Outbreak of Meningococcal Disease Caused by PorA-Deficient Meningococci

A. van der Ende,[1] C. T. P. Hopman,[1] W. C. M. Keijzers,[1] L. Spanjaard,[1] E. B. Lodder,[2] P. H. J. van Keulen,[3] and J. Dankert[1]

[1]Department of Medical Microbiology, Academic Medical Center, and The Netherlands Reference Laboratory for Bacterial Meningitis, National Institute of Public Health and Environmental Protection, Amsterdam, [2]Public Health Department, West Brabant, and [3]Department of Medical Microbiology and Infection Prevention, Amphia Hospital, Breda, The Netherlands

An outbreak of 7 cases of group C meningococcal disease occurred during the last week of July and the first week of August 2001 in the southwestern part of The Netherlands. Characterization of the 7 patients' isolates by various typing methods showed that the isolates were identical, except for the expression of PorA. Isolates from 5 patients were PorA deficient. These results show that transmission of PorA-deficient meningococci occurs and that PorA-deficient meningococci can cause invasive disease. PorA-based meningococcal vaccines may provide limited protection.

Life-threatening meningitis and septicemia caused by *Neisseria meningitidis* continue to cause serious public health problems worldwide. Thirteen meningococcal serogroups are recognized on the basis of serological variation of the capsular polysaccharide. Ninety percent of cases of meningococcal disease are due to meningococcal serogroups A, B, and C, whereas the remaining cases of disease are caused mainly by meningococcal serogroups W-135 and Y. Current meningococcal vaccines are based on the capsular polysaccharides of meningococcal serogroups A, C, W-135, and Y. None of these vaccines provides protection against disease due to serogroup B meningococci, which is the prevalent serogroup in Europe, North America, South America, and Australia. Protection by vaccination with group B capsular polysaccharide vaccine is difficult to achieve, because this polysaccharide is poorly immunogenic [1]; therefore, meningococcal outer membrane proteins (OMPs) are being investigated as possible vaccines to prevent disease due to meningococci, regardless of their serogroup [2].

In clinical trials with meningococcal OMP–based vaccines, the induced serum bactericidal activity was predominantly attributed to the presence of antibodies directed against PorA [3]. In addition, monoclonal antibodies directed against PorA proved to exert serum bactericidal activity and to confer protection against *N. meningitidis* infection in an animal model [4]. PorA is, therefore, considered to be an important component in protein-based vaccines against meningococcal disease. However, PorA shows a high degree of antigenic variation, which is used for serological differentiation of isolates (i.e., serosubtyping) [5, 6]. Consequently, newer PorA-based vaccines contain multiple antigenic variants of PorA, to prevent an acceptable percentage of cases of meningococcal disease [7]. Clinical trials of a hexavalent PorA-based vaccine already have been performed and reported elsewhere [8].

Another drawback for PorA-based vaccines might be the variable expression of PorA. Previous studies show that PorA expression can be varied in multiple ways [9]. Bacterial descendants with a PorA expression different from that of their parent bacterial cells are the product of slipped strand mispairing during replication in the homopolymeric tract of guanidine residues and/or thymidine residues in the *porA* promoter, as well as in the homopolymeric tract of adenine residues in the *porA* coding region [9, 10]. In addition, point mutations in the coding region may result in meningococci without PorA expression [9]. PorA expression may also be absent because of deletion of the complete *porA* gene [11] or insertion of an insertion sequence element in the *porA* coding region [12]. Until now, PorA-deficient isolates were cultured from sporadic cases of disease only. It is possible that these patients acquired infection with a meningococcal isolate that expresses PorA and that a mutation in *porA* occurred during the course of infection. In the present study, a recent outbreak of meningococcal disease caused by nonsubtypeable meningococci was examined.

Materials and methods. Meningococcal isolates were characterized in the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) by serotyping, multilocus sequence typing (MLST) [13], and sequencing of the variable regions of *porA* that encode the PorA epitopes on which the serosubtyping system is based, as well as sequencing of the complete *porA* [6, 9]. In addition, the OMP fraction of the isolates was analyzed by SDS-PAGE and Western blotting [9]. Pure cultures origi-

Received 24 September 2002; revised 22 October 2002; electronically published 18 February 2003.

Reprints or correspondence: Dr. A. van der Ende, Dept. of Medical Microbiology, Academic Medical Center, PO Box 22700, 1100 DE Amsterdam, The Netherlands (a.vanderende@amc.uva.nl).

The Journal of Infectious Diseases 2003; 187:869–71

0022-1899/2003/18705-0020$15.00

nating from single colonies of the isolates were obtained as described elsewhere [9].

Results. During the first week of August 2001, the NRLBM received group C meningococcal isolates from 7 patients, all residents of 4 small villages in a confined area in the southwestern part of The Netherlands (patients 1–7; table 1). Patients 1–5 belonged to the same age group (9–11 years old) and lived within the same social community. These patients had visited the same community swimming pool on 24 July 2001. Patients 6 and 7 were 2 and 23 years old, respectively, and lived near each in another village nearby. An epidemiological connection could not be established between patients 6 and 7 or between them and the other 5 patients in the cluster.

All isolates of the outbreak were determined to be sequence type 11 by MLST, which is commonly found among isolates of the ET-37 complex, a hypervirulent clone that often causes local outbreaks of meningococcal disease [13]. By serotyping, all outbreak isolates were serotype C:2a, but they differed by serosubtyping (i.e., the antigenicity of PorA). The isolates from patients 1–5 were nonserosubtypeable, whereas the isolates from patients 6 and 7 were serosubtype P1.5. However, sequencing of the *porA* variable regions of the 7 isolates revealed an identical PorA epitope (P1.5-1,10-8).

Differences in the level of PorA expression may explain the differences in serosubtyping [9]. Protein SDS-PAGE and Western blotting of the OMP fraction of the isolates indeed demonstrated P1.5-specific PorA expression in the 2 serosubtypeable isolates, whereas this protein was not detectable in the 5 nonserosubtypeable isolates. *porA* sequence data confirmed these results. The 5 PorA-deficient isolates had a premature stop codon in *porA*, which was caused by a single base-pair substitution (C→T) at position 259 of the *porA* coding region (GenBank accession nos. AY166656 and AY166657 for *porA* sequences of a PorA-deficient and -containing isolate, respectively).

From January 2001 through September 2001, a total of 544 isolates from patients with meningococcal disease were received at the NRLBM. Among these 544 isolates, 3 strains (patients 8–10; table 1) were typed as C:2a nonserosubtypeable with the P1.5-1,10-8 *porA* genotype. In 2000, this genotype was not encountered among the 539 isolates received by the NRLBM. The MLST type of the 3 additional nonserosubtypeable isolates was identical to that of the 7 outbreak isolates. Analysis of the *porA* sequence showed that 1 of these isolates (from patient 10) had the same point mutation as the PorA-deficient isolates from the 5 patients in the cluster. The other 2 isolates had a homopolymeric tract of 8, instead of 7, adenine residues in the *porA* coding region. Predicted translations of *porA* showed that full coding integrity is maintained with a repeat of 7 adenine, whereas expansion or reduction by 1 residue introduces a frame shift, which truncates the coding sequence [9].

Discussion. The present study describes an outbreak of meningococcal disease caused by PorA-deficient C:2a nonserosubtypeable meningococcal isolates with P1.5-1,10-8 *porA* sequence type and MLST genotype 11. The meningococcal isolates of the clustered cases lacked PorA expression because of a single base-pair substitution (C→T) at position 259 of the *porA* coding region. The results strongly suggest that spreading of PorA-deficient meningococci exists. Before the outbreak in July, 3 sporadic cases of meningococcal disease were also caused by PorA-deficient C:2a:P1.5-1,10-8 meningococci with MLST genotype 11. Of interest, 1 of the 3 isolates from these sporadic cases had a *porA* mutation identical to that of the isolates in the cluster. This case occurred ~2 months before the outbreak in West Brabant, but a relationship between this case and the cases in the cluster could not be established.

PorA is an important component of protein-based vaccines against meningococcal disease. However, the large antigenic diversity of PorA may jeopardize an acceptable efficacy level of PorA-based vaccines [5, 6, 14]. In addition, variation in PorA expression among meningococci may also limit the efficacy of

Table 1. Characteristics of meningococcal isolates from 10 patients with meningococcal disease in The Netherlands, 2001.

Patient	Date of disease onset	Sex	Age, years	City of residence	Source of isolate	Serotype	PorA expression
1	26 Jul 2001	M	11	Zevenbergen	Blood	C:2a:nt	−
2	26 Jul 2001	F	11	Zevenbergen	Blood	C:2a:nt	−
3	27 Jul 2001	M	11	Klundert	Blood	C:2a:nt	−
4	27 Jul 2001	F	11	Zevenbergen	Blood	C:2a:nt	−
5	27 Jul 2001	F	9	Zevenbergen	Blood	C:2a:nt	−
6	28 Jul 2001	F	2	Standdaarbuiten	Blood and CSF	C:2a:P1.5	+
7	1 Aug 2001	F	23	Etten-Leur	Blood and CSF	C:2a:P1.5	+
8	5 May 2001	M	17	Muiden	CSF	C:2a:nt	−
9	17 May 2001	M	41	Tilburg	Blood and CSF	C:2a:nt	−
10	2 Jun 2001	F	14	Valkenswaard	Blood and CSF	C:2a:nt	−

NOTE. All strains were of the 5-1,10-8 PorA genotype. CSF, cerebrospinal fluid; +, present; −, absent.

PorA-based vaccines. Virtually all meningococci are capable of PorA phase variation [9]. Hence, all PorA-containing meningococcal isolates have the potential to generate PorA-deficient progeny and vice versa, with a relative high frequency. The conditions in the host may then be the driving force for the selection for PorA variants, either positive or negative. On the one hand, PorA-deficient variants may escape the host's immune response when entered into the bloodstream. On the other hand, PorA may be beneficial to the meningococcus at some stages in the pathogenesis of meningococcal disease, although experimental evidence is lacking for this hypothesis.

PorA-deficient meningococci have been isolated previously from patients with meningococcal disease [15]. During the surveillance conducted by the NRLBM, a small proportion (1%–2%) of the meningococcal isolates analyzed had a stable point mutation in the *porA* operon, which leads to PorA deficiency (data not shown). However, those cases were sporadic, so it is possible that the patients acquired infection with a meningococcal isolate that expresses PorA and that a mutation in *porA* occurred during the course of infection. In such cases, disease would be prevented by a PorA-based vaccine.

In the present study, the 5 PorA-deficient outbreak isolates had an identical *porA* mutation, which was not phase variable. It is unlikely that these 5 isolates obtained the same *porA* mutation by chance; thus, it is probable that PorA-deficient meningococci were transmitted and caused invasive disease. Although the outbreak of PorA-deficient meningococcal disease was caused by serogroup C meningococci, PorA-deficient isolates with a stable point mutation in the *porA* operon leading to PorA deficiency were also observed among serogroup B meningococcal isolates by the NRLBM [9] and by Jelfs et al. [15]. It may be expected that, when any PorA-based vaccine is used, these PorA-deficient isolates will be selected. In addition, these results show that *porA* genotyping alone is insufficient to detect future vaccine failures. In conclusion, in concert with the great antigenic variability of PorA, these findings suggest that PorA-based vaccines are to be expected to provide limited protection against meningococcal disease.

References

1. Finne J, Leinonen M, Makela PH. Antigenic similarities between brain components and bacteria causing meningitis: implications for vaccine development and pathogenesis. Lancet **1983**; 2:355–7.
2. Herbert MA, Heath PT, Mayon-White RT. Meningococcal vaccines for the United Kingdom. Commun Dis Rep CDR Rev **1995**; 5:R130–5.
3. Bjune G, Hoiby EA, Gronnesby JK, et al. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet **1991**; 338:1093–6.
4. Saukkonen K, Abdillahi H, Poolman JT, Leinonen M. Protective efficacy of monoclonal antibodies to class 1 and class 3 outer membrane proteins of *Neisseria meningitidis* B:15:P1.16 in infant rat infection model: new prospects for vaccine development. Microb Pathog **1987**; 3:261–7.
5. Suker J, Feavers IM, Maiden MCJ. Monoclonal antibody recognition of members of the meningococcal P1.10 variable region family: implications for serological typing and vaccine design. Microbiology **1996**; 142:63–9.
6. Feavers IM, Fox AJ, Gray S, Jones DM, Maiden MCJ. Antigenic diversity of meningococcal outer membrane protein PorA has implications for epidemiological analysis and vaccine design. Clin Diagn Lab Immunol **1996**; 3:444–50.
7. van der Ley PA, van der Biezen J, Poolman JT. Construction of *Neisseria meningitidis* strains carrying multiple chromosomal copies of the *porA* gene for use in the production of a multivalent outer membrane vesicle vaccine. Vaccine **1995**; 13:401–7.
8. Peeters CC, Rumke HC, Sundermann LC. Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine. Vaccine **1996**; 14:1009–15.
9. van der Ende A, Hopman CT, Dankert J. Multiple mechanisms of phase variation of PorA in *Neisseria meningitidis*. Infect Immun **2000**; 68:6685–90.
10. van der Ende A, Hopman CT, Zaat S, Oude Essink BB, Berkhout B, Dankert J. Variable expression of class 1 outer membrane protein in *Neisseria meningitidis* is caused by variation in the spacing between −10 and −35 regions of the promoter. J Bacteriol **1995**; 177:2475–80.
11. van der Ende A, Hopman CT, Dankert J. Deletion of *porA* by recombination between clusters of repetitive extragenic palindromic sequences in *Neisseria meningitidis*. Infect Immun **1999**; 67:2928–34.
12. Newcombe J, Cartwright K, Dyer S, McFadden J. Naturally occurring insertional inactivation of the *porA* gene of *Neisseria meningitidis* by integration of IS*1301*. Mol Microbiol **1998**; 30:453–4.
13. Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA **1998**; 95:3140–5.
14. Sacchi CT, Whitney AM, Popovic T, et al. Diversity and prevalence of PorA types in *Neisseria meningitidis* serogroup B in the United States, 1992–1998. J Infect Dis **2000**; 182:1169–76.
15. Jelfs J, Munro R, Wedege E, Caugant DA. Sequence variation in the *porA* gene of a clone of *Neisseria meningitidis* during epidemic spread. Clin Diagn Lab Immunol **2000**; 7:390–5.