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Characterization of Two Distinct Opsonic and Protective Epitopes within the Alpha C Protein of the Group B *Streptococcus*

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Group B *Streptococcus* (GBS) is a major cause of neonatal sepsis, meningitis in early infancy, postpartum endometritis, and serious invasive infections in adults in the United States. We previously cloned, sequenced, and characterized the alpha antigen gene, *bca*, and showed that the alpha C protein of GBS is a trypsin-resistant, surface-associated polypeptide that contains a signal sequence, a unique N terminus, nine identical tandem repeats, and a C-terminal membrane anchor structure. Polyclonal antiserum raised to the recombinant alpha C protein and an opsonic monoclonal antibody, 4G8, raised to the native protein from GBS have been shown to be protective in a mouse model. The binding site of 4G8 has now been localized to the tandem repeat region of the alpha C protein. To determine whether the N terminus of the alpha C protein contains additional opsonic and/or protective epitopes, the sequence corresponding to the alpha C protein N terminus was subcloned into a pET vector, the expressed peptide from *Escherichia coli* was purified by Ni^{2+} affinity chromatography, and rabbit polyclonal antibodies were raised to the purified recombinant peptide. Antibodies to the alpha C protein N terminus were shown to be opsonic by an in vitro opsonophagocytosis assay. In addition, 69% of newborn mouse pups from mothers passively immunized with the antiserum to the recombinant N-terminal polypeptide of the alpha C protein were protected against lethal challenge with GBS A909. These data indicate that at least two distinct regions of the alpha C protein, the N terminus and the tandem repeat region, contain opsonic and protective epitopes.

Pathogenic streptococci express a number of surface-associated, opsonic, and protective polysaccharides and protein antigens (13, 14). The type-specific capsular polysaccharide by itself is not very immunogenic; however, antibodies to conjugates of the capsular polysaccharides and protein antigens elicit protection in animal models of infections with the group B *Streptococcus* (GBS) (12, 26, 34, 35). GBS also expresses a family of protective and well-characterized protein antigens called C proteins (alpha and beta) and R proteins (Rib) (9, 11, 16, 20–22, 31, 33). The sequence of the alpha C protein gene (*bca*) of GBS reveals four distinct domains: a signal sequence, an N-terminal region, a tandem repeat region, and a C-terminal anchor region (22). Identification and characterization of protective epitopes within the domains of the alpha C protein will help determine the immunological properties of these regions. These protective epitopes could be used to develop an alpha C protein-capsular polysaccharide conjugate vaccine to protect against a broad range of GBS strains.

An opsonic and protective epitope of the alpha C protein was identified with monoclonal antibody 4G8, which is specific for the alpha C protein (19). However, the location of the epitope detected by 4G8 on the alpha C protein had not been determined. To develop an effective alpha C protein-based conjugate vaccine, the protective epitopes within the alpha C protein need to be mapped, particularly since the antigen appears to undergo antigenic variation in isolates from neonates and their mothers (10, 18). Reductions in the number of tandem repeats within the *bca* gene may give rise to antigenically variable polypeptides due to conformational epitopes on the alpha C protein that vary as a function of the number of repeats (7).

If an effective conjugate GBS vaccine is to be developed, protective epitopes that are conserved in the parental strains and the deletion mutants need to be identified. In the isolates from neonates, there were deletions in the tandem repeat region, but not in the N and C termini, of the *bca* gene (4, 18). Therefore, conserved epitopes are likely to be localized to the N- and C-terminal regions. The N terminus of the alpha C protein is a likely location for protective epitopes of the alpha C protein that are conserved in spontaneous deletions and wild-type strains. However, the C terminus of the alpha C protein may not contain protective epitopes, since it is thought to be involved in the antigen's attachment to the cell wall peptidoglycan (22, 23, 29). This study addresses two issues: (i) the localization of the protective epitope on the alpha C protein as defined by the monoclonal antibody 4G8 and (ii) the identification of opsonic and protective epitopes in the alpha C protein N terminus.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains used in this study included GBS A909 and 090 (15) and *Escherichia coli* BL21(DE3) (8), DH5α (Gibco/BRL, Bethesda, Md.), and NK8032 (kindly provided by Nancy Kleckner). Alpha C protein subclones pJMS23-1 and pJMS23-9 have been described previously (21, 22), and plasmids pSKOF1-13, pDEK14, and pDEK15 are described below. Plasmid vectors included pET24a (Novagen, Madison, Wis.) and pGEM-7Zf(−) (Promega, Madison, Wis.). GBS strains were grown in Todd-Hewitt broth (Difco, Detroit, Mich.) and on tryptose soy agar with 5% sheep's blood plates (Becton Dickinson, Woburn, Mass.). *E. coli* strains were grown in L broth (Difco). Antibiotic concentrations were 100 μg/ml for ampicillin and 50 μg/ml for kanamycin.

DNA procedures: subclones of the *bca* gene. Restriction endonucleases, ligases, and calf intestine alkaline phosphatase were obtained from New England Biolabs (Beverly, Mass.) and Boehringer Mannheim (Indianapolis, Ind.). Staggered-end ligations were carried out at 14°C (32). Plasmids used in this study are shown in Fig. 1. pSKOF1-13 was developed by digesting pJMS23-9 [a derivative

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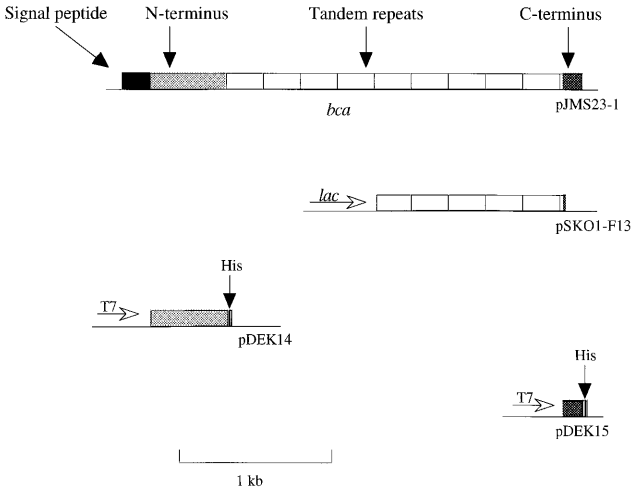

FIG. 1. *bca* gene subclones used to define the location of monoclonal antibody 4G8 binding within the alpha C protein. pJMS23-1 is the *bca* gene subclone (22). pSKOF1-13 was derived from a subclone of pJMS23-1 (pJMS23-9) by exonuclease digestion (24). This construct encodes the alpha C protein tandem repeat region and a single amino acid from the C terminus. Expression of this insert is driven by the *lac* promoter. pDEK14 encodes the alpha C protein N terminus, and pDEK15 encodes the alpha C protein C terminus. Both constructs are cloned into the pET24a expression vector, which facilitates IPTG-inducible expression of the cloned gene fragments. His represents a 6-residue histidine tag of vector pET24a that is used to facilitate purification of the recombinant gene products cloned into the vector.

of pJMS23-1 that contains an *Alu*I fragment of the *bca* gene cloned into pGEM-7Zf(−)] with *Hin*dIII and *Nsi*I, followed by exonuclease III digestion (22). This plasmid contains a 1.24-kb insert consisting of ~4.9 alpha C protein tandem repeats. In addition, pSKOF1-13 encodes 11 amino acids of the C-terminal partial repeat and the 1st amino acid from the C terminus. The cloned insert is expressed from the *lacZ* promoter of the pGEM-7Zf(−) vector. Thus, the gene product expressed from pSKOF1-13 contains only the alpha C protein repeat region and a single amino acid of the C terminus.

The DNAs encoding the alpha C protein N and C termini were PCR cloned into a pET24a overexpression vector containing a T7 polymerase promoter that facilitates isopropyl-β-D-thiogalactosidase (IPTG)-inducible, high-level expression of the recombinant gene fragment and a C-terminal 6-residue histidine tag that supports purification of the gene products by Ni^{2+} affinity chromatography. Oligonucleotide preparations for PCR were synthesized at an institutional core facility with the Expedite nucleic acid synthesis system, model 8909 (Millipore Corp., Bedford, Mass.). The DNA sequence encoding the alpha C protein N terminus was amplified from the *bca* gene subclone pJMS23-1 by PCR with the following oligonucleotide primers: 5′-GTATATGGATCCATAGTTGCTGCAT CTACA-3′ and 5′-GGGCTGAAGCTTCAATACTAACAATTTCTC-3′. The oligonucleotide primers used to amplify the DNA sequence encoding the alpha C protein C terminus are 5′-GTATATGGATCCAAAGCTCAGCAAGTCAA C-3′ and 5′-GGGCTGAAGCTTATCCTCTTTTTTCTTAGAAAC-3′. Conditions of the amplification were as follows: denaturation for 3 min at 94°C, annealing for 2 min at 39°C, and polymerization for 3 min at 72°C. Amplification was carried out with a Vent polymerase kit, with 1.5 mM $MgCl_2$ (New England Biolabs). The *Bam*HI and *Hin*dIII restriction endonuclease sites (underlined) were encoded in the primers to facilitate cloning into the pET24a vector.

A 542-bp fragment was amplified with the N terminus-specific primers by PCR from pJMS23-1 and ligated into the *Bam*HI- and *Hin*dIII-digested pET24a vector. Plasmid pDEK14 (Fig. 1) contained such a 542-bp insert and was verified to encode the alpha C protein N terminus by partial nucleotide sequence analysis. With C terminus-specific primers, a 144-bp fragment was amplified from pJMS23-1 and ligated into the pET24a vector. Recombinant clones were screened by colony blot hybridization and probed with the amplified C-terminal PCR product; insert size was determined by digestion with *Bam*HI and *Hin*dIII. Plasmid pDEK15 (Fig. 1) contained a 135-bp fragment encoding the alpha C protein C terminus, as confirmed by nucleotide sequence analysis.

Colony blot hybridization for *E. coli*. Colony blot hybridization was carried out as described previously (34). The amplified PCR fragments were labeled by random priming reaction with [α-^{32}P]dCTP (Amersham, Arlington Heights, Ill.) by use of a random priming kit (Boehringer Mannheim). The labeled probes were separated from unincorporated nucleotides on NucTrap columns (Stratagene, La Jolla, Calif.).

SDS-PAGE, Western immunoblotting, and antibodies. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8 or 15% polyacrylamide), with both Coomassie blue staining and Western immunoblots carried out by standard methods (6, 28). Primary antibodies and secondary alkaline phosphatase conjugates (Organon Teknika, West Chester, Pa.) were used at a dilution of 1/1,000. Blots were developed with alkaline phosphatase substrate buffer (Sigma, St. Louis, Mo.). Antibodies used in this study include polyclonal alpha C protein antibodies (7), antibodies to the alpha C protein N-terminal peptide (this study), and the alpha C protein monoclonal antibody 4G8 (19).

Ni^{2+} affinity chromatography. The recombinant alpha C protein N-terminal peptide was purified with a Ni^{2+} affinity column according to the Novagen pET system manual. Eluted fractions containing the largest amounts of protein were identified by Bradford assay (30) and desalted with an Amicon microconcentrator, P-10,000-molecular-weight cutoff.

Development of alpha C protein N terminus-specific antibodies. To obtain alpha C protein N terminus-specific antibodies, purified alpha C protein N-terminal peptides were lyophilized and sent to Lampire Biologicals (Malvern, Pa.) for rabbit immunization: 100 μg of the alpha C protein N-terminal peptide was resuspended in 2.5 ml of phosphate-buffered saline, emulsified in 2.5 ml of complete Freund's adjuvant, and injected subcutaneously at six sites on day 1. Booster immunizations given at 21 and 42 days consisted of solubilized antigen, emulsified with incomplete Freund's adjuvant. Blood was drawn on days 1 (preimmunization bleed), 21, and 42 for antibody testing. At day 56, a 50-ml blood sample was drawn (postimmunization bleed).

ELISA and opsonophagocytosis assay. Titers of the mouse monoclonal antibody 4G8 and of rabbit antiserum elicited to the alpha C protein N-terminal peptide were measured by enzyme-linked immunosorbent assay (ELISA) inhibition (7). The opsonophagocytosis assay to determine the functionality of the alpha C protein N terminus-specific antibodies was carried out as described previously (1). This assay requires human serum (used as a complement source), GBS (~1.5×10^6 CFU), polymorphonucleocytes (PMNs) (~3.0×10^6 cells), and antibodies (final dilution, 1/100) combined in a 500-μl volume. The amount of opsonophagocytic killing (log-kill) was determined by subtracting the log of the number of colonies surviving the 1-h assay from the log of the number of CFU at the 0 time point.

Neonatal mouse model. The following is a modification of the neonatal mouse model described by Rodewald et al. (27). CD-1 outbred mice arrived 17 to 18 days pregnant (Charles River Laboratories). On the 2nd day after arrival, mice were divided into three groups, with four pregnant mice per group. The mice were immunized intraperitoneally with 0.5 ml of the postimmunization antiserum raised to the N-terminal polypeptide, preimmunization serum, or antiserum raised to a protein-polysaccharide conjugate consisting of the Ia capsular polysaccharide covalently coupled to tetanus toxoid (Ia-TT) (33). Newborn mouse pups were challenged with 3×10^4 CFU of GBS A909 by intraperitoneal injection. After 48 h, numbers of dead and surviving mice were counted.

RESULTS

Expression of the recombinant alpha C protein N- and C-terminal peptides. The alpha C protein monoclonal antibody 4G8 binds a protective epitope of the alpha C protein (19). To map the location of this epitope, plasmid constructs consisting of the N terminus, repeat region, and C terminus were cloned and expressed in *E. coli*. Figure 1 shows the subclones of the *bca* gene that were used to map the protective epitope. PAGE was used to characterize the expressed gene products from the N- and C-terminal clones. Extracts of strain BL21(DE3) containing pET24a (negative control), pDEK14 (N-terminal clone), and pDEK15 (C-terminal clone) were prepared after IPTG induction and electrophoresed on 15% polyacrylamide gels. Figure 2 shows a band of ~23 kDa (the expected size of the N-terminal fragment) in the lane corresponding to the extract of *E. coli* containing pDEK14 (lane 2). Lane 3 shows a 6.2-kDa band in the *E. coli* extract containing pDEK15, the size of which is similar to the expected size of the recombinant alpha C protein C-terminal peptide.

Localization of the protective epitope(s) defined by 4G8. Western blot analysis was used to determine whether the epitope bound by 4G8 is localized to the N terminus, repeat region, or C terminus of the alpha C protein. Figure 3 shows a series of bands ranging in size from 27 to 40 kDa in the *E. coli* extract containing pSKOF1-13 (tandem repeat region) but not in the extracts of *E. coli* carrying pDEK14 (N terminus) or pDEK15 (C terminus). The top band (40 kDa) corresponds approximately to the expected size of the recombinant gene product (45 kDa). These data indicate that 4G8 specifically

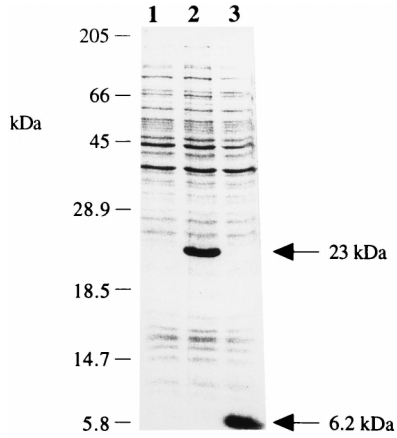

FIG. 2. Expression of the alpha C protein N and C termini as seen in Coomassie blue-stained 15% polyacrylamide gels of extracts of *E. coli* BL21(DE3) containing pET24a (lane 1), pDEK14 (lane 2), and pDEK15 (lane 3) after induction with IPTG. Arrows indicate a 23-kDa peptide that corresponds to the N-terminal fragment and a 10-kDa peptide that corresponds to the C-terminal fragment.

detects an epitope in the tandem repeat region of the alpha antigen. Bands ranging in size from 34 to 95 kDa were found in GBS A909 (used as a positive control for alpha C protein expression) but not in 090 (negative control). This result is similar to the previously observed size range of the alpha C protein bands from A909 (36 to 116 kDa) (19). In addition, bands ranging from 32 to 105 kDa were detected in the extract of *E. coli* containing pJMS23-1. These bands correspond to the approximate sizes of the recombinant alpha C protein (40 to 120 kDa) (21). No bands were detected in the *E. coli* extracts carrying pGEM-7Zf(−) or pET24a (negative controls). Thus, 4G8 detects the alpha C protein from both the native antigen (A909) and the recombinant antigen (pJMS23-1) (19, 21). These data indicate that 4G8 does not bind either terminus and is specific for the alpha C protein tandem repeat region.

ELISA inhibition. ELISA inhibition was used to study the relationship between the relative affinity of monoclonal antibody 4G8 and the numbers of repeats expressed by alpha C protein. Figure 4 indicates that 40-, 133-, and 222-fold higher concentrations of 1-repeat antigen were required than of 2-, 9-, and 16-repeat antigens, respectively, to obtain 50% inhibition of antigen-antibody binding. These data show a high affinity of 4G8 for 2-, 9-, and 16-repeat alpha C protein but a much lower affinity for the 1-repeat alpha C protein. These data are similar to results of other studies of the effects of repeat number on antibody binding (7).

Purification of the alpha C protein N-terminal peptide. The N-terminal peptide expressed from *E. coli* was purified by Ni^{2+} affinity chromatography. Figure 5A shows a Coomassie blue-stained polyacrylamide gel of the cell extracts of *E. coli* BL21 (DE3) containing pDEK14 before and after induction with IPTG, as well as a sample of the eluate from the Ni^{2+} column (lane 3). A single 22-kDa band is seen in the eluate from the Ni^{2+} column, which demonstrates that the recombinant alpha C protein N-terminal peptide was successfully induced, expressed, and purified by Ni^{2+} affinity chromatography.

Raising antibodies to the recombinant alpha C protein N-terminal peptide. Antibodies were raised in rabbits to the recombinant Ni^{2+}-purified N-terminal peptide. Western blot analysis of the postimmunization serum specifically detected a band of ~22 kDa in the extract of *E. coli* carrying pDEK14 after induction with IPTG, which corresponds to the expected size of the N-terminal peptide (data not shown). An ELISA was used to quantitate the titer of the antiserum to the N-terminal peptide. Titers of antiserum were found to be high, in excess of a 1/102,400 dilution.

Western blot analysis was used to determine whether antibodies specific for the alpha C protein N terminus can bind the native protein. Figure 5B shows a ladder pattern of bands with a size range from 36 to 116 kDa detected in extracts prepared from GBS A909, but not in extracts from strain 090 (negative control), probed with the postimmunization serum. This ladder pattern corresponds to the expected size range of the alpha C protein bands (17). Thus, antiserum to the alpha C protein N-terminal peptide can detect native alpha C protein.

Opsonophagocytosis assay using alpha C protein N terminus-specific antibodies. An in vitro opsonophagocytosis assay was used to determine whether antibodies to the alpha C protein N-terminal peptide are opsonic for GBS (6). Figure 6 shows that the postimmunization antiserum kills approximately 0.6 log unit more GBS than the preimmunization serum; this degree of killing is comparable to that by antiserum raised to the whole recombinant molecule (alpha C protein polyclonal serum). As expected, no killing is observed when PMNs are not added or when heat-killed complement is used. These data demonstrate that alpha C protein N-terminus-specific antibodies are opsonic for GBS.

Mouse protection by alpha C protein N terminus-specific antibodies. A mouse protection study was conducted to determine whether alpha C protein N-terminus-specific antibodies can protect neonatal mice against infection with alpha C protein-bearing strains of GBS. Pregnant dams were passively immunized with postimmunization rabbit antiserum raised to the N-terminal peptide, preimmunization rabbit serum (negative control), and rabbit antiserum to the Ia-TT protein-capsular polysaccharide conjugate (positive control) (35). Table 1 shows that 69% of the mice immunized with antibodies to the N-terminal peptide survived challenge with GBS A909, whereas only 15% of those immunized with the preimmunization serum survived. This protection was significant ($P < 0.0001$ by Fisher's exact test). These results show that the antibodies to the alpha C protein N-terminal peptide are significantly more protective than control serum. Therefore, the alpha C protein N terminus contains a protective epitope.

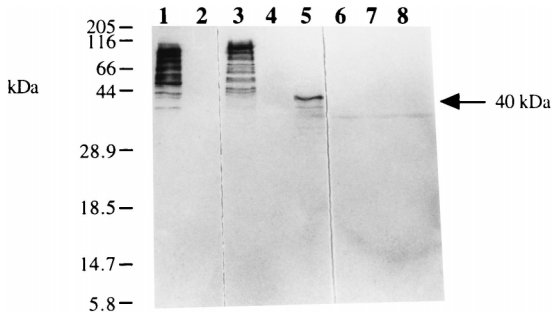

FIG. 3. Detection of epitopes located in the repeat region of the alpha C protein by monoclonal antibody 4G8. Shown are Western blots of extracts of GBS A909 (positive control) (lane 1) and 090 (negative control) (lane 2); *E. coli* DH5α containing pJMS23-1 (positive control) (lane 3), pGEM-7Zf(−) (negative control) (lane 4), and pSKOF1-13 (alpha C protein repeat region) (lane 5); and *E. coli* BL21(DE3) containing pET24a (negative control) (lane 6), pDEK14 (alpha C protein N terminus) (lane 7), and pDEK15 (alpha C protein C terminus) (lane 8). The arrow indicates the 40-kDa band that corresponds to the expressed gene product from pSKOF1-13.

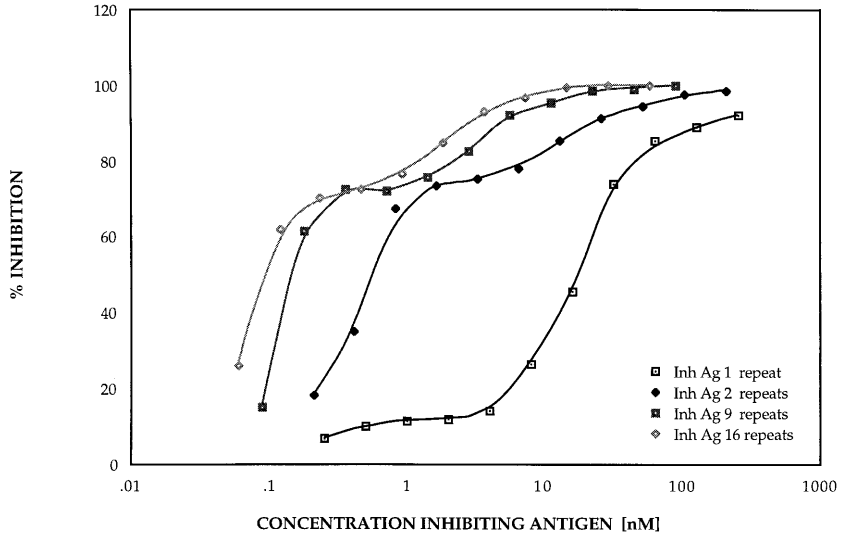

FIG. 4. ELISA inhibition showing relative binding affinities of monoclonal antibody 4G8 to purified alpha C proteins with 1, 2, 9, and 16 repeats. Plates were coated with 9-repeat alpha C protein (0.125 μg/ml). 4G8 was used as the primary antibody (dilution, 1:8,000), and alkaline phosphatase-conjugated antibody to rabbit immunoglobulin G Fc (1:2,000) was used as the secondary antibody. Twofold dilutions of 1-, 2-, 9-, and 16-repeat alpha C protein were made and used as inhibiting antigen (Inh Ag) (starting dilution, 5 μg/ml).

DISCUSSION

The alpha C protein of GBS is a surface-associated protein that is thought to play a role in the virulence of and immunity to GBS. The different structural domains of the alpha C protein, the N terminus, repeat region, and C terminus, may have different biological and immunologic properties. Thus, in order to develop a conjugate vaccine to protect against alpha C protein-bearing strains, the opsonic and protective epitopes of the antigen needed to be mapped. Epitopes of the alpha C protein that are both opsonic and protective have now been localized to the repeat region and the N terminus.

Studies of other surface-associated proteins of GBS that have repetitive sequences (e.g., Rib, epsilon, and type V strains) suggest that there is divergence from the alpha antigen in the N terminus (4, 5, 14, 33). To determine whether the alpha C protein N terminus contained protective epitopes, we raised specific antibodies to the N terminus that conferred passive protection against alpha C protein-bearing strains in a neonatal mouse model. It is interesting that antibodies specific to the alpha C protein N terminus conferred 70% passive protection, whereas the polyclonal antibodies to the 9-repeat alpha C protein conferred 41% passive protection (7). However, polyclonal antibodies elicited to recombinant 1- or 2-repeat alpha C proteins give greater than 75% protection (7). Hypothetically, antibodies directed at the full-length alpha C protein might select for deletions within the repeat region.

A mouse model of GBS infection was used to determine whether alpha C protein tandem repeat deletion mutants are selected in vivo (18). Mice immunized with antibodies to the alpha C protein were challenged with GBS strains expressing the alpha C protein. The sizes of the alpha C proteins in strains of GBS isolated from the spleens of the mice were determined by Western blot analysis. Fifty percent of the recovered GBS strains expressed truncated forms of the alpha C protein, a result suggesting the selection of deletion mutants within the repeat region of the *bca* gene in the presence of antibodies to alpha C protein. Because these mutants were isolated at a relatively high frequency, they may have been protected against opsonophagocytosis by the antibodies to alpha C protein.

In an in vitro opsonophagocytosis assay, strains of GBS with deletions in the *bca* gene were killed less frequently than parental strains by antibodies to the full-length alpha C protein (18). This apparent lesser susceptibility to opsonization may be explained by fewer tandem repeat epitopes in deletion mutants than are present in strains with a full-length alpha C protein. In addition to being fewer in number, the alpha C protein protective epitopes may be conformational. By ELISA inhibition the affinity for recombinant alpha C proteins containing a single repeat is shown to be reduced compared with that for

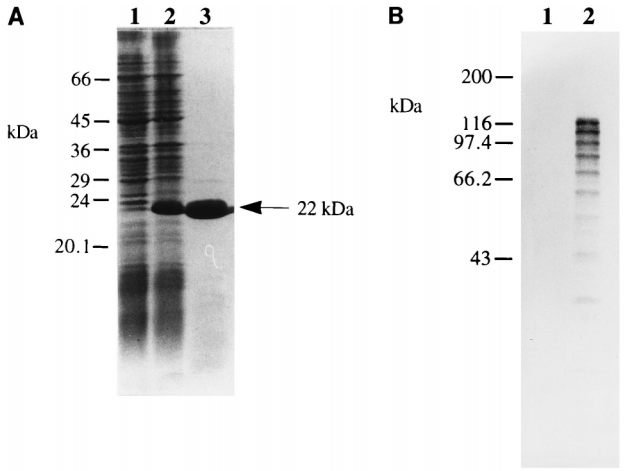

FIG. 5. (A) Induction of expression and purification of the alpha C protein N-terminal peptide. Lanes 1 and 2, Coomassie blue-stained gels of extracts of *E. coli* containing pDEK14 before and after induction with IPTG, respectively; lane 3, 10-μg sample of the eluate from a lysate of *E. coli* containing pDEK14 after Ni^{2+} affinity column chromatography. (B) Detection of native alpha C protein by antibodies to the gene product of pDEK14. Shown are Western blots of extracts of GBS 090 (negative control) (lane 1) and A909 (lane 2) probed with antibodies raised to the alpha C protein N terminus.

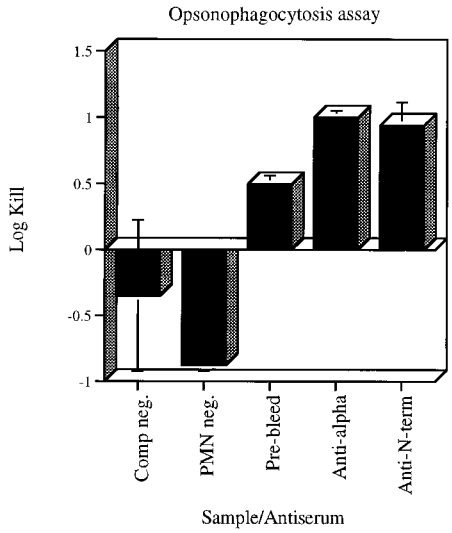

FIG. 6. Results of an opsonophagocytosis assay used to determine whether antibodies to the alpha C protein N-terminal peptide (anti-N-term) were opsonic. Negative controls included the presence of heat-killed complement (comp neg.), the absence of PMN (PMN neg.), and preimmunization sera (pre-bleed). Polyclonal antibodies to the alpha C protein (anti-alpha) served as the positive control. Opsonization is expressed as log-kill: the log number of GBS CFU at the start of the assay minus the log number of GBS CFU after 1 h of incubation.

recombinant proteins containing larger numbers of repeats (7). These antibodies recognize epitopes expressed by proteins containing more (9 or 16) repeats but lose their overall binding affinity for epitopes expressed by proteins containing fewer (1 or 2) repeats.

These observations support the possibility that the epitopes of the alpha C protein tandem repeat region are conformational. Alternatively, the reduced affinity for the single-repeat recombinant protein can also be explained if the recombinant alpha C protein is proteolytically processed. Thus, the 1-repeat recombinant protein may lose repeat region epitopes due to proteolytic processing within the single repeat and may have a reduced ability to inhibit antibody binding to these epitopes. In contrast to epitopes within the repeat region, the N-terminal epitopes are conserved in both parental and deletion mutant strains of GBS A909, and these mutants are susceptible to opsonophagocytosis with antiserum to the N-terminal peptide (18). Therefore, the recombinant alpha C protein N terminus may be a prime candidate for use in a protein-polysaccharide conjugate vaccine.

In this study we localized the protective monoclonal antibody 4G8 to the repeat region of the alpha C protein (19). Attempts to further localize the epitope bound by 4G8 within the repeat region have not been successful. Our unpublished studies using synthetic peptides corresponding to overlapping 10-amino-acid segments within the alpha C protein repeats did not reveal a binding site for 4G8. The inability to define a peptide binding site for 4G8 on the alpha C protein could be explained if the site detected by 4G8 were a conformational epitope or if the binding site contained noncontiguous segments from within the repeat region.

TABLE 1. Passive protection of neonatal mice with N terminus-specific antibodies to GBS

Antiserum	No. of pups surviving/ total no.	% Survival
Ia-TT[a]	45/46	98
Preimmunization[b]	6/39	15
N-terminus	29/42[c]	69

[a] Positive control.
[b] Negative control.
[c] $P < 0.0001$ compared with the preimmunization serum by Fisher's exact test.

Protective epitopes have been mapped within the closely related M protein of group A *Streptococcus* (GAS) (2). In studies assessing whether protective epitopes were localized to the N-terminal half, the N terminus of the M protein was liberated from the cell wall of GAS by pepsin cleavage. Antibodies were raised to the isolated pepsin-cleaved N-terminal fragments (3). Such antibodies have been demonstrated to be opsonic and protective in studies of several M proteins (13). Protective epitopes of the M5, M6, and M24 proteins were mapped to the extreme N-terminal regions of the mature proteins (2), and a synthetic peptide vaccine consisting of these epitopes was developed. This vaccine elicited opsonic antiserum that protected mice against challenge with M5, M6, and M24 strains of GAS.

The identification of two protective epitopes of GBS, the N terminus and the tandem repeat region, will facilitate the development of a conjugate vaccine against GBS. One vaccine strategy would be to use the recombinant alpha antigen containing the N terminus plus 2 tandem repeats (7). This recombinant protein confers passive protection in a neonatal mouse protection assay. However, to increase the efficacy of a vaccine based on the 2-repeat protein, this recombinant antigen can be coupled to several different N-terminal and repeat regions from other surface-associated proteins of GBS, such as the Rib protein, which is commonly found on many serotype III strains. By combining multiple, distinct, and conserved protective epitopes to form a multivalent vaccine, it should be possible to raise antibodies against the majority of clinically significant GBS isolates.

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