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A Large Portion of Meningococcal Antigen Typing System-Negative Meningococcal Strains from Spain Is Killed by Sera from Adolescents and Infants Immunized with 4CMenB

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A new vaccine (the 4CMenB 4-component protein vaccine [Bexsero], which includes PorA, factor H-binding protein [fHbp], neisserial heparin-binding antigen [NHBA], and *Neisseria* adhesin A [NadA]) against serogroup B meningococci has recently been approved for use in people older than age 2 months in Europe, Australia, and Canada. Preapproval clinical efficacy studies are not feasible for invasive meningococcal disease because its incidence is low/very low, and the serum bactericidal antibody (SBA) titer (or the human SBA [hSBA] titer when human complement is used in the assay) has been used as a surrogate marker of protection. However, the hSBA assay cannot be used on a large scale, and therefore, a meningococcal antigen typing system (MATS) was developed. MATS combines conventional PorA genotyping with an enzyme-linked immunosorbent assay (ELISA) that quantifies both the expression and the cross-reactivity of antigenic variants. The assay has been used to evaluate the potential of the 4CMenB meningococcal group B vaccine to cover group B strains in several countries. Some recent data suggest that MATS is a conservative predictor of strain coverage. We used pooled sera from adolescents and infants to test by the hSBA assay 10 meningococcal group B strains isolated in Spain that were negative for the 3 antigens ($n = 9$) or that had very low levels of the 3 antigens ($n = 1$) by MATS. We found that all strains were killed by sera from adolescents and that 5 of the 10 strains were also killed, although at a low titer, by sera from infants. Our data confirm that MATS underestimates vaccine coverage.

In spite of the development of effective conjugate vaccines against meningococci of serogroups A, C, Y, and W over the last 15 years (1), a vaccine against serogroup B was a challenge that had remained unsolved until recently, when the European Medicines Agency (EMA) approved a 4-component protein vaccine (4CMenB [Bexsero], containing porin A [PorA; presented as part of an outer membrane vesicle {OMV} derived from the New Zealand strain], factor H-binding protein [fHbp], neisserial heparin-binding antigen [NHBA], and *Neisseria* adhesin A [NadA]) for use in people older than 2 months (information on the vaccine is available on the European Medicines Agency web page [http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002333/human_med_001614.jsp&mid=WCOb01ac058001d124]). This vaccine has also been recently licensed in Australia and Canada.

Clinical efficacy studies required for the approval of vaccines are not feasible in the case of invasive meningococcal disease because its incidence is low/very low. For this reason, for the authorization of conjugate vaccines, the use of a surrogate marker of protection as a replacement for the findings of formal efficacy studies has been proposed. The marker used is the serum bactericidal antibody (SBA; or the human SBA [hSBA] when human complement is used in the assay), and SBA assays can be used to measure the ability of serum antibodies to kill a specific meningococcal strain (2). However, due to the diversity of the sequences of the antigens included in this novel vaccine and their different levels of expression, the use of hSBA requires the testing of many serum samples with large panels of isolates to evaluate the bactericidal killing of many different meningococcal strains, making this approach not feasible (3, 4). Therefore, the development of alternative methods that can give information about immunologic recognition, the level of expression of the antigens, and the association of those parameters with killing in the hSBA assay has been critical in this history. As a result, a meningococcal antigen typing system (MATS; which combines conventional PorA genotyping [*porA* variable region 2 {VR2}] with an enzyme-linked immunosorbent assay [ELISA] that quantifies both the expression and the cross-reactivity of antigenic variants for each antigen) was developed (3–5). MATS quantifies the relative expression and cross-reactivity of antigenic variants by assigning a relative potency (RP) against fHbp, NHBA, and NadA in each strain. The method establishes a positive bactericidal threshold (PBT) for each antigen, allowing prediction of whether a given serogroup B strain would be killed in the hSBA assay by antibodies elicited by the vaccine. The potential coverage of the vaccine against a meningococcal population can then be estimated.

The assay has been introduced in several European, American, and Australian reference laboratories through a strict interlaboratory standardization study (4) to ensure the comparability of strain coverage data collected worldwide. The potential coverage of the 4CMenB vaccine against meningococcal group B (MenB) strains collected in one or two epidemiological years in different

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TABLE 1 Strain characterization (genotype, MATS phenotype, and RP for each antigen) and hSBA titers

Strain	Genotype	RP		*nadA* presence[a]	MATS phenotype[b]	hSBA activity (titer) in sera from[c]:			
						Adolescents		Infants	
		fHbp	NHBA			PI	Post2	Control	Post4
E-18847	CC11, 1.p10/20/+/5-1, 10-8	0.023	0.313	+ (0)	+, +, −, −	<4	16	<2	2
E-19030	CC11, 1.p10/20/−/5-1, 10-8	0.016	0.226	−	−, −, −, −	<4	4	<2	<2
E-19749	CC60, 1.p13/25/−/21, 16	0.016	0.07	−	−, −, −, −	8	32	2	8
E-19759	NA,[d] 1.p13/468/−/21, 16	0.014	0.275	−	−, −, −, −	<4	32	<2	<2
E-19292	CC269, 1.p357/17/−/22, 9	0.001	0.198	−	−, −, −, −	4	32	<2	<2
E-19353	CC461, 3.p174/118/−/22, 14	0.011	0.273	−	−, −, −, −	16	64	<2	2
E-19290	CC213, 3.p45/18/+/22, 14	0	0.012	+ (0)	−, −, −, −	32	64	<2	<2
E-19528	CC213, 3.p45/18/+/22, 14	0	0.134	+ (0)	−, −, −, −	<4	4	<2	4
E-19809	NA, 1.p14/30/−/19, 15-1	0.012	0.152	−	−, −, −, −	<4	32	<2	4
E-19260	CC269, 2.p19/17/−/22, 9	0	0.287	−	−, −, −, −	<4	32	<2	<2

[a] Data in parentheses are the RP for samples with positive results.
[b] Strains showing an RP for the antigen that was greater than the PBT were defined as positive (+), and those with an RP for the antigen that was less than the PBT were defined as negative (−), following the order fHbp, NHBA, NadA, and PorA (for PorA, the result was defined as positive if the VR2 genosubtype was the 1.4 variant [that is included in the vaccine] or negative if the PorA variant expressed was different).
[c] PI, sera obtained preimmunization; Post2, sera obtained after the 2nd dose of the vaccine; Control, negative-control pooled sera from subjects receiving only 3 doses of routine vaccine; Post4, sera obtained after the 4th dose of the vaccine.
[d] NA, not assigned.

countries has been estimated to range from 66% to >90% (6–8). The vaccine was predicted to cover most circulating MenB strains in Europe, but some small differences (not significant) were observed (7), with lower rates of coverage of 69% appearing in Spain, whereas the average rate for the other countries was 78%.

A recent study with a representative panel of strains from the United Kingdom showed that MATS is a conservative predictor of strain coverage. In this study, 40 strains were tested by use of the MATS and the hSBA assay using pooled sera from infant and adolescent vaccinees. The number of strains killed in the hSBA assay was higher than the number of strains predicted to be killed by MATS (9). The reasons for that might be the use of very conservative algorithms in the MATS analysis (3, 4, 9) but also some level of synergy of the different antibodies raised against the different antigens (particularly between those raised against fHbp, NHBA, or OMV non-PorA antigens), augmenting the SBA response.

Considering all the information presented above, we designed a small study by selecting 10 strains from the overall panel of 300 Spanish MenB isolates previously analyzed by MATS (7) for analysis by the hSBA assay. In this study, the 10 strains did not represent the overall Spanish panel because just those isolates ($n = 93$) that were predicted to not be covered by MATS (with the exception of only 1 strain that was predicted to be covered but that had RP values close to the PBTs) were selected. The nine strains were selected in an attempt to cover the most common clonal complexes (CCs) among those that were not covered in the analysis of MATS.

MATERIALS AND METHODS

Definition of MATS phenotype. The PBTs used were 0.021 for fHbp, 0.294 for NHBA, and 0.009 for NadA. Strains showing an RP for the antigen greater than the PBT were defined to be positive, and those with an RP for the antigen less than the PBT were defined to be negative. None of them showed a PorA type 1.4, which is the PorA type of the OMV included in the vaccine. Only two different MATS phenotypes were obtained: fHbp positive, NHBA positive, NadA negative, and PorA negative (only one strain) and fHbp, NHBA, NadA, and PorA negative (in nine isolates).

Definition of genotypic profiles. The sequence types (STs) and CCs defined by multilocus sequence typing and genotypes for the *fHbp*, *nhba*, and *nadA* genes and *porA* variable region 2 (VR2) were assigned as previously described (8). The genotype of each isolate was defined by the CC, followed by the *fHbp* genotype/*nhba* genotype/*nadA* genotype/*porA* VR1 type, *porA* VR2 type (see Table 1).

Human serum samples and hSBA assay methodology. The hSBA assay for activity against the 10 meningococcal strains was performed using pooled sera from both infants and adolescents. Basically, pooled sera were derived from 12 randomly selected adolescent subjects from a phase 2b/3 clinical trial (10) in which the subjects received the 4CMenB vaccine in a two-dose regimen, and sera were collected both before vaccination and 30 days after the second dose of the vaccine and pooled (9). Pooled sera from infants were derived from 27 randomly selected subjects from a phase 2b clinical study (11) in which the subjects received a primary series of 3 doses of 4CMenB plus a booster dose at 12 months of age. Sera were collected 30 days after the booster dose was administered. Negative-control sera consisted of pooled sera from 40 randomly selected subjects infants who were part of the control group of the clinical study mentioned a above and who received only the routine vaccination, without 4CMenB (11).

hSBA assays were performed as previously described (9). We considered the hSBA assay to be positive for all strains against which an increase in the titer of hSBAs in the postvaccination pooled sera was seen but against which no activity by sera obtained from adolescents before vaccination (titer, <1:4) or control infants (titer, <1:2) was observed, even though the clinical relevance of this activity is not well-known; hSBA assay positivity was also defined for those strains against which a ≥4-fold rise in titer compared with the preimmune titer was observed.

RESULTS

The genetic profile and MATS phenotypes for the 10 analyzed strains are shown in Table 1. Only one strain was predicted to be covered (E-18847) by MATS, according to the RPs for both fHbp and NHBA. The panel composition corresponded to a small group of strains that was heterogeneous enough (Table 1), with

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8/10 strains having different genotypes and the strains showing a wide range of MATS RP values for each antigen.

Data on bactericidal activity are shown in Table 1. Preimmune sera from adolescents were positive (titer, ≥4) for antibodies against 4 strains. If we look at the control sera from infants (Table 1), there were no bactericidal antibodies against the tested strains with only one exception (strain E-19749), for which limited bactericidal activity (titer, 2) was detected.

If we look at the data obtained for pooled sera from adolescents (Table 1) who had received two doses of the vaccine, a high proportion of the strains (7/10) were killed in the hSBA assay with titers of ≥8 (when preimmunization titers were <4) or at least a 4-fold rise in titer was achieved (if preimmunization titers were ≥4).

Data for postvaccination pooled infant sera showed an evident increase in the titer of hSBAs against one strain (E-19749). A significant increase in titer against two isolates (E-19809 and E-19528) was also observed: a titer of <2 (that we might define as 1 or even 0) using the control pooled sera and a titer of 4 using postvaccination sera. A small increase in the titer of hSBAs against for two additional isolates (E-18847 and E-19353) was also found, and no activity at all against the other 5 meningococcal strains was seen (Table 1).

DISCUSSION

The proportion of sera from prevaccinated adolescents (4/10) with bactericidal activity found in this study was significantly higher than that found in the United Kingdom study (2/40) (10). Taking into account the fact that these sera were derived from adolescents participating in a clinical study conducted in Chile, this finding might suggest that the strains from meningococcal carriers in Chile might be similar to those circulating in Spain, although we do not have enough information to prove this suggestion.

Data obtained for pooled sera from adolescents (Table 1) who had received two doses of the vaccine showed that a high proportion of the strains (7/10) were killed by hSBAs at titers of ≥8 (when preimmunization titers were <4) or that at least a 4-fold rise in titer (if preimmunization titers were ≥4), which is associated with experimental coverage, was achieved (10). The proportion of strains killed rose to 100% if a titer of ≥4, which has been used in other studies (12, 13), was used to define positive bactericidal activity. This finding suggests that the predicted coverage by MATS might be extremely conservative, particularly in adolescents, and that these preliminary data should be confirmed with an extended panel of strains. Taking into account the positive hSBA response (titer, ≥4) against the tested strains in the sera from adolescents before vaccination, we could hypothesize that the vaccine might induce a very strong booster effect not only in subjects with titers of ≥4 before immunization but also in subjects with no measurable hSBA activity that could have been previously exposed to similar strains. It is worth noting that under certain circumstances the results obtained with pooled vaccine-elicited immune sera may not accurately describe how the individual serum samples that compose the pool would react against a given strain. These circumstances include the ability of sera from a few subjects who respond with unusually high or low hSBA titers to skew the overall pooled response or the potential of antibodies against multiple antigens with subbactericidal titers in different subjects to behave synergistically when combined in a pool (14). However, data that demonstrate that these phenomena do not significantly contribute to assessments of the hSBA titers of pooled sera have recently emerged. Studies in which the responses of individual infants to several MenB strains were compared to the response of the corresponding serum pool showed that individual hSBA responses to 4CMenB vaccination was distributed according to a regular pattern, with the titers for vaccine responders being clustered about the mean hSBA titer (15). Furthermore, the hSBA titers of pooled sera proved to be a robust approximation of the arithmetic average of the individual titers. Thus, in this age group, the hSBA titers of pooled sera accurately predicted the proportion of subjects that responded with protective hSBA titers.

However, the data obtained using sera from infants after they had received 4 doses of the vaccine look rather different (Table 1). The absence of hSBAs in pooled sera from the control infants is not surprising because there is a very low frequency or even an absence of meningococcal carriage in individuals in this age group (16). Data for postvaccination pooled infant sera showed different increases in the titers of hSBAs against 5 strains (Table 1). Although in a validated assay a titer of 4 is needed to consider a strain to be killed by the SBA assay, in an experimental setting in the laboratory, an hSBA titer of even 2 could be indicative of functional activity when there was no killing by preimmune sera (hSBA titer, <2). Therefore, the effect of immune sera on the different strains is clearly different, suggesting that the antibodies induced are specifically able to kill some of the strains. This study shows that adolescents will very likely be protected from invasive disease caused by the 10 strains tested. The question is whether the very low titers induced by the vaccine in infants are an indication of protection. According to Goldschneider et al. (12), while a positive hSBA assay result is indicative of protection, a negative hSBA assay result is not necessarily an indication of susceptibility. In fact, most people with negative hSBA assay results are still protected from invasive disease by an immunity that is below the threshold of detection by the hSBA assay (12, 17). In infants, the induction of measurable titers of hSBAs against 3 of the 10 strains suggests that the vaccine also induces some protection in infants. The use of an assay more sensitive than the hSBA assay for these sera with low but measurable bactericidal activity could provide more information, as has been suggested previously (17).

We do not know the clinical relevance (in terms of protection) of low hSBA titers, particularly for a low bactericidal titer of 2, but the results obtained with pooled sera suggest that MATS provides a conservative estimate of strain coverage, and this finding is particularly significant in adolescents and is not negligible in infants. Although the study of Frosi et al. (9) did not show differences between the bactericidal activity of sera from infants and that of sera from adolescents, a lower hSBA coverage in infants than in adolescents has already been suggested (3), and the potential synergy of antibodies elicited against different antigens has been proposed. Although the relationship between the low titers in the hSBA assay and the RPs obtained by MATS (Table 1) has not been defined, the results of this preliminary study support the hypothesis that MATS is a conservative tool for predicting strain coverage, particularly in the adolescent age group. Further studies will be needed to address questions about the relationship between pooled individual serum samples and the response rate to define more precisely the potential coverage of this vaccine.

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REFERENCES

1. **Tan LK, Carlone GM, Borrow R.** 2010. Advances in the development of vaccines against *Neisseria meningitidis*. N Engl J Med **362:**1511–1520. http://dx.doi.org/10.1056/NEJMra0906357.
2. **Borrow R, Miller E.** 2006. Subrogates of protection, p 323–351. *In* Frosch M, Maiden M (ed), Handbook of meningococcal disease. Wiley-VCH, Weinheim, Germany.
3. **Donnelly J, Medinia D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, Moxon ER, Stella M, Comanducci M, Bambini S, Muzzi A, Andrews W, Chene J, Santos G, Santini L, Boucher P, Serruto D, Pizza M, Rappuoli R, Giuliania MM.** 2010. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci U S A **107:**19490–19495. http://dx.doi.org/10.1073/pnas.1013758107.
4. **Plikaytis BD, Stella M, Boccadifuoco G, DeTora LM, Agnusdei M, Santini L, Brunelli B, Orlandi L, Simmini I, Giuliani M, Ledroit M, Hong E, Taha MK, Ellie K, Rajam G, Carlone GM, Claus H, Vogel U, Borrow R, Findlow J, Gilchrist S, Stefanelli P, Fazio C, Carannante A, Oksnes J, Fritzsønn E, Klem AM, Caugant DA, Abad R, Vázquez JA, Rappuoli R, Pizza M, Donnelly JJ, Medini D.** 2012. Interlaboratory standardization of the sandwich enzyme-linked immunosorbent assay designed for MATS, a rapid, reproducible method for estimating the strain coverage of investigational vaccines. Clin Vaccine Immunol **19:**1609–1617. http://dx.doi.org/10.1128/CVI.00202-12.
5. **Vogel U, Stefanelli P, Vazquez J, Taha MK, Claus H, Donnelly J.** 2012. The use of vaccine antigen characterization, for example by MATS, to guide the introduction of meningococcus B vaccines. Vaccine **30**(Suppl 2)**:**B73–B77. http://dx.doi.org/10.1016/j.vaccine.2011.12.061.
6. **O'Ryan M, Stoddard J, Toneatto D, Wassil J, Dull PM.** 2014. A multicomponent meningococcal serogroup B vaccine (4CMenB): the clinical development program. Drugs **74:**15–30. http://dx.doi.org/10.1007/s40265-013-0155-7.
7. **Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, Stefanelli P, Caugant DA, Kriz P, Abad R, Bambini S, Carannante A, Deghmane AE, Fazio C, Frosch M, Frosi G, Gilchrist S, Giuliani MM, Hong E, Ledroit M, Lovaglio PG, Lucidarme J, Musilek M, Muzzi A, Oksnes J, Rigat F, Orlandi L, Stella M, Thompson D, Pizza M, Rappuoli R, Serruto D, Comanducci M, Boccadifuoco G, Donnelly JJ, Medini D, Borrow R.** 2013. Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect Dis **13:**416–425. http://dx.doi.org/10.1016/S1473-3099(13)70006-9.
8. **Bettinger JA, Scheifele DW, Halperin SA, Vaudry W, Findlow J, Borrow R, Medini D, Tsang R, members of the Canadian Immunization Monitoring Program, Active (IMPACT).** 2013. Diversity of Canadian meningococcal serogroup B isolates and estimated coverage by an investigational meningococcal serogroup B vaccine (4CMenB). Vaccine **32:**124–130. http://dx.doi.org/10.1016/j.vaccine.2013.03.063.
9. **Frosi G, Biolchi A, Lo Sapio M, Rigat F, Gilchrist S, Lucidarme J, Findlow J, Borrow R, Pizza M, Giuliani MM, Medini D.** 2013. Bactericidal antibody against a representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage. Vaccine **31:**4968–4974. http://dx.doi.org/10.1016/j.vaccine.2013.08.006.
10. **Santolaya ME, O'Ryan ML, Valenzuela MT, Prado V, Vergara R, Muñoz A, Toneatto D, Graña G, Wang H, Clemens R, Dull PM, V72P10 Meningococcal B Adolescent Vaccine Study Group.** 2012. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. Lancet **379:**617–624. http://dx.doi.org/10.1016/S0140-6736(11)61713-3.
11. **Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, Principi N, Diez-Domingo J, Sokal E, Becker B, Kieninger D, Prymula R, Dull P, Ypma E, Toneatto D, Kimura A, Pollard AJ, European MenB Vaccine Study Group.** 2012. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. JAMA **307:**573–582. http://dx.doi.org/10.1001/jama.2012.85.
12. **Goldschneider I, Gotschlich EC, Artenstein MS.** 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med **129:**1307–1326.
13. **Pollard AJ, Galassini R, Rouppe Van Der Voort EM, Booy R, Langford P, Nadel S, Ison C, Kroll JS, Poolman J, Levin M.** 1999. Humoral immune responses to *Neisseria meningitidis* in children. Infect Immun **67:**2441–2451.
14. **Vu DM, Wong TT, Granoff DM.** 2011. Cooperative serum bactericidal activity between human antibodies to meningococcal factor H binding protein and neisserial heparin binding antigen. Vaccine **29:**1968–1973. http://dx.doi.org/10.1016/j.vaccine.2010.12.075.
15. **Budroni S, Kleinschmidt A, Ypma E, Boucher P, Medini D.** 2014. Pooled hSBA titers predict seroresponse rates of infants vaccinated with 4CMenB, poster 41. Abstr XIXth Int Pathogenic Neisseria Conf, Asheville, NC.
16. **Caugant DA, Maiden MCJ.** 2009. Meningococcal carriage and disease—population biology and evolution. Vaccine **27:**B64–B70. http://dx.doi.org/10.1016/j.vaccine.2009.04.061.
17. **Welsch JA, Granoff D.** 2007. Immunity to *Neisseria meningitidis* group B in adults despite lack of serum bactericidal antibody. Clin Vaccine Immunol **14:**1596–1602. http://dx.doi.org/10.1128/CVI.00341-07.

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