Three Doses of an Experimental Detoxified L3-Derived Lipooligosaccharide Meningococcal Vaccine Offer Good Safety but Low Immunogenicity in Healthy Young Adults

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Meningococcal diseases caused by Neisseria meningitidis are a significant health burden throughout the world, leading to death and permanent sequelae (15). Whereas polysaccharide or polysaccharide conjugate vaccines are effective against se-

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This open, randomized phase I study evaluated the safety and reactogenicity of an experimental meningococcal serogroup B (MenB) vaccine obtained from outer membrane vesicle detoxified L3-derived lipooligosaccharide. Healthy young adults (n = 150) were randomized to receive either experimental vaccine (provided in five formulations, n = 25 in each group) or VA-Mengoc-BC (control, n = 25) administered on a 0- to 6-week/6-month schedule. Serum bactericidal assays performed against three MenB wild-type strains assessed the immune response, defined as a 4-fold increase from pre- to postvaccination. No serious adverse events related to vaccination were reported. Pain at the injection site, fatigue, and headache were the most commonly reported adverse events. Solicited adverse events graded level 3 (i.e., preventing daily activity) were pain (up to 17% of the test subjects versus 32% of the controls), fatigue (up to 12% of the test subjects versus 8% of the controls), and headache (up to 4% of any group). Swelling graded level 3 (greater than 50 mm) occurred in up to 4% of the test subjects versus 8% of the controls. The immune responses ranged from 5% to 36% across experimental vaccines for the L3 H44-76 strain (versus 27% for the control), from 0% to 11% for the L3 NZ98/124 strain (versus 23% for the control), and from 0% to 13% for the L2 760676 strain (versus 59% for the control). All geometric mean titers were below those measured with the control vaccine. The five experimental formulations were safe and well tolerated but tended to be less immunogenic than the control vaccine.

MATERIALS AND METHODS

Subjects and ethical aspects. Male and female young adults 18 to 25 years of age were recruited in Argentina (Centro de Educación Médica e Investigaciones Clínicas, Buenos Aires) after giving written informed consent. Subjects had to have completed routine childhood vaccinations (to the best of their knowledge) and to be free from obvious health problems. They were not admitted to the study if they had a history of or known exposure to meningococcal serogroup B...
disease or were previously immunized with meningococcal serogroup B vaccine. Any abnormal laboratory test value at screening, chronic drug administration, or planned administration of a vaccine not foreseen in the study protocol was a reason for nonenrollment. Acute disease or a temperature of ≥37.5°C at a vaccination visit resulted in postponement of vaccination or withdrawal from the study. Female subjects with childbearing potential had to agree to avoid pregnancy (by adequate contraceptive measures) and to have a negative pregnancy test before each vaccination.

This research was approved by the study center ethics committee and performed in compliance with the October 2000 version of the Declaration of Helsinki, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use—Good Clinical Practice, and relevant institutional policies regarding the protection of human subjects. The clinical phase was carried out between April 2006 and June 2007.

**Vaccine composition.** To increase the yield of the LOS present in the OMVs during the extraction phase, an optimized process was developed using 0.1% Na-deoxycholate. However, high levels of LOS are not acceptable for human vaccination due to the toxicity of the lipid A moiety. To circumvent that toxicity, we developed recombinant N. meningitidis strains harboring a detoxified lipid A by inactivating the msbB gene (Fig. 1) (39, 40).

Two genes, coding for the immunodominant and variable PorA and FrpB proteins, have been deleted to improve cross-protective immunogenicity induced by minor outer membrane proteins (39, 40). We overexpressed the minor proteins Hsf and TbpA by genetic manipulation (40, 41) and addition of an iron chelator to the culture medium.

Furthermore, to avoid the theoretical risk of autoimmunity, as human red blood cells and wild-type MenB LOS have the same lacto-N-neotetraose [LNnT] epitope (23), the tetrasaccharidic chain of the LOS has been genetically modified (40, 41) and addition of an iron chelator to the culture medium.

**Vaccination visit.** The immunogenicity analysis was performed for the according-to-protocol (ATP) cohort (defined as all eligible subjects who received all three doses of vaccine in accordance with the protocol and for whom pre- and postvaccination results of at least one bacterial assay were available) and for the total vaccinated cohort, as more than 5% of the subjects in most of the groups were eliminated from the ATP cohort for life-threatening congenital anomaly, or an important medical condition) occurring from the first vaccine dose up to 1 month after the full vaccination course.

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**Safety monitoring.** All adverse events were recorded by the investigators according to clinical judgment. Adverse events were scored on a three-grade intensity scale. Grade 3 was assigned to symptoms preventing normal activity, to redness/swelling at the injection site >50 mm in diameter, and to a fever of >39.5°C (axillary route). All serious adverse events (SAEs; defined as hospitalization, disability, death, a life-threatening congenital anomaly, or an important medical condition) occurring from the first vaccine dose up to 1 month after the full vaccination course were also recorded. Pregnancies were recorded as SAEs and followed up to delivery.

**Statistical analysis.** This phase I study was set up to get a preliminary safety assessment of a new MenB vaccine. A sample size of 25 subjects per group was selected to detect an increase in the percentage of subjects with grade 3 symptoms (solicited or unsolicited) following vaccination from 20% in the control group to 61% in a MenB group with 80% power (two-sided alpha = 5% unadjusted for multiple comparison). The safety and reactogenicity analyses were performed for the total vaccinated cohort. The incidence of solicited (local and general) symptoms within 4 and 14 days after each vaccination and unsolicited symptoms within 30 days after each vaccination was calculated along with the 95% CI. The occurrence of grade 3 symptoms within 4 and 14 days postvaccination was also calculated for each vaccine group along with the 95% CI.

The immunogenicity analysis was performed for the according-to-protocol (ATP) cohort (defined as all eligible subjects who received all three doses of vaccine in accordance with their random assignments and in compliance with the protocol and for whom pre- and postvaccination results of at least one bacterial assay were available) and for the total vaccinated cohort, as more than 5% of the subjects in most of the groups were eliminated from the ATP cohort for immunogenicity. The percentages of subjects with SBA-MenB titers of ≥1:2 and ≥1:4 and geometric mean titers (GMTs) of three antibodies (L3 H44-76, L3 NZ98/124, and L2 760676) were determined for each vaccine group along with the 95% CIs. The percentage of SBA-MenB responders in each vaccine group was also computed; vaccine response was defined as a postvaccination titer of ≥1:8 for subjects with a prevaccination titer of <1:2 and as a ≥4-fold increase over the prevaccination titer for initially seropositive subjects.

Antibody titers below the assay cutoff were given an arbitrary value of half the cutoff for the purpose of GMT calculation. Calculation of GMTs was performed by taking the antilog of the mean of the log_{10} concentration or titer transformation. Data analysis was performed at GSK Biologicals using SAS software (version 8.2) and StatXact (version 5.0); all analyses were descriptive only.
RESULTS

A total of 150 subjects 18 to 26 years old were enrolled and vaccinated with either the experimental or the control vaccine. Twenty subjects withdrew from the study for the reasons provided in Fig. 2, and 13 additional subjects were also excluded from the ATP analysis of immunogenicity. The demographic profiles of all of the groups were similar (Table 1). As the immunogenicity results obtained from the ATP cohort were consistent with those from the total vaccinated cohort, only data from the ATP cohort are presented here.

Safety and reactogenicity. A total of six SAEs were reported (one in each study group), none of them considered by the investigator to be related to vaccination; all except one (pregnancy leading to stillbirth) were resolved without sequelae. The administration of each formulation was comparable with regard to local and general solicited symptoms (Table 2). The vast majority of symptoms occurred in the first 4 days of the follow-up period. Pain at the injection site, fatigue, and headache were the most commonly reported solicited symptoms. Rash occurred, at most, after 3 (4%) doses of the experimental vaccine formulations (in three subjects) and after 3 (5%) doses of the control vaccine (in 3 subjects); there were no reports of petechiae or purpura.

Grade 3 solicited adverse events reported during the 14-day follow-up period were uncommon and within the same range across all of the groups, except for pain, which tended to occur less frequently after the experimental formulations (after 1 to 2% of the TrL3 doses [in one subject] and after 6% of the L7 doses).

FIG. 2. Study flow chart. One hundred fifty subjects were randomized among five experimental formulations and a placebo. Group TrL3-4a (n = 25), TrL3 with 4 μg LOS (–25 μg protein), adsorbed formulation; group TrL3-8a (n = 25), TrL3 with 8 μg LOS (–50 μg protein), adsorbed formulation; group TrL3-8n (n = 25), TrL3 with 8 μg LOS (–50 μg protein), nonadsorbed formulation; group L7-8a (n = 25), L7 with 8 μg LOS (–50 μg protein), nonadsorbed formulation; control group VAMen (n = 25), licensed VA-Mengoc-BC. AE, adverse event; EX, excluded from the analysis; ICFW, informed consent form withdrawn; LFUC, lost to follow-up with complete vaccination; LFUI, lost to follow-up with incomplete vaccination; MV, moved; PV, protocol violation. +, one informed consent form, signed by the subject’s sister instead of the mother, led to the subject’s withdrawal from the study, and the subject was replaced.

TABLE 1. Demographics of vaccinated subjects in this study

| Characteristic     | TrL3-4a | TrL3-8a | TrL3-8n | L7-8a | L7-8n | VAMen |
|--------------------|---------|---------|---------|-------|-------|-------|
| Total no. vaccinated | 25      | 25      | 25      | 25    | 25    | 25    |
| Mean age, yr (SD)  | 21.6 (2.3) | 22.5 (2.0) | 21.8 (2.3) | 21.1 (2.6) | 22.2 (2.5) | 21.9 (2.2) |
| Female-to-male ratio | 19:6    | 12:13   | 7:18    | 15:10 | 11:14 | 13:12 |

*a* TrL3-4a, TrL3 with 4 μg LOS, adsorbed formulation; TrL3-8a, TrL3 with 8 μg LOS, adsorbed formulation; TrL3-8n, TrL3 with 8 μg LOS, nonadsorbed formulation; L7-8a, L7 with 8 μg LOS, adsorbed formulation; L7-8n, L7 with 8 μg LOS, nonadsorbed formulation; VAMen, licensed VA-Mengoc-BC (control group).
doses [in up to four subjects]) than after the control vaccine doses (after 21% of the doses [in eight subjects]) (Table 2). A fever of ≥39.5°C was only observed in one subject after two of the three doses of VA-Mengoc-BC in the control group.

Laboratory test values were within the acceptable clinical range for all subjects. Direct Coombs tests were positive (1+ over a 3+ scale) for three subjects (by gel test, but results were negative when the classical tube method was used for confirmation). Those positive gel test results were considered probably false positive for one subject in the control group, as a repeat test done on the same day was negative. The two other subjects were in the TrL3-8n group. One subject turned positive on the basis of the blood sample collected the day of the second vaccine dose (i.e., 6 weeks after the first dose) and returned to normal on the basis of a subsequent blood sample collected 30 days later; a full investigation could not identify any etiology. The second subject presented a positive direct Coombs test that was considered probably related to antibiotics received to treat an earlier SAE (pneumonia). Both subjects were withdrawn from further vaccination.

**Immunogenicity.** Prior to vaccination, the percentages of subjects with SBA-MenB titers of ≥1:4 against the three target strains were high: 94 to 100% for L3 H44-76, 100% for L3 NZ98/124, and from 0 to 13% for the L2 760676 strain. However, none of the five experimental formulations showed acceptable immunogenicity against any of the three strains. Approximately half of the subjects in the experimental groups and all except one subject in the control group had a titer of at least 1:4 against the L2 760676 strain. However, none of the five experimental formulations showed acceptable immunogenicity against any of the three strains tested, as GMTs did not increase significantly from pre- to postvaccination and all GMTs were below those obtained with the control vaccine (Table 3). Notably, we observed substantial immunogenicity against the L2 760676 strain with the control vaccine.

The vaccine response (defined as a 4-fold increase in the SBA-MenB titer) 1 month after the last injection ranged in the experimental groups and all except one subject in the control group had a titer of at least 1:4 against the L2 760676 strain. However, none of the five experimental formulations showed acceptable immunogenicity against any of the three strains tested, as GMTs did not increase significantly from pre- to postvaccination and all GMTs were below those obtained with the control vaccine (Table 3). Notably, we observed substantial immunogenicity against the L2 760676 strain with the control vaccine.

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TABLE 3. SBA GMTs by target strain

| Strain and time | TrL3-4a | TrL3-8a | TrL-8n | L7-8a | L7-8n | VAMen |
|----------------|---------|---------|--------|-------|-------|-------|
| Pre 20         | 12.4 (7.8–19.7) | 17 | 11.6 (6.8–19.9) | 15 | 15.8 (9.2–26.9) | 23 | 14.9 (9.9–22.4) | 19 | 17.5 (9.5–32.3) | 18 | 18.4 (10.1–33.5) |
| Post II 21     | 18.2 (13.1–25.3) | 18 | 17.8 (12.8–24.8) | 18 | 26.3 (17.0–40.8) | 23 | 27.4 (17.7–42.4) | 19 | 36.4 (18.6–64.3) | 17 | 49.1 (24.7–97.7) |
| Post III 23.6   | 23.6 (15.5–35.8) | 18 | 22.7 (16.1–32.0) | 16 | 35.6 (19.9–63.8) | 23 | 37.9 (23.9–60.1) | 19 | 52.9 (27.5–101.6) | 15 | 92.7 (37.8–227.6) |

NZ98/124

| Pre 20 | 46.1 (30.4–69.9) | 17 | 71.6 (45.2–113.2) | 18 | 59.6 (39.6–89.6) | 22 | 47.2 (30.1–74.0) | 18 | 60.3 (35.6–102.0) | 17 | 79.7 (46.8–135.9) |
| Post II 20 | 61.0 (40.8–91.2) | 18 | 78.2 (54.1–113.1) | 18 | 66.2 (47.1–93.1) | 23 | 54.3 (39.9–75.9) | 19 | 61.3 (42.0–89.6) | 16 | 113.9 (67.0–193.6) |
| Post III 21 | 53.2 (35.9–78.9) | 17 | 95.9 (69.3–132.6) | 16 | 67.1 (42.8–105.2) | 23 | 88.7 (60.7–129.0) | 19 | 83.0 (51.3–134.2) | 14 | 140.7 (62.4–317.3) |

760676

| Pre 20 | 3.5 (1.9–6.4) | 18 | 7.8 (3.2–19.2) | 16 | 2.9 (1.2–6.6) | 22 | 4.8 (2.5–9.4) | 18 | 2.8 (1.4–5.6) | 17 | 5.1 (2.4–10.8) |
| Post II 20 | 3.1 (1.8–5.5) | 18 | 6.2 (2.5–15.1) | 17 | 1.5 (0.9–2.5) | 23 | 5.2 (2.4–11.2) | 19 | 3.9 (1.9–8.0) | 18 | 19.4 (10.9–34.5) |
| Post III 21 | 3.6 (2.2–5.9) | 17 | 8.8 (3.6–21.4) | 15 | 3.0 (1.4–6.4) | 23 | 6.2 (3.0–12.9) | 19 | 4.2 (2.2–7.8) | 17 | 35.1 (19.9–61.9) |

a For study group compositions, see Table 1.
b Number of subjects whose antibody titers were available.
c Pre, prior to vaccination; Post II, 1 month after second vaccination; Post III, 1 month after third vaccination.

DISCUSSION

This study assessed five formulations of a MenB experimental vaccine expressing non-PorA, non-FrpB proteins and L3-derived LOS. Although safety and reactogenicity were acceptable, none of the experimental formulations induced sufficient immunogenicity to deserve further development.

Rather unexpected and in contrast to preclinical findings, where the experimental L3.7 formulations induced good immunogenicity in mice against the majority of L3,7 MenB strains (39), those results led us to believe that murine Toll-like receptor 4 (TLR4) and human TLR4 act differently. This is akin to recent findings reported by Steeghs et al. (32), who suggested that the msbB lipid A-LOS is a TLR4 agonist in mice but may act as a TLR4 antagonist in humans. In our study, the msbB mutation curtailed immunogenicity in humans, which was not the case in mice. An ongoing clinical study performed by the Walter Reed Army Institute of Research using detoxified OMV (also based on an msbB knockout strategy) (41) should allow us to confirm or not confirm the low adjuvant effect of msbB LOS on human TLR4. Moreover, the slender immunogenicity profile could also result from the small amount or the absence of aluminum salt used in the experimental formulations, even if preclinical experiments demonstrated that nonadsorbed OMVs display an immunogenicity profile similar to that of adsorbed OMVs.

We observed prevaccination SBA-MenB titers (≥1:4 in more than 94% of our test subjects for the L3 strains and in 31 to 61% of our test subjects for the L2 strain) higher than those reported previously in United Kingdom adults (≥1:4 in more than 45% of the test subjects for L3 H44-76) (13) or in Belgian and Spanish adolescents (≥1:4 in 23 to 33% of the test subjects for L3 H44-76) (2). Our study was conducted with young adults, and it is well known that seropositivity spontaneously increases with age (12). However, high variability in SBA-MenB according to the complement sources and strains is not uncommon and was described by Findlow et al. (10), and differences between laboratories cannot be ruled out (1, 2). Another plausible explanation for the high prevaccination seropositivity seen could be the residual immunity conferred by MenB strains circulating in Argentina (6). Whatever the case, this pleads for relying on more than one type of assay to assess the predictive value of vaccine effectiveness (10, 38).

One limitation of our study was the exclusive use of SBA, particularly as it is a less sensitive measure than clinical efficacy, which can be achieved even with SBA titers of <1:4 (26, 38). Other mechanisms, such as opsonophagocytosis, are probably involved in clinical protection, as antibodies can be protective without being bactericidal (2, 26, 36). Moreover, Welsch and Granoff have reported bactericidal activity induced by whole blood in SBA-negative subjects, for some of them independently from white blood cells (14, 38).

Considering the very low immunogenicity observed with the experimental vaccines based on modified LNnT and in line with preclinical results (40), we can still hypothesize that the removal of the sialyl groups (in the TrL3 and L7 formulations) and the fourth sugar (in the TrL3 formulations) did not impact the antigenicity of the LOS molecule and the ability of induced antibodies to mediate the killing of L3 MenB strains.

A potential concern was the theoretical risk of induction of autoimmunity, due to the presence of the LNnT epitope on antibodies to mediate the killing of L3 MenB strains.

TABLE 4. Vaccine responses by target strain

| Strain | TrL3-4a | TrL3-8a | TrL-8n | L7-8a | L7-8n | VAMen |
|--------|---------|---------|--------|-------|-------|-------|
| H44-76 | 20.5 (0.1–24.9) | 17.17 | 17.6 (3.8–43.4) | 13 | 30.8 (9.1–61.4) | 22 | 36.4 (17.2–59.3) | 19 | 31.6 (12.6–56.6) | 15 | 26.7 (7.8–55.1) |
| NZ98/124 | 20.5 (0.1–24.9) | 15 | 0.0 (0.0–21.8) | 16 | 0.0 (0.0–20.6) | 22 | 9.1 (1.1–29.2) | 18 | 11.1 (1.4–34.7) | 13 | 23.1 (5.0–53.8) |
| 760676 | 20.0 (0.0–16.8) | 17 | 5.9 (0.1–28.7) | 15 | 13.3 (1.7–40.5) | 22 | 4.5 (0.1–22.8) | 18 | 5.6 (0.1–27.3) | 17 | 58.8 (32.0–81.6) |

a The vaccine response was defined as a ≥4-fold increase from pre- to postvaccination 1 month after the third injection. For study group compositions, see Table 1.
both MenB OMV and human erythrocytes. Although the experimental vaccine was genetically modified to prevent the expression of this LNnT epitope, Coombs tests (with a gel test as the primary test) were performed throughout this study as a precautionary measure. Three subjects had positive direct Coombs test results by this method, but none of them were confirmed by the classical tube method. There was a plausible etiology other than the vaccine for two subjects, but a possible relationship with vaccination could not be ruled out for one subject who was administered a TrL3 formulation, as no other plausible cause could be identified. Owing to the low immunogenicity of the vaccine, it is, however, highly improbable that the slightly positive gel test was induced by vaccine antibodies.

The Cuban VA-MenC-BC vaccine was chosen as a control since it was proven effective in subjects ≥4 years old, including against meningococcal B strains that are PorA heterologous to the vaccine (7, 24). In our study, VA-MenC-BC induced a weaker immune response (27%) than those observed in Iceland (44%) (26) and Chile (56%) (34) to the H44-76 strain, and reasonable explanations may be the high levels of preexisting antibodies in our study and differences in the assays or study populations used. Although the Cuban vaccine is made of OMV of the L3 immunotype, the response to the heterologous L2 strain (760676) was high in our study (59%); the absence of cross-reactivity between the L2 and L3 immunotypes (40) suggests that this cross-protective response is mediated by bactericidal antibodies directed against minor outer membrane proteins still to be identified, as the Cuban strain and the 760676 strain do not have homologous PorA and FrpB proteins.

The experimental vaccine has an acceptable safety profile, with fewer grade 3 symptoms (especially pain and signs of toxicity) than the control, supporting the benefit of LOS detoxification for the experimental formulations. The lower reactivity observed could also be the consequence of reduced aluminum content in the experimental vaccine or a combination of both factors.

In conclusion, the five experimental formulations were safe and well tolerated but tended to be less immunogenic than the control vaccine; hence, further clinical development of these candidate vaccines was stopped.

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