

NOTES

Heterogeneity of the PorB Protein in Serotype 22 *Neisseria meningitidis*

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Received 11 June 1998/Returned for modification 4 August 1998/Accepted 25 August 1998

The genetic diversity of *porB* genes from meningococcal isolates characterized as serotype 22 was investigated by gene sequencing. This procedure identified seven distinct *porB* sequences, demonstrating variation in the PorB protein recognized by the serotype 22 monoclonal antibody. This is consistent with the genetic heterogeneity of serotype 22 meningococci reported previously.

Neisseria meningitidis causes bacterial meningitis and septicemia worldwide (3). For routine epidemiological surveillance, meningococci are classified by immunological reagents into serogroups (by type of capsular polysaccharide), serotypes (PorB, class 2 or 3 outer membrane protein [OMP]), and serosubtypes (PorA, class 1 OMP) (7). Many meningococcal isolates are nonserotypeable (NT), or nonserosubtypeable (NST), as a result of antigenic variation in the PorA and PorB proteins (5, 11, 14) and the assay-dependent reactivity of some of the monoclonal antibodies (MAbs) used (17, 20, 23).

A MAb identifying a new serotype, 22, was produced in the Czech Republic in 1994 to combat the large proportion (50 to 80%) of NT meningococci isolated there between 1973 and 1994 (9). Use of this MAb in the National Reference Laboratory for Meningococcal Infections, Prague, Czech Republic, showed that 44% of the meningococci previously characterized as B:NT during 1995, and 37% of such isolates obtained between 1973 and 1994, were serotype 22 (10). Testing of meningococcal isolates in other European countries gave the following rates of serotype 22 for isolates previously classified as NT: Austria, 5.4%; Germany, 11.3%; and Greece, 9.5% (19). Addition of this reagent to the serotyping panel used at the Meningococcal Reference Unit for England and Wales in 1995 identified serotype 22 organisms among invasive and noninvasive serogroup B and C isolates of diverse serosubtype. A study of 22 Czech serogroup B, serotype 22 (B:22), meningococci by PCR-restriction endonuclease pattern analysis of the *pilA* gene and multilocus enzyme electrophoresis concluded that these organisms were highly heterogeneous, with 17 clonal complexes and 14 *pilA* alleles identified among serotype 22 isolates (16).

In the present work, the antigenic heterogeneity of the PorB proteins recognized by the serotype 22 MAb was examined by nucleotide sequence determination of the *porB* genes of serotype 22 meningococci. The study included 10 Czech B:22 isolates (isolates 312204 to 312213) and 3 United Kingdom (U.K.)

isolates, 1 B:22 (isolate 312664) and 2 C:22 (isolates 312472 and 312597) (Table 1). The meningococcal template DNA preparation was as described previously (20). Amplification of *porB* genes by PCR (in 100- μ l reaction mixtures) was carried out with reaction buffer (Gibco BRL); 200 μ M (each) dATP, dCTP, dGTP, and dTTP; 1 μ M concentrations of PCR primers PB1 (5'-TAAATGCAAAGCTAAGCGGCTTG-3') and PB2 (5'-TTTGTGATACCAATCTTTTCAG); 0.5 U of *Taq* polymerase (Gibco BRL); and 1 μ l of template DNA (approximately 50 ng μ l⁻¹). Reaction conditions were 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, followed by incubation at 72°C for a further 2 min. Purification and direct nucleotide sequence determination of amplified *porB* genes were done as described previously (20).

Two class 2 OMP-encoding *porB* allele sequences were identified among the 13 isolates (GenBank accession no. AF065125 and AF065126). The peptide sequences deduced from these alleles were consistent with the porin model of PorB structure (22), with eight surface exposed loop regions (loops I to VIII) in which the serotype-specific peptide sequences reside (6). The new sequences were aligned with 92 meningococcal PorB protein sequences covering all known serotypes, including a distinct sequence from an additional serotype 22 isolate (GenBank accession no. U92906 [17]) and many PorB sequences from isolates described as NT, most of which had not been tested with the serotype 22 MAb (2, 6, 17, 21).

Comparison of the alignment showed that the only peptide sequence unique to the serotype 22 PorB sequences was AKNNDGTANQGKKH, located in putative loop VI, all other loop sequences being diverse among serotype 22 isolates or shared with PorB sequences from isolates with different serotypes (Fig. 1 and data not shown). This loop VI sequence was also encoded by the *porB* genes from isolates 315/85, EG 011, NG H38, and 528 (GenBank no. AF065127, AF065128, AF065129, and AF065130, respectively) (Fig. 1). These isolates had not been typed with a reagent panel including the serotype 22 MAb and were classified as NT. To test the hypothesis that the loop VI peptide sequence was required for serotype 22 MAb recognition, the isolates were reserotyped, in a blinded fashion, with a MAb panel including the serotype 22 MAb. All four meningococci were characterized as serotype 22

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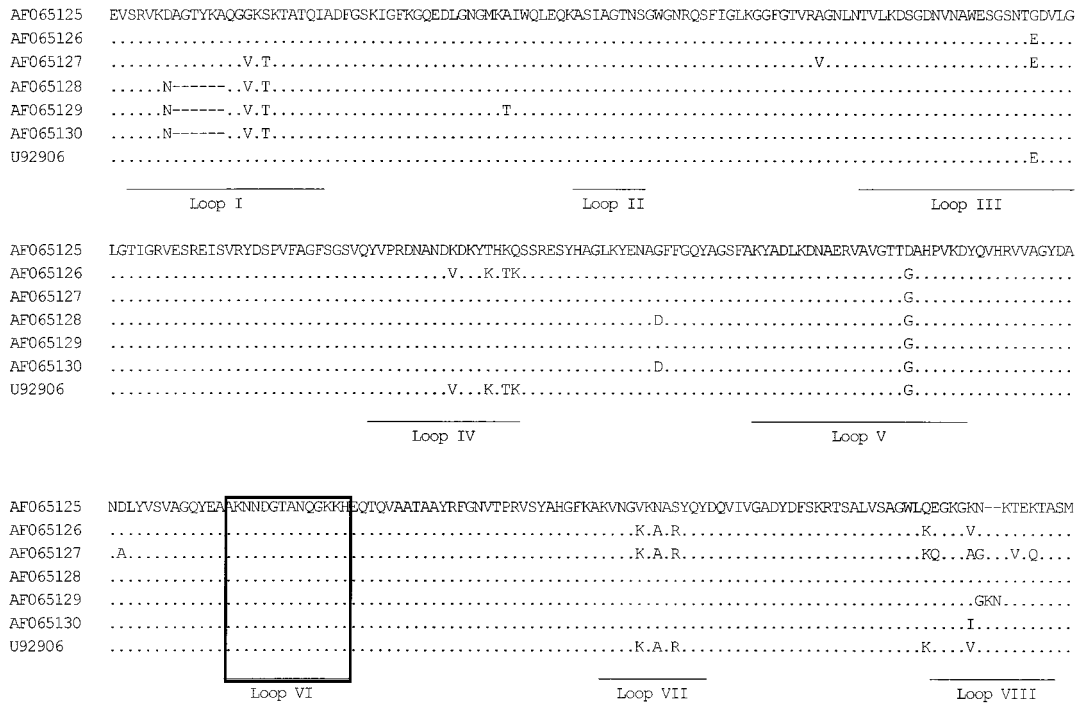


FIG. 1. Alignment of seven meningococcal PorB protein sequences obtained by translation of the nucleotide sequences of each of the *porB* alleles identified among serotype 22 meningococci. The locations of the putative surface loops (I to VIII) of the porin are indicated. The boxed area defines the putative surface loop VI that is likely to be important for recognition of the PorB proteins by the serotype 22 MAb. Sequences AF065126 and U92906 were different at the nucleotide sequence level but possessed identical peptide sequences.

(Table 1), strengthening the evidence that loop VI is the critical loop for serotype 22 recognition.

The relationships among the seven *porB* allele sequences identified in serotype 22 meningococci were represented graphically by the split-decomposition method (1) (Fig. 2). The split graph obtained illustrates a network of possible pathways linking the *porB* allele sequences obtained from serotype 22

meningococci, suggesting that genetic recombination, which occurs within and between *Neisseria* species (12, 15, 18), has resulted in the circulation of a sequence encoding the serotype 22 epitope among the *porB* alleles present in populations of *N. meningitidis*.

TABLE 1. Meningococcal serotype 22 strains examined and GenBank accession numbers of their *porB* gene sequences

Isolate no.	Country of origin	Serological data	<i>porB</i> sequence accession no.
312204	Czech Republic	B:22:NST	AF065125
312205	Czech Republic	B:22:NST	AF065126
312206	Czech Republic	B:22:P1.9,10	AF065125
312207	Czech Republic	B:22:NST	AF065126
312208	Czech Republic	B:22:P1.4	AF065126
312209	Czech Republic	B:22:NST	AF065125
312210	Czech Republic	B:22:NST	AF065126
312211	Czech Republic	B:22:P1.14	AF065126
312212	Czech Republic	B:22:P1.14	AF065126
312213	Czech Republic	B:22:P1.1,7	AF065126
312472	U.K.	C:22:P1.15,10	AF065126
312597	U.K.	C:22:NST	AF065126
312664	U.K.	B:22:NST	AF065126
315/85	Germany	B:22:P1.7,14	AF065127
EG 011	Germany	B:22:P1.3	AF065128
NG H38	Norway	NG:22:P1.3,6	AF065129
528	USSR	B:22:P1.14	AF065130
503/93	Czech Republic	B:22:NST	U92906 ^a

^a Published previously (17).

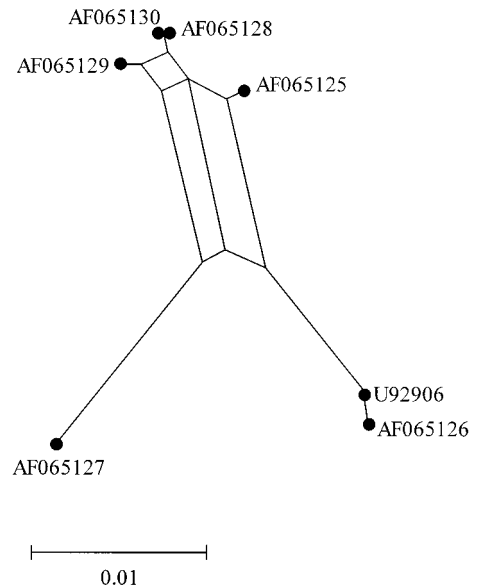


FIG. 2. Split-decomposition analysis of the seven *porB* sequences obtained from serotype 22 meningococcal isolates. Split graphs were drawn from Hamming (uncorrected) distance matrices of aligned *porB* allele sequences by using SplitsTree version 2.4 (8). Branch lengths are drawn to scale.

In conclusion, these data demonstrate that while meningococcal strains that react with the serotype 22 MAb possess an identical peptide sequence in variable surface loop VI of the PorB protein, the PorB proteins may be encoded by mosaic gene structures that are highly diverse in one or more of the other variable surface loops. Furthermore, the serotype 22 epitope is encoded by only a small part of the meningococcal genome, the remainder of which has been shown previously to be highly heterogeneous among serotype 22 meningococci (16). Positive reactions with the serotype 22 MAb therefore do not provide a robust indication of the genetic relatedness of meningococcal isolates unless they are supported by additional epidemiological information, obtained, for example, from multilocus sequence typing (13) or multilocus enzyme electrophoresis analyses (4).

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited in the GenBank database under accession no. AF065125 to AF065130.

M.C.J.M. is a Wellcome Senior Research Fellow in Biodiversity, and R.U. and M.C.J.M. are grateful to the Wellcome Trust for financial support. P.K. and M.M. acknowledge the support of research grant 310/96/K102 from the Grant Agency of the Czech Republic and research grant 3982-3 from the Internal Grant Agency of the Ministry of Health of the Czech Republic.

We thank Steve Gray at the Meningococcal Reference Unit for England and Wales for serological characterization of meningococcal isolates.

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