Immunogenicity of Two Efficacious Outer Membrane Protein–Based Serogroup B Meningococcal Vaccines among Young Adults in Iceland

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Serum bactericidal activity (SBA) and ELISA antibody levels elicited by two efficacious serogroup B meningococcal vaccines were measured in a controlled trial involving 408 15- to 20-year-olds. Subjects were given two doses at a 6-week interval of a serogroup B or control vaccine. Response was defined as ⩾4-fold rise in antibody level. After two doses of the Finlay Institute (Havana) vaccine at 12 months, the proportions of SBA and ELISA responders were not different from those of the control group (15% and 17% [vaccine] vs. 13% and 9% [control], $P > .05$). After two doses of the National Institute of Public Health (Oslo) vaccine, there were more SBA and ELISA responders than in the control group (47% and 34% [vaccine] vs. 10% and 1% [control]) or the Finlay Institute vaccine group ($P < .05$ for both). SBA and ELISA may be insensitive correlates for protective efficacy for some outer membrane protein–based serogroup B meningococcal vaccines.

This is the first study to compare directly the immunogenicity of two serogroup B meningococcal vaccines shown to be effective for older children and adults in large clinical efficacy trials [1, 2]. Each vaccine is based on partially purified outer membrane proteins from a specific epidemic *Neisseria meningitidis* serogroup B strain, presented as proteoliposome vesicles adsorbed onto aluminum hydroxide. The first vaccine was developed by the Finlay Institute in Havana. A two-dose regimen was tested in a cluster randomized trial over a 16-month period in 1988 and 1989 in Cuba, and its efficacy for prevention of serogroup B meningococcal disease was estimated to be 83% (95% confidence interval [CI], 42%–95%). The second vaccine was developed by the National Institute of Public Health (NIPH) in Oslo. A two-dose regimen was evaluated over 29 months in 1989 through 1991 in Norway, and its efficacy was estimated to be 57% (lower 95% confidence limit, 27%).

The results from these trials among older children and adults do not provide data on efficacy in young children, a group at high risk for endemic and epidemic serogroup B meningococcal disease, nor do they clarify whether the vaccines will be effective in situations in which the circulating strains are different from those used to make the vaccines. Predicting efficacy would be facilitated if there were a validated immunologic correlate for protection from serogroup B meningococcal disease. Serum bactericidal activity (SBA) has been the primary serologic correlate used in development of serogroup B meningococcal vaccines. It was a useful immunologic correlate for protection from serogroup C meningococcal disease and helped establish the protective role of anti–capsular polysaccharide antibodies for serogroup C and A meningococcal disease [3]. However, its applicability to *N. meningitidis* serogroup B, for which capsular polysaccharide is poorly immunogenic and protective antibodies must be directed at subcapsular antigens, is not established. Studying vaccines with demonstrated efficacy provided an opportunity to examine SBA assay and ELISA as immunologic correlates for protection from serogroup B meningococcal disease.

There is some evidence that SBA correlates with protection from serogroup B meningococcal disease [4–6]. In addition to the Finlay- and NIPH-produced vaccines, another outer membrane protein–based serogroup B meningococcal vaccine

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Informed consent for participation in this study was obtained from enrollees and a parent or guardian. Human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this clinical research.

F.S., G.S., and H.C.C., at the Finlay Institute in Havana, have a commercial association with production and sales of the Finlay-produced vaccine. The National Institute of Public Health (Oslo) vaccine is not commercially available. This study was blinded and was monitored by an international monitoring committee composed of persons with no commercial or other association with the vaccines. In addition, the trial was conducted by persons in Iceland with no commercial interests or other associations with the vaccine producers. Similarly, much of the laboratory testing for this study was done at the Centers for Disease Control and Prevention (CDC), and the first author of this paper, responsible for much of the data interpretation, is from CDC. None of the persons involved in this study from CDC, the World Health Organization, or the National Institute of Biologic Standards and Control (UK) have commercial interests or other associations with the vaccine producers.

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(based on a B:15:P1.3 strain from Chile) was developed by persons at the Walter Reed Army Institute of Research (Bethesda, MD). It was evaluated for efficacy by using a two-dose regimen in a large trial in northern Chile among persons 1–21 years of age over a 20-month period during 1987 through 1989 [4]. The point estimate for efficacy in the whole group was 51% ($P = .11$); for persons 5–21 years of age, it was 70% ($P = .04$); and for children 1–4 years old, it was −39%. SBA in a sample of these persons showed similar age-dependence, with minimal or no increases among children 1–4 years old but marked increases in those 5–18 years old. The inverse was seen with the ELISA-measured IgG antibody response. The highest levels were seen in children 1–4 years old and lower levels in those 5–18 years old.

Age-dependent differences in vaccine efficacy and SBA were also seen in epidemiologic and laboratory studies following a vaccination campaign involving children 3 months to 6 years of age with the Finlay-produced vaccine in São Paulo, Brazil, during 1989 and 1990. Efficacy was estimated in a case-control study for children 3–23 months, 24–47 months, and 48–83 months old; estimated efficacies were −37%, 47%, and 74%, respectively [5]. A similar pattern was seen when serum samples from vaccinees were evaluated by SBA assay. With the same age groups as for estimation of vaccine efficacy, the proportions of children with ⩾4-fold increases in SBA were 22%, 45%, and 52%, respectively [6]. When fold antibody increase was measured by ELISA, all age groups had 4- to 5-fold increases and there were no significant differences between them. These studies, as well as some earlier ones, support a relationship between SBA and clinical protection from serogroup B meningococcal disease.

The current study had three major objectives. The first was to compare bactericidal activity and ELISA-measured antibodies elicited by the serogroup B vaccines to those elicited by a control vaccine and to each other. The second objective was to compare immune response elicited by two- and three-dose regimens of the serogroup B vaccines. This was included because there was evidence of waning protection during the latter part of the Norwegian clinical trial; data from other studies in Norway suggested that a third dose might significantly increase levels of antibody at 1 year and later [7]. The third objective was to determine bactericidal activity elicited by the serogroup B vaccines against heterologous or non–vaccine type strains. This was included because of concern that protection provided by outer membrane protein–based vaccines may be limited to the vaccine type strain or other closely related strains.

After preliminary analyses of results on sera from the first 12 months of the study, we decided to obtain an additional blood sample at 20 months (after the study had been unblinded). In addition, we attempted to ''bridge'' or link results of this study to the earlier clinical protection trials in Cuba and Norway by testing a sample of representative sera from those trials. The results of the 20-month blood sample, the ''bridging study,'' and some *N. meningitidis* carriage data are included in this report, although they were not part of the original study design.

Methods

Study design. This was a randomized, double-blind, controlled trial among students attending three secondary schools in Reykjavik, Iceland. The control vaccine was a licensed serogroup A/C polysaccharide meningococcal vaccine (provided by Pasteur Mérieux Connaught, Lyon, France). Vaccines were scheduled to be given in two- (0 and 6 weeks) or 3- (0 and 6 weeks and 10 months) dose regimens. SBA and ELISA-measured antibody levels were determined by testing 6 (scheduled for 0, 6, and 9 weeks and 10, 12, and 20 months) blood samples (study blinded through 12 months). A vaccine responder was defined as an individual with a ⩾4-fold rise in antibody titer compared with the prevaccination titer (time 0).

Finlay Institute and NIPH serogroup B meningococcal vaccine groups were designed to include 150 participants who would receive a dose of vaccine at study enrollment (time 0) and 6 weeks later; 75 participants in each of these groups would receive a third (booster) dose of vaccine 10 months after study enrollment. The control group was planned to include 100 participants who would receive a dose of control vaccine at the time of enrollment and 6 weeks later.

Vaccines were provided by their respective manufacturers and stored at 4°C in a monitored cooling room at the Icelandic State Import of Drugs and Medicine (Reykjavik). The serogroup B meningococcal vaccines were provided in single-dose vials, and the control vaccine was provided in 10-dose vials. Vaccines were loaded into opaque syringes; a 2.54-cm (1-inch) 21-gauge needle was used to inject vaccine intramuscularly into the deltoid muscle of the nondominant arm.

Blood samples were collected in 15-mL sterile tubes (Vacutainer; Becton Dickinson, San Jose, CA) with serum separation gel (marbleized red-top). Serum was allowed to separate, and 0.5-mL was aliquoted into 2-mL cryovials with screw-top caps and o-rings. Cyrovials were stored in a monitored freezer at −70°C. After the fifth blood sample was obtained, aliquots of sera sufficient to complete all planned testing (SBA and ELISA) from all participants were sent from Iceland to the Finlay Institute, NIPH, and the Centers for Disease Control and Prevention (CDC, Atlanta). The sixth blood sample was sent later, after laboratory testing on the first 5 samples was completed and the study was unblinded.

Monitoring. In addition to study collaborators, local and international monitoring committees oversaw the study. The local committee monitored study enrollment, vaccination, collection of information about adverse events associated with vaccination, specimen collection and handling, and data processing. The international monitoring committee maintained study blinding, evaluated adverse events, and archived all laboratory results before unblinding of the study (with the exception of the sixth blood collection).

Data management and analysis. All data were entered into standardized electronic databases. Demographic data were entered in Iceland (by H.B.) and laboratory data were entered by personnel at the Finlay Institute, NIPH, and CDC. As individual laboratory data sets were completed, they were sent to CDC and merged by one of us (B.D.P.) with the other laboratory data sets and the demographic and adverse events data sets, creating a master study data file. When this file was completed, it was redistributed to participating laboratories and to the chairperson of the international monitoring committee before unblinding of study codes.

A mixed model analysis of variance (ANOVA) procedure was used to examine differences in geometric mean SBA and ELISA values among the vaccine and control groups. As many as 5 serum samples were obtained from each individual during the first 12 months of the study. A sample of the study subjects volunteered for a sixth serum sample at 20 months. The mixed model ANOVA was performed, taking into account the repeated-measures design of this study and the inherent colinearity among blood collections from an individual. The vaccine and control groups and the serum number accounted for the fixed effects portion of the study; the selection of individuals included in the study and their assignment to vaccine or control group accounted for the random effect. A resultant longitudinal data analysis was performed using the MIXED procedure in SAS (SAS Institute, Cary, NC). Three groups were defined for the analysis: the two- and three-dose vaccine groups and the control group. The ANOVA was performed separately for the Finlay and NIPH vaccine groups versus their respective controls. Because the third dose was administered after 10 months, the two- and three-dose results for each vaccine group should theoretically be equivalent through the fourth blood sampling. Linear contrasts were developed to test this hypothesis; if the two- and three-dose results were not significantly different, they were combined before making the final comparisons with the control group.

Laboratory evaluation. All sera were tested in CDC, NIPH, and Finlay Institute laboratories using SBA assay only (CDC), or SBA assay and ELISA (Finlay Institute and NIPH). For both SBA assay and ELISA, a vaccine responder was defined as a person with a $\geq$4-fold rise in antibody titer compared with the prevaccination titer.

Four serogroup B *N. meningitidis* strains were used in testing of sera: Finlay vaccine type strain (CU385/83, B:4:P1.15:L3,7,9), NIPH vaccine type strain (44/76-SL, B:15:P1.7,16:L3,7,9, and Opc-positive) [7], an epidemic strain from South Africa (8069, provided by C. E. Frasch [Center for Biologics Evaluation and Research, Bethesda, MD], B:2b:P1.2,5:L3,7,9), and a strain responsible for increased rates of disease in Austria (G1963, provided by J. T. Poolman [National Institute of Public Health, Bilthoven, The Netherlands], B:4:P1.4,[7],14:L3,7,9). By multilocus enzyme electrophoresis, the Finlay and NIPH vaccine type strains were closely genetically related to each other and belonged to the enzyme type 5 (ET-5) complex [8]. The strains from South Africa and Austria (lineage III) were not closely genetically related to each other or to the vaccine type strains and did not belong to the ET-5 complex.

For this study, vaccine type strains were considered homologous when testing sera from persons who were vaccinated with the vaccine based on that strain (e.g., CU385/83 is homologous for a person vaccinated with the Finlay vaccine). Other comparisons were considered heterologous (e.g., CU385/83 for a person vaccinated with the NIPH vaccine and 8069 and G1963 for persons vaccinated with Finlay and NIPH vaccines).

CDC laboratorians evaluated SBA using the Finlay and NIPH vaccine type strains as well as strains 8069 and G1963 (from South Africa and Austria, respectively) on all sera. At the Finlay Institute, SBA and ELISA-measured antibodies were evaluated using the Finlay vaccine type strain and outer membrane vesicle vaccine preparation, respectively, on all sera. At the NIPH, SBA and ELISA-measured antibodies were evaluated against the NIPH vaccine type strain and outer membrane vesicle vaccine preparation, respectively, on all sera.

Aliquots of all strains sufficient to complete evaluation of study sera were flash frozen and stored at −70°C before testing of study sera. All laboratories periodically did colony blot or dot blotting [9] to monitor epitopic expression of strains during testing of study sera.

SBA. Methods and reagents used for determining SBA in each of the laboratories were compared before testing the study sera. All laboratories used the agar overlay technique in microtiter plates [10]. Serum (50 μL) was placed in the first well of a microtiter plate, and 10 serial 2-fold dilutions were made in Hanks' balanced salt solution with 0.1% bovine serum albumin. CDC and NIPH laboratories used the target strain at a concentration of 70−80 cfu/well in log-phase growth harvested from a 4-h agar plate. The Finlay Institute used 50 cfu/well, diluted from a frozen log-phase culture stored at −70°C.

All laboratories used human plasma obtained via plasmapheresis as a complement source. At CDC, serum and plasma were compared as a source of complement, and no differences were found in bactericidal activity. Also, heparin (2.5 U/μL) was added to the buffer of the cell suspension to prevent clot formation; there was no evidence that this affected SBA. All laboratories tested complement sources for complement-dependent and -independent killing using the target strain. Complement sources from participating laboratories were compared at CDC using two quality control sera (KME, provided by NIPH, and QC36, provided by the Finlay Institute). Complement sources were found to yield comparable bactericidal activities. CDC and NIPH laboratories used a 25% complement source, and the Finlay Institute used a 33% complement source. Serum, cells, and complement were incubated for 30 min at 35°C ± 2°C. For cell growth, tryptic soy broth agar was used at CDC and NIPH and Mueller-Hinton Broth agar plus supplements at the Finlay Institute. KME and QC36 quality control sera were run in each group of microtiter plates (usually four to six) in all laboratories.

ELISA. At NIPH and the Finlay Institute, outer membrane protein vesicles from their respective vaccine preparations were used as the solid-phase antigen [11].

Carriage of N. meningitidis among study participants. Study participants at two schools underwent posterior pharyngeal swab for culture of *N. meningitidis* at the time of the fourth and fifth blood collections. Swabs were streaked on modified New York City (NYC) agar plates within 2 h of being obtained and were incubated overnight at 37°C with 5% CO_2. Colonies suspected of being *N. meningitidis* were subcultured, confirmed as *N. meningitidis,* and serogrouped at the National University Hospital (Reykjavik). Selected strains (the first isolated from each participant) also underwent serotyping and subtyping with selected monoclonal antibodies (serotype: 2a, 4, 15, and 21; subtype: P1.3, P1.7, P1.14, P1.15, and P1.16) on protein extracts.

Bridging (or linking) study. SBA was measured using existing samples of pre- and postvaccination (after second vaccine dose) sera from studies in which protective efficacy was measured or in which the same vaccine lot used in the Iceland trial was used in a different population.

Finlay Institute and NIPH personnel first determined how many pre- and postvaccination (after the second vaccination) samples were available from 4 groups: (1) Cuban subjects who received the Finlay vaccine lot used during the double-blind placebo-controlled

efficacy trial in 1987 through 1989 [1]; (2) Norwegian subjects who received the NIPH vaccine lot used during the double-blind placebo-controlled efficacy trial done in Norway in 1989 through 1991 [2]; (3) Cuban subjects who received the Finlay-produced vaccine lot used in Iceland; and (4) Norwegian subjects who received the NIPH vaccine lot used in Iceland.

A scheme for systematic sampling of available sera was designed on the basis of number of desired specimens (75 paired sera per group) and the organization of original records. Specimen records and storage conditions were inspected on-site (Cuba and Norway) by a member of or designated representative of the World Health Organization Steering Committee on Encapsulated Bacteria (F.S., J.H., B.A.P.). All specimens were transported frozen to CDC, where they were tested by SBA assay against respective serogroup B vaccine type-strains (CU385/83 and 44/76-SL). Methods for SBA were as described earlier.

Results

Study enrollment and follow-up. Study group numbers were assigned to 445 students. Of these, 408 (92%; 149 from school 1, 241 from school 2, and 18 from school 3) were enrolled (14–24 February); 153 were randomized to each of the serogroup B meningococcal vaccine groups, and 102 were randomized to the control vaccine group. Almost all participants (97%) were 16–19 years of age; 1 was 15 years of age and 9 were 20; 56% were female (table 1). There were no significant differences in age distributions between study groups, but there were more female subjects in the NIPH group than in the Finlay or control group.

Vaccinations or blood collections were planned at enrollment (time 0), 6 weeks (or 42 days), 9 weeks (or 63 days), 10 months (304 days), and 12 months (365 days). Actual mean times were 0, 6 weeks (41 days), 11.6 weeks (82 days), 10.7 months (325 days), and 12 months (360 days).

Thirty-six students (9%) withdrew (for at least 1 blood sample), were lost to follow-up, or were excluded. Three persons receiving the Finlay vaccine developed sterile abscesses at the site of vaccination.

SBA and ELISA against homologous N. meningitidis strains. Analyses of laboratory data were done for 403 students. On the basis of preliminary results from the first 5 blood samples, students were asked to provide a sixth blood sample at ~20 months after enrollment; 241 (60%) consented (Finlay two-dose, $n = 47$; Finlay two-dose + booster, $n = 47$; NIPH two-dose, $n = 44$; NIPH two-dose + booster, $n = 48$; and control two-dose, $n = 55$).

Table 2 shows the proportions of SBA and ELISA responders by time and study group against respective homologous strains (SBA) and vaccine preparations (ELISA). For analysis of the second through fourth blood collections, Finlay two-dose and two-dose + booster were combined and NIPH two-dose and two-dose + booster were combined. For analysis of the fifth and sixth blood collections, all groups were considered separately. The increasing proportion of SBA responders in the control group to the Finlay and NIPH vaccine type strains over the study period is presumably due to new acquisition of *N. meningitidis* carriage or carriage of another organism(s) stimulating production of cross-reacting antibodies. This effect appeared to be less pronounced with ELISA measurements, especially among the NIPH study groups.

The Finlay and NIPH two-dose + booster groups had significantly higher proportions of SBA and ELISA responders at 12 months after enrollment than did the two-dose groups. In the 20-month blood sample, these differences were no longer significant with the NIPH vaccine and were less pronounced with the Finlay vaccine. The proportion of SBA responders in the NIPH two-dose + booster group declined from 84% (12 months) to 69% (20 months), while the proportion of SBA responders in all other groups, including the two-dose groups, increased. The proportions of SBA responders in the control group increased from 13% to 20% for the Finlay group and from 10% to 13% for the NIPH group.

Figures 1 and 2 show mean CDC $\log_2$ SBA titers for all blood collections among all students and only those with no detectable SBA at the time of enrollment (insets in figures 1, 2) for the Finlay and NIPH vaccine groups versus the control group, respectively. The control group started with slightly higher titers than the Finlay and NIPH groups. After the first (time 0) and second vaccinations (6 weeks), titers rose in the Finlay and NIPH groups. SBA titers declined by 11 months in all vaccine groups except the Finlay two-dose + booster group. The difference between the Finlay two-dose + booster and

Table 1. Characteristics of study participants by study group.

Characteristic	Finlay 2-dose, no booster/booster ($n = 79/n = 74$)	NIPH 2-dose, no booster/booster ($n = 78/n = 75$)	Control, 2-dose ($n = 102$)
Mean age, years	18.1	18.0	18.0
Female sex, %	52.3	64.7	48.0
Mean (range) days from enrollment to 2nd vaccination	41.3 (38–57)	41.2 (38–46)	41.4 (38–61)
Mean (range) days from enrollment to bleed 3	81.7 (73–99)	81.4 (72–96)	81.5 (73–98)
Mean (range) days from enrollment to bleed 4 ± booster vaccination	325.0/324.5 (319–334)/(319–333)	325.3/323.9 (319–334)/(318–332)	325.0 (317–334)
Mean (range) days from enrollment to bleed 5	360.2/360.6 (353–370)/(355–371)	360.6/360.4 (355–376)/(354–371)	360.3 (353–374)
Did not complete study follow-up, %	7.8/6.7	12.8/6.7	7.1

Table 2. Serum bactericidal activity (SBA) and ELISA responders tested against respective vaccine type strains (SBA) and vaccine preparations (ELISA) by time and study group (compared with control group).

	% SBA responders (control)[†]				% ELISA responders (control)[‡]			
Time after enrollment*	Finlay 2-dose	Finlay + booster	NIPH 2-dose	NIPH + booster	Finlay 2-dose	Finlay + booster	NIPH 2-dose	NIPH + booster
6 w	18[‖] (1)		51[‖] (3)		20[‖] (0)		43[‖] (0)	
12 w	25[‖] (2)		71[‖] (3)		54[‖] (4)		74[‖] (3)	
11 mo	22[‖] (12)		40[‖] (12)		24[‖] (3)		33[‖] (0)	
12 mo	15 (13)	44[‖] (13)	47[‖] (10)	84[‖] (10)	17 (9)	68[‖] (9)	34[‖] (1)	89[‖] (1)
20 mo[§]	43[‖] (20)	62[‖] (20)	64[‖] (13)	69[‖] (13)			32[‖] (0)	54[‖] (0)

NOTE. w, weeks; mo, months.

* Rounded to nearest week or month.

[†] CDC laboratory results using respective vaccine type strains as target strains.

[‡] Finlay Institute laboratory results for Finlay vaccine study groups and NIPH laboratory results for NIPH vaccine study groups, using respective vaccine preparations as ELISA targets.

[§] 20-month blood sample was not part of original study design. This sample was collected and tested after study had been unblinded and is based on 60% of students initially enrolled.

[‖] $P < .05$ vs. control group, Fisher's exact test.

Finlay two-dose at 11 months cannot be explained by study design; both these groups had been treated identically until this time. Patterns of response among the students in the Finlay and NIPH groups with no detectable SBA activity at the time of enrollment were similar to those among all students.

SBA results in table 2 and figures 1 and 2 are based on data from CDC.

Table 3 shows the geometric mean SBA titers from CDC and ELISA values from the Finlay and NIPH laboratories against their respective vaccine preparations.

SBA against heterologous N. meningitidis strains. The proportions of SBA responders were generally lower when tested against heterologous *N. meningitidis* strains. Table 4 shows the proportions of SBA responders in the Finlay and NIPH vaccine groups against heterologous strains. Data are shown for the Finlay vaccine group tested against the NIPH vaccine type strain 44/76-SL (a heterologous strain in this instance) and strains 8069 and G1963. Finlay vaccine group titers were sometimes higher against the NIPH vaccine type strain 44/76-SL than against the Finlay vaccine type stain CU385/83. The NIPH

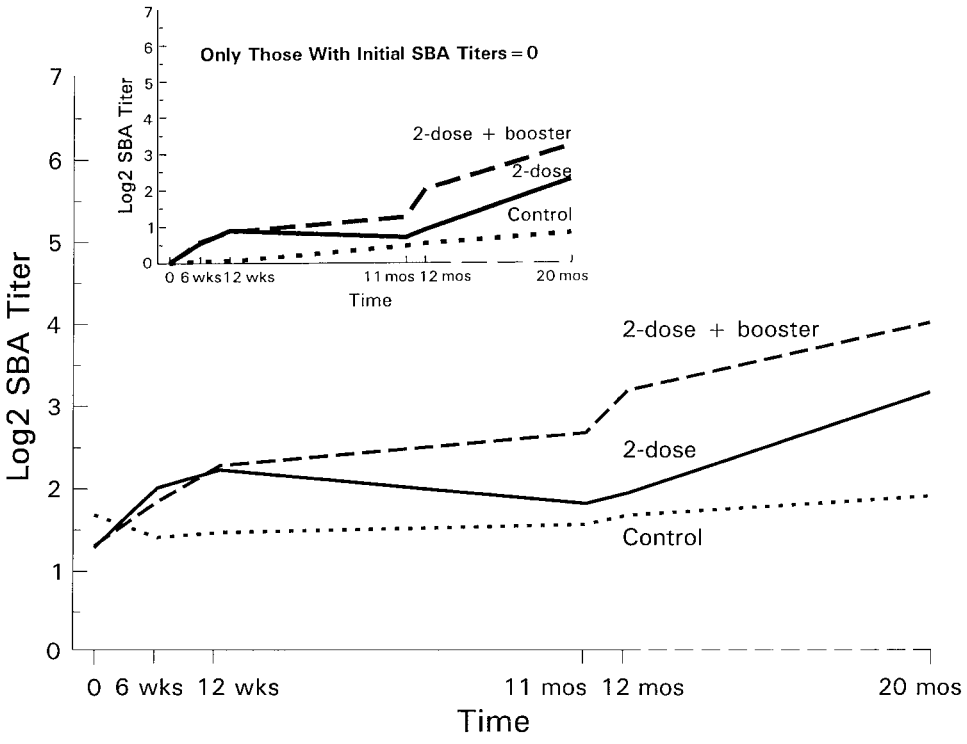

Figure 1. Finlay Institute (Havana) vaccine groups. Mean $\log_2$ serum bactericidal activity (SBA) titers for 1st through 6th blood samplings among all students and only those with no detectable SBA at time of enrollment (inset).

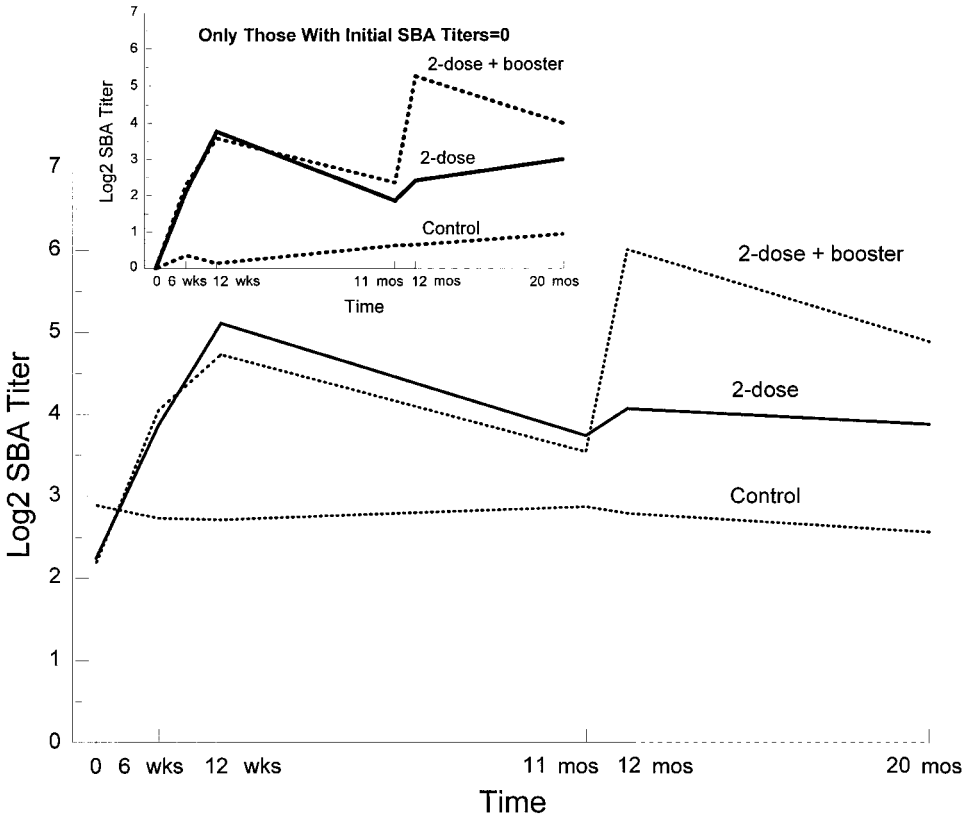

Figure 2. National Institute of Public Health (Oslo) vaccine groups. Mean $\log_2$ serum bactericidal activity (SBA) titers for 1st through 6th blood samplings among all students and only those with no detectable SBA at time of enrollment (inset).

vaccine groups were tested against the Finlay vaccine type strain CU385/83 (a heterologous strain in this instance) and strains 8069 and G1963.

Carriage. Of 358 students, 111 (31%) tested had oropharyngeal cultures yielding *N. meningitidis* at 11 or 12 months after enrollment (or at both times). *N. meningitidis* serogroup B was identified in 44 of these persons (40% of carriers and 12% of students tested); 7 isolates (6%) were serogroup C, 5 (4%) were serogroup Y, 1 (1%) was serogroup Z, and 51 (46%) were *N. meningitidis* that could not be serogrouped (''nonsero-

Table 3. Geometric mean serum bactericidal activity (SBA) titers and ELISA values by time and study group compared with control.

	Geometric mean SBA titers (control), CDC laboratory				Geometric mean ELISA values (control)			
					Finlay laboratory*		NIPH laboratory†	
Time after enrollment	Finlay 2-dose	Finlay + booster	NIPH 2-dose	NIPH + booster	Finlay 2-dose	Finlay + booster	NIPH 2-dose	NIPH + booster
0	2.4 (3.2)		4.6 (7.4)		732 (714)		90 (113)	
6 w	3.8‡ (2.6)		15.6‡ (6.6)		1635‡ (606)		325‡ (112)	
12 w	4.7‡ (2.8)		30.2‡ (6.5)		3457‡ (671)		662‡ (121)	
11 mo	3.5 (3.0)	6.4‡ (2.9)	12.4‡ (7.31)		1691‡ (631)		273‡ (117)	
12 mo	3.9 (3.2)	9.2‡ (3.2)	16.8‡ (6.9)	64.6‡ (6.9)	1308‡ (642)	7847‡ (642)	316‡ (117)	1344‡ (117)
20 mo	9.0‡ (3.8)	16.2‡ (3.8)	14.8‡ (5.9)	29.8‡ (5.9)			270‡ (86)	385‡ (86)

NOTE. w, weeks; mo, months.

* Using Finlay Institute vaccine preparation.

† Using NIPH vaccine preparation.

‡ $P < .05$ vs. control group, mixed model analysis of variance.

Table 4. Serum bactericidal activity (SBA) responders against heterologous *N. meningitidis* strains.

Time after enrollment	2-dose	2-dose + booster	2-dose	2-dose + booster	2-dose	2-dose + booster
	Finlay vaccine groups, % SBA responders (control)					
	44/76-SL		8069		G1963	
6 w	21 (2)		11 (1)		8 (6)	
12 w	34 (3)		18 (6)		16 (10)	
11 mo	31 (12)		14 (13)		19 (14)	
12 mo	27 (10)	48 (10)	8 (13)	30 (13)	12 (13)	36 (13)
20 mo	45 (13)	51 (13)	26 (11)	43 (11)	36 (22)	45 (22)
	NIPH vaccine groups, % SBA responders (control)					
	CU385/83		8069		G1963	
6 w	20 (1)		16 (1)		17 (6)	
12 w	28 (2)		24 (6)		21 (10)	
11 mo	25 (12)		23 (13)		25 (14)	
12 mo	34 (13)	31 (13)	22 (13)	38 (13)	31 (13)	27 (13)
20 mo	43 (20)	48 (20)	34 (11)	38 (11)	39 (22)	31 (22)

NOTE. w, weeks; mo, months.

groupable''). There were no significant differences in distributions of serogroups, serotypes, or subtypes by study group (data not included).

Bridging (or linking) study. Sera were available from 1068 Cuban subjects who had participated in the Finlay Institute–produced vaccine, double-blind, placebo-controlled efficacy trial. For the bridging study, every sixth person vaccinated with the Finlay vaccine was selected, resulting in 142 participants. A sample of the original records documenting collection of sera was reviewed. These specimens had been moved many times since collection; it was not possible to determine the number of times they had been thawed and refrozen. Pre- and postvaccination sera were available from 87 persons; 72 samples were free of bacterial contamination and had sufficient volume for SBA testing. Of these 72, 3 (4% [95% CI, 1%–12%]) had a ⩾4-fold rise in SBA titer against the Finlay vaccine type strain.

Eighty-eight Cuban subjects were given two doses of the Finlay vaccine lot used in the Iceland trial but only 31 matched sera were available. The original records pertaining to collection of these samples were reviewed. These specimens had undergone not more than three freeze-thaw cycles. Twenty-two were free of bacterial contamination and had sufficient volume for SBA testing. Of these 22 (30% [95% CI, 12%–54%]) had a ⩾4-fold rise in SBA titer against the Finlay vaccine type strain.

Sera were available from 542 Norwegian subjects who received the NIPH vaccine during a double-blind, placebo-controlled efficacy trial done in Norway. Every sixth individual was selected, resulting in 87 participants. The original records pertaining to collection of the serum specimens were reviewed; we estimated they had undergone two or three freeze-thaw cycles. Seventy-one samples were free of bacterial contamination and had sufficient volume for SBA testing. Of the 87 study participants, 40 (46% [95% CI, 34%–59%]) had a ⩾4-fold rise in SBA titer against the NIPH vaccine type strain.

Pre- and postvaccination serum samples were available from 140 Norwegian subjects who had enrolled in a controlled and blinded study of the NIPH vaccine lot used in the Iceland trial. All original records pertaining to collection of these specimens were reviewed; these specimens had not been thawed since collection. Eighty-seven subjects were identified by excluding every fifth person; 85 had received the NIPH vaccine and were included in SBA testing. Of these 85, 37 (44% [95% CI, 33%–54%]) had a ⩾4-fold rise in SBA titer against the NIPH vaccine type strain.

Discussion

The proportions of SBA and ELISA responders among the Finlay vaccine recipients were generally lower than in those who received the NIPH vaccine. This is the converse of what would be expected on the basis of published estimates of vaccine efficacies (83% and 57% for Finlay and NIPH vaccines, respectively).

The estimated efficacy of 57% for the NIPH vaccine might be misleading for comparison because of differences in duration of follow-up in our study (12 months and an additional unblinded blood collection at 20 months) and the trial from which this estimate of vaccine efficacy was derived (29 months). Data from the NIPH vaccine trial suggest that vaccine efficacy may have been higher during the early part the trial

period [12]. Only 1 case occurred among persons in the NIPH vaccine group before 11 months compared with 7 cases in the placebo group. The estimated vaccine efficacy was 87% for the first 10 months of the trial. This was the basis for selecting 10 months as the time for a third dose of vaccine in this study.

If the expected efficacy of the NIPH vaccine over our 12-month study period is between 57% and 87%, the proportions of SBA seroconversions in the NIPH two-dose group—71% (after second vaccination), 40% (11 months), and 47% (12 months)—seem to correlate with estimated efficacy. For the Finlay two-dose group, however, the proportions of SBA responders were much lower than would be predicted on the basis of the estimated efficacy of 83% (95% CI, 42%–95%)—18% (after second vaccination), 25% (11 months), and 15% (12 months). The ELISA data were similar to SBA data for both vaccines.

One possible reason for this discrepancy could be a higher background of serologic reactivity to the NIPH than the Finlay vaccine type strain in Iceland. This could result from carriage of particular *N. meningitidis* strains that matched or were more similar to the NIPH than the Finlay vaccine type strain. Intrinsic differences in susceptibility of the vaccine type strains to killing could also account for discrepant SBA results. Both these explanations seem unlikely since the SBA and ELISA activity against the 2 vaccine type strains (SBA) and vaccine preparations (ELISA) was so similar in the control group, with very low seroconversion rates at enrollment (0–3%), which increased to 10%–13% over 1 year.

Several other possible explanations were considered for discordance between estimated efficacy and proportions of SBA and ELISA responders. The first was possible differences between vaccine lots used in our study and those used in the earlier efficacy trials. However, after an extensive effort to compare vaccine lots used in these studies to the earlier lots, only minor differences were found. The only clear difference identified was an apparently greater amount of 70-kDa protein in the NIPH vaccine lot used in Iceland compared with lots used in the efficacy trial. Differences in study populations also may have accounted for the discrepancy. For example, genetic factors could have allowed Icelandic persons to respond differently from Cuban persons to the Finlay vaccine. The results of the bridging study suggest that this was not the case. When sera from efficacy studies of both vaccines were tested by SBA assay, the proportions of responders were similar to (for NIPH vaccine) or lower (for Finlay vaccine) than those in Iceland.

Methods used for analysis of our SBA and ELISA data could also result in mistaken conclusions about their relationship to vaccine efficacy. We tried to avoid this by using several different analytic approaches. These included 4-fold rises in SBA and ELISA antibody titers compared with prevaccination levels, geometric mean titers, and proportions of persons achieving specific threshold titers of antibody (data not included). A 4-fold rise in SBA antibody titer was also separately analyzed for the group of study participants who had no detectable SBA antibody at study enrollment. The conclusions reached using all these methods were similar in comparing the serogroup B vaccine group with the control group and comparing the serogroup B vaccine groups to each other.

The most likely reason for the differences between our immunogenicity data and estimates of efficacy, especially for the Finlay vaccine, is that SBA (as we have measured it) is a less sensitive measure of protection than clinical efficacy. When SBA is present, it is likely to correlate with protection (against the assay target strain, at least), but the converse may not be true. Antibodies that are not bactericidal may be protective through other mechanisms such as opsonophagocytosis. The low sensitivity of our measurements of SBA for protection is also supported by our low prevaccination titers. Almost one-third of our study participants had no detectable SBA at the time of study enrollment. It is unlikely that these persons are at high risk of disease, given the relatively high carriage rates identified in this study and low rates of disease.

Another objective of our study was to evaluate the possible usefulness of a third (booster) dose of the vaccines, given 10 months after enrollment. In our study, the booster dose was actually given closer to 11 months. The proportions of SBA and ELISA responders in the two-dose + booster Finlay and NIPH groups were significantly higher than among those who received only two doses of vaccine. Although the booster dose clearly provided a benefit at 12 months, at 20 months the differences between the two-dose and two-dose + booster groups were less pronounced. A third dose of the Finlay and NIPH vaccines may be useful, but further studies will be needed to determine an optimal time for boosting, and vaccinees may need to be followed for a longer period.

SBA against non–vaccine type (heterologous) strains was evaluated in an attempt to predict how the efficacy of these vaccines might be affected if circulating *N. meningitidis* serogroup B strains are different from those used to produce the vaccines. The 2 heterologous strains used were selected because they had caused increased rates of serogroup B meningococcal disease but had different serologic characteristics and electrophoretic enzyme types than the vaccine type strains. For the NIPH groups, the proportion of SBA responders to heterologous strains was less than one-half of those who responded when tested against the NIPH vaccine type strain.

For the Finlay groups, the proportion of SBA responders to the NIPH vaccine type strain was sometimes higher than to the Finlay vaccine type strain; against the other 2 strains, the proportions were reduced by about one-half. There was some evidence that both vaccines were able to boost responses to one (NIPH-produced) or more (Finlay-produced) of the heterologous strains. These data suggest that these vaccines may not be as effective against non–vaccine type strains as they would against the vaccine type strains. These findings may offer support for current efforts to develop multivalent outer membrane protein meningococcal vaccines, which include the most common class 1 (or subtype) meningococcal proteins [13].

Until the components of these vaccines that are responsible for conferring protection are more carefully defined, investiga-

tors will need to rely on SBA for comparison of Finlay and NIPH vaccines with newly developed candidate vaccines, possibly in combination with other functional (e.g., opsonophagocytic assay) and nonfunctional techniques [14]. Animal models of meningococcal disease such as the infant rat model may be useful in more fully elucidating the relationship between SBA and protection; such models may also be useful in evaluation of other possible laboratory correlates for protection [15].

The protective efficacy of the Finlay-produced serogroup B meningococcal vaccine among children <4 years of age remains uncertain, and the NIPH-produced vaccine has only recently undergone immunogenicity testing among children in this age group [5, 16, 17]. These children are usually the most severely affected by endemic and epidemic serogroup B meningococcal disease. Identification of a vaccine that provides protection against the most commonly circulating *N. meningitidis* serogroup B strains among young children remains an important public health priority.

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