Persisting Immune Responses Indicating Long-Term Protection after Booster Dose with Meningococcal Group B Outer Membrane Vesicle Vaccine

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MenBvac is an outer membrane vesicle vaccine against systemic meningococcal disease caused by serogroup B Neisseria meningitidis. In this placebo-controlled double-blind study including 374 healthy adolescents, the safety and immunogenicity of a schedule of three primary doses 6 weeks apart followed by a fourth dose a year later were evaluated. Antibody responses to the vaccine strain and heterologous strains (non-vaccine-type strains) and the persistence of these antibodies were measured by the serum bactericidal assay (SBA) and enzyme-linked immunosorbent assay up to 1 year after the last dose. The proportion of subjects with SBA titers of ≥4 against the vaccine strain increased from 3% prevaccination to 65% after the third dose. Ten months later, this proportion had declined to 28%. The fourth dose induced a booster response demonstrated by 93% of subjects achieving a titer of ≥4. One year after the booster dose, 64% still showed SBA titers of ≥4. Cross-reacting antibodies were induced against all heterologous strains tested, although the magnitude of SBA titers differed widely between the different strains. All four doses of MenBvac were safe. Both MenBvac and the placebo had reactogenicity profiles of mild to moderate local and systemic reactions. Pain, the most common reaction, was reported with similar frequencies in both groups. No serious adverse events occurred in the MenBvac group. This study confirmed the good immunogenicity of the primary course of MenBvac and demonstrated prolonged persistence and increased cross-reactivity of functional antibodies elicited by a booster dose.

Meningococcal disease represents a substantial public health problem in different parts of the world. The disease, which is usually manifested as cerebrospinal meningitis and/or septicemia, is most prevalent in infants and young children, but teenagers also are at increased risk. Septicemic cases are characterized by a rapid course and, not infrequently, despite immediate treatment with effective antibiotics, permanent sequelae (e.g., developmental delay, skin, digit or limb loss) or a fatal outcome. During the past 30 years, serogroup B meningococci have been the cause of epidemics in, e.g., Norway (3, 13), Cuba (24), Brazil (23), Chile (7), and New Zealand (2, 14). In contrast to serogroups A, C, Y, and W135, for which effective polysaccharide-based vaccines are available, no such vaccine is available for serogroup B meningococci, due to the poor immunogenicity of the serogroup B capsular polysaccharide (27). As a response to the epidemic starting in Norway in the mid 1970s, the Norwegian Institute of Public Health (NIPH) developed a meningococcal group B outer membrane vesicle (OMV) vaccine, MenBvac. This vaccine is based on outer membrane proteins in the form of vesicles and is prepared from a clinical isolate representative of that epidemic (10).

MenBvac has been shown to be safe and immunogenic and to confer protection against meningococcal serogroup B disease (3, 18, 22). An efficacy study performed with students in secondary schools showed that the estimated rate of protection against serogroup B meningococcal disease after two doses was 57% over the entire 29-month follow-up period (3). However, it was observed that the estimated protection rate after 10 months was as high as 87% (11). After that there was a decline in protection, and therefore it was concluded that a third dose would be needed for long-term protection. A third dose, given either as a third primary dose (1, 25) or as a booster given from 6 months to several years after the primary immunization (11, 17, 20, 22), has since been tested in several studies and found to be beneficial.

The aim of this study was to evaluate the immune responses of teenagers after a primary immunization with three doses of MenBvac and additionally to evaluate the persistence of antibody levels after 10 months and the effect of a fourth dose given 10 months after the primary immunization.

Serogroup B strains are responsible for an increasing proportion of systemic meningococcal disease in Europe, and the strains causing disease show large differences (6, 9). Therefore, the cross-reactivity of MenBvac against other meningococcal group B strains (isolates of different serotypes and/or subtypes) from various countries was also investigated in this study.

MATERIALS AND METHODS

Study design. This study was a double-blind, randomized, controlled multicenter phase II study. Subjects were assigned according to a randomization list to receive either MenBvac or a placebo in a ratio of 2:1. The vaccines were admin-

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istered intramuscularly in the deltoid region of the nondominant arm at weeks 0, 6, and 12 and approximately 10 months after the third dose.

**Vaccines.** MenBvac was manufactured at NIPH from a B:15:P1.7,16 meningococcal strain (44/76) by fermentor growth and extraction of the OMVs with the detergent deoxycholate. OMVs were purified by ultracenrifugation and adsorbed to aluminum hydroxide. One dose (0.5 ml) of MenBvac contained 25 μg outer membrane protein and 1.67 mg aluminum hydroxide (10).

One dose (0.5 ml) of the placebo vaccine contained 1.67 mg aluminum hydroxide in vaccine solvent.

**Vaccines.** Healthy 12- to 17-year-old secondary school students were eligible for participation in the study. The students were recruited from four schools in Oslo, Norway. Participants were excluded if they had experienced a hypersensitivity reaction following previous vaccinations or if they had an acute or chronic systemic illness. Other reasons for exclusion from the study were immunosuppressive therapy, receipt of blood products within 3 months, or earlier immunization with a group B meningococcal vaccine of any kind.

**Immunogenicity.** Blood samples (10 ml) were collected by venipuncture at the time of the first dose, 6 weeks after the second dose, 6 weeks and 10 months after the third, and 6 weeks and 1 year after the booster dose. The blood samples were fractionated and the serum aliquoted and stored at −20°C.

(i) SBA. The serum bactericidal assay (SBA) is a functional assay that measures the ability of serum antibodies to induce lysis of bacteria in the presence of complement.

The analyses were performed in microtiter plates using the “tilt” method (5). After heat inactivation, the sera to be tested were serially diluted twofold (starting with 1:2) and incubated for 60 min (37°C) in the presence of the bacterial inoculum and human complement (25% human serum from one donor without bactericidal activity). The bacterial colonies were counted using a colony counter, and the bactericidal titer was defined as the reciprocal of the serum dilution killing at least 50% of the organisms. A responder was defined as a subject achieving at least a fourfold increase in the SBA titer.

The bactericidal activity against the vaccine strain 44/76-SL (B:15:P1.7,16) (22) was measured for all subjects. A more recently isolated strain from Norway (N 14/00), of the same serotype (PorB) and subtype (PorA) as the vaccine strain, was also evaluated for a randomly selected subset of 34 subjects receiving MenBvac. In addition, SBA titers against a panel of heterologous strains were evaluated for the same subset of sera. Among the strains of different serotypes and subtypes were two recently recovered clinical isolates from Norway (N 11/03 [B:4:P1.7-2,4] and N 13/99 [B:4:P1.7-2,4]) and two strains representative of the ongoing group B epidemic in New Zealand: the epidemic strain used for production of the OMV vaccine MenZB (19) (NZ 98/254 [B:4:P1.7-2,4]) and a different isolate with similar antigenic characteristics (NZ 94/167 [B:4:P1.7-2,4]). A strain of a different serotype but of the same subtype, isolated during a recent outbreak in Normandy, France (LNP20404 [B:14:P1.7,16]), was also tested in the SBA. The detailed characteristics of the strains are presented in Table 1.

(ii) ELISA. The immunoglobulin G (IgG) antibodies against serogroup B OMV were analyzed by an enzyme-linked immunosorbent assay (ELISA). The OMV used for coating ELISA microtiter plates was OMV from strain 44/76 prepared as for vaccine production (10). The results were given in arbitrary units (U/ml).

**Safety monitoring.** Selected local and systemic adverse events were monitored after each dose. These events were termed “local and systemic reactions,” irrespective of relation to the study vaccine. The subjects were instructed to complete a diary card to describe local (i.e., injection site) reactions (pain, redness, swelling, other) and systemic reactions (headache, malaise, other) during the first and second 24-h periods after each dose. The subjects were also asked to record the body temperature if they felt feverish. Other adverse events after the first 48 h as well as medication taken during the study were recorded in interviews performed on the planned visit days. All serious adverse events were collected throughout the study until 6 weeks after the fourth dose (i.e., during a period of approximately 18 to 20 months).

**Ethics.** This study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization guideline for Good Clinical Practice, and other national legal and regulatory requirements. The trial was approved by the Regional Committee for Ethics and Medical Research and the Norwegian Medicines Agency. Each subject and the subject’s parent(s) or legal guardian(s) gave informed consent in writing before enrollment.

**Statistical methods.** The number and proportion of subjects with SBA titers of ≥4 against the homologous and heterologous strains of group B meningococci and associated 95% Clopper-Pearson confidence intervals (CIs) were calculated at the different time points. *P* values for differences in proportions between time points within the MenBvac group were calculated using Pearson’s chi-square test. Furthermore, the following variables were studied: response rate in SBA,
results

A total of 374 subjects were enrolled and given at least one dose of MenBvac or placebo (Fig. 1). The treatment groups were similar with regard to age and sex. Of the 248 subjects in the MenBvac group, 55% were males and 45% were females, and of the 126 subjects in the placebo group, 49% were males and 51% were females. The mean age at the time of inclusion was 13.6 years in both groups, ranging from 13 to 15. In the subset of subjects tested for responses to heterologous strains, the age distribution was similar (mean age, 13.7 years), while the proportion of males was somewhat higher (62%) than that in the whole MenBvac group.

Immunogenicity. (i) Proportion of subjects with SBA titers of ≥4. In the MenBvac group, the proportion of subjects with SBA titers of ≥4 against the vaccine strain (44/76) was 3% before vaccination, 53% at 6 weeks after the second dose, and 65% at 6 weeks after the third dose. The proportion of subjects with SBA titers of ≥4 was statistically significantly higher after the third dose than after the second dose \( (P = 0.009) \). This proportion declined to 28% at 10 months after the third dose. At 6 weeks after the booster dose, the proportion of subjects with SBA titers of ≥4 against the vaccine strain had increased to 93% in the MenBvac group, a figure that declined more slowly than after the third dose and amounted to 64% 1 year after the booster dose. Both at 6 weeks and at 1 year after the booster dose, the proportions of subjects with SBA titers of ≥4 were statistically significantly higher than those at the corresponding time points after the third dose \( (P < 0.0001) \) (Fig. 2).

The immune response to a more recently isolated strain \( (N_{14/00}) \) with the same serotype and subtype as the vaccine strain was found to be similar to that of the vaccine strain isolated in 1976, with 79% of the subjects achieving a GMT of ≥4 at 6 weeks after the third dose, 90% at 6 weeks after the booster dose, and 62% 1 year after the booster dose.

The proportion of subjects with SBA titers of ≥4 against the French strain LNP20404 was similar to that observed for strain 44/76, reaching 90% at 6 weeks after the booster dose and 72% 1 year after the booster dose (Table 1).

The other strains evaluated showed kinetics similar to that observed for the vaccine strain, with an increase in the proportion of subjects with SBA titers of ≥4 after the primary series of three doses and a further increase after the booster dose. However, the proportion of subjects with SBA titers of ≥4 differed between the individual strains from 21% for strain \( N_{11/03} \) to 76% for strain \( N_{13/99} \) at 6 weeks after the booster dose (Table 1).

In the placebo group, the proportion of subjects with SBA titers of ≥4 against the vaccine strain ranged between 3% and 7% at the different time points.

(ii) GMTs (SBA) against the vaccine strain. Before vaccination, the GMT was 1.1 both in the MenBvac group and in the placebo group. At 6 weeks after the second dose, the GMT had increased to 3.8 in the MenBvac group; it was further statistically significantly increased at 6 weeks after the third dose, to 5.4 \( (P = 0.001) \). The GMT declined to 2.1 at 10 months after the third dose. The booster dose resulted in an increase in GMT to 18 (significantly higher than the corresponding value at 6 weeks after the third dose \( [P < 0.0001] \)), which then declined more slowly than after the third dose, resulting in a GMT of 5.2 at 1 year after the booster dose.
GMT 1 year after the booster dose was statistically significantly higher than the value recorded 10 months after the third dose ($P < 0.0001$). Throughout the study, the GMTs remained at prevaccination levels in the placebo group (Fig. 3).

(iii) Proportion of SBA responders. The proportion of responders in the MenBvac group was 49% at 6 weeks after the second dose, increased significantly to 61% at 6 weeks after the third dose ($P = 0.01$), and then declined to 25% at 10 months after the third dose. At 6 weeks after the booster dose, the proportion of responders had increased to 90%. This figure declined more slowly than after the third dose, and 1 year after the booster dose, 59% of the subjects were still responders. After the booster dose, the proportions of responders in the MenBvac group were statistically significantly higher than the corresponding proportions after the third dose ($P < 0.0001$).

In the placebo group, the proportion of responders ranged from 0% to 4% at the different time points (Table 2). The proportion of responders to the evaluated heterologous strains followed the same pattern as that for the vaccine strain (data not shown), with a pronounced increase after the booster dose.

(iv) Antibody responses measured by ELISA. The ELISA results for geometric mean IgG concentrations against OMVs from strain 44/76 showed a strong IgG response in the vaccine group after only two doses. The IgG levels had decreased at 10 months after the third dose but were still 10-fold higher than prevaccination levels. A booster response was observed after the fourth dose (Table 3).

Safety. The incidence of a majority of the recorded local and systemic reactions was similar in the MenBvac group and the placebo group, and the incidence of reactions, both local and systemic, was similar after the first, second, third, and fourth doses. Most of these reactions were reported as mild or moderate in intensity. The incidence of all reactions was lower in the second than in the first 24-h period following vaccination. Overall, the results from this study demonstrated that a four-dose regimen of MenBvac was safe with an acceptable reactogenicity profile.

(i) Local reactions. Local pain at the site of vaccination was the most common reaction, reported by 97% of the subjects in both the MenBvac group and the placebo group after the first dose, and was similar or somewhat lower after the following doses. Pain of severe intensity was reported by 5 to 20% of the subjects in the MenBvac group and by 5 to 13% in the placebo group.
Redness and swelling were reported by approximately 50 to 60% of the subjects in the MenBvac group after each dose, compared to 20 to 30% in the placebo group (Table 4).

(ii) Systemic reactions. After the first dose, malaise was reported by 46% of the subjects in the MenBvac group compared to 32% in the placebo group, and headache was reported by 34% of the subjects in the MenBvac group compared to 28% in the placebo group. Fever was reported by 2 to 5% of the subjects after each dose in the MenBvac group compared to 0 to 1% in the placebo group. No temperature of 38.5°C was reported (Table 4).

(iii) Other adverse events. No serious adverse events occurred in the group receiving MenBvac, whereas three unrelated serious adverse events occurred in the placebo group. A total of 15 subjects (6%) in the MenBvac group reported adverse events classified as at least possibly related to MenBvac (headache, rash, and influenza-like illness being most common among these); none were of severe intensity. In the placebo group three subjects (2%) reported events assessed as at least possibly related to the study drug. One subject in each group (1%) was withdrawn by the investigator after presenting a type I allergic reaction after the second dose. Approximately 3% of the subjects in both groups withdrew due to local pain, and one subject (<1%) in the MenBvac group was withdrawn by the investigator due to severe headache.

**DISCUSSION**

Although the serogroup B meningococcal epidemic starting in 1974 in Norway has abated, the need for preparedness to prevent future outbreaks of group B meningococcal disease in Norway or elsewhere (e.g., New Zealand or, more recently, Normandy, France) warrants further studies and development of MenBvac. This was the first study evaluating a regimen with three primary doses of MenBvac followed by a booster dose. It was shown that there was a statistically significant increase in bactericidal antibodies after the third dose compared to the second. Nonetheless, the response to the homologous strain after the booster dose reached SBA levels much higher than after the third dose, and the subsequent decline was slower

**TABLE 3. Geometric mean IgG concentrations against OMVs from strain 44/76 as measured by ELISA**

| Vaccine group | Pre-vacc.† | 6 wk after 2nd dose | 6 wk after 3rd dose | 10 mo after 3rd dose | 6 wk after 4th dose | 1 yr after 4th dose |
|---------------|------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|               | GMC (95% CI) n | GMC (95% CI) n | GMC (95% CI) n | GMC (95% CI) n | GMC (95% CI) n | GMC (95% CI) n |
| MenBvac       | 18 (16–19) 227 | 995 (902–1,097) 227 | 1,136 (1,027–1,256) 221 | 251 (223–282) 207 | 1,738 (1,574–1,920) 202 | 460 (409–518) 187 |
| Placebo       | 18 (15–20) 120 | 17 (15–19) 120 | 16 (14–19) 116 | 41 (35–48) 110 | 41 (35–48) 107 | 40 (35–47) 94 |

† Pre-vacc., prevaccination.

TABLE 4. Local (injection site) and systemic reactions

| Reaction | No. (%) of subjects with the indicated reaction† after: |
|----------|-----------------------------------------------------|
|          | 1st dose | 2nd dose | 3rd dose | 4th dose |
|          | MenBvac (n = 229) | Placebo (n = 117) | MenBvac (n = 204) | Placebo (n = 111) | MenBvac (n = 188) | Placebo (n = 105) | MenBvac (n = 183) | Placebo (n = 90) |
| Pain     |          |          |          |          |          |          |          |          |
| Present  | 222 (97) | 113 (97) | 192 (94) | 103 (93) | 169 (90) | 88 (84) | 178 (97) | 84 (93) |
| Severe   | 45 (20)  | 15 (13)  | 16 (8)   | 6 (5)    | 10 (5)   | 6 (6)   | 33 (18)  | 6 (7)   |
| Redness  |          |          |          |          |          |          |          |          |
| Present  | 107 (47) | 25 (21)*** | 101 (50) | 14 (13)*** | 98 (52) | 7 (4) | 10 (10)*** | 99 (54) | 11 (12)*** |
| Severe   | 4 (2)    | 1 (1)    | 9 (4)    | 0        | 7 (4)    | 0 | 12 (7)   | 0        |
| Swelling |          |          |          |          |          |          |          |          |
| Present  | 116 (51) | 38 (32)**  | 118 (58) | 28 (25)** | 113 (60) | 9 (5) | 22 (21)** | 118 (64) | 25 (28)** |
| Severe   | 9 (4)    | 1 (1)    | 15 (7)   | 0        | 9 (5)    | 0 | 14 (8)   | 0        |
| Headache |          |          |          |          |          |          |          |          |
| Present  | 78 (34)  | 33 (28)  | 56 (27)  | 26 (23)  | 53 (28)  | 19 (18) | 45 (25)  | 15 (17)  |
| Severe   | 4 (2)    | 2 (2)    | 4 (2)    | 2 (2)    | 4 (2)    | 2 (2) | 3 (2)    | 0        |
| Malaise  |          |          |          |          |          |          |          |          |
| Present  | 106 (46) | 37 (32)**  | 77 (38)  | 35 (32)  | 64 (34)  | 31 (30) | 75 (41)  | 20 (22)** |
| Severe   | 10 (4)   | 5 (4)    | 8 (4)    | 1 (1)    | 4 (2)    | 1 (1) | 9 (5)    | 0        |
| Temp     |          |          |          |          |          |          |          |          |
| ≥38 and <40°C | 11 (5)   | 1 (1) b | 11 (5)   | 0 b | 3 (2)   | 0 b | 7 (4)   | 0 b |
| ≥40°C     | 0        | 0        | 0        | 0        | 0        | 0 | 0        | 0 |

* P < 0.05; ** P < 0.01; *** P < 0.001 (based on P values from Pearson's chi-square test for vaccine group differences). If any expected cell count was <1 or if >20% of the cells had an expected cell count of <5, then Fisher's exact test was used.

b Vaccine group difference not evaluable.
than after the third dose, resulting in a longer-lasting response, thus indicating an increased duration of protection after the booster dose.

Sera from bactericidal antibodies, the functional antibodies measured in this study, have generally been accepted as the best surrogate for protection for all serogroups of meningococci. For serogroup B, the proportion of vaccinees with SBA titers of \( \geq 4 \) has been suggested to correlate to clinical efficacy (4, 11).

Following vaccination with OMV vaccines, antibody responses against a wide range of outer membrane proteins (e.g., PorA and PorB porins, reduction modifiable protein [Rmp], OpcA invasin) and lipopolysaccharide (LPS) are induced (11, 22). However, it is the specific PorA proteins that are the most abundant and the immunodominant antigens in OMV vaccines; they are a main target for SBA activity (4, 16, 22). MenBvac has earlier been shown to induce bactericidal antibodies against several heterologous strains (20, 25). In this trial we have included more recently isolated strains and shown that MenBvac gives rise to functional antibody responses also to strains currently causing disease and strains responsible for ongoing epidemics, although these strains differ in PorA or PorB. The panel of heterologous strains tested was, however, limited with regard to diversity of subtypes.

The Norwegian isolate N13/99 has an LPS immunotype different from that of the other P1.7-2,4 strains, L8 instead of L3,7 (Table 1). The differences between these strains (measured as the proportion of subjects with SBA titers of \( \geq 4 \)) indicate that the immunotype may influence the level of bactericidal activity. Possible differences in the expression of other antigens, e.g., OpcA, may also contribute to the different susceptibilities to bactericidal activity.

The SBA analyses showed an increase in cross-reactive bactericidal antibodies after the third primary dose compared with the second, confirming that a primary immunization schedule of three doses is advantageous. The booster dose resulted in further increased levels of bactericidal antibodies against the heterologous strains tested. At 6 weeks after the booster dose, the percentage of subjects with titers of \( \geq 4 \) against the tested strains ranged from 21% to 90%. The results indicate that MenBvac gives rise to antibody responses against outer membrane proteins other than PorA and that at least among teenagers, MenBvac may have the potential to protect also against meningococcal group C conjugate vaccine could confer protection also against group C meningococci (1).

The SBA levels observed in this study were lower than those reported from studies with MenBvac conducted during the epidemic in Norway (11). This may be due to the different epidemiological situation. At the start of this study, the incidence of meningococcal disease in Norway was about 1.7 per 100,000 (12), much lower than the incidence observed during the epidemic (13). The change of SBA method from the agar overlay assay to the tiff assay may also contribute to the somewhat lower titers observed in studies analyzed by the tiff method, although the overall results with the two methods are similar (5). Independently of the number of primary doses administered and the epidemiological situation, a booster dose is of benefit for a long-lasting response.

In accordance with earlier studies with this age group, MenBvac was found to be moderately reactogenic without any safety concerns. Local and systemic reactions are very common for intramuscularly administered aluminum hydroxide-containing vaccines, and therefore MenBvac was expected to have such a profile. The most common adverse reaction was local pain, which is consistent with earlier data (18). The frequency of some of the reactions was high in the placebo group also. Most of the local and systemic reactions were of mild or moderate intensity. Among those subjects who experienced local reactions of severe intensity after the first vaccine dose, only a few reported local reactions of severe intensity after the subsequent doses. No serious adverse events occurred in the group receiving MenBvac, and the four-dose regimen was found to be safe.

In conclusion, the results from this study demonstrated that MenBvac was safe with an acceptable reactogenicity profile. The immunogenicity profile with the large increase in SBA titers and in the proportion of subjects with titers of \( \geq 4 \) after the booster dose demonstrates immunological memory and a strong booster response after priming with three doses. Furthermore, the slower decline in antibody response after the booster dose compared with the response after the primary immunization indicates that a booster dose could significantly extend the persistence of serum bactericidal antibodies against the vaccine-type strains as well as that of cross-reactive antibodies against some heterologous strains, presumably resulting in prolonged protection against meningococcal serogroup B disease.

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