bca, Beta Gene, and Gene Product Divergency in Reference and Prototype Strains of Streptococcus agalactiae

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Reference and prototype strains of Streptococcus agalactiae (GBS) were originally selected on the basis of phenotypic traits which, however, do not always mirror genotypic traits. A total of 14 reference and prototype GBS strains were examined by PCR designed to detect the bca and beta genes, encoding the c proteins cα and cβ, respectively. The cognate proteins were detected by whole-cell-based fluorescent antibody testing and Western blotting. The PCR for beta gene detection and the antibody-based cβ protein detection showed concordant results with all of the isolates, whereas 7 of 14 strains which did not express cα protein at detectable levels contained bca gene elements, consistent with bca gene and gene product divergency in these strains. The results emphasize the importance of genetic characterization of reference and prototype strains of GBS which, in the past, have been selected on the basis of phenotypic traits.

Streptococcus agalactiae (group B streptococci) (GBS) is an important cause of infections in humans, particularly neonatal infections. Serotyping is widely used in epidemiological studies of GBS infections and has traditionally been based on capsular polysaccharide antigens of which the antigens Ia, Ib, and II through VIII are presently known. In addition, the surface-anchored c proteins cα and cβ (5) and the R proteins R1 through R4 (20) are important GBS serotype markers. Serotyping based on both the capsular polysaccharide and the protein antigens enable subdivision of GBS into a variety of serovariants (8). Both the capsular antigens and the surface-localized proteins function as virulence factors and as targets of protective antibodies (1, 10, 11, 17, 18).

The genes encoding the GBS protein serotype markers cα, cβ, and the protein Rib, which may be identical to the R4 protein (3), have been sequenced (7, 15, 19). The beta gene product, the cβ protein, has a signal sequence, an N terminus, a cell wall- and cell membrane-spanning C-terminal domain, and two functional domains (A and B) which mediate binding of immunoglobulin A (7). The bca gene encoding the cα protein is different and, in the GBS strain A909, encodes a signal sequence, an N terminus, nine identical tandem repeats (82 amino acids in each repeat), a partial repeat, and a C terminus (15). The bca gene may undergo mutational deletion of repeats, and this results in mutants which may escape immune clearance (9). PCRs for detection of both the bca and beta genes have been described (9, 13, 14).

Over the years, reference and prototype strains of GBS have been selected solely on the basis of phenotypic traits such as surface-exposed proteins defined on the basis of immunoprecipitation testing. However, as the technology makes it possible, data concerning phenotypes of these strains should be supplemented by data concerning genotypes. The importance of molecular analyses of such strains has recently been emphasized by the findings that clinical GBS strains which are negative in fluorescent antibody testing (FAT) of proteins cα and cβ nevertheless may harbor corresponding gene elements (12, 13). In particular, bca/cα protein discrepancy occurred quite frequently (12). Since properties of strains are crucial in the context of research and reagent developments, such as generation of antibodies for serotyping, we have tested our collection of reference and prototype GBS isolates for the presence of the bca and beta genes and expression of the products of these genes.

GBS was cultured on blood agar plates or in Todd-Hewitt broth. The beta gene PCR was performed as described previously using a primer set which resulted in the amplification of a 620-bp product (13). The PCR product included a part of each of the domains A and B of the beta gene (7). For the bca gene, primers were designed that supported amplification of a 202-bp region within the bca gene, consistent with bca gene and gene product divergency in these strains. The results emphasize the importance of genetic characterization of reference and prototype strains of GBS which, in the past, have been selected on the basis of phenotypic traits.

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TABLE 1. Testing by PCR of \textit{S. agalactiae} reference and prototype strains for the beta and \textit{bca} genes

| Strain          | Serotype | Beta PCR result | \textit{bca} PCR result (category) |
|-----------------|----------|-----------------|-----------------------------------|
| ATCC 12400; 090 | Ia       | –               | + (C)                             |
| NCTC 11078; A909| Ia/c\textit{c}\textsubscript{b}/c\textsubscript{p} | + | + (A) |
| NCTC 12906; 335 | Ia\textit{c} | –               | + (A)                             |
| NCTC 12907; 70339| Ia\textit{c}/R4 | + | + (B) |
| ATCC 12401; H36B | Ib\textit{c}/c\textsubscript{p} | + | + (A) |
| NCTC 11079; 18RS21 | II      | –               | – (D)                             |
| ATCC 12403; D136C | III/R1  | –               | + (C)                             |
| 65604 (3)       | III/R4   | –               | – (D)                             |
| BM110; Rib\textit{b}  | III/R4 (Rib) | – | – (D) |
| 71-735\textsuperscript{a} | III/R1 | – | + (C) |
| ATCC 49446; 1/82 | IV      | –               | + (B)                             |
| 15626/81 (3)   | IV/c\textsubscript{p} | + | + (B) |
| ATCC 49447; 10/84 | V/R3   | –               | – (D)                             |
| NCTC 9828; Pattison R | NT/R3/R4\textsuperscript{d} | – | + (B) |

\textsuperscript{a} Categories A to D indicate the following: strains with positive \textit{c} FAT and positive \textit{bca} PCR with two or more amplimers (A); negative FAT and two or more amplimers (B); negative FAT and a single amplimer (C); and negative both FAT and \textit{bca} PCR (D).

\textsuperscript{b} Received from G. Lindahl, Lund, Sweden.

\textsuperscript{c} Received from P. Ferriero, Minneapolis, Minn.

\textsuperscript{d} Nontypable for capsular antigen type.

PCR products of the C category (12). Previously, we found that the category C pattern was consistent with a \textit{bca} gene with only one repeat unit and that category A and B patterns were compatible with two or more repeats, the multiplicity of PCR products probably resulting from amplification which extended over a variable number of repeats (12).

Other investigators have shown that mutational deletion of \textit{bca} repeats may occur both in vitro and in vivo (9). Conceivably, the mutations have adaptive importance since the resulting \textit{c} proteins, with a reduced number of repeats, show loss of protective epitopes and the mutants show reduced susceptibility to opsonophagocytic killing by \textit{c} antibodies (6, 9). We assume that all or some of the seven category B and C strains described in this study have been subjected to similar mutational deletions of \textit{bca} repeats. These strains, including that single clone, have been repeatedly subcultured for years. It is possible that the mutational changes have occurred during the subculturing and that these changes have benefitted the bacteria when growing in vitro. Alternatively, the mutational changes could have been present at the time of the original isolation. Recently, we showed that of 40 clinical GBS isolates which were negative in the \textit{c} FAT, 16 strains showed positive PCR for \textit{bca} elements (12). None of the seven prototype and reference GBS strains with \textit{c} FAT and \textit{bca} PCR discrepancy showed \textit{c} protein expression at levels which could be detected by the antibody-based methods used in this study, consistent with the results of earlier serotyping. This may be due to additional mutational changes, for instance in a regulatory region. Alternatively, one or more of these strains may express a \textit{c} protein with conformational changes that excluded recognition by the anti-\textit{c} antibodies used by us (6). These antibodies were raised against a \textit{c} protein with many repeats (2) which is antigenically different from \textit{c} with few repeats (6). In contrast to the genotype and phenotype divergence of \textit{c}, PCR testing of the beta gene showed complete agreement with the testing of the \textit{c} protein, although beta gene/\textit{c} protein discrepancy may occur (13).

Our findings underscore the need for characterization of reference and prototypes strains of GBS at the genetic level and highlight problems involved when selecting strains for research purposes or for the generation of diagnostic reagents such as antibodies against serogroup or serotype markers. For instance, the possibility cannot be excluded that one or more of the strains which contained \textit{bca} without \textit{c} protein expression may express the protein when exposed to environmental changes in vitro or in vivo.

REFERENCES

1. Baker, C. J., and M. S. Edwards. 1990. Group B streptococcal infections, p. 742–811. In J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infants. The W. B. Saunders Co., Philadelphia, Pa.

2. Bevanger, L., O.-J. Iversen, and A. I. Naess. 1992. Characterization of the alpha antigen of the c protein of group B streptococci (GBS) using a murine monoclonal antibody. \textit{APMIS} \textbf{100}:57–62.

3. Bevanger, L., A. I. Kvm, and J. A. Maeland. 1995. \textit{A Streptococcus agalactiae} R protein analysed by polyclonal and monoclonal antibodies. \textit{APMIS} \textbf{103}: 73–78.

4. Bevanger, L., and J. A. Maeland. 1977. Type classification of group B streptococci by a fluorescent antibody test. \textit{Acta Pathol. Microbiol. Immunol. Scand. Sect. B} \textbf{85}:357–362.

5. Bevanger, L., and J. A. Maeland. 1979. Complete and incomplete \textit{bc} protein fraction in group B streptococci. \textit{Acta Pathol. Microbiol. Immunol. Scand. Sect. B} \textbf{87}:51–54.

6. Gravekamp, C., D. H. Hensens, J. L. Michel, and L. C. Madoff. 1996. Variation in repeat number within the \textit{c} protein of group B streptococci alters antigenicity and protective epitopes. \textit{Infect. Immun.} \textbf{64}:3576–3583.

7. Jerlstrom, P. G., G. S. Chhatwal, and K. N. Timmis. 1991. The IgA-binding \textit{\beta} antigen of the \textit{c} protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions. \textit{Mol. Microbiol.} \textbf{5}:843–849.

8. Kvm, A. I., E. Elstratou, L. Bevanger, B. D. Cookson, I. F. Marticorena, R. C. George, and J. A. Maeland. 1995. Distribution of serovarintants of group B streptococci in isolates from England and Norway. \textit{J. Med. Microbiol.} \textbf{42}:246–250.

9. Madoff, L. C., J. L. Michel, E. W. Gong, D. E. Kling, and D. L. Kasper. 1996. Group B streptococci escape host immunity by deletion of tandem repeat elements of the alpha \textit{c} protein. \textit{Proc. Natl. Acad. Sci. USA} \textbf{93}:4131–4136.

10. Madoff, L. C., J. L. Michel, E. W. Gong, A. K. Rodewall, and D. L. Kasper. 1992. Protection of neonatal mice from group B streptococcal infection by maternal immunization with beta \textit{c} protein. \textit{Infect. Immun.} \textbf{60}:4989–4994.

11. Madoff, L. C., J. L. Michel, and D. L. Kasper. 1991. A monoclonal antibody identifies a protective \textit{c} protein alpha-antigen epitope in group B streptococci. \textit{Infect. Immun.} \textbf{59}:234–240.

12. Maeland, J. A., O. G. Brakstad, L. Bevanger, and S. Krookstad. Distribution and expression of \textit{bca}, the gene encoding the \textit{c} alpha protein, by \textit{Streptococcus agalactiae}. \textit{J. Med. Microbiol.}, in press.

13. Maeland, J. A., O. G. Brakstad, L. Bevanger, and A. I. Kvm. 1997. \textit{Streptococcus agalactiae} \textit{bca} gene and gene product variations. \textit{J. Med. Microbiol.} \textbf{46}:999–1005.
14. Mawn, J. A., A. J. Simpson, and S. R. Heard. 1993. Detection of the c protein gene among group B streptococci using PCR. J. Clin. Pathol. 46:633–636.
15. Michel, J. L., L. C. Madoff, K. Olson, D. E. Kling, D. L. Kasper, and F. M. Ausubel. 1992. Large, identical tandem repeating units in the c protein alpha antigen gene, bca, of group B streptococci. Proc. Natl. Acad. Sci. USA 89:10060–10064.
16. Naess, A. I., L. Bevanger, O., J. Iversen, and J. A. Maeland. 1991. Evaluation of monoclonal antibodies in serovar classification of group B streptococci (GBS). APMIS 99:1058–1060.
17. Payne, N. R., and P. Ferrieri. 1985. The relation of the lbc protein antigen to the opsonization differences between strains of type II group B streptococci. J. Infect. Dis. 151:672–681.
18. Payne, N. R., Y. Kim, and P. Ferrieri. 1987. Effect of differences in antibody and complement requirements on phagocytic uptake and intracellular killing of “c” protein-positive and -negative strains of type II group B streptococci. Infect. Immun. 55:1243–1251.
19. Wästfelt, M., M. Stålhammar-Carlemalm, A. M. Delisse, T. Cabezon, and G. Lindahl. 1996. Identification of a family of streptococcal surface proteins with extremely repetitive structure. J. Biol. Chem. 271:18892–18897.
20. Wilkinson, H. W. 1972. Comparison of streptococcal R antigens. Appl. Microbiol. 24:669–670.