infection by injection of rabbit antiserum to the α, β, and Rib proteins (8, 15, 22, 28). However, it should be noted that the mouse models used by us and others (26, 28) depend on i.p. injection of pathogenic bacteria, which is not the normal route of infection in GBS disease (6). In humans, it seems possible that mucosal immunity is also of importance, since such immunity might prevent vaginal colonization by GBS and thereby prevent infection of the newborn child (6). It is not known whether the immunization procedures currently used in GBS research confer mucosal immunity, but a possible way to elicit such immunity might be to express GBS proteins in salmonella or some other heterologous host (1).

In summary, we have presented evidence that immunization with the purified cell surface proteins Rib and α can protect mice from infections caused by encapsulated GBS strains of several serotypes. This finding supports the notion that protein vaccines may confer immunity to encapsulated bacteria and encourages further studies of surface proteins not only in GBS but also in other encapsulated human pathogens (1, 2, 16). In addition, the characterization of surface proteins expressed by GBS is of interest for analysis of the pathogenic mechanisms used by this important cause of neonatal disease.

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C.L. and M.S.-C. contributed equally to this work.

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