The VR2 Epitope on the PorA P1.7-2.4 Protein Is the Major Target for the Immune Response Elicited by the Strain-Specific Group B Meningococcal Vaccine MeNZB

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A protracted epidemic of group B meningococcal disease in New Zealand led to the testing of a strain-specific tailor-made vaccine, MeNZB. Immunogenicity levels achieved during age group trials enabled New Zealand’s regulatory authority to grant licensure to deliver MeNZB to all individuals under age 20. During the trials target strains for serum bactericidal antibody measurements included the vaccine target strain NZ98/254 and two comparator epidemic-type strains (NZ94/167 and NZ02/09). In this study, 12 other strains differing variously from the vaccine strain by their capsular group, PorB type, and PorA variable region specificities, or PorA expression, were used as target strains. The PorA specificity of the serum bactericidal antibody responses to the vaccine was determined for 40 vaccinees. Sets of 10 pre- and postvaccination sera were chosen randomly from the young infant, older infant, toddler, and school-age group trials. Antibody recognition of linearized PorA proteins was also determined using immunoblotting. Across all age groups vaccine-induced serum bactericidal antibodies specifically targeted the VR2 P1.4 epitope of the PorA P1.7-2.4 protein irrespective of the PorB type and/or capsular type of the target strain. Deletion of amino acids within the VR2 epitope or replacement of the epitope through genetic exchange allowed strains variously to resist antibody-directed complement-mediated lysis and negated PorA-specific antibody recognition in immunoblots. The demonstration that the immunodominant antibody response was specifically for the VR2 P1.4 epitope of the PorA protein supports the public health decision to use a strain-specific vaccine for the control of New Zealand’s epidemic of meningococcal disease.

Since 1991, New Zealand has experienced an epidemic of meningococcal disease caused by meningococci belonging to the ST41/ST44 complex, lineage III, with the signature strain type B:4:P1.7-2,4 (2, 3, 7, 8). This strain type accounted for 85.9% of 2,511 group B case isolates identified from 1991 through 2004 (1, 8). To control the epidemic, a strain-specific outer membrane vesicle (OMV) vaccine, MeNZB, was developed by Chiron Vaccines, in association with the Norwegian Institute of Public Health (5, 14, 20). Age group clinical trials were conducted with school-age children enrolled between ages 8 and 12 years, toddlers between 16 and 24 months, older infants between 6 and 8 months, and young infants between 6 and 10 weeks. The safety and immunogenicity of MeNZB were evaluated (15). Total immunoglobulin G (IgG) was determined by enzyme-linked immunosorbent assay using antibody capture OMVs derived from the vaccine strain. Vaccine immunogenicity was determined using a validated serum bactericidal assay (SBA) with the vaccine strain NZ98/254 and two other epidemic strain isolates, NZ94/167 and NZ02/09, as target strains (9). Serial serum dilutions in the SBA started at 1:2. The lower limit of quantitation for this assay was a titer of 4 (reciprocal of 1:4) with a fourfold rise (seroresponse) requiring a minimum titer of 8 (≥1:8) from a baseline titer of 2 (<1:4) (9). The target strain for immunogenicity was the vaccine strain, NZ98/254. In the trials, >70% of the older infants, toddlers, and schoolchildren attained a fourfold rise in serum bactericidal antibody (SBAb) to a titer of ≥8, and >90% achieved a titer of ≥4 against NZ98/254 (15). Results for the young infants are still to be published. The results for the SBAb responses to the comparator B:4:P1.7-2,4 strains (NZ94/167 and NZ02/09) are reported in this study.

PorA has previously been shown to be the immunodominant protein targeted by antibodies following vaccination with group B meningococcal OMV vaccines (22, 27). The PorA protein consists of eight surface-exposed loops, with loops 1 and 4 each containing variable region epitopes labeled VR1 and VR2, respectively (25). The VR1 of the New Zealand strain is designated P1.7-2, and the VR2 is designated P1.4. The P1.7-2 designation recognizes a deletion of three amino acids (VTK) on the carboxy side of the linear epitope that shortens the VR1 loop, masking detection of the P1.7 epitope by antibodies (11). Strains therefore serotype only as P1.4, but the presence of the P1.7-2 epitope can be identified using DNA sequencing analysis. Meningococci with the PorA VR2 P1.7-2 are variously described in the literature as P1.7b (26) and P1.7b (29), where P1.7-2, P1.7b, and P1.7b denote the same genetic variation (www.neisseria.org).

From 1991 through 2003, sequence variations involving deletions (n = 11) or single nucleotide substitutions (n = 2) in the VR2 region of the P1.7-2,4 protein were found in New Zealand case isolates (1). An additional deletion variant was identified in 2004. Most deletions were of different sizes and positions in the encoded epitope. Three nonserosubtypeable isolates had intact P1.7-2,4 DNA sequences but impaired ex-
pression of the PorA protein due to sequence variation in the promoter region of the PorA protein (1). As delivery of MenZB was aimed at halting a strain-specific epidemic, it was important to determine the impact of such variations on recognition by PorA-specific antibodies elicited by the vaccine. A range of target strains derived from meningococcal disease cases was selected for use in the SBA and in immunoblotting. These included strains with deletions in VR2 of the P1.7-2,4 protein, with an alternative VR2, with impaired expression of PorA due to sequence variation in the promoter region of the protein, and differing from the vaccine strain in both VR1 and VR2 regions of the PorA protein. Also tested were unrelated strains from different clonal complexes that expressed alternative capsular antigens in association with the P1.7-2,4 PorA protein. This study reports only PorA-specific antibody responses measured by SBA or by immunoblotting. Responses to other antigens contained in the MenZB vaccine will be reported independently.

### MATERIALS AND METHODS

**Target strains.** Target strains with various phenotypes were selected from meningococcal disease isolates referred to the Meningococcal Reference Laboratory, Institute of Environmental Science and Research (ESR), under New Zealand’s national surveillance program (8). Each strain, assigned a unique code, was collected at a single ‘time point’ in relation to the meningococcal surveillance program. Strains were grown on 50 sheep Columbia blood agar plates overnight (30°C, 5% CO2). Growth on all plates was harvested into 0.2 M LiCl-0.1 M Na acetyl buffer (pH 5.6) before being centrifuged at 15,000 × g for 15 min, and the supernatant discarded, and the cells were further resuspended in Tris-glycine buffer (pH 8.0) before being heated at 56°C for 60 min to inactivate PorB and PorA VR1 and VR2 antigen types, in addition to the control strains, NZ98/254 (vaccine strain) and F91, were used to define epitope positions. Sera were tested at a 1:200 dilution and antibody binding was detected using horseradish peroxidase-labeled goat anti-human IgG (catalog no. P0214; DAKO). All bands were visualized with 4-chloro-1-napthol color development (Bio-Rad).

**Immunoblotting.** Immunoblots were scanned with the DS-800 calibrated densitometer (Bio-Rad). Lanes with the molecular weight marker were aligned. Band recognition by PorA-specific antibodies elicited by the vaccine. A validated SBA (9) was used to determine the serum bactericidal antibody responses against each of the target strains. Human serum complement was predetermined as suitable for each individual target strain. As in the trials, interpolated titer values were measured using a formula that calculates the level of antibodies based on the percentage kill in dilutions immediately on either side of the 50% cutoff. The titer value is the actual dilution of serum at the intersection of the 50% kill/survival cutoff (9). Interpolated titers are reported as log2 titers.

**Outer membrane vesicle preparations.** Outer membrane vesicle (OMV) preparations were prepared by growing each strain on sheep blood agar plates overnight (30°C, 5% CO2). Growth on all plates was harvested into 0.2 M LiCl-0.1 M Na acetyl buffer (pH 5.6) before being centrifuged at 15,000 × g for 15 min, and the supernatant discarded, and the cells were further centrifuged at 100,000 × g for 90 min. The pellet was resuspended in sterile water containing 0.02% azide. The protein concentration was determined using a bicinchoninic acid protein assay kit (catalog no. 23227; Pierce). OMV preparations were stored at 4°C. In-house OMV preparations were validated for protein expression against the Chiron-supplied OMV preparation as used in the MenZB vaccine.

**SDS-PAGE.** Outer membrane proteins prepared for each strain were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a Mini-PROTEAN 3 electrophoresis cell (Bio-Rad, Hercules). Gels were loaded with 1 µg protein per well. For size reference the side wells and the central well were loaded with 1 µl of molecular weight marker (MagicMark; Invitrogen, Carlsbad, Calif.). Samples were loaded in a 4% acrylamide gel and separated in a 12% acrylamide gel using Tris-glycine buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) Gels were run at 200 V for 45 min. Protein bands were visualized by staining for 1 h in Coomassie brilliant blue (0.1% [wt/vol] in 50% [vol/vol] methanol, 10% [vol/vol] acetic acid) and destaining overnight in 10% (vol/vol) methanol-10% (vol/vol) acetic acid. Protein sizes were compared to the prestained SDS-PAGE broad-range standard (Bio-Rad) or the MagicMark molecular weight marker (Invitrogen, Carlsbad, Calif.). Immunoblots were scanned with the DS-800 calibrated densitometer (Bio-Rad). Lanes with the molecular weight marker were aligned. Band densities in all lanes were measured and plotted as density against the relative amount of protein.

### TABLE 1. Description of strains

| Strain       | Phenotype | PorA epitope | VR2 sequencea | MAb recognitionb |
|--------------|-----------|--------------|---------------|-------------------|
| NZ98/254     | B:4:P1.4  | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ94/167     | B:4:P1.4  | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ02/09      | B:4:P1.4  | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ97/47      | B:14:P1.4 | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ97/122     | B:1:P1.4  | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ02/199     | B:4:NT    | P1.7-2,4-10  | HVVN–NKNVKVATHVP | –                 |
| NZ02/68      | B:4:NT    | P1.7-2,4     | HVVN–NKNVKVATHVP | –                 |
| NZ97/27      | B:4:NT    | P1.7-2,4     | HVVN–NKNVKVATHVP | –                 |
| NZ96/142     | B:4:NT    | P1.7-2,4     | HVVNKNVKVATHVP | –                 |
| NZ00/265     | B:NT:P1.15| P1.7-2,15    | HYTRQNNADVVFVP | –                 |
| NZ00/41      | B:4:P1.19,15| P1.19,15    | HYTRQNNADVVFVP | –                 |
| NZ02/38      | C:2a:P1.5 | P1.5-1,10-4  | HVVN–NKNQ0OPPLTV | –                 |
| NZ96/211     | C:2b:P1.4 | P1.7-2,4     | HVVNKNVKVATHVP | +                 |
| NZ04/117     | W135:2a:P1.4| P1.7-2,4   | HVVNKNVKVATHVP | +                 |
| F91          | P1.4      | P1.7-2,4     | HVVNKNVKVATHVP | +                 |

a—, missing amino acid. Letters denote amino acids.
b Monoclonal antibody P1.4(NIBSC 02/148); +, positive; -, negative.
TABLE 2. Number of individuals with seroresponses and their serum bactericidal antibody titer range against comparator B:4:P1.7-2,4 target strains in subjects vaccinated with MeNZB

| Measure                      | Young infants (6 to 8 wk) | Older infants (6 to 10 mo) | Toddlers (8 to 24 mo) | Schoolchildren (8 to 12 yr) |
|------------------------------|---------------------------|---------------------------|-----------------------|-----------------------------|
| nz98/254                     |                           |                           |                       |                             |
| No. with seroresponses/total | 126/239                   | 148/201                   | 173/231               | 369/486                     |
| % with seroresponses          | 53                        | 74                        | 75                    | 76                          |
| Titer range<sup>c</sup>       | 3.0–11.0                  | 3.0–9.8                   | 3.0–9.9               | 3.0–12.6                    |
| nz94/167                     |                           |                           |                       |                             |
| No. with seroresponses/total | ND                        | 136/201                   | 172/231               | 358/486                     |
| % with seroresponses          | ND                        | 53                        | 74                    | 74                          |
| Titer range<sup>c</sup>       | 3.0–9.8                   | 3.0–10.3                  | 3.0–12.2              |                             |
| nz02/09                      |                           |                           |                       |                             |
| No. with seroresponses/total | ND                        | 107/201                   | 122/231               | 277/486                     |
| % with seroresponses          | ND                        | 53                        | 53                    | 57                          |
| Titer range<sup>c</sup>       | 3.0–8.5                   | 3.0–9.5                   | 3.0–11.5              |                             |

<sup>a</sup> Number of individuals achieving a minimum log<sub>2</sub> 3.0 titer from a baseline titer of log<sub>2</sub> 1.0. ND, not determined.

<sup>b</sup> Range of postvaccination log<sub>2</sub> interpolated titers achieved by those with seroresponses.

front. The data were exported to Microsoft Excel for further analysis. To determine the postvaccination change, the density obtained for the prevaccination serum was subtracted from the density obtained postvaccination. This was calculated for approximately 800 relative front points and plotted on a graph with the average peak position of the molecular weight markers defined. PorA peak densities were scored on a scale of 0 to 4, with 0 indicating no antibody recognition and 4 (>2.0 units) indicating considerable antibody increase. Validation of this method with 10 serum samples tested on three different days showed that the greatest density variation in repeat testing was 0.2 (standard deviation, 0.07). Peak densities of >1 were considered increases in antibody concentration.

RESULTS

Comparison of SBA titers obtained during trials using comparator epidemic strains as target strains. Serum bactericidal antibody responses to strains NZ94/167 and NZ02/09 were not undertaken for the 6- to 8-week-old infant trial due to the shortage of sera and the need to undertake alternative antibody testing. Across the three older age groups (older infants, toddlers, and schoolchildren), similar SBA titers and seroresponse rates (≥8, greater than or equal to log<sub>2</sub> 3) were obtained when using either NZ98/254 or NZ94/167 as a target strain. When NZ02/09 was used as the target strain, lower seroresponse rates (P < 0.02) were recorded for most subjects in the three age groups, although the titer range was similar to those obtained with both NZ94/167 and NZ98/254 (Table 2).

Serum antibody responses to target strains expressing P1.7-2,4 PorA regardless of their group or PorB type. SBA titers obtained using NZ98/254 as the reference target strain for this study were consistent with those that had been obtained in the trials. Across the age groups similar SBA titers were obtained when using NZ98/254 (B:4:P1.7-2,4), NZ97/47 (B:14:P1.7-2,4), and NZ96/211 (C:2b:P1.7-2,4) as target strains (Table 3). Post-vaccination titers (≥8, greater than or equal to log<sub>2</sub> 3) were achieved in 39/40 (97.5%) sera tested against target strain NZ98/254 (log<sub>2</sub> range, 2.3 to 9.6) and 38/40 (95.0%) sera tested with NZ97/47 (B:14:P1.7-2,4) (log<sub>2</sub> range, 1.0 to 10.6). All 40 sera showed titers of ≥8 against target strain NZ96/211 (C:2b: P1.7-2,4) (log<sub>2</sub> range, 3.3 to 10.4) (Table 3). Sera with the highest SBA titers showed the strongest differential band.
than or equal to log2 3 (log2 range, 1.0 to 5.7). Deletions in the tested against strain NZ97/27 (B:4:P1.7-2,-) had titers greater almost totally deleted (B:4:P1.7-2,-). Only 6/23 (26.1%) sera to 10.5) with target strain NZ02/68, which had the VR2 epitope.

PorA VR2 epitope. When strains with deletions in the VR2 determinations. The latter two strains were not used as target strains in SBAb says by 33/40 (82.5%) and 14/23 (60.9%) sera, respectively. The NZ04/117 (W:2a:P1.7-2,4) were recognized in immunoblot as-protein of strains NZ97/122 B:1:P1.7-2,4; 11, NZ97/27 B:4: P1.7-2,- (VR2 deletion); 12, H44/76 B:15:P1.7,16; 13, NZ00/41 B:4: P1.19,15.

$$\text{protein of strains NZ99/254 (Fig. 1, lane 10)}$$ and against the PorA of strain NZ96/211 (Fig. 1, lane 8). The PorA proteins of strains NZ97/122 B:1:P1.7-2,4, 37/40 (92.5%) sera bound to the PorA in immunoblotting. Strong band densities were obtained with 36/40 (90%) sera against the PorA of strain NZ98/254 (Fig. 1, lane 6), 37/40 (92.5%) sera against the PorA of strain NZ97/47, and 33/40 (82.5%) sera against the PorA of strain NZ96/211 (Fig. 1, lane 8). The PorA protein of strains NZ97/122 (B:1:P1.7-2,4) (Fig. 1, lane 10) and NZ04/117 (W:2a:P1.7-2,4) were recognized in immunoblot assays by 33/40 (82.5%) and 14/23 (60.9%) sera, respectively. The latter two strains were not used as target strains in SBAb determinations.

Serum antibody responses to strains with deletions in the PorA VR2 epitope. When strains with deletions in the VR2 P1.4 epitope were used as target strains, SBAb titers varied depending on the nature of the deletion in PorA (Table 3). Thirty-one of the 40 (80.0%) sera showed at least a fourfold rise in titer with the use of target strain NZ01/199 with a one-amino-acid deletion in the P1.4 epitope (B:4:P1.7-2,4-10). The postvaccination log2 titer range for the 40 sera was 1.0 to 10.5. In contrast, only 15/40 (37.5%) sera showed at least a fourfold elevation in titer postvaccination (log2 titer range, 1.0 to 10.5) with target strain NZ02/68, which had the VR2 epitope almost totally deleted (B:4:P1.7-2,-). Only 6/23 (26.1%) sera tested against strain NZ97/27 (B:4:P1.7-2,-) had titers greater than or equal to log2 3 (log2 range, 1.0 to 5.7). Deletions in the VR2 of the PorA protein in strains NZ01/199, NZ02/68, and NZ97/27 resulted in weak or no recognition of the PorA protein by antibodies in immunoblots (Fig. 1, lanes 3, 5, and 11). Differential band densities of >1 demonstrating antibody binding to the PorA protein were shown by 22/40 (55%) sera with strain NZ01/199 (3-bp deletion) compared with 10/40 (25.0%) sera with strain NZ02/68 and 5/40 (12.5%) sera with strain NZ97/27.

Serum antibody responses to a strain not expressing the PorA protein. Although the strain had a complete VR2 sequence, no SBAb responses were detected against target strain NZ96/142 (B:4:P1.7-2,4). Impaired expression of the PorA protein of strain NZ96/142 was due to a reduction of guanidine residues in the polyguanidine track of the porA promoter region. The failure to express PorA by strain NZ96/142 resulted in limited recognition of the PorA protein by antibodies in immunoblots (Fig. 1, lane 9). Only 23/40 (57.5%) sera bound to the PorA in immunoblots.

Serum antibody responses to a strain with alternative VR2. When strain NZ00/263 (B:NT:P1.7-2,15), which had a different VR2 epitope (P1.15), was used as the target strain, only 4/40 (10.0%) sera showed a postvaccination log2 titer greater than or equal to log2 3. The overall titer range was 1.0 to 4.6 (Table 3). Only 4/40 (10.0%) sera showed weak binding (≥1) of antibody to PorA in immunoblots (Fig. 1, lane 2).

Serum antibody responses to heterologous strains. Only five subjects tested showed weak SBAb levels when heterologous strains expressing PorA proteins were unrelated to the epidemic strain. Two of these five sera (9.5%) had weak SBAb titers against NZ02/38 (C:2a:P1.5-1,10-4), giving postvaccination log2 titers of 3.6 and 4.7; 2/20 sera had log2 titers of 4.0 and 4.8 to strain NZ00/41 (B:4:P1.19,15); and 2/23 (8.7%) sera, including one that also had SBAb to NZ00/41, responded to H44/76 with postvaccination log2 titers of 3.5 and 5.7, respectively (Table 3). Only weak (≤1.5) heterologous immunoblotting band densities were recorded by 1/26 (3.8%) sera against strain NZ02/38 (C:2a:P1.5-1,10-4), 1/40 (2.5%) against strain NZ00/41 (B:4:P1.19,15), and 3/40 (7.3%) against strain H44/76 (Fig. 1, lanes 11 and 12). Failure to recognize alternative PorA types in immunoblot assays coincided with the failure of the same sera to effect SBAb killing of strains unrelated to the vaccine strain.

DISCUSSION

The threshold of a titer of ≥8 for a minimum fourfold rise in SBAb used in this study (9) is more stringent than the titer of ≥4 used in other published studies (17, 22). Thus, no attempt was made to compare levels or geometric mean titers (GMTs) of SBAb. Although all three strains used in the trials typed as B:4:P1.7-2,4, results showed that the percentages of cases achieving a fourfold rise in SBAb to a titer of ≥8 following the primary vaccination course were similar across the age groups with strains NZ98/254 and NZ94/167 but were lower with NZ02/09 (Table 2). Strains NZ98/254 and NZ94/167 express small amounts of Opc protein and immotype as L3,7,9. Strain NZ02/09 does not express Opc and immotypes as L3,7,7. This strain required an alternative human complement source to that used by the other two strains. Vermont and coworkers (28) similarly reported unexplained variability in SBAb titers when different New Zealand epidemic strains, NZ91/40, NZ92/53, and NZ97/181, were used as target strains against a range of sera obtained from three different vaccine trials conducted in Europe. Subjects had received either the RIVM HexaMen vaccine or the RIVM MonoMen vaccine, both containing the P1.7-2,4 PorA protein. These workers reported GMTs and percentage seroresponse based on a titer of ≥4 when using target strains NZ92/53 and NZ97/181 that were significantly higher than GMTs recorded using either the P1.7-2,4 isogenic mutant of H44/76 (MonoMen vaccine strain) or the New Zealand strain NZ91/40 (28). Undetermined differences in protein configuration or antigen presentation may explain the variation in responses to different strains of the same strain.
type observed in the two different studies. The MonoMen and HexaMen vaccines used by Vermont and coworkers are designed to elicit only a PorA immune response.

Multiple mechanisms have been shown to alter PorA expression (23). Previously, we reported that reduced expression of PorA due to promoter region deletions in strain NZ97/142 prevented uptake of monoclonal antibodies and thus failure to identify the serotype of this strain (1). Antibodies in the sera of vaccinees also failed to effect complement-mediated bactericidal killing of NZ97/142 although the P1.4 epitope was recognized by type-specific antibody in sera when the PorA protein was linearized in immunoblot assays. Gorla et al. (4) showed that strains isolated from some vaccine failure cases following immunization with the Cuban vaccine (MENGOCBC) in Brazil had a poly(G) tract with only 10 residues and expressed low levels of PorA protein. A cluster of five cases caused by a group C strain not expressing PorA protein was recently reported (24). While failure to express PorA by a group C strain should not interfere with the effectiveness of a C-conjugate vaccine, the effectiveness of a group B PorA-specific vaccine could be compromised by the emergence of such a strain.

Using synthetic peptides, McGuinness et al. (12) showed that the minimum VR2 epitope recognized by P1.4 monoclonal antibody has the peptide sequence VNNKV. Previously, we have reported the failure of P1.4 monoclonal antibody to recognize the P1.4-8 epitope of strain NZ98/214, which has a single amino acid change from K to Q within the minimum epitope (1). In contrast, monoclonal antibody did recognize the P1.4-11 variant on strain NZ01/56, which has a T instead of a P amino acid beyond the minimum described epitope sequence (1). These strains were not used as target strains in this study. Strains NZ02/68 (B:4:P1.7-2,-) and NZ92/27 (B:4:P1.7-2,-), which have an almost complete deletion of the VR2, including the minimum peptide sequence, showed greater resistance to bactericidal killing than strain NZ01/199 (B:4: P1.7-2, P1.4-10), which has a one-amino-acid deletion within the minimum peptide sequence of the VR2 (Table 1). With target strains NZ02/68 and NZ97/27, only 37.5% (15/40) and 26% (6/23) of the sera tested demonstrated a 4-fold rise in SBAb compared with 77.5% (31/40) for target strain NZ01/199. Deletions in VR2 also had an impact on antibody recognition of linearized proteins in immunoblotting, with 10/40 (25.0%) and 5/40 (12.5%) sera showing PorA antigen recognition for strains NZ02/68 and NZ97/27, respectively, compared with 22/40 (50.0%) for strain NZ01/199 (Table 3). In two trials undertaken following vaccination with MonoMen, Vermont and coworkers (28) reported that only 15% and 6% of children had significant SBAb responses to a variant strain with P1.7-8,4-1 PorA, which had an amino acid change from K to N within the P1.4 minimum epitope, compared with a 100% response rate against the vaccine strain containing the intact P1.7-2,4 PorA. Similarly, Martin et al. (10) showed that SBAb titers in children vaccinated with the RIVM recombinant hexavalent vaccine containing the intact P1.10 antigen were significantly different from those obtained when tested against the variant target strain with P1.10-1 (P < 0.0001) or the variant target strain with P1.10-6 (P < 0.0001). Both of these PorA variants had a change from R to P in the minimum peptide sequence for the P1.10 VR2 epitope.

The complete exchange only of the VR2 P1.4 epitope for a P1.15 epitope in strain NZ00/263 resulted in the failure of vaccine-induced antibody either to activate complement-mediated bactericidal killing of this strain or to recognize the PorA protein in immunoblot assays (Table 3). With target strains NZ02/38 (C:2a:P1.5,-1,10-4), NZ00/41 (B:4:P1.19,15), and H44/76 (B:15:P1.7,16) having quite distinct PorA types, vaccine-induced antibody demonstrated little activation of complement-mediated bactericidal killing although strain NZ00/41 had the same PorB as NZ98/254. The PorA proteins of these heterologous strains were recognized by few sera in the immunoblotting of samples from MeNZB vaccinees. Antibodies recognized in immunoblotting as having been elicited against other antigens present in MeNZB vaccine, such as PorB and Rmp, were shown to have no significant bactericidal activity, as they failed to effect significant killing of target strains deficient in the VR2 P1.4 PorA antigen (Table 3).

The results from this study using natural mutant strains from cases of disease confirm that the VR2 P1.4 epitope is immunodominant on the New Zealand epidemic strain, B:4:P1.7-2,4. This study clearly shows that bactericidal killing depends on the interaction between the intact P1.4 epitope and P1.4-specific antibody, irrespective of the expression of other antigens on the target strain or the clonal derivation of the strain. This was demonstrated by bactericidal killing of the target strain NZ96/211 (C:2b:P1.7-2,4) by serum from infants and children vaccinated with MeNZB. That the P1.4 (VR2) epitope of the P1.7-2,4 PorA protein is the major target for serum bactericidal antibodies is consistent with the findings of Vermont and coworkers (28), who showed that the majority of antibodies induced by the P1.7-2,4-specific MonoMen vaccine were directed against VR2 P1.4. These workers also suggested that limited recognition of the PorA P1.4 epitope on a strain expressing the PorA protein P1.12-1,4 may have been related to the inaccessible of VR2 on that strain (28).

Idanpaan-Heikkila and coworkers (6), using purified P1.7 and P1.16 peptides in immunoblotting and enzyme-linked immunosorbent assays, showed strong IgG responses only to the P1.16 (VR2) peptide in the convalescent-phase sera from four cases following B:15:P1.7,16 disease. They also showed that previous vaccination with the B:15:P1.7,16 OMV vaccine was associated with a strong anti-P1.16 VR2 booster response. The dominance and specificity of the PorA immune response following vaccination with OMV vaccines were also demonstrated in a study of T-cell responses after vaccination with the Norwegian OMV vaccine MenBVac (13). MenBVac induced strong proliferative human T-cell responses against OMV and the PorA protein P1.7,16 (γ = 0.50, P < 0.0001), whereas responses against the PorB protein were considerably lower. It has also been demonstrated in an infant rat model that only class I outer membrane PorA protein antibodies were highly protective against bacterial challenge (19).

This study was undertaken using sera collected during the trials of a strain-specific vaccine, MeNZB, in New Zealand. This vaccine is currently being delivered throughout New Zealand to those under the age of 20 years. Concerns have been expressed that use of a vaccine specifically targeting the immunodominant PorA on an epidemic strain carries a risk of selecting for escape mutants that would evade immune recog-
nition (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification resulted in three strains represented 0.6% of 2,511 group B culture-confirmed cases (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification resulted in three strains represented 0.6% of 2,511 group B culture-confirmed cases (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification resulted in three strains represented 0.6% of 2,511 group B culture-confirmed cases (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification resulted in three strains represented 0.6% of 2,511 group B culture-confirmed cases (10, 16, 21). From 1991 through 2004, PorA variant strains represented 0.6% of 2,511 group B culture-confirmed cases (8). This study has shown that SAb elicited by MeNZB specifically targeted the VR2 P1.4 epitope of the P1.7,2-4 PorA protein and that either a lack of expression or genetic modification of the amino acid sequence in the VR2 epitope resulted in a loss of antibody recognition. In contrast, differences in the VR2 epitope protein and that either a lack of expression or genetic modification resulted in three strains represented 0.6% of 2,511 group B culture-confirmed cases (10, 16, 21).