Letters to the Editor

Interruption of \textit{siaD} in a Meningococcal Carrier Isolate Mediated by an Insertion Sequence

\textit{Neisseria meningitidis}, an important human pathogen causing invasive disease such as meningitis and sepsis, is normally a harmless commensal bacterium that colonizes the human nasopharynx. Dissemination occurs from this ecological niche, which is colonized in up to 10\% of the human population (1).

The capsule expressed on the surface of \textit{N. meningitidis} is associated with virulence, mediating resistance to both phagocytosis and complement-mediated killing (4). Molecular analysis has shown that expression of meningococcal capsules is regulated by the 24-kb \textit{cps} gene complex (7). On the basis of structural variation in the capsular polysaccharide, 13 serogroups are recognized, although only 3 (serogroups A, B, and C) produced most of the cases of meningococcal disease worldwide.

Traditionally, the serogrouping of meningococcal strains has been done by immunologic techniques like agglutination or coagglutination with polyclonal and monoclonal antibodies. When these techniques are used, the percentage of nongroupable (NG) meningococcal strains is high among carrier isolates (2). However, the serogroups of some strains which have become phenotypically nongroupable can be determined by PCR (8).

In order to determine the capsular genotypes of NG meningococcal strains isolated in two wide meningococcal-carrier surveys conducted in Galicia, Spain, between 1996 and 1998 (2), 254 NG meningococcal strains were analyzed by PCR for B and C capsular genotypes. Two pairs of primers, which amplify a region of the gene (\textit{siaD}) encoding the sialic acid capsule polymerase were used: 5'-CTCTCACCCTCAACCCATTGTC-3' and 5'-TGTCGGCGGAATAGTAATAATGT-3' for serogroup B and 5'-GCACATTCAGGCGGGATTA-3' and 5'-TCTCTTGTTGGGCTGTATGGTGTA-3' for serogroup C.

Bacterial suspensions were boiled for 10 min and then centrifuged at 12,000 \( \times g \) for 10 min. PCR amplifications were performed with 5 \( \mu l \) of each sample, 1.5 mM MgCl\(_2\), 200 \( \mu M \) each deoxynucleoside triphosphate, the corresponding oligonucleotides at 0.4 \( \mu M \), and 2.5 U of Taq polymerase (Perkin-Elmer) in the buffer recommended by the manufacturer.

The PCR conditions were as follows: initial denaturation at 95°C for 1 min, followed by 39 cycles of annealing at 55°C for 40 s, extension at 72°C for 1 min, and denaturation at 94°C for 25 s and a final extension at 72°C for 3 min. Amplicons were analyzed by electrophoresis on a standard 0.8% agarose gel, stained with ethidium bromide, and photographed under UV light.

In our PCR assay with specific serogroup B primers, one strain generated a 1.5-kb PCR product instead of the expected product of 457 bp (Fig. 1). In order to characterize the genetic event that had generated this PCR product, we decided to sequence the product.

DNA sequencing was performed with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit, and completed reactions were run on an ABI 377 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems). Nucleotide sequence homology searches were performed with the BLAST program on the National Center for Biotechnology Information (Bethesda, Md.) server.

The DNA sequence matched the sequence of the meningococcal serogroup B \textit{siaD} gene amplified with the PCR assay used, and within this sequence there was a copy of an insertion sequence (IS) recently described (5), tentatively named IS\textit{4351}N1 (Silke Klee, personal communication). The insertion of this mobile genetic element was accompanied by the duplication of the target DNA flanking the IS. Although this IS has been described as an insertion in a locus in the chromosome other than one of the capsule gene loci (5), in our study the IS led to inactivation of the \textit{siaD} capsular gene and it consistently determined loss of encapsulation.

In vitro studies have suggested that the modulation of capsule expression is mediated by transposable genetic elements (3, 6). However, to our knowledge, this is the first report of this phenomenon in clinical isolates.

The presence of the IS (or another transposable genetic element) in another position in \textit{siaD} or another gene of the \textit{cps} gene complex is critical for \textit{siaD} gene expression. This study suggests that the IS is a common mechanism for \textit{siaD} gene inactivation in meningococcal isolates.

FIG. 1. PCR amplification of the serogroup B meningococcus \textit{siaD} gene. Lanes 1 and 5, molecular size markers; lane 2, PCR negative control; lane 3, meningococcal carrier isolate with the IS in the \textit{siaD} gene; lane 4, PCR serogroup B positive control.
complex would explain the lack of encapsulation in some meningococcal carrier isolates. Additional studies are needed to verify this hypothesis.

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