Antigenic and/or phase variation of PorA protein in non-subtypable Neisseria meningitidis strains isolated in Spain

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The PorA protein is a potential candidate as a vaccine component against meningococcal disease. However, this protein experiences antigenic variation and is subject to phase variations to evade immune selective pressure. In this study, the mechanisms responsible for altered expression of the PorA protein were analysed in 50 non-subtypable strains isolated from patients with meningococcal disease in Spain. The porA gene was amplified from 47 of the 50 strains. The majority of isolates were not recognized by the subtyping panel, as a result of non-synonymous base changes in the variable regions of the porA gene. Two of these strains revealed a premature stop codon before the variable region VR1 of PorA due to a single base-pair substitution at position 109 of the porA coding region. Another two presented a homopolymeric tract of eight adenine residues in the coding region, producing a DNA strand-slippage mechanism and PorA phase variation.

INTRODUCTION

The major outer-membrane protein PorA of Neisseria meningitidis is the product of the porA gene, being expressed by most clinical isolates but with variation in the levels of expression (Hopman et al., 1994; Van der Ende et al., 1995; Sawaya et al., 1999). Despite its antigenic variation, PorA is a potential component of N. meningitidis vaccines. Previous studies have shown that mAbs directed against serosubtype-specific epitopes on PorA have bactericidal activity in serum and confer protection against N. meningitidis infection in an animal model (Saukkonen et al., 1987, 1989). Moreover, immunization of volunteers with experimental vaccines based on outer-membrane vesicles demonstrated that bacterial polyclonal antibodies are mainly directed against this protein, and the presence of these antibodies has been accepted as a marker for protection against infection caused by strains with the same serosubtype(s) as those of the vaccine strain (Rosenqvist et al., 1995; Van der Voort et al., 1997; Longworth et al., 2002). During the past decade, single and multivalent vaccines composed of different PorA epitopes have been developed according to the most prevalent circulating serosubtypes in different geographical areas (Peeters et al., 1996; Cartwright et al., 1999; De Kleijn et al., 2000; Martin et al., 2001).

Because the serosubtype prevalence of meningococci changes over time, continuous monitoring of the circulating serosubtypes is necessary to adapt the vaccine composition in a particular region. Therefore, knowledge of the genetic mechanisms behind the variability of PorA expression is important for designing these kinds of vaccines.

However, previous studies showed that PorA expression could be altered by multiple mechanisms. Slipped-strand mispairing during replication in the homopolymeric tract of guanidine (polyG) and/or thymidine residues between the −10 and −35 domains of the porA promoter, as well as the homopolymeric tract of adenine (polyA) residues in the porA coding region are the principal mechanisms responsible for altered PorA expression (Van der Ende et al., 1995, 2000; Sawaya et al., 1999). In addition, point mutations (Arhin et al., 1997; Van der Ende et al., 2000, 2003) or insertion of an IS element (Newcombe et al., 1998) in the porA coding region or deletion of the complete porA gene (Van der Ende et al., 1999) may result in meningococci lacking PorA expression.

In this study, 50 non-subtypable (NST) strains isolated from patients with meningococcal disease in Spain were analysed to identify the mechanisms responsible for altered expression of the PorA protein.

METHODS

Bacterial strains and chromosomal DNA isolation. Isolates were recovered from blood or cerebrospinal fluid of patients with meningococcal disease in Spain. They were serogrouped/serosubtyped as

Abbreviation: NST, non-subtypable.

The GenBank accession number for the sequence of porA from Neisseria meningitidis isolate 9688 is AY319969.
RESULTS AND DISCUSSION

The porA gene was amplified and sequenced from 47 of the 50 strains analysed (Table 1). The majority of isolates were not recognized by the subtyping panel as a result of non-synonymous base changes in the variable regions of the porA gene. Among strains from which the porA gene could be amplified, only three VR1/VR2 combinations had both epitopes identical to the corresponding mAb, while the rest had a variant of either VR1 or VR2 or both. Another possible reason for mAb subtyping failure may be the limited accessibility of the mAbs to the corresponding epitopes because of a large amount of capsular polysaccharide or lipopolysaccharide masking the PorA VR epitopes, as other authors have suggested previously (Sacchi et al., 2000). This possibility could explain the lack of recognition of epitopes identical to their prototype mAbs. Finally, lack of expression remained another important explanation for some cases of serotyping failure. It has been described previously that meningococci can down- or upregulate expression of the porA gene as a result of non-synonymous base changes in the variable regions of the porA gene (Sacchi et al., 1995; Arhin et al., 1997; Sawaya et al., 1999). In this study, strains with a polyG tract ranging between 9 and 12 guanidine residues were found (Table 2). SDS-PAGE analysis showed the presence of PorA in all strains with a polyG tract.

Table 1. Distribution of PorA types among N. meningitidis strains

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Serotype</th>
<th>VR1</th>
<th>VR2</th>
<th>Strains (n)</th>
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<td>2b</td>
<td>5</td>
<td>2–1</td>
<td>7*</td>
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<tr>
<td>C</td>
<td>2b</td>
<td>19</td>
<td>15–1</td>
<td>3</td>
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<td>–</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
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*Two of these strains presented a stop codon before VR1, and two a homopolymeric tract of eight adenine residues in the coding region.
†Among serogroup C strains, three isolates were NST by genotyping.
ranging between 10 and 12 nt. Only one exception was found for a strain showing nine nucleotide residues in the −10 − 35 region. In this isolate, SDS-PAGE detected no PorA. However, analysis of total RNA by RT-PCR showed that porA mRNA transcript was present, although its expression was lower than that found for other strains analysed that showed 10 or more nt residues.

In relation to the replacement of a guanidine residue by an adenine residue in the polyG tract, there are two contradictory hypotheses, Sawaya et al. (1999), by in vitro mutagenesis experiments, suggested that the replacement of guanidine by adenine residues decreased the promoter activity. On the other hand, van der Ende et al. (1995) suggested that substitutions in the polyG tract increased levels of PorA expression in these strains compared with others that presented identical number of residues in the polyG tract.

In our study, seven strains had mutations in the porA promoter region, other types of phase variations affecting the coding region of the porA gene have been described (van der Ende et al., 1999). We found four subtypable strains corresponding to the P1.5, 2−1 antigenic combination by PorA sequencing, with no PorA expression by SDS-PAGE analysis. Two of these strains revealed a premature stop codon before the variable region VRI caused by a single base-pair substitution (C→T) at position 109 of the porA coding region (GenBank accession no. AY319969). The other two present eight adenine residues in the homopolymeric tract of adenine in the coding region of porA, producing a DNA strand-slippage mechanism and PorA phase variation. SDS-PAGE and Western blotting using the correspondent mAbs confirmed the absence of PorA in these strains.

Finally, we describe the appearance of three NST strains by PorA typing. In these isolates, the PorA protein could not be amplified by PCR using any set of primers, and SDS-PAGE analysis did not detect PorA. Moreover, RT-PCR confirmed no porA mRNA transcript was present, suggesting that the porA gene in these strains was deleted, as other authors have suggested previously (van der Ende et al., 1999).

All these mechanisms have important implications for epidemiological analysis and vaccine design and demonstrate the need for genetic characterization, rather than phenotypic characterization using mAbs, for the identification of meningococcal strains. Moreover, the magnitude of micro-evolutionary mechanisms in meningococci and the remarkable genetic plasticity of these bacteria are important aspects to be considered in relation to PorA vaccine development and its potential efficacy.

**ACKNOWLEDGEMENTS**

B. A. and R. A. hold post-doctoral and pre-doctoral fellowships from Instituto de Salud Carlos III, respectively. Two pre-doctoral fellowships from Wyeth Farma supported C. S. and R. E. M. J. U. holds a pre-doctoral fellowship from FIS-02/9260. PorA type designation was performed by Janet Suker from NIBSC (National Institute for Biological Standards and Control).

**REFERENCES**


**Table 2. Alterations found in the promoter region of the porA gene among NST N. meningitidis strains**

The putative −35 and −10 domains are underlined and the polyG tract is in bold.

<table>
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<tr>
<th>Promoter sequence</th>
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<th>PorA presence by SDS-PAGE</th>
<th>porA mRNA presence by RT-PCR</th>
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<td>+</td>
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<td>1</td>
<td>+</td>
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