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Outbreak of Meningococcal Disease Caused by PorA-Deficient Meningococci

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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 guanine 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-
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\(\rightarrow\)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 meningococci 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\(\rightarrow\)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:2aP1.5-1,10-8 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.

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

**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