NOTES

Genetic Heterogeneity of Strains of Neisseria meningitidis Belonging to Serotype 22 Isolated in the Czech Republic

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Strains of Neisseria meningitidis of serogroup B isolated in the Czech Republic frequently belong to serotype 22. We analyzed the genetic relationships among strains of this serotype by using the multilocus enzyme electrophoresis technique and the polymorphism of the pilA gene. Our results indicate that these strains correspond to a highly heterogeneous population rather than to the expansion of a single clone.

Meningococcal infections provoked by Neisseria meningitidis occur either as endemic or epidemic outbreaks. The characterization of strains involved in these infections is an important aspect of epidemiological surveillance. Current serological methods of typing are based on the immunospecificity of bacterial surface structures. These methods define the antigenic formula (serogroup:serotype:serosubtype) by using antibodies directed against the capsule, the outer membrane protein PorB, and the outer membrane protein PorA, respectively. However, the antibodies used in this characterization are not comprehensive, and a variable proportion of strains remains nontypeable (NT) and/or nontypable (NST). This was the case in the Czech Republic, where 50 to 80% of meningococcal strains were NT and/or NST (9).

Recently, a new monoclonal antibody (MAb) directed against PorB was developed by immunizing BALB/c mice with the whole-cell antigen of an N. meningitidis B:NT:NST strain isolated in the Czech Republic (9). The new MAb has permitted the description of a new serotype among NT strains. This serotype was named 22, as the last available serotype-specific MAb was 21 (9). Serotype 22 accounted for 37% of the NT strains of meningococcal strains were NT and/or NST (9).

Among the 22 strains tested in this study, 14 groups corresponding to 14 alleles of pilA were characterized (Table 1). One group (the group of the allele pilA26) contained six strains; it represents the most common allele (27%) among the strains tested. A combined numerical analysis of different restriction profiles was performed with the Taxotron software package as previously described (6). In comparison with our collection of strains (6), the strains of serotype 22 were distributed all over the phylogenetic tree, indicating the high heterogeneity of these strains (Fig. 1). A weak correlation was observed between the distribution of pilA alleles and serosubtypes. The serosubtype P1.2,P1.5 was found in strains from four different groups, as determined by the polymorphism of pilA (pilA2, pilA4, pilA14, and pilA29). Moreover, strains from the same group (pilA26) showed two different serosubtypes (P1.14 and P1.15 [Table 1]). Indeed, several previous studies have shown the lack of correlation between genetic typing and serological characterization of meningococcal strains (6, 11).

To provide more insights into the genetic relationships among these strains, we studied them further by the multilocus enzyme electrophoresis (MLEE) method. This technique is based on the difference in electrophoretic mobility of isoenzymes encoded by the different alleles of a given gene. Several enzymes are usually analyzed, and their electrophoretic profile is called the electrophoretype (ET). This method defines “clonal complexes,” which are composed of closely related clones corresponding to ETs which differ by the migration of no more than two enzymes (3). A distinctive group of clones are clustered into the ET-5 complex. These clones usually belong to serogroup B, serotype 15. The ET-37 complex is composed of closely related clones belonging to serogroup C, serotype 2a (2).
The following enzymes were assayed: malic enzyme (ME; EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6P; EC 1.1.1.49), leucine aminopeptidase (PEP; EC 3.4.11.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), aconitase (ACO; EC 4.2.1.3), glutamate dehydrogenase (NADP dependent) (GD1; EC 1.4.1.2), glutamate dehydrogenase (NAD dependent) (GD2; EC 1.4.1.4), alcohol dehydrogenase (ADH; EC 1.1.1.1), fumarase (FUM; EC 4.2.1.2), alkaline phosphate (ALP; EC 3.1.3.1), superoxide dismutase (SOD; EC 1.15.1.1), and adenylate kinase (ADK; EC 2.7.4.3). Preparation of enzyme extracts, horizontal starch gel electrophoresis of FUM, and enzyme-staining procedures were performed as previously described (10) with the exception of the substitution of 0.2 M Tris-HCl, pH 8.3, at 500 V, 4°C, for 2.5 h (12 h for PEP, GD1, and GD2 assays) in standard Tris-glycine buffer and in Protean II (Bio-Rad). Enzyme extracts were diluted 1:4 in 40% glycerol-water solution and loaded on the gels as 10-μl samples. This setting provided better isozyme discrimination than starch gel electrophoresis. The results obtained by the MLEE analysis also showed a high degree of heterogeneity among the strains of serotype 22 tested in this study. Indeed, 19 different ETs were observed (Table 1). They clustered into 17 different clonal complexes (ETs) which differ by no more than two enzymes clustered together. The identified clusters did not correspond to any major clonal complex, such as ET-5 or ET-37 complexes. A good, but not perfect, correlation was observed between MLEE analysis and the polymorphism of pilA. Indeed, strains 15-95, 442-95, 478-95, and 481-95 (but not 319-95 and 269-96) are clustered together by the two methods. This was also the case for strains 350-93, 546-94, and 547-94 (Table 1).

It is interesting to note that two strains (65-95 and 355-95) have the allele pilA4, which has been shown to be correlated with the ET-37 complex (6). However, the MLEE analysis did not cluster these strains into the ET-37 complex. Moreover, strains belonging to the ET-37 complex were not reported in the Czech Republic before 1993. Indeed, an endemic situation currently characterized are shown to the right of the dendrogram. The results of serotype 22 tested in this study are indicated to the right of the corresponding pilA alleles.
was observed in the Czech Republic from 1970 to 1993, with sporadic cases. Strains of *N. meningitidis* of serogroup B and serotype 22 were predominant during this period (8, 9). By the spring of 1993, a new epidemiological situation occurred; the meningococcal strains involved showed the antigenic formula C:2a:P1.2 (P1.5) and belonged to ET-15, a member of the ET-37 clonal complex (8). Bacteria isolated during this epidemic have the allele *pilA4* (data not shown). The acquisition of the allele *pilA4* by strains of serotype 22 may have occurred by horizontal DNA exchange between the endemic strains of serotype 22 and the new epidemic clone of the ET-37 complex. Alternatively, capsule and serotype switching of the epidemic clone may account for the appearance of strains such as 65-95 and 355-95. Capsule switching of *N. meningitidis* has been proposed to occur during an epidemic (reference 12 and data not shown). The fact that these strains did not belong to ET-37, as indicated by the MLEE analysis, is in favor of the first explanation. The study of the polymorphism of other genes may be needed to better analyze such strains.

Our data clearly demonstrate a high degree of heterogeneity among strains of serotype 22. These results support the hypothesis that the high frequency of this serotype of *N. meningitidis* in the Czech Republic did not result from the expansion of a single clone but rather from the local adaptation of the meningococcal strains to their hosts.

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