Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 4131–4136, April 1996
Microbiology

Group B streptococci escape host immunity by deletion of tandem repeat elements of the alpha C protein

(*Streptococcus agalactiae*/antigenic variation/bacterial antigens/repetitive sequences)

LAWRENCE C. MADOFF*†‡, JAMES L. MICHEL*, ELIZABETH W. GONG*, DAVID E. KLING*, AND DENNIS L. KASPER*

*Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and †Division of Infectious Diseases, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115

Communicated by Frederick M. Ausubel, Massachusetts General Hospital, Boston, MA, December 28, 1995 (received for review November 15, 1995)

ABSTRACT **Group B streptococci (GBS) are the most common cause of neonatal sepsis, pneumonia, and meningitis. The alpha C protein is a surface-associated antigen; the gene (*bca*) for this protein contains a series of tandem repeats (each encoding 82 aa) that are identical at the nucleotide level and express a protective epitope. We previously reported that GBS isolates from two of 14 human maternal and neonatal pairs differed in the number of repeats contained in their alpha C protein; in both pairs, the alpha C protein of the neonatal isolate was smaller in molecular size. We now demonstrate by PCR that the neonatal isolates contain fewer tandem repeats. Maternal isolates were susceptible to opsonophagocytic killing in the presence of alpha C protein-specific antiserum, whereas the discrepant neonatal isolates proliferated. An animal model was developed to further study this phenomenon. Adult mice passively immunized with antiserum to the alpha C protein were challenged with an alpha C protein-expressing strain of GBS. Splenic isolates of GBS from these mice showed a high frequency of mutation in *bca*—most commonly a decrease in repeat number. Isolates from nonimmune mice were not altered. Spontaneous deletions in the repeat region were observed at a much lower frequency (6×10^{-4}); thus, deletions in that region are selected for under specific antibody pressure and appear to lower the organism's susceptibility to killing by antibody specific to the alpha C protein. This mechanism of antigenic variation may provide a means whereby GBS evade host immunity.**

Group B streptococci (GBS) are the leading cause of pneumonia, sepsis, and meningitis in neonates in the United States and are increasingly recognized as a cause of invasive infection in immunocompromised and debilitated adults (1–3). GBS are part of the normal vaginal microflora of many women and colonize neonates during birth. Neonatal resistance to invasive GBS infection is related, in part, to maternal antibodies to the type-specific polysaccharides acquired transplacentally (4). On the basis of these findings, maternal immunization has been suggested as a strategy for the prevention of GBS infection in neonates (5).

The alpha C protein of GBS is a surface-expressed antigenic determinant present on many clinical isolates that is capable of eliciting protective antibody-mediated immunity in experimental animals (6–8). The alpha C protein, like the capsular polysaccharide of GBS and the M proteins of group A streptococci, may confer resistance to opsonophagocytic killing in the absence of type-specific antibody (9). The gene, *bca*, encoding the prototype alpha C protein from strain A909 has been sequenced, and several domains have been defined, including a signal sequence and a C-terminal membrane anchor domain that are typical of those found in other surface proteins of Gram-positive cocci (10). An N-terminal region encoding 185 aa is followed by a series of nine identical tandem repeats of 246 nt. The antigen appears as a series of evenly spaced bands on SDS/PAGE. Clinical isolates of GBS that express the alpha C protein display antigens of molecular size that varies with the number of tandem repeats contained within the gene; we have reported strains of GBS that express alpha C proteins ranging in size from 62.5 to 167 kDa (9).

We have previously shown that isolates of GBS vary in their susceptibility to opsonophagocytic killing in the presence of monoclonal antibody to the alpha C protein and that the extent of killing is proportional to the size of the alpha C protein expressed and to the number of tandem repeats within the gene (9). We also previously described a series of paired strains of GBS obtained from mothers and neonates in Majorca, Spain. Among 14 pairs that expressed the alpha C protein, two isolates from neonates showed a diminution in molecular size of the alpha C protein when compared with the corresponding maternal isolate (11).

Because nucleotide sequence repeats are hot spots for genetic recombination, we hypothesized that size variability in the alpha C protein results from recombination of intragenic repeats. We thus considered the possibility that this size change represented a form of antigenic variation, perhaps selected for by the presence of maternal antibody. Diminution in repeat number and in the corresponding size of the alpha C protein might reduce the ability of alpha-specific serum to opsonize a given strain for phagocytic killing.

We demonstrate that the neonatal isolates with altered alpha C proteins arose from the maternal isolate by intragenic deletion in *bca*. We further show that such deletions occur frequently in a mouse model of GBS infection in the presence of immune serum raised to the alpha C protein. Thus, deletions within the repeat-containing alpha C protein of GBS appear to play a role in evasion of type-specific host immunity in GBS infection by causing antigenic variation.

MATERIALS AND METHODS

Bacterial Strains. Strain A909 is the prototype Ia/C GBS strain originally obtained from the collection of Rebecca Lancefield (Rockefeller University, New York) (12). Clinical isolates of GBS were obtained from vaginal culture of parturient women prospectively studied at a single maternity hospital in Majorca, Spain, and from surface sites on their neonates as described (11). The isolates that were found by immunoblot with monoclonal antibody to express the alpha C protein were chosen for further study.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GBS, group B streptococci; cfu, colony-forming unit(s).

‡To whom reprint requests should be addressed at: Channing Laboratory, Brigham and Women's Hospital, 180 Longwood Avenue, Boston, MA 02115.

Antisera. To generate antiserum to the alpha C protein, that protein was expressed in a phage T7 polymerase system. The *bca* gene encoding the prototype alpha C protein from strain A909 of GBS was subcloned into plasmid pT7–7 (13). PCR mutagenesis was used to create a new *Nde* I site at the ATG start site of the *bca* gene and thus ligate the start site of the gene with the optimal spacing from the T7 promoter and ribosome-binding site on the plasmid (14). Purification of recombinant alpha C protein to homogeneity was achieved with ion exchange followed by gel filtration chromatography. The final product (r-alpha) was >99% pure as assessed by silver-stained polyacrylamide gel and compared with Western blot by monoclonal antibody (data not shown). Two New Zealand White rabbits (6–8 kg) were immunized s.c. with 50 μg of r-alpha emulsified in complete Freund's adjuvant and then immunized 3 and 6 weeks later with the same dose in incomplete Freund's adjuvant. Immune serum was obtained 2 weeks after the final boost. Western blot analysis used 4G8, a protective monoclonal antibody directed toward the repeat region of the alpha C protein as described (15).

Isolation of Spontaneous Mutants. To determine the frequency of spontaneous alpha C protein deletions, a single colony of GBS strain A909 was inoculated into Todd–Hewitt broth (THB) and grown overnight at 37°C. A 10^{-4} dilution of overnight cultures (10 μl) was spread on blood agar plates and incubated overnight at 37°C. Individual colonies were transferred onto Todd–Hewitt broth plate grids and incubated overnight at 37°C; the colonies were screened for alpha antigen expression by colony blot hybridization.

Colony Immunoblots. Disks of nitrocellulose were placed on the plates containing colonies of GBS and lifted to remove surface proteins (16). The disks were placed in a blocking buffer {[phosphate-buffered saline (PBS)]/0.5% Tween 20/5% skim milk} and gently rocked for 30 min at room temperature. The blocking solution was removed, and ascites containing the alpha antigen monoclonal antibody 4G8 was added at a 1:1000 dilution in PBS/0.5% Tween 20. After a 1-h incubation, blots were washed three times with PBS/0.5% Tween 20 and incubated with alkaline phosphatase-conjugated anti-mouse antibodies at a 1:1000 dilution in PBS/0.5% Tween 20 for 1 h at room temperature. Blots were then washed twice in PBS/0.5% Tween 20 and once in $MgCl_2/Na_2CO_3$. Alkaline phosphatase development reagent (Sigma) was added to detect the conjugate.

Opsonophagocytic Assay. The functional capacity of antibodies to the alpha C protein of GBS was assessed by an opsonophagocytic assay measuring *in vitro* killing of GBS (17). In brief, a 300-μl vol of human polymorphonuclear leukocytes ($\approx 3 \times 10^6$ cells) was mixed with the test GBS strain [$\approx 1.5 \times 10^6$ colony-forming units (cfu)], 50 μl of human serum (as a complement source), and 100 μl of antibody diluted 1:100. Viable GBS cells were enumerated as 10-fold dilutions on blood agar plates immediately and after 60-min incubation at 37°C; the difference was calculated as killing. The result is reported as the "log kill," which is the difference between log cfu before and after incubation for at least two measurements per strain. Human serum was prepared for use as a complement source by absorption on ice for 30 min with GBS of homologous serotype. The bacteria were removed by centrifugation and filter sterilization, and the absorbed serum was stored in aliquots at −80°C.

Isolation of DNA from GBS. Chromosomal DNA was prepared from GBS strains by a modification of published methods (16). Briefly, late stationary cultures of GBS in Todd–Hewitt broth (100 ml) were washed with 0.2 M sodium acetate, suspended in 10 ml of Tris/EDTA and glucose [0.1 M Tris (pH 7.0), 0.01 M EDTA, and 25% glucose], and incubated with 200 μg of *N*-acetylmuramidase (Mutanolysin; Sigma) for 1 h at 37°C. The suspension was pelleted and then resuspended in 9 ml of Tris/EDTA with 0.5% Sarkosyl; 10 μg of RNase and 5 mg of Pronase were added sequentially, each for 10 min at 37°C. The mixture was extracted with phenol/chloroform/isoamyl alcohol, ethanol precipitated, and spooled onto a glass rod. The DNA was air-dried, rinsed with 70% ethanol, and dissolved in 1 ml of H_2O.

Restriction Enzyme Analysis of Chromosomal DNA. Chromosomal DNA was analyzed for restriction fragment length polymorphisms by the method of Fasola *et al.* (18). Briefly, 10-μg samples of chromosomal DNA were incubated with *Hae* III, 60–100 units at 37°C for 18 h. Agarose gel electrophoresis was performed, and the bands were visualized with ethidium bromide.

Mouse Passage. A mouse model of infection was developed to demonstrate selection for GBS strains with deletions or truncations of the alpha C protein gene. CD-1 female outbred mice (6–8 weeks of age; Charles River Breeding Laboratories) were rendered immune to lethal challenge with GBS by passive immunization with alpha-specific antiserum (or preimmune control antiserum), 0.25 ml i.p. Mice were challenged 24 h later with lethal doses of the A909 (prototype Ia/C) strain of GBS [10^5 for preimmune or 10^6–10^7 for postimmune grown to mid-logarithmic phase (A_{650} = 0.3) diluted in Todd–Hewitt broth] delivered i.p. After 24 h, mice were killed, and the spleens were homogenized and plated. GBS isolates were then examined for expression of alpha C protein by Western blot analysis with alpha-specific monoclonal antibody.

Southern Blot Hybridization. Digests of chromosomal DNA were separated on agarose gel, denatured, and transferred onto GeneScreen hybridization transfer membranes (NEN) by UV cross-linking (Stratalinker 1800, Stratagene). Hybridizations were performed with a commercial kit (enhanced chemiluminescence; Amersham) according to the manufacturer's instructions. Briefly, the support is prehybridized to reduce nonspecific binding and then probed with peroxidase-labeled single-stranded DNA fragments, which are then oxidized to emit light. Fragments were probed with the entire *bca* gene in the expression plasmid pT7–7 (10, 13).

PCR. PCR was used to analyze the gene size and better define the region of the gene where deletions occurred. The forward primer was chosen within the region encoding the N terminus of the gene: 5′-TGCAGAGTACAGGAAGGGCT-3′, and the reverse primer began 30 bases 3′ to the gene: 5′-TGTTCACACCAATAAATGGTGA-3′ (Fig. 1). Chromosomal DNA from each mutant or clinical isolate (200 ng) was used as template, 50 pM of each primer was added, and PCR was performed with *Thermus aquaticus* (*Taq*) DNA polymerase (5 units), its accompanying buffer, dNTP (0.05 mM), and $MgCl_2$ (1 mM) (Boehringer Mannheim) in a total volume of 100 μl. The reaction was cycled through 94°C for 1 min, 63°C for 1 min, 72°C for 2 min 25 times, with a terminal extension at 72°C for 7 min in a DNA thermal cycler model 480

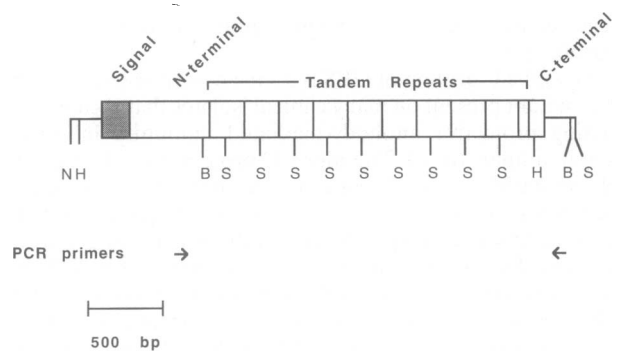

FIG. 1. Map of *bca* gene showing partial restriction endonuclease map, regions of the protein from the deduced amino acid sequence, and locations of primers used for PCR-amplification of the repeat region. N, *Nde* I; H, *Hin*dIII; S, *Sty* I; B, *Bsa*BI (*Mam* I).

(Perkin–Elmer/Cetus) (14). PCR products were examined by electrophoresis on 1.5% agarose gel.

RESULTS

Opsonophagocytic Killing of Maternal and Infant Isolates in the Presence of Alpha-Specific Antiserum. As previously noted (11), 2 of the 14 GBS isolates from infants expressed alpha C proteins of smaller molecular size than the protein expressed in isolates recovered from their mothers (Fig. 2). To determine whether the observed change in the phenotype of the alpha C protein would confer a biologic change to the organism, the discordant maternal and neonatal isolates were incubated with antibody specific for the alpha C protein in the presence of human serum complement and human peripheral blood leukocytes for 1 h. In each case, the maternal isolates were susceptible to opsonic killing by antiserum to the alpha C protein, but the neonatal isolates resisted such killing and proliferated (Table 1). Thus, the neonatal isolates, with deletions in alpha, were resistant to killing in the presence of alpha-specific antibody. In the absence of alpha-specific antibody, all strains were able to proliferate (data not shown).

Restriction Enzyme Analysis of Chromosomal DNA and Southern Blot Hybridization. All 14 pairs were subjected to restriction analysis of chromosomal DNA by digestion with *Hae* III and electrophoresis on agarose gel. Corresponding maternal–infant isolates appeared identical, except for those with discrepant alpha C protein size (representative strains shown in Fig. 3*A*). In each of these two pairs of isolates, a single band differed between maternal and infant isolates (arrows). Each pair of isolates could be distinguished from the others by the restriction fragment pattern.

Southern hybridization performed with the *bca* gene delineated a single band in each strain (Fig. 3*B*). In the two discrepant strain pairs, this band comigrated with the altered band seen on the ethidium bromide-stained gel. This result indicated that the band accounting for the discrepancy on restriction enzyme analysis of chromosomal DNA contained the gene for the alpha C protein and was consistent with an alteration in the size of the gene causing the discrepancy in restriction fragment pattern. Among the concordant strains, the migration of the hybridizing band was the same within each pair.

Frequency of Alpha Antigen Spontaneous Deletions in GBS. Colonies of GBS were screened for the lack of alpha antigen expression to determine the frequency of alpha antigen spontaneous deletion mutants. Derivatives of a single colony of A909 expressing the nine-repeat alpha C protein were screened for alpha expression by colony immunoblot; 3.06×10^3 cfu were screened with the alpha antigen monoclonal antibody 4G8 as a probe (7). Two colonies were not detected with this antibody. These independent isolates were confirmed to contain deletions of five tandem repeats by Western and Southern blot analyses (data not shown), thereby indicating that the frequency of alpha spontaneous deletions is $\approx 6.5 \times 10^{-4}$.

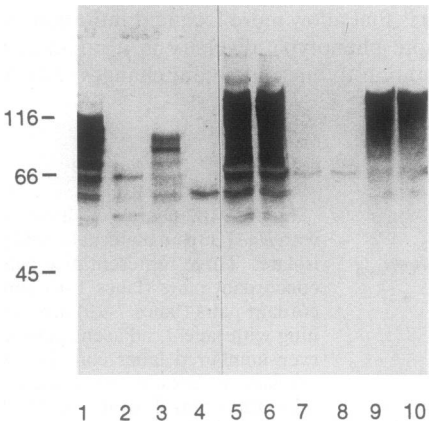

FIG. 2. Western blots with alpha-specific monoclonal antibody of mother–infant paired isolates from Majorca, Spain. Strain 73 (lane 1) is the maternal isolate and strain 72 (lane 2) is the corresponding neonatal isolate. Strain 144 (lane 3) is the maternal isolate and strain 140 (lane 4) is the corresponding neonatal isolate. Lanes 5–10 show representative maternal–neonatal pairs in which the sizes of the alpha C protein were the same. The size discrepancies in lanes 1–4 were previously noted (11) but are illustrated here for clarity.

Mouse Passage Experiments. To determine whether selective pressure could account for the high observed frequency of deletions among neonatal GBS, GBS were passaged through mice rendered immune to the alpha C protein. Mice were passively immunized with either immune rabbit antiserum raised to the recombinant alpha C protein or preimmune rabbit serum. They were then challenged with the prototype alpha C protein-positive GBS strain. Isolates recovered from the spleens of four immune and four nonimmune mice were randomly chosen (28 per mouse) for Western blot analysis with alpha-specific antiserum. Of the isolates from immune mice, 49 (44%) showed no alpha C protein, 34 (30%) showed immunoreactive alpha C protein of smaller molecular mass (range 46–100 kDa), and 29 (26%) showed full-sized alpha C protein (representative Western blots shown in Fig. 4). All 112 isolates from the control animals immunized with nonimmune serum showed full-sized alpha C protein. To determine the consequences of the observed mutations for antibody-mediated opsonophagocytic killing of the strains, representative mouse-passaged isolates were incubated in the presence of human peripheral blood leukocytes, serum as a complement source, and rabbit antibody specific for the alpha C protein for 1 h (as for the human isolates). Each of the truncated and deleted mutants showed decreased killing relative to the parent strain (Table 1). One isolate (number 25) with intact, full-sized alpha was also tested and remained susceptible to opsonophagocytic killing.

Analysis of GBS Strains by PCR of the *bca* Repeat Region. To further define the location of the mutations occurring in the passaged GBS strains and in the human maternal–neonatal strain pairs, the tandem repeat region of the *bca* gene was amplified using PCR. Five of the strains isolated after mouse passage, which represented the diversity of strains recovered (including one strain that did not express immunoreactive alpha, one apparently unaltered strain, and three expressing differing sizes of alpha C protein), were further examined by PCR of the repeat region of *bca*. The discrepant maternal–neonatal paired clinical isolates were also examined by PCR of the alpha repeat region. The sizes of the repeat region of the alpha C protein genes from the Majorca isolates and the mouse-passaged isolates were determined by PCR with primers flanking this region. The size of the PCR products correlated with the molecular mass of the largest protein band as seen on Western blot analysis with 4G8 antibody (Table 1). The smallest PCR fragment generated (mutants 1 and 5) corresponded to one full repeat and the additional 33 nt of a partial repeat.

DISCUSSION

The alpha C protein of GBS is a known target of protective immunity (6–9, 12, 19). Although the biological function is unknown, its most striking structural feature is a series of nine tandem repeats that are identical at the nucleotide level (10). Repeat sequences in surface proteins from Gram-positive bacteria have been suggested to play a role in bacterial binding to host structures (20, 21). However, the identical nature of the repeat sequences at the DNA level, rather than allowing for conservative substitutions, suggests that the repeats play an important role at the nucleotide level. We hypothesized that this degree of conservation between repeats must have adaptive importance to have been preserved through evolution.

Table 1. Characteristics of GBS strains and opsonophagocytic killing in the presence of alpha antigen-specific rabbit antiserum

Strain number	Alpha C protein molecular mass (kd)	Source of isolate	PCR fragment size (kb)	Calculated number of full repeats	Killing (log cfu ± SD)*
A909†	116	Prototype	2.30	9	1.3 ± 0.03
A909 M1	None	Mouse passaged	0.18	1	−0.13 ± 0.6
A909 M5	38	Mouse passaged	0.17	1	0.39 ± 0.3
A909 M21	46	Mouse passaged	0.45	2	0.76 ± 0.4
A909 M10	70	Mouse passaged	1.03	4	0.02 ± 0.6
A909 M25	116	Mouse passaged	2.37	9	1.40 ± 0.09
73 (Mother A)‡	116	Human maternal	2.31	9	0.76 ± 0.07
72 (Neonate A)	74	Human neonatal	1.34	5	−0.096 ± 0.06
144 (Mother B)	100	Human maternal	1.76	7	0.34 ± 0.12
140 (Neonate B)	62	Human neonatal	1.02	4	−0.23 ± 0.1

*Amount of test strain killed after 1-h incubation with antiserum, serum complement, and human PMNs.
†Prototype Ia/C strain and parent for mouse-passaged mutants.
‡The size discrepancies between the alpha C proteins in these human maternal and neonatal isolates have been previously noted (11) and are listed here for clarity.

Repeating sequences of DNA are common among many proteins of Gram-positive bacteria and indeed among antigens of many other species of prokaryotes and eukaryotes (22–28).

We have shown that strains of GBS passed from mother to neonate, an important step in the pathogenesis of neonatal GBS infection, undergo mutations in the repeat region of the *bca* gene and that these mutations coincide with a loss of susceptibility to antibody-mediated killing by polymorphonuclear leukocytes. The resulting repeat number variants are presumably less well-recognized by antibody and thus less susceptible to opsonophagocytic killing. We further demonstrate that these mutations can occur both *in vitro* and *in vivo* and are readily selected for in an animal model of invasive GBS infection. All of the mutants studied contain fewer repeats than the parent strain and are less susceptible to alpha-specific antibody-mediated opsonophagocytic killing than the wild-type strain. However, there does not appear to be exact direct correlation between the size of the deletion and the magnitude of antibody-mediated opsonophagocytic killing. This variability in killing may be due to the inherent imprecision of the assay (which comprises several biologic components), to unobserved phenotypic changes occurring in the mutants, or to other immunologic factors such as conformational presentation of the antigen. Many of the mutants obtained *in vivo* appear to contain one full repeat and one partial repeat, a finding that supports (but does not prove) the hypothesis that homologous recombination of intragenic repeats is the mechanism of mutation. It is interesting that one of the mutants (strain 1) generates a PCR product of the repeat region that would correspond to one full and one partial repeat. Yet the strain fails to express a protein that reacts with antiserum to intact alpha C protein, which suggests that, after deletion, another mutation completely blocks expression of the alpha C protein.

The M proteins of group A streptococci are the best characterized of Gram-positive surface proteins and have similarities with the alpha C protein (29). Like the alpha C protein, they are targets of protective immunity, protect the organism from phagocytic killing, and share sequence homology in signal and C-terminal membrane anchor regions. The M6 protein of group A streptococci is a surface antigen important in virulence of and immunity to this bacterium that contains intragenic repeating sequences not identical at the nucleotide level (23, 30). These organisms undergo spontaneous mutations in the *emm6* gene by homologous recombination at intragenic repeats that result in variation in size and in antigenic and opsonic epitopes. These variants appear to occur at a lower frequency than do variants of the alpha C protein ($\approx 10^{-6}$ isolates per cfu), perhaps owing to the nonidentical nature of the tandem repeats in the *emm6* gene (31). Other M proteins also contain nonidentical repeats and probably exhibit this behavior (30). However, the biologic consequences of these mutations in natural infections are not known.

Moxon has proposed the concept of "contingency" loci; these are sites that allow more frequent mutations to occur so as to promote phenotypic diversity in a population and enhance adaptation to environmental changes (32). Many such

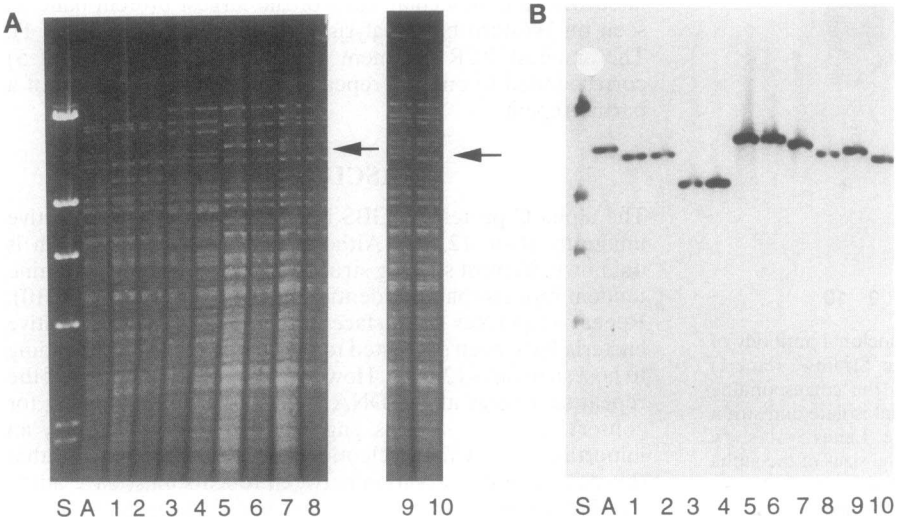

FIG. 3. (*A*) Digests of chromosomal DNA with *Hae* III from maternal–child paired GBS isolates. Three representative pairs of the 12 concordant pairs (lanes 1–6) and the 2 discordant pairs (lanes 7–10) are shown. Beginning with lane 1, adjacent pairs of odd- and even-numbered lanes contain maternal and neonatal isolates, respectively. Lane A contains the control strain A909. The arrows show aberrant bands present in lanes 7 and 9 (maternal strains 144 and 73) not visible in lanes 8 and 10 (corresponding neonatal strains 140 and 72). Concordant strains were identical. (*B*) Southern blot of the same gel hybridized with a probe for the alpha C protein gene *bca*. The aberrant bands are caused by a change in the size of the restriction fragment containing the *bca* gene.

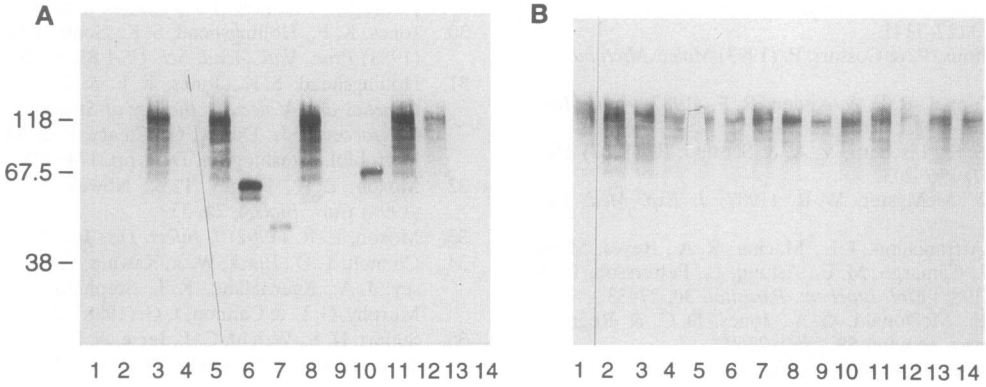

FIG. 4. Sequential strains of GBS isolated from the spleens of a mouse that received (*A*) alpha-specific immune serum or (*B*) received preimmune serum followed by challenge with alpha-positive GBS. Extracts of the strains are immunoblotted with alpha C protein-specific monoclonal antibody. All of the strains recovered from the nonimmune animals expressed the wild-type alpha phenotype. A high proportion of those from the immune animals expressed an altered phenotype.

highly mutable loci in pathogenic bacteria incorporate repeated DNA elements that may affect the expression of a phenotypic element. For example, strains of *Haemophilus influenzae* may either express or lack fimbriae. The switching mechanism was found to lie in a dinucleotide repeat (33). The number of repeats upstream of the gene controls the spacing between the −10 and −35 consensus sequence elements and thereby controls the efficiency of RNA polymerase binding and thus the level of fimbrial protein expression. Antigenic variation is a widely described phenomenon among pathogenic microorganisms, and a variety of genetic mechanisms underlie these phenotypic changes. Other methods of antigenic variation in bacterial proteins have been described that involve the introduction of DNA from outside the gene. For example, gonococcal pilin genes undergo homologous recombination with partial pilin genes outside of the region of expression, thus producing an antigenically altered pilin protein (34, 35). *Borrelia* species produce variable major protein variation by replacement of an active gene with an inactive gene (36). Antigenic variation appears to be an important and common mechanism of adaptation for pathogens that, to our knowledge, has not previously been described in GBS.

The striking identity of the repeats in the alpha C protein and the existence of a known protective epitope within the repeat lead us to hypothesize that the alpha C protein gene undergoes mutation, most likely by homologous recombination of identical intragenic repeats, which induces an alteration in the size (and repeat content) of the antigen. This change must affect the protective epitope in its interaction with antibody and thus allows the organism to elude antibody-mediated host immunity. This antigenic change could result from a decrease in the number of antibody-binding sites, alteration in the conformation of antibody-binding sites, and/or by decreased exposure of the antigen on the surface of the organism.

The observation of decreases in tandem repeat number of the alpha C protein under selective antibody pressure raises the question of what conditions might select for a larger number of tandem repeats. Such conditions must exist; otherwise one would expect all strains to display a small number of repeats, whereas, in fact, wild-type clinical isolates typically display 9 or 10 tandem repeats (9). Because other repeat-containing proteins are involved in binding to host factors (21), perhaps the repeat region of the alpha C protein is involved in colonization by GBS, and replication under colonizing conditions would favor a larger number of repeats.

A more thorough study of the phenomenon of antigenic variation by repeat number alteration has practical significance in the rational design of protein-based bacterial vaccines. A better understanding of the immunologic characteristics that allow protective epitopes to be altered should improve our ability to develop vaccines protective against the numerous pathogens expressing repeat-containing antigens.

We gratefully acknowledge the participation of Juan Hervas and Vincent Benedi in providing the maternal and neonatal clinical isolates. We thank Michael Wessels for valuable advice and discussion and Joanna Goldberg for thoughtful review of the manuscript. This research was supported in part by U.S. Public Health Service Contract N01-AI-25152 and National Institutes of Health grants AI00981, AI28500, AI08222, and AI23339.

1. Baker, C. & Edwards, M. (1990) in *Infectious Diseases of the Fetus and Newborn Infant*, eds. Remington, J. S. & Klein, J. O. (Saunders, Philadelphia), pp. 742–811.
2. Farley, M. M., Harvey, R. C., Stull, T., Smith, J. D., Schuchat, A., Wenger, J. D. & Stephens, D. S. (1993) *N. Engl. J. Med.* **328,** 1807–1811.
3. Madoff, L. C. & Kasper, D. L. (1993) in *Obstetric and Perinatal Infections*, ed. Charles, D. (Mosby, St. Louis), pp. 210–224.
4. Baker, C. J. & Kasper, D. L. (1976) *N. Engl. J. Med.* **294,** 753–756.
5. Baker, C. J. & Kasper, D. L. (1985) *Rev. Infect. Dis.* **7,** 458–467.
6. Bevanger, L. & Naess, A. I. (1985) *Acta Pathol. Microbiol. Immunol. Scand. B* **93,** 121–124.
7. Madoff, L. C., Michel, J. L. & Kasper, D. L. (1991) *Infect. Immun.* **59,** 204–210.
8. Michel, J. L., Madoff, L. C., Kling, D. E., Kasper, D. L. & Ausubel, F. M. (1991) *Infect. Immun.* **59,** 2023–2028.
9. Madoff, L. C., Hori, S., Michel, J. L., Baker, C. J. & Kasper, D. L. (1991) *Infect. Immun.* **59,** 2638–2644.
10. Michel, J. L., Madoff, L. C., Olson, K., Kling, D. E., Kasper, D. L. & Ausubel, F. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10060–10064.
11. Hervas, J. A., Gonzalez, L., Gil, J., Paoletti, L. C., Madoff, L. C. & Benedi, V. J. (1993) *Clin. Infect. Dis.* **16,** 714–718.
12. Lancefield, R. C., McCarty, M. & Everly, W. N. (1975) *J. Exp. Med.* **142,** 165–179.
13. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
14. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1993) *Current Protocols in Molecular Biology* (Wiley, New York).
15. Madoff, L. C., Michel, J. L. & Kasper, D. L. (1991) *Infect. Immun.* **59,** 204–210.
16. Wanger, A. R. & Dunny, G. M. (1987) *Infect. Immun.* **55,** 1170–1175.
17. Baltimore, R. S., Kasper, D. L., Baker, C. J. & Goroff, D. K. (1977) *J. Immunol.* **118,** 673–678.
18. Fasola, E., Livdahl, C. & Ferrieri, P. (1993) *J. Clin. Microbiol.* **31,** 2616–2620.
19. Stalhammar, C. M., Stenberg, L. & Lindahl, G. (1993) *J. Exp. Med.* **177,** 1593–1603.

20. Gaillard, J. L., Berche, P., Frehel, C., Gouin, E. & Cossart, P. (1991) *Cell* **65,** 1127–1141.
21. Dramsi, S., Dehoux, P. & Cossart, P. (1993) *Molec. Microbiol.* **9,** 1119–1122.
22. Kemp, D. J., Coppel, R. L. & Andeu, R. F. (1987) *Annu. Rev. Microbiol.* **41,** 181–208.
23. Hollingshead, S. K., Fischetti, V. A. & Scott, J. R. (1987) *Mol. Gen. Genet.* **207,** 196–203.
24. Wallis, A. E. & McMaster, W. R. (1987) *J. Exp. Med.* **166,** 1814–1824.
25. Ibanez, C. F., Affranchino, J. L., Macina, R. A., Reyes, M. B., Leguizamon, S., Camargo, M. E., Aslund, L., Pettersson, U. & Frasch, A. C. (1988) *Mol. Biochem. Parasitol.* **30,** 27–33.
26. Anderson, B. E., McDonald, G. A., Jones, D. C. & Regnery, R. L. (1990) *Infect. Immun.* **58,** 2760–2769.
27. Yother, J. & Briles, D. E. (1992) *J. Bacteriol.* **174,** 601–609.
28. Zheng, X., Teng, L. J., Watson, H. L., Glass, J. I., Blanchard, A. & Cassell, G. H. (1995) *Infect. Immun.* **63,** 891–898.
29. Fischetti, V. A. (1991) *Sci. Am.* **264,** 58–65.
30. Jones, K. F., Hollingshead, S. K., Scott, J. R. & Fischetti, V. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8271–8275.
31. Hollingshead, S. K., Jones, K. F. & Fischetti, V. A. (1991) in *Genetics and Molecular Biology of Streptococci, Lactococci, and Enterococci*, eds. Dunny, G., Cleary, P. & McKay, L. (Am. Soc. Microbiol., Washington, DC), pp. 174–178.
32. Moxon, E. R., Rainey, P. B., Nowak, M. A. & Lenski, R. E. (1994) *Curr. Biol.* **4,** 24–33.
33. Moxon, E. R. (1992) *J. Infect. Dis.* **165,** Suppl. 1, S77–S81.
34. Connell, T. D., Black, W. J., Kawula, T. H., Barritt, D. S., Dempsey, J. A., Kverneland, K. J., Stephenson, A., Schepart, B. S., Murphy, G. L. & Cannon, J. G. (1988) *Mol. Microbiol.* **2,** 227–236.
35. Seifert, H. S., Wright, C. J., Jerse, A. E., Cohen, M. S. & Cannon, J. G. (1994) *J. Clin. Invest.* **93,** 2744–2749.
36. Barbour, A. G., Carter, C. J., Burman, N., Freitag, C. S., Garon, C. F. & Bergstrom, S. (1991) *Infect. Immun.* **59,** 390–397.