Comparison of Phenotypically Indistinguishable but Geographically Distinct *Neisseria meningitidis* Group B Isolates in a Serum Bactericidal Antibody Assay[▽]

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The "gold standard" assay for measuring serologic protection against *Neisseria meningitidis* group B (MenB) is the serum bactericidal antibody (SBA) assay. Of vital importance to the outcome of the SBA assay is the choice of the target strain(s), which is often chosen on the basis of phenotype or genotype. We therefore investigated the effect on the results produced by the SBA assay of using phenotypically indistinguishable but geographically distinct MenB isolates. Nine PorA P1.19,15 and 11 PorA P1.7-2,4 MenB isolates were incorporated into the SBA assay using human complement and were assayed against sera obtained either before or after outer membrane vesicle vaccination. Large differences in the results produced by the isolates in the SBA assay were demonstrated. These included differences as great as 5.8-fold in SBA geometric mean titers and in the proportions of subjects with SBA titers of ≥ 4. Ranges of as many as 9 SBA titers were achieved by individual sera across the panels of isolates. To determine the reasons for the differences observed, investigations into the expression of capsular polysaccharide, PorA, PorB, Opc, and lipooligosaccharide (LOS) and into LOS sialylation were completed. However, minor differences were found between strains, indicating similar expression and no antigen masking. These results have implications for the choice of MenB target strains for inclusion in future studies of MenB vaccines and highlight the requirement for standardization of target strains between laboratories.

Neisseria meningitidis group B (MenB) remains a major cause of bacterial meningitis and septicemia, associated with high mortality and morbidity. The poor immunogenicity of the MenB polysaccharide (28) and fears about the possible induction of autoimmune antibodies (8) have hindered the development of an effective MenB polysaccharide vaccine. Consequently, the development of vaccines to confer protection against MenB disease has focused on subcapsular antigens either as outer membrane vesicles (OMV) or as individual antigens.

Numerous candidate OMV vaccines have been developed, including monovalent vaccines in response to single-strain epidemics in Norway (9), Cuba (21), Chile (4), and New Zealand (12). However, the ability of these vaccines to afford protection against heterologous meningococci in young children is questionable (15, 16, 22, 27), and this issue has led to the development of multivalent OMV vaccines, including bivalent (1, 5), hexavalent (25), and nonavalent (24) vaccines, for use in areas with multiclonal MenB disease. Protein vaccines, either as individual or as multivalent formulations, have also been de-

veloped and may be broadly cross-reactive against diverse meningococci. Early studies using a vaccine containing five proteins has produced promising results (10), and further development is awaited.

The immunogenicity of OMV vaccines has been evaluated primarily by the determination of functional activity using the serum bactericidal antibody (SBA) assay, which has been recommended as the primary end point for MenB vaccine evaluation (3). The MenB SBA assay was recently standardized between four laboratories (2), and a tentative protective titer of ≥ 4 has been proposed for assays using human complement (12). Prior to this proposal, MenB vaccine studies generally did not rely on SBA titer cutoffs but used ≥ 4-fold rises in SBA titers from before vaccination to 1 month following the last scheduled vaccination. Despite the inherent variation in the SBA assay, with the magnitude of titers varying, proportions of subjects with both ≥ 4-fold rises and SBA titers of ≥ 4 remain relatively constant between laboratories (2).

Of vital importance to the outcome of the SBA assay is the choice of the target strain(s), which is often chosen on the basis of phenotype or genotype. Candidate subcapsular-antigen-based MenB vaccines have been evaluated using a large variety of target strains, including wild-type and isogenic strains. Vaccine evaluation is further complicated by the necessity of using numerous target strains to determine the cross-reactive response or the responses to all components of multivalent vaccines. The use of phenotypically indistinguishable target strains

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TABLE 1. MenB P1.19,15 wild-type isolates investigated in the SBA assay

Isolate	Phenotype	Immunotype	Sequence type	Clonal complex	Isolation site[a]	Date of isolation (day/mo/yr)	Geographical location
M00 242888	B:4:P1.19,15	L3,7,9	749	32	Nasal swab	31/10/2000	Malta
M00 243202	B:4:P1.19,15	L3,7,9	34	32	CSF	11/12/2000	Rochdale, United Kingdom
M01 240075	B:4:P1.19,15	L3,7,9	749	32	CSF	06/01/2001	North Tyneside, United Kingdom
M01 240121	B:4:P1.19,15	L3,7,9	1398	32	Blood	09/01/2001	Milton Keynes, United Kingdom
M01 240265	B:4:P1.19,15	L3,7,9	749	32	Blood	25/01/2001	Malta
M01 240400	B:4:P1.19,15	L3,7,9	749	32	Blood	07/02/2001	Malta
M01 241017	B:4:P1.19,15	L3,7,9	41	41/44	Blood	10/04/2001	Nottingham, United Kingdom
M01 241117	B:4:P1.19,15	L3,7,9	749	32	Blood	27/04/2001	Malta
M01 241535	B:4:P1.19,15	L3,7,9	749	32	CSF	28/06/2001	Liverpool, United Kingdom

[a] CSF, cerebrospinal fluid.

in the SBA assay has been shown to significantly affect SBA geometric mean titers (GMTs) and proportions of subjects with ≥4-fold rises in SBA titers from before vaccination to after vaccination (26). Additionally, it was concluded that the immunogenicity of the hexavalent vaccine Hexamen against subtype P1.7-2,4 strains may have been underestimated, because the responses to wild-type isolates were significantly greater than that to the original, isogenic vaccine strain (26). More recently, similar findings have been reported following vaccination with both the "Norwegian" vaccine MenBvac and the "New Zealand" vaccine MeNZB, with subtype P1.7-2,4 (7, 13).

Due to the importance of these findings, coupled with suggestions for standardization of target strains for the evaluation of MenB vaccines (2, 3, 6), the effects of using different, phenotypically indistinguishable isolates in the MenB SBA assay were investigated. Wild-type MenB isolates with subtypes P1.7-2,4 and P1.19,15 were investigated because these are prevalent in the United Kingdom (11) and are contained in current OMV vaccine formulations (1, 5, 12, 21, 24, 25). To determine the true level of variation, isolates recovered from geographically distinct locations within the United Kingdom and Malta were chosen. Additionally, two P1.7-2,4 strains previously used in the evaluation of MenB vaccines were also used as a comparator.

MATERIALS AND METHODS

Isolates. Wild-type disease-causing MenB isolates with subtypes P1.19,15 and P1.7-2,4 that were isolated from geographically distinct locations or distinct in date of isolation were obtained from the Health Protection Agency (HPA)

Meningococcal Reference Unit (MRU). Isolates from Malta were available because the HPA MRU provides assistance to Malta in the absence of a Maltese MRU. On arrival at the MRU, isolates were cultured on Columbia horse blood agar (CHBA) plates (Oxoid, Basingstoke, United Kingdom) and stored as frozen cultures in Microbank cryovials containing glycerol broth (ProLab Diagnostics, Ontario, Canada) at −80°C.

Following investigations into the compatibility of wild-type isolates with the SBA assay methodology, nine P1.19,15 and nine P1.7-2,4 isolates were used in the study; they are listed and characterized in Tables 1 and 2, respectively. Two P1.7-2,4 strains previously used in the evaluation of MenB vaccines, M01 240149 and NZ 98/254, were also utilized (7, 18).

Isolate characterization. Isolates were characterized at the HPA MRU as previously reported (11), with the exception of lipooligosaccharide (LOS) immunotyping, which was carried out as previously described (20).

SBA assay. The standardized SBA assay was performed as previously described (6), incorporating a starting dilution of 1/4. Briefly, human serum at 25% was used as an exogenous source of human complement, with titers expressed as the reciprocal of the final dilution giving ≥50% killing by SBA at 60 min compared to the control column (inactive complement/no test sera). Validation determined that the interassay variation was ≤2 titer dilutions; therefore, results were considered different if they were >2 titer dilutions apart.

Serum samples. Samples were from a study of a bivalent OMV vaccine (P1.19,15 and P1.7-2,4) with young adults (ages, 18 to 25 years) (5) and comprised samples taken before and after vaccination. The panel consisted of 15 samples with positive and negative SBA titers against either P1.19,15 or P1.7-2,4 vaccine strains (data not presented).

Data analysis. Results from the SBA assay were log transformed, and GMTs with 95% confidence intervals (CI) were calculated. In the SBA assay, titers of <4 were assigned a value of 2 for data analysis. Significant differences in the $\log_{10}$ SBA titers were calculated using regression with factors for samples and isolates, and differences in GMTs were compared using Bonferroni's correction.

Comparison of capsular polysaccharide, PorA, PorB, LOS, and Opc levels. To determine differences in expression, monoclonal antibodies (MAbs) from the National Institute of Biological Standards and Controls (NIBSC; Hertfordshire, United Kingdom) against MenB capsular polysaccharide (95/750), type P3.4 (01/536), subtypes P1.4 (95/700), P1.19 (04/248), and P1.15 (02/114), immuno-

TABLE 2. MenB P1.7-2,4 wild-type isolates investigated in the SBA assay

Isolate	Phenotype	Immunotype	Sequence type	Clonal complex	Isolation site[a]	Date of isolation (day/mo/yr)	Geographical location
M00 242922	B:4:P1.7-2,4	L1,8	41	41/44	Blood	01/11/2000	London, United Kingdom
M00 243221	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	13/12/2000	Pinderfield, United Kingdom
M01 240017	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	31/12/2000	Horton, United Kingdom
M01 240554	B:1:P1.7-2,4	L3,7,9	41	41/44	Blood	26/02/2001	Ipswich, United Kingdom
M01 240566	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	24/02/2001	Bury St Edmonds, United Kingdom
M01 240805	B:4:P1.7-2,4	L1,8	41	41/44	Blood	15/03/2001	Birmingham, United Kingdom
M01 240938	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	03/04/2001	London, United Kingdom
M01 241245	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	10/05/2001	Leeds, United Kingdom
M01 241607	B:4:P1.7-2,4	L3,7,9	41	41/44	Blood	01/07/2001	Camberley, United Kingdom
M01 240149	B:4:P1.7-2,4	L1,8	41	41/44	Blood	12/01/2001	Maidstone, United Kingdom
NZ 98/254	B:4:P1.7-2,4	L1/L3,7,9	42	41/44	CSF	1998	New Zealand

[a] CSF, cerebrospinal fluid.

TABLE 3. SBA GMTs for P1.19,15 isolates and significant differences in SBA GMTs between isolates

Isolate	GMT (95% CI)	Isolate(s) with significantly different GMTs
M01 241535	25.4 (8.9–72.8)	M01 240121, M01 240265, M01 240075
M01 241117	26.6 (10.1–70.0)	M01 240121, M01 240265, M01 240075
M00 243202	40.3 (13.1–123.7)	M01 240265, M01 240075
M01 240400	42.2 (13.5–132.1)	M01 240265, M01 240075
M00 242888	50.8 (16.7–154.3)	M01 240075
M01 240017	50.8 (19.3–133.9)	M01 240075
M00 240121	67.0 (24.7–182.1)	M01 241535, M01 241117
M01 240265	101.6 (29.5–349.9)	M01 241535, M01 241117, M00 243202, M01 240400
M01 240075	147.0 (39.1–552.3)	M01 241535, M01 241117, M00 243202, M01 240400, M00 242888, M00 240121

type L3,7,9 (01/412), and Opc (trial fill; therefore, no NIBSC code [19]) were incorporated into the SBA assay or a whole-cell enzyme-linked immunosorbent assay (ELISA). The level of LOS sialylation was determined by using the same assays with MAb 3F11 (29), kindly provided by M. Apicella (University of Iowa, Iowa City).

Whole-cell ELISA. Meningococci were cultured overnight on CHBA (Oxoid) at 37°C under 5% CO_2. Approximately 50 colonies were subcultured onto fresh CHBA plates and incubated under the same conditions for a further 4 h. Following incubation, meningococci were suspended in a buffer consisting of 15 mM Na_2CO_3 (Sigma-Aldrich, Dorset, United Kingdom) and 35 mM $NaHCO_3$ (Sigma-Aldrich) at pH 9.6. The suspension was adjusted to an optical density (OD) of 0.1 at 650 nm and was incubated at 60°C for 60 min. Killed suspensions of meningococci were added to Immulon 2 HB (Thermo Labsystems, Franklin, MA) ELISA plates, and the plates were coated overnight at 4°C. Twofold serial dilutions of MAbs in the coated plates were completed in 10 mM phosphate-buffered saline, 0.05% Tween 20 (Sigma-Aldrich), and 5% newborn bovine serum albumin (ICN Phamaceuticals, Basingstoke, United Kingdom). Plates were incubated overnight at 4°C. Following incubation, plates were washed, MAbs were detected using alkaline phosphatase-conjugated anti-mouse immunoglobulin G (Sigma-Aldrich), and plates were incubated at room temperature for 2.5 h. The assay was developed using a p-nitrophenol phosphatase substrate (Sigma-Aldrich) and stopped after 2 h with 3 M NaOH (Sigma-Aldrich). All experiments were completed a minimum of three times, and the average ODs with equal concentrations of MAb were compared. Validation determined an interassay reproducibility of ≤25% (based on OD). Therefore, expression levels for strains were deemed different if the difference in the OD between strains was >25%.

RESULTS

Determination of suitability of isolates for use in the SBA assay. Initially, 17 MenB P1.19,15 and 12 P1.7-2,4 wild-type isolates from the HPA MRU from the epidemiological year 2000 to 2001 that met selection criteria were screened for suitability as target strains in the SBA assay (not including previously used vaccine evaluation strains M01 240149 and NZ 98/254). Screening included the determination of an acceptable morphology to allow counting using the semiautomated

counting procedure, expression of capsule and PorA, compatibility with human complement sources, and increase in the number of CFU over the reaction mixture incubation. Of the 29 P1.19,15 and P1.7-2,4 wild-type isolates originally investigated, 9 P1.19,15 and 9 P1.7-2,4 isolates were acceptable for use in the MenB SBA assay. For the majority of isolates deemed not suitable, the reasons were incompatibility with human complement sources ($n = 9$) and poor growth ($n = 2$).

No single human complement source for use with all isolates (wild-type and vaccine evaluation strains) was obtained; therefore, three different sources were used with isolates from each of the two isolate panels.

Comparison of isolates in the SBA assay. Regression analysis with a factor for the sample and a factor for the isolate indicated that isolates within each of the P1.19,15 and P1.7-2,4 panels produced different SBA titers ($P < 0.001$). After Bonferroni's correction was applied, differences in GMTs of ≥2.4- and 3-fold for the P1.19,15 and P1.7-2,4 results, respectively, constituted significant differences. The SBA GMTs (95% CI) against each of the isolates, as well as significant differences, are shown in Table 3 for P1.19,15 and in Table 4 for P1.7-2,4 isolates. On average, isolates were significantly different from 2.7 and 2.4 other isolates in the P1.19,15 and P1.7-2,4 panels, respectively. The differences between the isolates producing the lowest and highest GMTs were 5.8-fold for P1.19,15 and 5.5-fold for P1.7-2,4 isolates.

Proportions of serum samples with SBA titers of ≥4 against isolates from each subtype are given in Table 5. The results demonstrate as much as 13.3 and 20% variation in the number of serum samples with SBA titers of ≥4 against P1.19,15 and P1.7-2,4 isolates, respectively. The number of SBA titer dilutions between the lowest and highest results for each of the 15

TABLE 4. SBA GMTs for P1.7-2,4 isolates and significant differences in SBA GMTs between isolates

Isolate	GMT (95% CI)	Isolates with significantly different GMTs
M01 240607	11.6 (5.5–24.2)	M01 241245, M01 240554, M01 240566, M00 243221, M01 240805
M01 240149	11.6 (6.5–20.6)	M01 241245, M01 240554, M01 240566, M00 243221, M01 240805
NZ 98/254	14.6 (5.4–39.4)	M01 240566, M00 243221, M01 240805
M00 242922	23.1 (8.1–66.5)	None
M01 240017	23.2 (7.8–69.2)	None
M01 240938	29.2 (12.5–67.9)	None
M01 241245	35.1 (11.2–109.9)	M01 240607, M01 240149
M01 240554	40.3 (13.8–117.9)	M01 240607, M01 240149
M01 240566	46.3 (20.4–105.0)	M01 240607, M01 240149, NZ 98/254
M00 243221	50.8 (17.5–147.0)	M01 240607, M01 240149, NZ 98/254
M01 240805	64.0 (16.3–251.6)	M01 240607, M01 240149, NZ 98/254

TABLE 5. Numbers and percentages of sera showing SBA titers of ≥4 against P1.19,15 and P1.7-2,4 isolates

Isolate	No. (%) of sera with SBA titer of ≥4
P1.19,15 isolates	
M00 242888	12/15 (80.0)
M01 240075	14/15 (93.3)
M01 240265	14/15 (93.3)
M01 241017	14/15 (93.3)
M01 241535	12/15 (80.0)
M00 243202	13/15 (86.7)
M01 240121	14/15 (93.3)
M01 240400	13/15 (86.7)
M01 241117	13/15 (86.7)
P1.7-2,4 isolates	
M00 242922	11/15 (73.3)
M01 240017	10/15 (66.7)
M01 240566	13/15 (86.7)
M01 240938	13/15 (86.7)
M01 241607	11/15 (73.3)
M00 243221	13/15 (86.7)
M01 240554	12/15 (80.0)
M01 240805	12/15 (80.0)
M01 241245	11/15 (73.3)
M01 240149	13/15 (86.7)
NZ 98/254	10/15 (66.7)

TABLE 6. Ranges of SBA titers against P1.19,15 and P1.7-2,4 isolates

Sample no.	SBA titer range (no. of dilutions between lowest and highest values)	
	P1.19,15 isolates	P1.7-2,4 isolates
1	<4–8 (3)	<4–<4 (1)
2	4–64 (5)	<4–16 (4)
3	<4–8 (3)	16–256 (5)
4	8–256 (6)	8–512 (7)
5	16–256 (5)	<4–<4 (1)
6	<4–16 (4)	8–256 (6)
7	64–8,192 (8)	16–4,096 (9)
8	16–64 (3)	64–512 (4)
9	64–512 (4)	32–512 (5)
10	512–4,096 (4)	16–256 (5)
11	16–64 (3)	16–256 (5)
12	32–1,024 (6)	8–128 (5)
13	32–128 (3)	32–1,024 (6)
14	32–4,096 (8)	<4–128 (7)
15	64–2,048 (6)	<4–128 (7)

against all isolates except M01 240805, where the SBA titer for the MAb against capsular polysaccharide was 3 SBA titer dilutions greater than the median.

serum samples against each of the isolates is shown in Table 6 and ranges from 1 to 9 SBA titer dilutions. Samples with the lowest range in SBA titers were those with the lowest/negative SBA titers. The average differences in SBA titers for all samples were 4.7 for P1.19,15 isolates and 5.1 for P1.7-2,4 isolates.

Comparison of capsular polysaccharide, PorA, PorB, Opc, and LOS levels, and LOS sialylation levels, by whole-cell ELISA. For P1.19,15 isolates, capsular polysaccharide, PorB, and LOS levels, as well as LOS sialylation levels, were comparable for all isolates. Expression of P1.19 and expression of P1.15 were comparable except for M01 240075, which had an OD approximately 30% lower than the average for all isolates (P1.19 and P1.15), and M00 242888, which had an OD approximately 60% greater than the average for all isolates (P1.19 and P1.15). Expression of Opc was similar (ODs within 20%) except for M01 240075 and M01 240400, which produced ODs almost 3 times greater than the average for all isolates. For P1.7-2,4 isolates, capsular polysaccharide, PorA, PorB, LOS, Opc, and sialylation levels were comparable (ODs within 25%) with the exception of M01 240017, which gave an OD with the anti-P1.4 MAb approximately 30% lower than the average for all isolates (not including non-P3.4/L3,7,9 isolates).

Comparison of capsular polysaccharide, PorA, PorB, Opc, LOS, and LOS sialylation levels in the SBA assay. SBA titers for each of the MAbs with each of the isolates from a single run are given in Tables 7 and 8 for the P1.19,15 and P1.7-2,4 isolates, respectively. Almost identical results were achieved with repeat runs of the investigation (data not presented). For P1.19,15 isolates, similar SBA titers were obtained for the MAbs against capsular polysaccharide, PorA, PorB, LOS, and sialylation (not including non-P3.4/L3,7,9 isolates). For the anti-Opc MAb, negative SBA titers (<4) were obtained for all isolates except M01 240075 and M01 240400. For P1.7-2,4 isolates, similar SBA titers were obtained for each MAb

DISCUSSION

The choice of meningococcal target strain(s) is vital to the outcome of the SBA assay. This has been demonstrated previously for the group A and C SBA assays (14, 23), which detect anticapsular antibodies. Because after OMV vaccination the MenB SBA assay determines levels of functional antibodies to mainly subcapsular antigens, which may be subject to considerable variation, the effect of using different target strains may be more pronounced. Vermont and coworkers demonstrated differences between both wild-type and isogenic MenB P1.7-2,4, P1.5-1,2-2, and P1.5-2,10 isolates, which affected SBA GMTs and proportions of subjects attaining ≥4-fold rises in SBA titers (26). More recently, Martin and coworkers have also demonstrated differences between wild-type MenB P1.7-2,4 isolates, which affected the proportions of subjects with ≥4-fold rises (13). In this study we have confirmed similar differences with MenB P1.7-2,4 isolates, using a larger number of isolates, and also with the P1.19,15 subtype.

Significant differences between GMTs for isolates, proportions with SBA titers of ≥4, and large ranges in SBA titers were noted for individual serum samples across both panels of isolates (up to 9 SBA titer dilutions). Because sera used in this study were not paired before and after vaccination, it was not possible to determine the effect on ≥4-fold rises. However, it is likely that proportions of subjects with ≥4-fold rises in SBA titers would be affected as previously reported (7, 13, 26). Wild-type isolates were selected using criteria to ensure the greatest level of variation by choosing those that were geographically distinct. All isolates were either from the United Kingdom or from Malta (with the exception of NZ 98/254), and showed significant variation in the results they gave. The studies of Martin et al. (13) and Vermont et al. (26) investigated isolates either from New Zealand alone or from New Zealand and The Netherlands, respectively. Therefore, all

TABLE 7. SBA titers of MAbs against capsular polysaccharide, PorA, PorB, Opc, L3,7,9, and LOS sialylation for P1.19,15 isolates

MAb target	SBA titer of the indicated MAb for:								
	M00 242888	M01 240075	M01 240265	M01 241017	M01 241535	M00 243202	M01 240121	M01 240400	M01 241117
MenB	8,192	4,096	16,384	8,192	4,096	2,048	8,192	8,192	16,384
P1.19	8,192	8,192	16,384	8,192	8,192	4,096	8,192	8,192	16,384
P1.15	32,768	32,768	16,384	32,768	32,768	16,384	16,384	16,384	32,768
P3.4	131,072	131,072	65,536	65,536	131,072	65,536	131,072	131,072	131,072
Opc	<4	8	<4	<4	<4	<4	<4	16	<4
L3,7,9	8	8	4	8	4	8	8	8	8
3F11 (LOS sialylation)	32	32	16	64	32	32	64	32	32

studies incorporated isolates obtained from relatively localized areas of the world. If isolates were obtained from more-dispersed global locations, greater variations in SBA titers might result.

The sera used in the study contained samples with SBA titers across the full range of the assay (including negative sera). If only positive sera with similar SBA titers were used, the differences in GMTs may have been greater than those presented and would also not be skewed by isolates/samples with negative titers (<4). Similarly, the effect on the proportion of subjects with SBA titers of ≥4 may have also been influenced by the use of samples with SBA titers across the full range of the assay. If samples with low-positive SBA titers (4 to 8) were used, the variation demonstrated between isolates might have a more significant impact on the proportions with SBA titers of ≥4.

Differences between isolates were emphasized during the screening process. Two of the isolates gave poor growth with low numbers of CFU, making them incompatible with the assay methodology, and nine isolates were not used, because acceptable human complement sources were not found. This highlights the importance of validation of target strains prior to use in the SBA assay, since inclusion of these isolates with greater complement sensitivity would undoubtedly result in greater differences between SBA assay results. Validation of the strains investigated also adds confidence to the observed differences in results, since we can conclude that they were not due to assay features such as poor growth or minor differences in complement sensitivity.

For the isolates used in this investigation, differences were further emphasized by the necessity to use three human complement sources. However, we are confident that the different complement sources were not responsible for the differences reported, because some isolates were assayed with multiple sources and gave identical results (data not presented). Additionally, there was no correlation/cluster between the complement source used and the GMTs for the isolates (data not presented). This is in agreement with our previous findings that different validated human complement sources did not affect SBA results (2).

The P1.19,15 isolates were indistinguishable except for three isolates that had a different sequence type (ST), resulting in a different clonal complex (CC) for one of the isolates. Nonetheless, these differences are unlikely to account for the differences in SBA results, because greater variation was shown among the other six isolates with the same ST and CC. Similarly, differences in the site of isolation had no determinable relationship with SBA results. For P1.7-2,4 isolates, a single isolate had a different PorB type and a single isolate had a different ST and CC from the others; these were unlikely to account for the observed differences. Greater variation was demonstrated among the immunotypes of P1.7-2,4 isolates, with L1,8 and L3,7,9 expressed. Such differences could be important, because L8 expression has previously been associated with increased susceptibility to SBA activity (17). However, in our study, L1,8 isolates gave a dispersed magnitude of GMTs, which included both the lowest and the highest GMT of all P1.7-2,4 isolates.

We utilized the standardized MenB SBA assay methodology (2), which uses solid medium for the growth of isolates prior to inclusion into the assay. To investigate whether systematic differences in the metabolism of isolates were affecting assay results, we investigated various liquid media and incubation lengths. However, results almost identical to those achieved with the standardized methodology were obtained (data not presented), indicating that neither isolate preparation nor metabolism differences caused differences in assay results.

TABLE 8. SBA titers of MAbs against capsular polysaccharide, PorA, PorB, Opc, L3,7,9, and LOS sialylation for P1.7-2,4 isolates

MAb target	SBA titer[a] of the indicated MAb for:										
	M00 242922	M01 240017	M01 240566	M01 240938	M01 241607	M00 243221	M01 240554	M01 240805	M01 241245	M01 240149	NZ 98/254
MenB	2,048	2,048	2,048	2,048	4,096	8,192	8,192	32,768	1,024	4,096	4,096
P1.4	65,536	32,768	32,768	32,768	16,384	131,072	65,536	65,536	65,536	32,768	32,768
P3.4	131,072	131,072	131,072	131,072	65,536	131,072	ND	131,072	65,536	65,536	131,072
Opc	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
L3,7,9	ND	8	8	8	8	8	8	ND	8	ND	8
3F11 (LOS sialylation)	128	64	32	32	64	64	64	32	64	64	64

[a] ND, not done (because the isolate did not express that antigen).

Investigations into the levels of sialylation and expression of capsular polysaccharide, PorA, and PorB indicated only minor differences between all isolates from each of the two isolate groups, suggesting that these were not the causes of the differences observed. This was in agreement with the findings of Vermont and coworkers (26) and not only indicated that expression of PorA was comparable but also suggested that the PorA epitopes were not being masked. Interestingly, the P1.7-2,4 isolate with the lowest GMT (M01 240805) reproducibly gave the highest SBA titer with the MenB capsular MAb. This could lead to suggestions that increased capsular polysaccharide levels could be masking underlying epitopes. However, this seems unlikely, because the SBA titers of other MAbs were similar to those obtained with the other isolates. Two P1.19,15 isolates had increased levels of Opc, one of which gave the greatest GMT. However, the P1.19,15 isolate with the greatest level of Opc expression had a GMT below the average of all GMTs for those isolates. It is unlikely that a single difference in expression accounts for the observed differences between isolates; it is probable that differences in configuration and/or expression of the huge array of other antigens, which could include Opa, RmpM, NspA, and many others, were responsible for the differences in SBA assay results. Furthermore, it is likely that the sum of numerous small differences in these other antigens may account for the observed effects.

In the present study, the effects of these isolates were not investigated in other immunologic assays. However, we have previously shown that two phenotypically indistinguishable isolates gave different results in the OMV ELISA, opsonophagocytic assay, and surface labeling assay, including differences in proportions of subjects achieving ≥4-fold rises (7). Therefore, differences similar to those demonstrated in the SBA assay would be expected with other immunologic assays.

These results, in conjunction with those of previous studies (7, 13, 26), suggest that the standardization of strains between laboratories is crucial for the compatibility of data. This highlights the requirement for a standardized, internationally available panel, which was one of the findings of the recent MenB meeting for correlates of protection and assay standardization (3). It would also be necessary to provide detailed instructions on the storage and growth of such strains to prevent any problems such as that recently reported with heterologous/homologous populations of 44/76-SL (2).

These results also raise the question of whether currently used target strains are truly "representative." Strains that are not representative may lead to either the overestimation or the underestimation of a vaccine's immunogenicity, as suggested with the P1.7-2,4 subtype of Hexamen (26). Equally, the effect on the proportion of subjects with SBA titers of ≥4 may have implications for future determination of absolute cutoffs and correlates of protection, matters that require future clarification. Care must also be taken in attempting to compare data obtained using different target strains, such as the B:15:P1.7,16 isolates 44/76 and MC58, which may be inappropriate. Prior to the future inclusion of target strains in immunologic assays, it may be necessary to ensure that they are "representative," or at least to determine comparative susceptibility to complement-mediated lysis in the MenB SBA assay, by completing investigations such as those we have reported. These findings are likely to be applicable to the evaluation of all subcapsular MenB vaccines.

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