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# A Protective Surface Protein from Type V Group B Streptococci Shares N-Terminal Sequence Homology with the Alpha C Protein

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**Infection by group B streptococci (GBS) is an important cause of bacterial disease in neonates, pregnant women, and nonpregnant adults. Historically, serotypes Ia, Ib, II, and III have been most prevalent among disease cases; recently, type V strains have emerged as important strains in the United States and elsewhere. In addition to type-specific capsular polysaccharides, many GBS strains possess surface proteins which demonstrate a laddering pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and resistance to trypsin digestion. These include the alpha C protein, the R proteins, and protein Rib. Some of these proteins elicit protective antibodies in animals. We demonstrate a trypsin-resistant laddering protein purified from a type V GBS strain by mutanolysin extraction and column chromatography. This protein contains a major 90-kDa band and a series of smaller bands spaced approximately 10 kDa apart on SDS-PAGE. Cross-reactivity of the type V protein with the alpha C protein and with R1 was demonstrated on Western blot (immunoblot). N-terminal sequence analysis of the protein revealed residue identity with 17 of 18 residues at corresponding positions on the alpha protein. Western blot of SDS extracts of 41 clinical type V isolates with rabbit antiserum to the protein demonstrated a homologous protein in 25 isolates (61%); two additional strains exhibited a heterologous pattern which was also demonstrated with 4G8, a monoclonal antibody directed to the alpha C protein repeat region. Rabbit antiserum raised to the type V protein conferred protection in neonatal mice against a type V strain bearing a homologous protein. These data support the hypothesis that there exists a family of trypsin-resistant, laddering GBS surface proteins which may play a role in immunity to GBS infection.**

Infection by group B streptococci (GBS) remains a leading cause of perinatal morbidity and mortality, despite advances in diagnosis and treatment. In the United States, infection occurs at a rate of approximately 1.8/1,000 total live births (27), striking infants lacking protective antibody who are perinatally exposed to this organism. The mortality rate among affected neonates is approximately 6% (27); survivors are at risk for serious sequelae in up to 50% of cases. GBS is also responsible for significant maternal peripartal disease, including bacteremia, chorioamnionitis, endometritis, and urinary tract infections, and for serious bacterial illness in nonpregnant adults with underlying illness. Farley et al. reported an incidence in nonpregnant adults of 4.4/100,000 in the Atlanta, Ga., area in 1989 to 1990; the mortality rate within this group was 25% (3). Data from a 1986 multistate surveillance study indicate that, in adults, invasive disease from GBS may be more common than that from *Haemophilus influenzae*, *Neisseria meningitidis*, or *Listeria monocytogenes* (23).

GBS strains can be serotyped according to the presence of specific capsular polysaccharides, which are known to be principal components of immunity. To date, nine distinct serotypes have been described. Historically, serotypes Ia, Ib, II, and III have been most prevalent among disease cases (12), with serotype III alone accounting for approximately 50% of neonatal disease. Recently, however, type V strains have emerged as important strains in the United States and elsewhere (25). Maryland surveillance data from the early 1990s showed that 12 and 31% of invasive isolates from neonates and adults, respectively, were type V isolates (5). From metropolitan Atlanta, Blumberg et al. reported type V isolates from 11, 25, and 31% of neonates, pregnant women, and nonpregnant adults, respectively, with invasive GBS disease (2). The reason for the emergence of type V isolates among GBS disease cases is unclear. Using ribotyping and restriction enzyme analysis of chromosomal DNA, Blumberg et al. demonstrated a high degree of relatedness among type V isolates from Atlanta, which suggests the dissemination of genetically related strains.

Prevention of neonatal GBS disease through maternal immunization is a promising area of active investigation. Although antibody to type-specific capsular polysaccharide is protective, a vaccine composed of type III polysaccharide alone elicited suboptimal immunity (1). Vaccination of animals with GBS polysaccharide conjugated to tetanus toxoid resulted in higher levels of antibody to homologous polysaccharide (18, 24). More recently, attention has focused on the development of a capsular polysaccharide-GBS surface protein conjugate vaccine as a means of providing both an enhanced immune response and protection against a variety of serotypes.

Previous work has demonstrated, on GBS strains of various serotypes, distinct protein antigens that share the properties of trypsin resistance and a characteristic ladder-like pattern on Western blot (immunoblot). These proteins include the alpha C protein (11, 16), the R proteins (4, 9), protein Rib (20), and an alpha-like protein purified from a type III strain (8). Generally, each of these proteins is present on strains of only one or two serotypes. These proteins may play an important role in

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GBS infection; in 1983, Linden et al. reported a correlation between low levels of maternal immunoglobulin G antibody to R protein and neonatal septicemia with type II and III R-protein-bearing GBS strains (10). Several of these proteins elicit protective immunity in animal models (10, 16, 20). These data imply the presence of a family of related GBS proteins that are of interest because of their possible role in immunity or virulence and the potential for one or more to serve as a protein carrier in a conjugate vaccine.

In this paper, we demonstrate an alpha-like protein purified from a type V GBS strain. This protein is resistant to trypsin degradation, shows a laddering pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), shares N-terminal sequence homology with the alpha C protein, and is immunologically related to the alpha C protein and to other GBS surface proteins. Rabbit antiserum raised to the protein provides protective immunity in an animal model; immunoblotting of clinical type V strains with this antiserum identifies a high prevalence of homologous proteins. These data are further evidence for a family of laddering GBS surface proteins that may play an important role in GBS infection.

## MATERIALS AND METHODS

**Bacterial strains, antigens, and antisera.** Strain A909, a type Ia strain that is the prototype alpha C protein strain (15); strain JM9-130013 (21, 22), a type VIII GBS strain reactive with antisera raised to R1 and R4 proteins; and strain 18RS21 (11), a type II strain containing no known surface proteins, were obtained from the Channing Laboratory collection. Strain D136C, a type III strain reactive to antiserum raised to R1 (26), and the Compton 25/60 strain, a nontypeable strain which is reactive to antiserum raised to R3 and to R4 (26), were kindly provided by Patricia Ferrieri, University of Minnesota School of Medicine, Minneapolis. Strain CJB-110, a type V strain isolated from the cerebrospinal fluid of a neonate, and a collection of 41 additional type V clinical isolates were generously provided by Carol Baker, Baylor College of Medicine, Houston, Tex. The collection included 9 neonatal and 10 maternal blood isolates, 3 neonatal cerebrospinal fluid isolates, 2 infant throat isolates, 2 infant rectal isolates, and 15 maternal vaginal isolates. Included in this collection was strain H1A-00001, a neonatal blood isolate, which was used in the mouse protection study.

Purified alpha C and beta proteins have been described previously (16). Rabbit anti-R1 and anti-R4 antisera were kindly provided by P. Ferrieri and have been previously described (4). Rabbit antiserum raised to protein Rib was kindly provided by G. Lindahl, University of Lund, Lund, Sweden. Purified type V polysaccharide (25) and rabbit antisera raised to formalin-treated strains A909 (15) and JM9-130013 (7), to purified alpha C protein (15), and to purified type V polysaccharide coupled to tetanus toxoid (25) were obtained from investigators at the Channing Laboratory. 4G8, a monoclonal antibody directed to the repeat region of the alpha protein (unpublished data), has been described elsewhere (15).

**Purification of an alpha-like protein from strain CJB-110.** Protein purification was adapted from previous methods (15, 20). GBS type V strain CJB-110 was grown overnight in 16 liters of Todd-Hewitt broth. The bacterial pellet was washed three times and then resuspended to five times the pellet volume in 50 mM Tris, pH 7.3. Mutanolysin dissolved in potassium phosphate buffer, pH 6.2, was added to a concentration of 350 U/ml, and digestion was allowed to proceed at 37°C overnight. Protease inhibitors were added at the indicated concentrations: benzamidine chloride (5 mM), iodoacetic acid (5 mM), and phenylmethylsulfonyl fluoride (2 mM). The mixture was ultracentrifuged at 140,000 × *g* for 30 min. The cell wall-rich supernatant component was filter sterilized, and the filtrate was dialyzed overnight against 10 mM Tris, pH 8.0. The dialyzed material was subjected to ion-exchange chromatography (ResourceQ; Pharmacia, Piscataway, N.J.) on a 0 to 0.3 M NaCl gradient. $A_{280}$ was read. Fractions were analyzed by Western blot with rabbit antiserum raised to formalin-treated GBS strain JM9-130013. Positive fractions were pooled, dialyzed overnight against water, and lyophilized. Lyophilized fractions were resuspended in water and subjected to gel filtration chromatography (Superose12; Pharmacia). Fractions were analyzed by SDS-PAGE with silver staining by the Morrisey method and Western blotting with antiserum raised to formalin-treated JM9-130013.

**Analysis of type V polysaccharide content by ELISA inhibition.** The ability of the type V protein preparation to inhibit binding of anti-capsular polysaccharide antibody to polysaccharide-coated plates was used to measure the presence of residual polysaccharide in the protein sample. An enzyme-linked immunosorbent inhibition assay (ELISA inhibition) was modified from previous methods (11). In brief, serial dilutions of the sample extract were incubated with rabbit antibody raised to type V capsular polysaccharide and added to microtiter plates coated with purified type V polysaccharide. After incubation with an enzyme-linked secondary antibody and development with substrate, inhibition [($A_{405}$ − $A_{405}$ of uninhibited control)/$A_{405}$ of uninhibited control] was compared with a standard curve of purified type V polysaccharide assayed in the same way.

**Rabbit antiserum.** Two New Zealand White rabbits were injected subcutaneously with 50 μg of the purified type V alpha-like protein emulsified in complete (first injection) or incomplete (subsequent injections) Freund's adjuvant at 3-week intervals for three injections. Serum was collected 2 weeks after the third injection. Antibodies to the protein were measured by ELISA as previously described (14).

**Immunoblotting.** SDS-PAGE and Western blotting were performed on purified protein and SDS extracts of whole GBS as previously described (11). SDS extracts were prepared by suspending bacteria from a blood agar plate into phosphate-buffered saline until a concentration giving a reading of 0.9 at $A_{650}$ was reached. A two-thirds volume of this suspension was added to a one-third volume of 187 mM Tris buffer (pH 6.8) containing 15% SDS, 30% glycerol, and 0.006% bromphenol blue, and the mixture was boiled for 3 min. Rabbit antiserum raised to formalin-treated GBS strain A909 or JM9-130013; rabbit anti-R1 or anti-R4 antiserum; rabbit antiserum raised to purified alpha, beta, Rib, or type V alpha-like protein; or 4G8, the monoclonal antibody directed to the repeat region of the alpha C protein, was used at a dilution of 1:500 (4G8) or 1:1,000 (immune sera).

**N-terminal sequence analysis.** N-terminal amino acid sequencing of the major 90-kDa band of the purified type V protein was performed at the Brigham and Women's Hospital Biopolymer Laboratory by the method of sequential cycles of Edman degradation followed by high-pressure liquid chromatography, with an automated sequencer (model 470A; Applied Biosystems, Inc., Foster City, Calif.).

**Neonatal mouse protection study.** A neonatal mouse model was used to test the ability of antiserum raised to the type V alpha-like protein under study to confer protection against infection by a GBS strain containing a homologous protein (19). Six- to eight-week-old pregnant female outbred CD-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.) were injected intraperitoneally (i.p.) with 0.5 ml of one of the following rabbit sera 48 to 72 h before the delivery of pups: nonimmune serum, antiserum raised against type V capsular polysaccharide coupled to tetanus toxoid, or antiserum raised against the purified type V protein. Pups were challenged i.p. within 48 h of birth with 50 μl of Todd-Hewitt broth containing approximately $1.4 \times 10^7$ CFU of strain H1A-00001, a type V clinical strain that demonstrated a homologous protein by immunoblot performed with antiserum raised to the type V alpha-like protein. Survivors were counted at 48 h.

## RESULTS

**Purification of a trypsin-resistant, laddering protein from type V GBS.** Western blots of SDS extracts of type V isolates from the Channing Laboratory collection were performed with antisera reactive to a variety of GBS proteins. Strain CJB-110, originally isolated from a neonate with meningitis, showed a laddering pattern on immunoblot with antiserum raised to strain JM9-130013 (a type VIII strain reactive with antisera raised to R1 and R4) and was chosen for further study. Overnight culture of strain CJB-110 was subjected to mutanolysin extraction followed by ion-exchange chromatography and gel filtration to extract and purify surface proteins. The gel filtration elution profile showed a single peak detected at $A_{280}$ (Fig. 1). Peak fractions were pooled, dialyzed, and lyophilized. The resulting product showed a laddering pattern on silver-stained SDS-PAGE, with a predominant band of approximately 90 kDa and a size difference between bands of approximately 10 kDa. This protein was relatively resistant to degradation by trypsin but sensitive to degradation by pepsin (Fig. 2). No residual polysaccharide was detected by ELISA inhibition. The sensitivity of the assay was 10 μg/ml; the lower limit of detection of the assay was 10 μg of polysaccharide per mg of protein.

**N-terminal sequencing data.** N-terminal sequencing of the major band of the type V protein preparation yielded identification or tentative identification of 18 of the first 20 amino acids; 17 of 18 were identical to residues at the corresponding positions of the mature alpha N terminus (Fig. 3) (17). Comparison of the N-terminal sequence analysis of the type V alpha-like protein with the published N-terminal sequence of protein Rib demonstrated 50% homology (20).

**Immunoreactivity with other GBS surface proteins.** The type V protein preparation was analyzed by immunoblot against a panel of antisera to determine cross-reactivity with

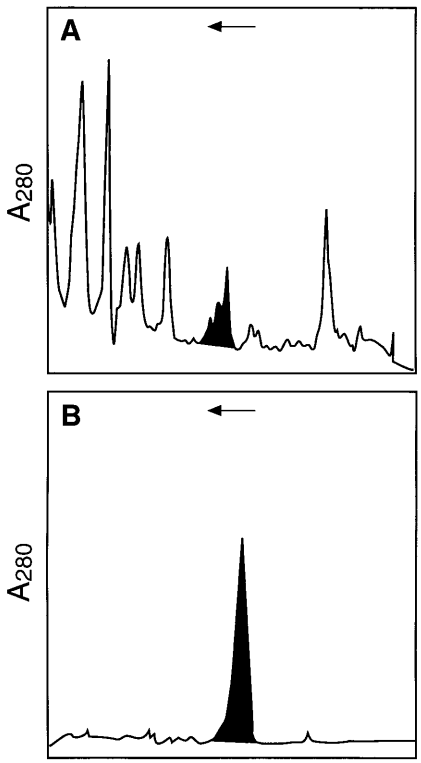


FIG. 1. Separation of an alpha-like protein from a mutanolysin extract of GBS strain CJB-110. (A) Mutanolysin surface extract of strain CJB-110 was subjected to ion-exchange chromatography using a 6-ml Resource Q column (Pharmacia Biotech, Uppsala, Sweden). The column was eluted with a 200-ml linear gradient of NaCl in 10 mM Tris, pH 8.0. The shaded area refers to the fractions that demonstrated the alpha-like protein by Western blot antiserum raised to strain JM9-130013, a type VIII strain which is reactive with antiserum raised to R1 and R4. These fractions eluted at a NaCl concentration of 0.13 to 0.18 M. (B) Pooled fractions containing the type V alpha-like protein were dialyzed against distilled water, concentrated, and subjected to gel filtration on a Superose 12 column (Pharmacia Biotech) in 10 mM Tris, pH 8.0. The shaded area refers to fractions which demonstrated the alpha-like protein by Western blot using antiserum raised to strain JM9-130013.

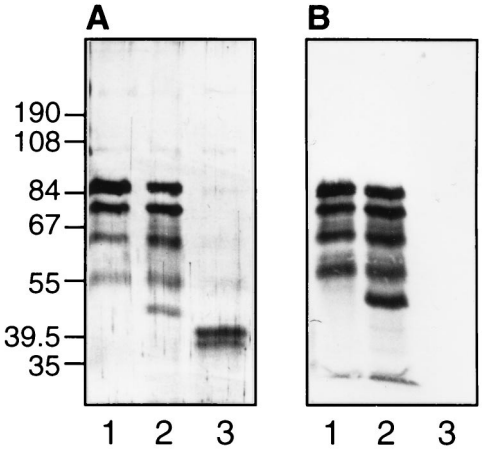


FIG. 2. (A) Silver-stained SDS-PAGE gel. (B) Western blot demonstrating the alpha-like protein purified from strain CJB-110. Following the gel filtration step, an aliquot of the protein was treated with 25% (wt/wt) trypsin (Sigma) in 50 mM Tris buffer, pH 8.0, or pepsin (Sigma) in 0.25 M sodium acetate buffer, pH 4.0, at 37°C for 2 h. The pepsin-treated aliquot was neutralized prior to further analysis. Equal amounts of untreated, trypsin-treated, and pepsin-treated protein were subjected to SDS-PAGE followed by silver staining by the Morrisey method or to Western blot using rabbit antiserum raised to the purified protein. Following trypsin treatment, one additional low-molecular-weight band was seen; otherwise, the structure and antigenicity of the alpha-like protein remained intact. However, pepsin treatment resulted in complete loss of immunoreactivity of the alpha-like protein. Lanes 1, untreated protein; lanes 2, trypsin-treated protein; lanes 3, pepsin-treated protein. Numbers to the left of panel A indicate molecular mass in kilodaltons.

other GBS surface proteins. The panel included R1- and R4-specific rabbit antisera; antiserum raised to purified alpha C or Rib; and 4G8, a murine monoclonal antibody directed to the alpha C protein repeat region. The purified type V protein reacted with antiserum raised to the alpha C protein and with R1-specific antiserum (Fig. 4A). Reactivity with 4G8 was fainter and present only in high-molecular-weight bands. There was no reaction with anti-R4 antiserum or antiserum raised to protein Rib (data not shown). Antiserum raised to the purified type V protein reacted with purified alpha C protein and with an SDS extract of strain D136C (R1) (Fig. 4B). This antiserum did not react with the Compton strain (R3/R4) or with purified beta protein (data not shown).

**Presence of homologous proteins in a panel of type V clinical strains.** The prevalence of homologous proteins among clinical type V isolates is of interest for defining relatedness among strains as well as for vaccine development. Using rabbit antiserum raised to the purified type V protein preparation, we screened by Western blot SDS extracts of 41 clinical type V GBS isolates for the presence of immunoreactive proteins. A laddering protein was demonstrated in 27 strains (66%) (Fig. 5), including 9 of 15 maternal vaginal or rectal isolates, 2 of 4 infant rectal or throat isolates, 8 of 10 maternal blood isolates, and 8 of 12 infant blood or cerebrospinal fluid isolates. The laddering pattern of 25 of the 27 appeared identical to that of the prototype protein, except for variations in the size of the largest band. Two additional strains, strains H4A-0114 (lane 10) and G-105 (lane 22), showed a different laddering pattern which was also apparent when immunoblotting was performed with monoclonal antibody to the repeating unit of the alpha C protein. None of the type V strains studied reacted with only the alpha C protein monoclonal antibody.

**Neonatal mouse protection against type V GBS challenge conferred by passive maternal immunization with antiserum raised to the type V purified protein.** A neonatal mouse protection model was used to determine whether antibody raised to the purified type V protein confers protection against infection by a GBS strain bearing a homologous protein. Pregnant mice were injected i.p. with 0.5 ml of one of the following rabbit sera 48 to 72 h before the delivery of pups: nonimmune serum, antiserum raised against type V capsular polysaccharide coupled to tetanus toxoid, or antiserum raised against the purified type V protein. Pups were challenged i.p. within 48 h of birth with a lethal dose of strain H1A-00001, a type V strain which demonstrated a homologous alpha-like protein by Western blot. Forty-eight hours after challenge, 14 of 18 (78%) pups born to mothers given antiprotein antiserum and 18 of 25 (72%) pups born to mothers given antipolysaccharide antiserum had survived, compared with 2 of 21 (10%) pups born to mothers injected with nonimmune serum (Table 1) ($P < 0.00002$, nonimmune versus antiprotein, Fisher's exact test).

## DISCUSSION

Previous work has demonstrated, on GBS of various serotypes, surface proteins that share the properties of trypsin resistance and a laddering phenotype on SDS-PAGE. These include the alpha C protein, the R proteins, and protein Rib. As a group, these proteins suggest a family of distinct but

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Alpha                ser-thr-ile-pro-gly-ser-ala-ala-thr-leu-asn-thr-ser-ile-thr-lys-asn-ile-gln-asn
                        |   |   |   |       |   |   |   |   |       |   |   |   |   |   |   |
Type V protein         X-thr-ile-pro-gly- X -ala-ala-thr-leu-asn-thr-lys-ile-thr-lys-asn-ile-gln-asn
                           |       |       |       |   |   |
Rib             ala-glu-val-ile-ser-gly-asp-ala-val-thr-leu-asn
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FIG. 3. N-terminal sequences of alpha C protein, the type V alpha-like protein, and protein Rib (20). The amino acid sequence of the alpha C protein shown here corresponds to residues 57 through 76 of the deduced amino acid sequence (17). Italics denote amino acids that are tentatively identified. Vertical lines denote identity.

related proteins, each of which is relatively specific to strains of a particular serotype. The best characterized of this group is the alpha C protein, the gene for which has been cloned and sequenced from strain A909, revealing an open reading frame of 3,060 nucleotides that contains a series of nine identical tandem repeats (17). The distances between the ladder steps of the alpha C protein correspond to the size deduced from the length of the repeating nucleotide units. Lower-molecular-weight variants of the alpha C protein contain correspondingly fewer numbers of nucleotide repeats and may reflect a type of antigenic variation by which GBS evades host immunity (13). Similar phenotypic patterns of other GBS surface proteins may reflect similar genetic structures.

This is the first report of an alpha-like protein purified from a type V GBS strain, an important emerging serotype that now accounts for up to 15% of disease-related strains in some geographic areas. The protein reported here appears structurally related to the alpha C protein and to other proteins within this group. Like the alpha C and Rib proteins, this is a high-molecular-weight, trypsin-resistant laddering protein, the size of which varies among different strains. N-terminal amino acid sequence analysis of this protein showed greater than 90% homology with the alpha C protein and 50% homology with protein Rib. Also like other proteins in this group, the type V alpha-like protein elicited antibodies that were protective against infection by a homologous protein-bearing GBS strain in an animal model. Protection of mouse pups born to dams passively immunized against other GBS antigens has been previously shown to correlate with transplacental transfer of specific immunoglobulin G (19). This is the presumed mechanism of protection in our study and supports a potential role for the type V alpha-like protein or a similar protein in a conjugate vaccine.

The cross-reactivity of the type V alpha-like protein with other trypsin-resistant laddering proteins is further evidence of a family of related but distinct antigens. The type V protein reacted with antiserum raised to the purified alpha C protein, and antiserum raised to the purified type V protein reacted with the alpha C protein. However, the type V protein reacted only weakly with 4G8, the monoclonal antibody to the alpha C protein repeat region, and Western blot patterns of the two proteins with antiserum raised to the type V protein are clearly different, indicating distinct antigens with a common epitope or epitopes. On the basis of N-terminal sequence homology between these two proteins, cross-reactivity at the N terminus was predicted. The reactivity, albeit weak, between the type V protein and 4G8 suggests additional homology elsewhere. The purified type V protein also reacted with R1-specific antiserum, and antiserum raised to the purified type V protein reacted with a cell surface extract of strain D136C, an R1-bearing strain, suggesting that these two proteins may be identical. However, the disparate Western blot patterns (Fig. 4B) of the SDS extracts of strains CJB-110 and D136C suggest that the type V alpha-like protein and R1 may be distinct proteins and, furthermore, illustrate the difficulty in distinguishing, on the basis of immunological assays, identical antigens from those which merely contain cross-reactive epitopes.

The type V protein was present in 25 (61%) of 41 clinical type V strains tested by Western blot with homologous antiserum. A second protein was identified in two additional strains by Western blot using antiserum raised to either the type V protein or 4G8, the monoclonal antiserum directed to the alpha C protein. These data are consistent with previous prevalence data indicating that the majority of GBS strains contain at least one trypsin-resistant, laddering protein (11, 20). In this and other regards, the alpha-like proteins may be analogous to the M proteins of group A streptococci, the best-characterized family of gram-positive surface proteins. Like the M proteins, the alpha C protein elicits protective antibodies in animals and may be important in the evasion of phagocytic killing (6, 11). Both the alpha C and the M proteins contain a signal sequence and a C-terminal membrane anchor region which are highly conserved among gram-positive proteins (17). And, like the alpha C protein, the M proteins contain tandem genetic repeats (though not identical), mutations of which are likely an important source of antigenic variation (6, 13). The identification of more than 75 different M-protein types hints at the potential for a large number of alpha-like proteins remaining to be defined.

The finding of two strains (strains H4A-0114 and G-105)

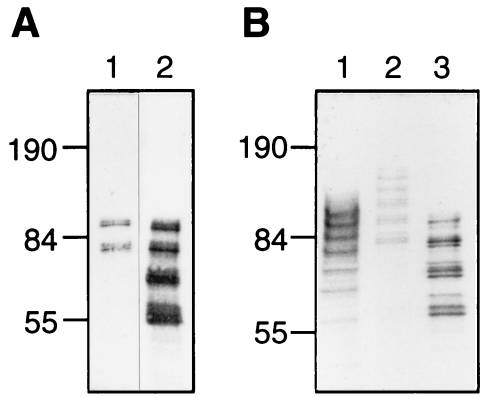


FIG. 4. Demonstration of immunoreactivity of the type V protein with other GBS surface proteins by Western blot. (A) The purified type V protein reacted with antiserum raised to purified alpha C protein (lane 1) and with antiserum raised to R1 (lane 2). (B) Antiserum raised to the purified type V protein reacted with an SDS extract of strain A909, the alpha C prototype strain (lane 1), and with an SDS extract of strain D136C, an R1 reference strain (lane 2). Western blot of an SDS extract of strain CJB-110 with the same antiserum is shown in lane 3. Molecular mass markers are in kilodaltons.

TABLE 1. Protection of neonatal mice against GBS infection after passive maternal immunization[a]

| Antiserum | No. of pups surviving/ total no. of pups | % Survival |
|---|---|---|
| Nonimmune | 2/21 | 10 |
| Anti-V-TT | 18/25 | 72 |
| Antiprotein | 14/18 | 78 |

[a] Anti-V-TT, antiserum to type V capsular polysaccharide coupled to tetanus toxoid. $P = 0.00002$ (nonimmune versus antiprotein; Fisher's exact test).

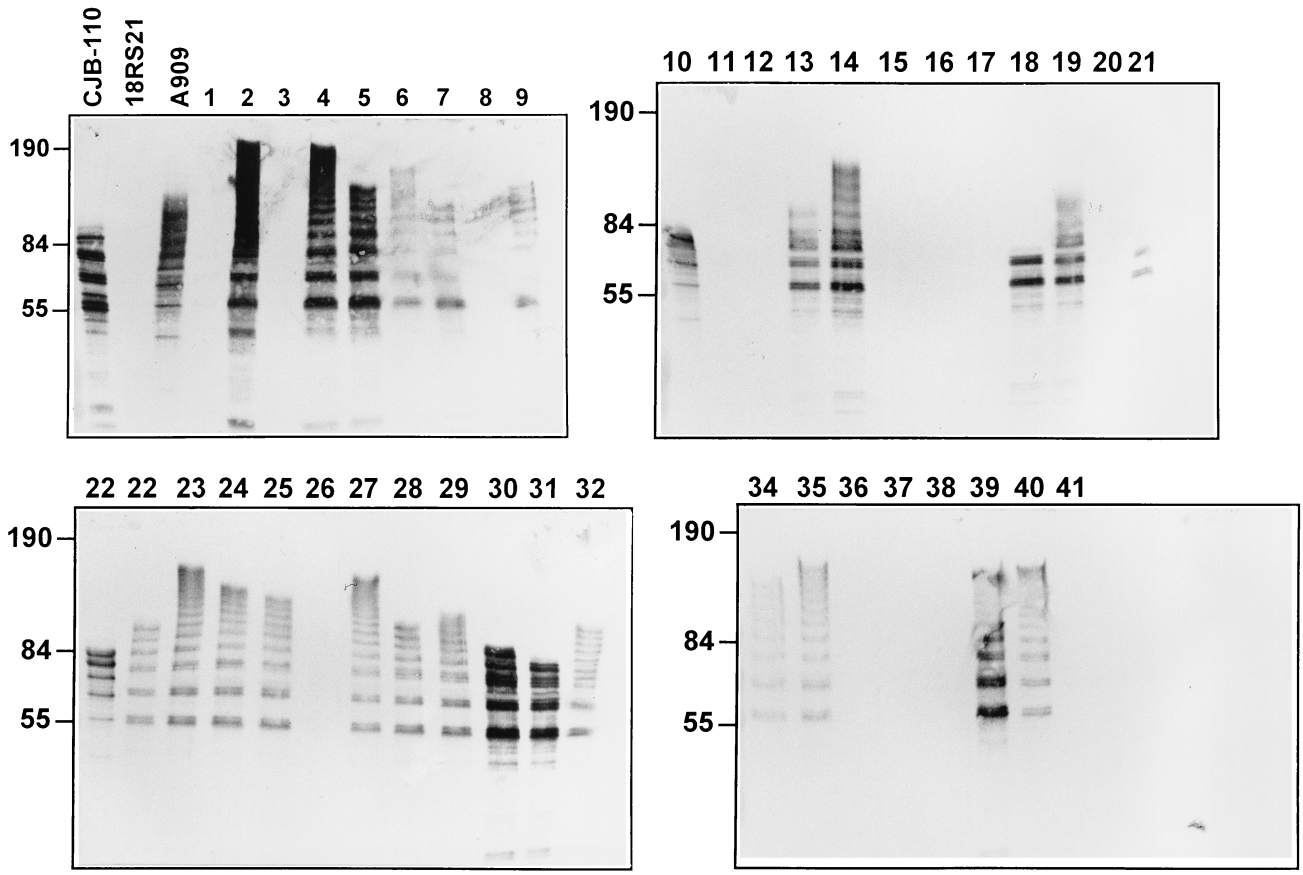


FIG. 5. Demonstration of alpha-like proteins in a panel of type V clinical strains by Western blotting with rabbit antiserum raised to the purified alpha-like protein from strain CJB-110. CJB-110, SDS extract of CJB-110, the type V protein parent strain; 18RS21, SDS extract of strain 18RS21, a type II GBS strain containing no known laddering surface proteins; A909, SDS extract of strain A909, the alpha C prototype strain; lanes 1 to 41, SDS extracts of 41 clinical type V GBS isolates. Twenty-five of the 41 strains demonstrated a pattern which was homologous to that of the prototype type V alpha-like protein. Lanes 10 and 22 show extracts of two additional strains, strains H4A-0114 and G105, which demonstrated a different pattern that was also visible on immunoblot with 4G8. Numbers to the left of each panel indicate molecular mass in kilodaltons.

whose cell surface extracts reacted to antiserum raised to the purified type V protein and to 4G8 underscores the inherent difficulty in categorizing strains containing proteins of this family. Strains have traditionally been described as alpha positive or R positive, for example, on the basis of immunoreactivity between immune serum and a cell surface extract, but this classification is limited by the concentration and specificity of the antiserum as well as the quantity of antigen present. Western blotting of surface extracts from strains H4A-0114 and G105 with antiserum raised to the type V protein showed a pattern which was clearly different from that of the purified type V protein; moreover, these two extracts reacted strongly with 4G8, giving a pattern more suggestive of the alpha protein. These strains may contain the alpha C protein or yet another alpha-like protein with an identical or highly homologous epitope.

The degree and nature of the relatedness among these proteins will likely be elucidated through genetic characterization. Such work may reveal a mechanism of antigenic variation important in GBS pathogenesis that can serve as a model for other gram-positive proteins. In addition, definition of a conserved protective epitope could lead to the identification of a candidate protein for use in a GBS conjugate vaccine.

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