INFECTION AND IMMUNITY, July 2007, p. 3683–3685 Vol. 75, No. 7
0019-9567/07/$08.00+0 doi:10.1128/IAI.01721-06

PorA Variable Antigenic Regions VR1, VR2, and VR3 of *Neisseria meningitidis* Serogroups B and C Isolated in Brazil from 1999 to 2004▽

Ivano de Filippis,[1,2]* Claudia Ferreira de Andrade,[1] Luciete Silva,[3] D. Rebecca Prevots,[2] and Ana Carolina P. Vicente[4]

National Institute for Quality Control of Health, Oswaldo Cruz Foundation, Rio de Janeiro 21045-900, Brazil[1]; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892[2]; Leonidas and Maria Deane Research Center, Oswaldo Cruz Foundation, 69057-070 Manaus, Brazil[3]; and Genetics Department, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro 21045-900, Brazil[4]

Received 26 October 2006/Returned for modification 24 January 2007/Accepted 2 May 2007

The high genetic diversity found among the PorA regions VR1 and VR2 of 101 *Neisseria meningitidis* isolates from patients with meningococcal disease and healthy carriers in Brazil contrasts with the stability found in the PorA VR3 of these isolates. The presence of VR3 epitope variant 35 or 36 on the surfaces of 87% of the strains analyzed suggests that these antigens should be considered for inclusion in new formulations of vaccines against serogroup B meningococci in Brazil.

Effective vaccines against the diverse group of heterologous *Neisseria meningitidis* serogroup B strains remain under development. Formulations using different outer membrane proteins (OMP), including vaccines containing multiple PorA variants expressed by genetically engineered meningococci, have been proposed previously (18). However, the interstrain variability of surface-exposed proteins has restricted the protective efficacy of these vaccines to a limited number of antigenically related strains. The inclusion of several OMP, including variants of surface-exposed proteins, in new vaccine formulations to induce a broader immune response has been suggested previously (23). The class 1 transmembrane protein PorA has been one of the most commonly used targets in vaccine trials with serogroup B meningococci derived from locally prevalent epidemic strains in several countries (2, 3, 4, 9, 12, 13, 17, 21). The PorA surface-exposed loops containing variable regions 1 and 2, named VR1 (loop I) and VR2 (loop IV), are used for the determination of meningococcal subtypes (24). According to the PorA sequence database (http://neisseria.org/nm/), the level of amino acid sequence variation among VR1 and VR2 is considerably high; these regions correspond to 11 and 18 sequence families, respectively, each with a large number of genetic variants, for a total of 148 variants of VR1 and 389 of VR2 identified to date. Because of the high level of heterogeneity found in these regions, the PorA vaccines show a narrow range of specificity for *N. meningitidis* strains. A third variable region, designated VR3, is present on the top of loop V (24). The genetic variability of this region has also been studied previously (1, 6, 7, 10, 14, 19), and research has revealed a lower level of genetic variability than that found within VR1 and VR2. One potential explanation is that loop V, where VR3 is located, is slightly shorter than loops I and IV, where VR1 and VR2 are located. For this reason, the VR3 is less exposed to the immune system and therefore subjected to less selection pressure; however, the production of bactericidal antibodies to loop V of PorA after immunization with synthetic peptides has been described previously (5, 15).

In this study, we considered the deduced amino acid sequences of VR1, VR2, and VR3 of 78 strains collected from patients with invasive disease reported through the Brazilian meningitis disease surveillance system and diagnosed in different geographic regions of the country from 1999 to 2004 and those of 23 strains isolated from a cluster of healthy carriers associated with a single index case of invasive disease in the Amazonas state in 2002. Among the strains included in this study, 86 belonged to serogroup B and 15 belonged to serogroup C (Table 1). All the strains isolated from carriers belonged to serogroup B. VR1 and VR2 of the same set of invasive strains described here were analyzed in a recent study (8). In the present study, we analyzed the VR3 of this set of invasive strains and VR1, VR2, and VR3 of the 23 carrier strains.

The amplification of the *porA* gene was performed using a set of primers described previously (11, 14, 20). To assign variable region sequences to families and variants, deduced amino acid sequences of VR1 and VR2 were submitted to the *N. meningitidis* PorA variable regions database (http://neisseria.org/nm/typing/pora). The VR3 variants were classified according to the sequences described by Clarke et al. (7).

Overall, 87.1% of the strains (88% of the invasive strains and 82% of the carrier strains) analyzed had one of two VR3 variants (Table 2). In contrast, 8 VR1 and 12 VR2 families corresponding to these strains had been identified. Of the seven VR3 families (designated 35 to 41) described to date, only four (35, 36, 37, and 38) were found among these Brazilian isolates. The most common VR3 family was 36, with 54 strains (53.4%) belonging to this family. The remaining strains were grouped into VR3 families 35 (34 strains; 36.6%), 37 (5

* Corresponding author. Mailing address: Fundacao Oswaldo Cruz, INCQS, Av. Brasil, 4365, Manguinhos, Rio de Janeiro 21045-900, Brazil. Phone: 55-21-3865-5236. Fax: 55-21-2290-0915. E-mail: ivano.defilippis@incqs.fiocruz.br.

▽ Published ahead of print on 14 May 2007.

TABLE 1. *porA* genosubtypes of *N. meningitidis*

No. of isolates	Genosubtype[a]			Source[b]	No. in serogroup:	
	VR1	VR2	VR3		B	C
2	7	16	35	CSF	2	0
1	5a	1	35-1	Nasoph	1	
10	7-1	1	35-1	CSF	10	0
2	7-2	1	35-1	CSF	2	0
1	7-2	13-1	35-1	CSF	0	1
1	7-4	1	35-1	CSF	1	0
15	12-5a	1	35-1	Nasoph	15	0
1	20	9	35-1	CSF	1	0
1	22	14-6	35-1	CSF	0	1
1	5	2	36	CSF	1	0
1	7-4	1	36	CSF	1	0
1	12-5a	15a	36	Nasoph	1	0
2	12-5a	15	36	Nasoph	2	0
35	19	15	36	CSF	35	0
1	22	14-6	36	CSF	1	0
1	22	14	36	CSF	1	0
3	5	2	36-2	CSF	0	3
2	5-1	10-1	36-2	CSF	1	1
1	5-1	2-2	36-2	CSF	1	0
1	5-1	10-8	36-2	CSF	1	0
4	22	14-6	36-2	CSF	0	4
1	19	15	36-2	CSF	0	1
1	19	15	37	CSF	1	0
2	5-2	10	37-1	CSF	0	2
2	21	16	37-1	CSF	2	0
1	7-2	3	38	CSF	1	0
1	18	14	38	CSF	1	0
2	18-1	3	38	CSF	0	2
1	7a	25-3	38-1	Nasoph	1	0
1	12-5a	25	38-1	Nasoph	1	0
1	12-5a	25-3	38-1	Nasoph	1	0
1	12-5a	4a	38-1	Nasoph	1	0

[a] An "a" at the end of a genosubtype designation denotes a new variant with more than 50% similarity to the indicated variant.
[b] CSF, cerebrospinal fluid; Nasoph, nasopharyngeal sample.

TABLE 2. Distribution of genosubtypes among VR3 families

VR3 family	No. of strains (% of total)	Genosubtypes	
		VR1	VR2
35	34 (36.6)	5, 7, 7-1, 7-2, 7-4, 12, 12-5, 20, 21, 22	1, 1-3, 9, 13-1, 14-6, 16
36	54 (53.4)	5, 5-1, 7-4, 12-5, 19, 22	1, 2, 2-2, 10-1, 10-8, 14, 14-6, 15
37	5 (4.9)	5-2, 19, 21	10, 15, 16
38	8 (7.9)	7, 7-2, 12-5, 18, 18-1	3, 4, 14, 25

strains; 4.9%), and 38 (8 strains; 7.9%). The great majority of isolates (87.1%) belonged to VR3 family 35 or 36 (Table 1). The lower genetic diversity among VR3 than among VR1 and VR2 in strains from carriers as well as patients suggests that this epitope is actually under less selection pressure. This finding confirms the higher stability of VR3 than of VR1 and VR2, which can be explained in part by the possibly lower level of VR3 loop exposure to the extracellular environment, allowing VR3 to evade selection pressure from the host immune system.

Our finding also suggests the existence of two specific genosubtypes each corresponding exclusively to invasive (P1.19,15,36) or carrier (P1.12-5,1,35-1) strains. The population of carriers from which these strains were isolated is not directly comparable to the population from which the invasive disease strains were collected, however, in that the invasive strains were isolated in states throughout Brazil whereas the carriers were all contacts associated with a single index case in the state of Amazonas. For this reason, further studies with invasive disease patients and carriers from the same outbreak will be needed.

Among the isolates, 32 potential antigenic profiles based on the combinations of VR1, VR2, and VR3 were found (Table 1). The three most common profiles were P1.19,15,36, P1.12-5,1,35-1, and P1.7-1,1,35-1 (Table 1). These three profiles represent 58.7% of the strains analyzed. Among the carrier strains, we found three new VR1 and two new VR2 variants. Finally, a number of strains ($n = 7$) would have appeared to be identical if VR3 typing had not been performed. The antigenic potential of these profiles has to be determined further.

Several OMP vaccines are used worldwide but show low levels of functional antibody cross-reactivity (22). The wide range of antigenic diversity within VR1 and VR2 has been demonstrated by the emergence of many variants of these two regions. Interestingly and in agreement with results of other studies, we found that among the 101 strains presenting previously described VR3 variants, there were 8 different VR1 families, 12 different VR2 families, and only 4 different VR3 families. Most of the strains with the Cuban vaccine-related genosubtype P1.19,15 presented VR3 family 36, but interestingly, two variants of this antigenic region in these strains were observed, which is probably a consequence of the exposition and selection of this epitope.

Finally, we believe that the inclusion of VR3 families 35 and 36 in new vaccines against serogroup B and C meningococcal strains isolated in Brazil would provide protection against more than of 80% of the genosubtypes found among carriers and invasive disease patients in Brazil. It has been indicated previously that VR1 and VR2 are immunodominant over other PorA loops, but they also show a higher level of genetic diversity, and thus, a vaccine containing a stable epitope such as VR3 may show a higher degree of efficacy against a wider number of strains. For this reason, it would be advisable to study new vaccine formulations using synthetic peptides 35 and 36 of VR3, since the potential of synthetic peptide immunogens to induce a protective immune response against serogroup B meningococci has already been established (5, 15, 16). Additionally, the present study shows the importance of maintaining a surveillance of the PorA variable regions to rapidly and accurately detect the emergence of genetic variations among circulating strains.

Nucleotide sequence accession numbers. The GenBank accession numbers for *porA* sequences reported here are DQ094010 to DQ094082 and DQ177161 to DQ177183.

We thank Rachel Urwin and Martin Maiden for their guidance with *porA* genotyping carried out at the University of Oxford. This study made use of the *N. meningitidis* PorA typing website (http://neisseria.org/nm/typing/) hosted by the University of Oxford and developed by Keith Jolley and Martin Maiden.

This work was funded by the Instituto Nacional de Controle de Qualidade em Saude (Manguinhos, Brazil) and the Wellcome Trust (United Kingdom).

REFERENCES

1. **Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills.** 1998. Sequencing of porA from clinical isolates of *Neisseria meningitidis* defines a subtyping scheme and its genetic regulation. Can. J. Microbiol. **44:**56–63.
2. **Bjune, G., E. A. Hoiby, J. K. Gronnesby, O. Arnesen, J. H. Fredriksen, A. Halstensen, E. Holten, A. K. Lindbak, H. Nokleby, E. Rosenqvist, et al.** 1991. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet **338:**1093–1096.
3. **Boslego, J., J. Garcia, C. Cruz, W. Zollinger, B. Brandt, S. Ruiz, M. Martinez, J. Arthur, P. Underwood, W. Silva, E. Moran, W. Hankins, J. Gilly, J. Mays, and the Chilean National Committee for Meningococcal Disease.** 1995. Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. Vaccine **13:**821–829.
4. **Cartwright, K. A. V., R. Morris, H. Ruemke, A. Fox, R. Borrow, N. Begg, P. Richmond, and J. Poolman.** 1999. Immunogenicity and reactogenicity in UK infants of a novel meningococcal vesicle vaccine containing multiple class 1 outer membrane proteins. Vaccine **17:**2612–2619.
5. **Christodoulides, M., B. T. McGuinness, and J. E. Heckels.** 1993. Immunization with synthetic peptides containing epitopes of the class 1 outer-membrane protein of Neisseria meningitidis: production of bactericidal antibodies on immunization with a cyclic peptide. J. Gen. Microbiol. **139:**1729–1738.
6. **Clarke, S. C., M. A. Diggle, and G. F. Edwards.** 2001. Semiautomation of multilocus sequence typing for the characterization of clinical isolates of *Neisseria meningitidis*. J. Clin. Microbiol. **39:**3066–3071.
7. **Clarke, S. C., M. A. Diggle, P. Molling, M. Unemo, and P. Olcen.** 2003. Analysis of PorA variable region 3 in meningococci: implications for vaccine policy? Vaccine **21:**2468–2473.
8. **de Filippis, I., and A. C. Vicente.** 2005. Multilocus sequence typing and repetitive element-based polymerase chain reaction analysis of Neisseria meningitidis isolates in Brazil reveal the emergence of 11 new sequence types genetically related to the ST-32 and ST-41/44 complexes and high prevalence of strains related to hypervirulent lineages. Diagn. Microbiol. Infect. Dis. **53:**161–167.
9. **de Moraes, C. J., B. A. Perkins, M. C. Camargo, N. T. Hidalgo, H. A. Barbosa, C. T. Sacchi, I. M. Landgraf, V. L. Gattas, and H. G. Vasconcelos.** 1992. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. Lancet **340:**1074–1078.
10. **Diggle, M. A., and S. C. Clarke.** 2003. Detection and genotyping of meningococci using a nested PCR approach. J. Med. Microbiol. **52:**51–57.
11. **Feavers, I. M., and M. C. Maiden.** 1998. A gonococcal porA pseudogene: implications for understanding the evolution and pathogenicity of *Neisseria gonorrhoeae*. Mol. Microbiol. **30:**647–656.
12. **Findlow, J., S. Taylor, A. Aase, R. Horton, R. Heyderman, J. Southern, N. Andrews, R. Barchha, E. Harrison, A. Lowe, E. Boxer, C. Heaton, P. Balmer, E. Kaczmarski, P. Oster, A. Gorringe, R. Borrow, and E. Miller.** 2006. Comparison and correlation of *Neisseria meningitidis* serogroup B immunologic assay results and human antibody responses following three doses of the Norwegian meningococcal outer membrane vesicle vaccine MenBvac. Infect. Immun. **74:**4557–4565.
13. **Martin, S. L., R. Borrow, P. van der Ley, M. Dawson, A. J. Fox, and K. A. Cartwright.** 2000. Effect of sequence variation in meningococcal PorA outer membrane protein on the effectiveness of a hexavalent PorA outer membrane vesicle vaccine. Vaccine **18:**2476–2481.
14. **Molling, P., M. Unemo, A. Backman, and P. Olcen.** 2000. Genosubtyping by sequencing group A, B and C meningococci; a tool for epidemiological studies of epidemics, clusters and sporadic cases. APMIS **108:**509–516.
15. **Oomen, C. J., P. Hoogerhout, A. M. Bonvin, B. Kuipers, H. Brugghe, H. Timmermans, S. R. Haseley, L. van Alphen, and P. Gros.**2003. Immunogenicity of peptide-vaccine candidates predicted by molecular dynamics simulations. J. Mol. Biol. **328:**1083–1089.
16. **Oomen, C. J., P. Hoogerhout, B. Kuipers, G. Vidarsson, L. van Alphen, and P. Gros.** 2005. Crystal structure of an anti-meningococcal subtype P1.4 PorA antibody provides basis for peptide-vaccine design. J. Mol. Biol. **351:**1070–1080.
17. **Oster, P., D. Lennon, J. O'Hallahan, K. Mulholland, S. Reid, and D. Martin.** 2005. MeNZB: a safe and highly immunogenic tailor-made vaccine against the New Zealand Neisseria meningitidis serogroup B disease epidemic strain. Vaccine **23:**2191–2196.
18. **Peeters, C. C., I. J. Claassen, M. Schuller, G. F. Kersten, E. M. van der Voort, and J. T. Poolman.** 1999. Immunogenicity of various presentation forms of PorA outer membrane protein of *Neisseria meningitidis* in mice. Vaccine **17:**2702–2712.
19. **Riesbeck, K., P. Orvelid-Molling, H. Fredlund, and P. Olcen.** 2000. Long-term persistence of a discotheque-associated invasive *Neisseria meningitidis* group C strain as proven by pulsed-field gel electrophoresis and *porA* gene sequencing. J. Clin. Microbiol. **38:**1638–1640.
20. **Russell, J. E., K. A. Jolley, I. M. Feavers, M. C. Maiden, and J. Suker.** 2004. PorA variable regions of *Neisseria meningitidis*. Emerg. Infect. Dis. **10:**674–678.
21. **Sierra, G. V. G., H. C. Campa, N. W. Varcacel, I. L. Garcia, P. L. Izquierdo, P. F. Sotolongo, G. V. Casanueva, C. O. Rico, C. R. Rodriguez, and M. H. Terry.** 1991. Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. NIPH Ann. **14:**195–207.
22. **Tappero, J. W., R. Lagos, A. M. Ballesteros, B. Plikaytis, D. Williams, J. Dykes, L. L. Gheesling, G. M. Carlone, E. A. Hoiby, J. Holst, H. Nokleby, E. Rosenqvist, G. Sierra, C. Campa, F. Sotolongo, J. Vega, J. Garcia, P. Herrera, J. T. Poolman, and B. A. Perkins.** 1999. Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. JAMA **281:**1520–1527.
23. **Urwin, R., J. E. Russell, E. A. Thompson, E. C. Holmes, I. M. Feavers, and M. C. Maiden.** 2004. Distribution of surface protein variants among hyperinvasive meningococci: implications for vaccine design. Infect. Immun. **72:**5955–5962.
24. **van der Ley, P., J. E. Heckels, M. Virji, P. Hoogerhout, and J. T. Poolman.** 1991. Topology of outer membrane porins in pathogenic *Neisseria* spp. Infect. Immun. **59:**2963–2971.

Editor: V. J. DiRita