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Safety and immunogenicity testing of an intranasal group B meningococcal native outer membrane vesicle vaccine in healthy volunteers☆

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Abstract

An intranasal vaccine composed of native outer membrane vesicles (NOMV) not exposed to detergent or denaturing agents was prepared from the group B meningococcal strain 9162 SynX(-)(:15:P1.3:P5.10,11:L3,7,9) and tested in 32 healthy adult volunteers. Four groups of 8 volunteers were vaccinated intranasally with three doses of vaccine. The vaccine was very well tolerated in all dosing groups, despite the presence of lipo-oligosaccharide in the vaccine at a level of 25% relative to protein. The antibody response as measured by ELISA in serum, saliva and nasal wash fluids was relatively low in all 4 groups, but the induced serum antibodies had strong bactericidal activity. Persistent bactericidal antibodies (≥4-fold increase) were produced in 75% of the recipients. Some of the bactericidal antibodies were cross reactive against divergent group B strains. Most of the bactericidal antibodies appeared to be specific for PorA and L3,7,9 LOS. The vaccine also produced a local antibody response which was detected in the nasal wash fluids of volunteers. These data suggest that nasal immunization with NOMV is a safe and effective approach to induce systemic and local immunity against the group B meningococcus and deserves further study. Published by Elsevier Science Ltd.

Keywords: Meningococcus; *Neisseria meningitidis*; Vaccine; Intranasal; Immunogenicity; Mucosal

1. Introduction

Meningococcal meningitis continues to pose a worldwide threat both in endemic and epidemic form. The currently licensed vaccines for meningococcal disease do not induce immunity to Group B meningococci which currently account for nearly one half of cases in many countries including the Americas and Europe [1]. The emergence of a virulent clone of Group B *Neisseria meningitidis*, know as ET5, from Norway in the mid-1970's has since been responsible for prolonged epidemics in Norway, Chile, Cuba and Brazil [2]. A second epidemic group B clone (lineage 3) that first appeared in the Netherlands [3] is now common in England and Wales [4] and is responsible for an epidemic in New Zealand [5].

Clearly, an effective group B vaccine is needed. Obstacles that have delayed the development of an effective vaccine to group B meningococci include: (a) the molecular mimicry exhibited by the B capsule and the dominant lipo-oligosaccharide (LOS) immunotype; (b) the antigenic variation and phase variation exhibited by many of the outer membrane proteins (OMPs) and the LOS; (c) the lack of a predictive animal model in which to test candidate vaccines and (d) the difficulty in removing or detoxifying the tightly bound LOS (endotoxin) without impairing the immunogenicity of the OMPs [6].

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Parenteral meningococcal Group B vaccines based on the OMPs have been shown to be 50 to 80% effective in several phase III trials conducted in Cuba, Norway and Brazil [7–10]. A similar Group B OMP–Group C polysaccharide vaccine was tested in a hyperendemic focus in Iquique, Chile in 1987–1990 [11]. An efficacy of 51% was noted ($p = 0.1$) overall and 70% in children over the age of 4 ($p = 0.045$). There was no protection demonstrated in children under the age of 4 who are at most risk for serious meningococcal disease. Interestingly, it was observed that young children had geometric mean IgG antibody responses by EIA that surpassed those of older individuals, but most of these antibodies were not bactericidal. It has been suggested that the OMP needs to be presented in a lipid environment to avoid exposure of normally unexposed and irrelevant epitopes. Such epitopes can induce antibodies that lack protective capability and may down-regulate the induction of protective bactericidal antibodies [11]. Clearly, a better means of presenting the OMP and other potentially protective epitopes is required to make a more effective vaccine for the target population [12].

Meningococci induce disease only after first colonizing the nasopharynx [13]. Induction of an immunologic response at the mucosal surface would be an attractive approach to interdict infection. To mimic the natural colonization of the nasopharynx by the intact organism, we prepared an intranasal vaccine based on native outer membrane vesicles (NOMV), which we delivered to the nasopharynx as a spray via an atomizer. Meningococci have been shown to bleb off pieces of their outer membrane which spontaneously form vesicles called native outer membrane complex [14] or NOMV. The blebs are representative of the intact outer membrane of the meningococci that elaborate them. Equivalent vesicles can be obtained from pelleted cells using mild methods that do not involve the use of detergent or denaturing solvents. The NOMV may contain multiple antigens capable of inducing bactericidal antibodies, for example, PorA, LOS, Opa, Opc and TbpB (if the cells are grown on limiting iron) [14,15]. Although NOMV, which contain LOS at the same concentration as in the intact outer membrane, would be too toxic to use as a parenteral vaccine, we have shown that they can be safely used as a mucosal vaccine and induce a high quality antibody response.

2. Materials and methods

2.1. Protocol development

The protocol was developed by the investigators and took place in the Clinical Trials section of the Walter Reed Army Institute of Research (WRAIR), a licensed facility which follows Good Clinical Practices. The study was reviewed and approved by local institutional review boards and the United States Food and Drug Administration (BB-IND 6993).

2.2. Volunteer characteristics

Healthy male and female volunteers (military or civilian), ages 18 to 50, were sought out non-coercively from the Washington, DC area. Exclusion criteria included: (a) history of severe organ/system disease; (b) history of allergy to any vaccine; (c) history of allergic rhinitis or chronic sinusitis; (d) presence of clinically significant abnormalities on screening laboratory tests; (e) fever (>38°C) or upper respiratory tract infection on the day of immunization; (f) use of nasal medications on a regular basis; (g) HIV seropositivity; (h) history of meningococcal disease or receipt of any vaccine containing meningococcal OMPs; (i) bactericidal antibody titer to the vaccine strain >1:8 on initial screening; (j) nasopharyngeal carriage of meningococcus on initial screening and (k) pregnancy

2.3. Vaccine production and description

The clinical grade lot (#0123) of NOMV vaccine used in this study was produced under cGMP at the Walter Reed Army Institute of Research in the Forest Glen Pilot Vaccine Production Facility in March 1995. The strain used for vaccine production was 9162 synX(-)(–:15:P1.3:P5.10,11:L3,7,9), which was derived by deletion of the *synX* (also called *siaA*) gene from a case isolate of group B *Neisseria meningitidis* isolated in Iquique, Chile [11,16]. The basic method of preparing NOMV (called OMC in the reference) has been described previously [17,18]. Briefly, liquid phase cultures of the parent organism were grown on low iron medium in a 300 l fermenter and the culture inactivated with 0.5% phenol. The cells were collected by centrifugation and stored frozen until processed. Cells were thawed, suspended in Tris-buffered saline with EDTA at pH 7.5 and warmed to 56°C for 30 min. The suspension was sheared in a Waring blender for 3 min and the cells pelleted by centrifugation. NOMV was collected from the supernatant by ultracentrifugation, washed once with water by recentrifugation and sterile filtered. The ratio of protein to LOS in the NOMV was about 4:1 w/w. The vaccine was stored at 5°C and was stable for at least 2 years. The lot passed sterility and general safety tests and was nonpyrogenic in rabbits at 0.05 μg in the standard intravenous test and at >400 μg when administered intranasally.

The doses chosen for this study were based on preclinical experience which suggested that about 10-fold more vaccine was required with intranasal immuniz-

ation as compared to parenteral immunization. The dosing was also based on the results of a Norwegian group B vaccine given intranasally [19].

2.4. Study design and immunizations

A total of 32 healthy volunteers were enrolled in the study after informed consent was obtained. The volunteers were randomly allocated into 4 groups of 8 and immunized intranasally with three doses of either 160 μg of vaccine (groups 1 and 2) or 416 μg of vaccine (groups 3 and 4). Groups 1 and 3 received the vaccine at weeks 0, 2 and 4; and groups 2 and 4 received the vaccine at weeks 0, 4 and 8.

The vaccine was delivered by means of commercially manufactured metered pumps (Valois of America, Greenwich, CT) that deliver fixed volumes of fluid (100 or 130 μl per spray) as an atomized spray. The spray was delivered by a physician into each nostril while the volunteer sniffed strongly and occluded the other nostril. To document the accuracy of vaccine delivery, the containers were weighed before and after vaccine delivery for every vaccination.

2.5. Specimen acquisition

Volunteers had phlebotomy performed at entry and on days 0, 14, 28, 42, 56, 70 and 98. Sera were stored frozen for batch testing at the conclusion of the study. Nasopharyngeal swabs (in duplicate) to detect meningococcal colonization were performed with each phlebotomy. Saliva collection was also performed with each phlebotomy and done using a commercially available device (Salivette, Sarstedt, Newton, NC) according to the manufacturer's directions. The saliva was collected from the chewable swabs by centrifugation and immediately frozen for storage and later batch testing. Nasal washes for antibody determinations were performed on entry and on days 42 and 98 for groups 1 and 3 and days 70 and 98 for groups 2 and 4. Nasal washes were performed by having the volunteer hyperextend the neck. With the glottis closed and the head tilted back, 5 ml of sterile saline was instilled into each nostril and held for 10 s. The head was then tilted forward and the saline expelled through the nares into a clean container. The wash fluids were then immediately frozen and stored for batch testing. Protocol allowance was ±3 days for all specimen acquisition.

2.6. Safety and reactogenicity

Reactogenicity to immunization was monitored for an hour after each immunization and at 24 and 48 h following each immunization by interview, questionnaire, physical examination and a temperature recording. Volunteers were instructed to report any symptom that they may experience on a separate symptom diary and were provided with disposable thermometers. Reactions were graded according to severity: 0 = not present, 1 = barely noticeable (minimal), 2 = noticeable but does not impair normal activities (mild), 3 = severe enough to impair normal activities (moderate) and 4 = life or health threatening (severe).

2.7. Antibody determinations in serum, saliva and nasal wash

IgA, IgG and IgM antibodies to homologous NOMV or to purified L3,7,9 LOS were determined by quantitative ELISA as previously described [20,21]. Prior to assay, the nasal washes were concentrated about 10-fold using Amicon Centriplus-50 Concentrators (Amicon, Beverly, MA). ELISA values for the concentration of specific antibody of a given isotype in saliva and nasal wash specimens were normalized by dividing the amount of specific antibody by the concentration of total Ig of that isotype in the sample. The amount of total IgG and secretory IgA in saliva and nasal washes was measured using a radial immunodiffusion kit according to the manufacturer's instructions (NANORID and BIND A RID Kits for human IgG and secretory IgA, The Binding Site, Birmingham, UK). Mucosal antibody levels were expressed as the ratio of specific to total IgA or IgG.

2.8. Bactericidal activity in serum

Bactericidal assays were performed as previously described [22] using a pool (3–5 individuals) of fresh frozen normal human serum lacking bactericidal activity against the test strain as a source of complement. The test strains used in the bactericidal assay included the parent of the vaccine strain, which is a case isolate from Chile, [9162(B:15:P1.7^{h},3:P5.10,11:L3,7,9)]; isogenic PorA positive and negative variants of a closely related case strain from the Chilean epidemic [8532(B:15:P1.7^{h},3:P5.5,10:L3,7,8,9) and 8532(B15:P1.–:P5.5,10:L3,7,8,9)]; a case isolate from the epidemic in Norway [44/76(B:15:P7,16:P5.5,c:L3,7,9)]; and a case isolate from Miami that is similar to the Cuban epidemic strain [8566(B:4:P1.19,15:L3,7,9)].

2.9. Absorption of bactericidal antibodies

A microabsorption procedure was used to study the specificity of bactericidal antibodies. Wells of a 96-well EIA microplate were coated with 2-fold serial dilutions of LOS or NOMV in the range of 0.2 to 100 μg/ml. The antigens were serially diluted in Dulbecco's phosphate buffered saline and 100 μl volumes of the dilutions incubated in the wells overnight at room

Table 1
Summary of NOMV Vaccine reactogenicity in human phase I study[a]

Symptom all groups ($N = 32$)	< 1 h			< 1 h		
	dose 1	dose 2	dose 3	dose 1	dose 2	Dose 3
Fever						
Tiredness		1 (2)	2 (1)	6 (1.5)	5 (1.6)	4 (1.5)
Achiness			1 (1)	3 (1.33)	2 (1.67)	3 (1.33)
Headache		1 (1)	1 (1)	11 (1.36)	4 (1.5)	5 (1.6)
Nasal discharge		1 (1)		5 (1.6)	3 (1.67)	2 (1.5)
Nasal Stuffiness	1 (1)			8 (1.5)	5 (1.4)	2 (1)
Nasal burning				1 (1)		
Nasal itch/tingle	1 (1)	1 (1)		2 (1)		
Sore throat	1 (1)	1 (1)		2 (1)	2 (1.5)	3 (1.67)
Postnasal drip	4 (1.25)	2 (1)		6 (1.17)	4 (1.25)	
Cough					4 (1.5)	3 (1)
Nose bleeding				2 (1)	1 (1)	1 (1)
Numbness	1					
Lightheadedness	1 (1)	3 (1.33)				
Sinus stinging			1 (1)			
Sinus dryness	1 (1)			1 (1)		1 (1)
Ear pain/clog				1 (1)		2 (1.5)
Sneezing			1 (1)	1 (1)		1 (1)
Itching eyes					2 (1)	

[a] Data are the number of volunteers who reported the indicated symptom with the mean grade of the symptom in parentheses. Reactions were graded on a scale of 0 to 4. Grading scale: 0 = not present, 1 = barely noticed, 2 = noticed more constantly, but did not impair normal activities, 3 = severe enough to impair normal activities, 4 = life or health threatening. All reactions were grade 1 or 2 except one grade 3 reaction (tiredness) reported by a single individual.

temperature. The solutions were removed and the wells were washed once with Gey's Balanced Salt Solution with 0.2% gelatin (GBSS/G), which is the buffer used in the bactericidal assay, and then incubated with 100 μl of the same buffer for 30 min at room temperature. The solution was removed and 60 μl of human serum, diluted to a point that would be expected to kill about 60–70% of the organisms (based on previous assays with the serum), was placed in each well corresponding to the antigen dilution series. The diluted sera were incubated in the antigen-coated wells for 4 h at room temperature. Following this incubation, 50 μl of the serum dilution from each well was transferred to a corresponding well in a bactericidal test plate. Organisms (about 2000 in 25 μl of GBSS/G) and then complement (25 μl) were added to the wells and the assay completed according to the standard procedure for the bactericidal test [22]. Purified LOS used as an absorbing antigen in the test was first complexed noncovalently to an equal weight of bovine serum albumin. This was done by combining equal amounts of bovine serum albumin and LOS in a detergent solution (1% Empigen BB, 0.15 N NaCl, 0.05 M Tris–HCl, 0.01 M EDTA, pH 8.0) at about 1 mg/ml of LOS and co-precipitating the LOS and albumin with 4 volumes of absolute ethanol. The precipitate was recovered by centrifugation and washed once with ethanol. The final pellet was suspended in about the original volume of normal saline.

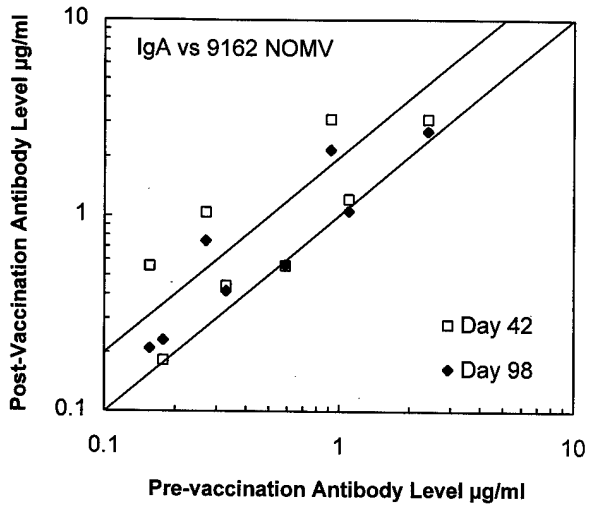
2.10. Statistical analysis

Data computation, including determination of geometric means and standard error of the mean, were done using the Microsoft Excel spreadsheet. Tests for statistical significance of post vaccination versus pre-vaccination ELISA antibody levels were done on log-transformed data with the Student *t*-test for paired samples and also with the Wilcoxon Matched-Pairs Signed-Ranks Test on untransformed data. The two analyses agreed except for one data set. The Bonferoni Adjustment was applied where multiple comparisons were made.

3. Results

3.1. Accuracy of vaccine delivery, safety and reactogenicity

The study was completed without incident. Forty-eight potential volunteers were screened. Twelve were excluded for possessing one or more exclusion criteria: (7 had high baseline titers to the vaccine strain, 1 had received another experimental meningococcal vaccine, 3 were excluded for medical conditions discovered on screening). Three withdrew before immunizations began and one alternate volunteer was not required. All but two of the 32 enrolled volunteers completed



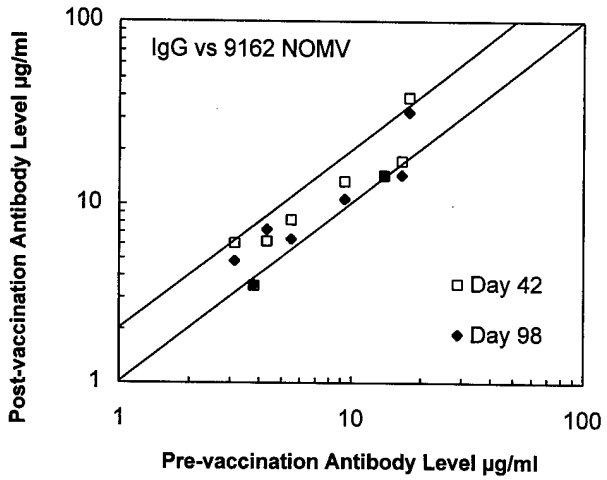
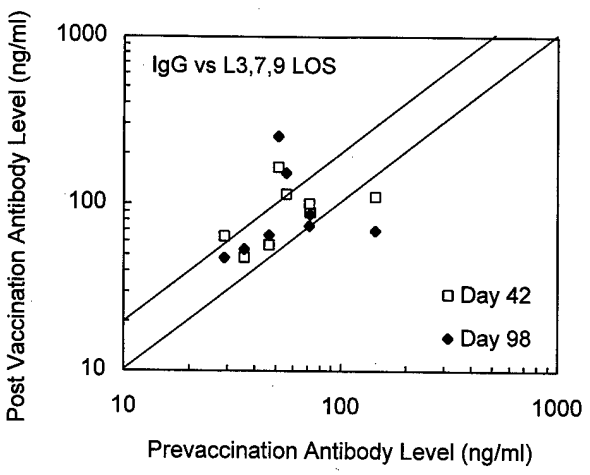


Fig. 1. Quantitative ELISA determination of specific serum IgA and IgG antibody responses of group 4 volunteers to homologous NOMV before and after intranasal vaccination with NOMV vaccine. Other groups showed little if any serum antibody response by ELISA.



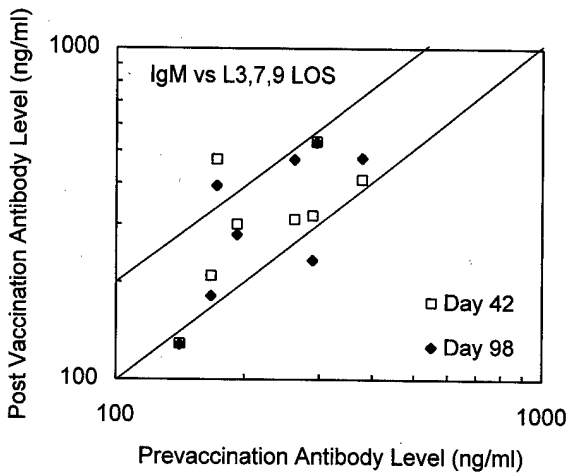


Fig. 2. Quantitative ELISA determination of specific serum IgG and IgM antibody responses of group 4 volunteers to homologous purified LOS before and after intranasal vaccination with NOMV vaccine. Other groups showed little if any serum antibody response to LOS by ELISA.

the study. These two had both received 2 of the 3 scheduled immunizations but withdrew due to unanticipated schedule conflicts requiring relocation, which precluded continuation. Nineteen women and 13 men participated; the mean (median) age was 32.3 [30] years with a range of 18 to 49. Compliance with immunizations and specimen acquisition was excellent. The metered nasal atomizer pumps were very accurate. There was less than 3% deviation of the actual delivered dose from the predicted dose.

The vaccine was well-tolerated. There were no grade 4 symptoms and only one report of a Grade 3 symptom (tiredness) at 24 h after the second immunization in one volunteer. The most common complaints were headache, tiredness, nasal stuffiness and post-nasal drip with an average grade between 1 and 2. There was no relation of reported symptoms to the group or particular immunization and no reproducibility was noted. Most volunteers reported no symptoms. No volunteer declined further immunizations including the 2 who withdrew for personal reasons. Reactogenicity data are summarized in Table 1. No volunteers were excluded for asymptomatic carriage of meningococci and none became colonized during the course of the study.

3.2. Induction of specific antibodies in serum, saliva and nasal wash

Antibody levels to homologous NOMV and to purified L3,7,9 LOS in serum, saliva and nasal wash fluids were determined by quantitative ELISA. The serum

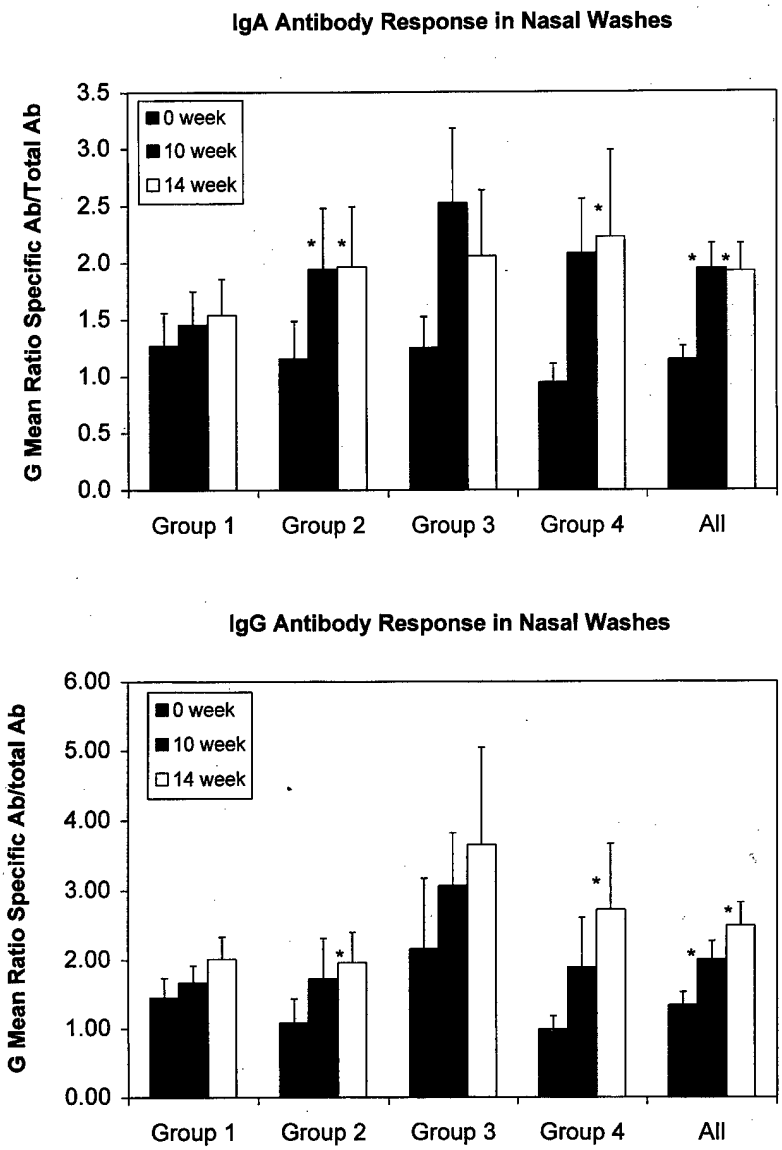


Fig. 3. Specific IgA and IgG antibodies to meningococcal 9162 NOMV in nasal washes of human volunteers vaccinated intranasally with NOMV vaccine. Bars represent the standard error of the mean. Asterisks indicate a statistically significant ($p < 0.05$) increase over the pre-vaccination levels. For assay the nasal washes were concentrated and normalized based on the total concentration of IgA or IgG in the sample.

antibody response as measured by ELISA was low or undetectable except in a few individuals. The volunteers in group 4 appeared to have the best response. In this group only 2 or 3 of the 8 volunteers had a 2-fold or greater increase in IgA or IgG antibodies to NOMV (Fig. 1). For group four, the mean 42 and 98 day post-vaccination IgA antibody levels were significantly higher ($p = 0.023$ and $p = 0.039$, respectively, Wilcoxon Matched-Pairs Signed-Ranks Test) than the pre-vaccination levels.

Increases in serum antibody to the purified, homologous LOS could also be detected for a few volunteers (Fig. 2), but the geometric mean pre- and post-vaccination levels for the entire groups were not significantly different.

The mucosal antibody response was evaluated by measurement of antibody levels in nasal wash fluids and in saliva. Analysis of nasal wash fluids, which were concentrated approximately 10-fold, was done by quantitative ELISA using homologous NOMV as antigen. Data were normalized by dividing the specific antibody concentration by the total concentration of immunoglobulin of the same isotype in the sample. The results for IgA and IgG are shown in Fig. 3. Asterisks indicate the post vaccination levels that are significantly higher than the pre-vaccination levels (Student t-test with the Bonferroni adjustment). Groups two and four showed significant increases and the mean increase for all groups combined was highly significant ($p < 0.001$). Very little change in normal-

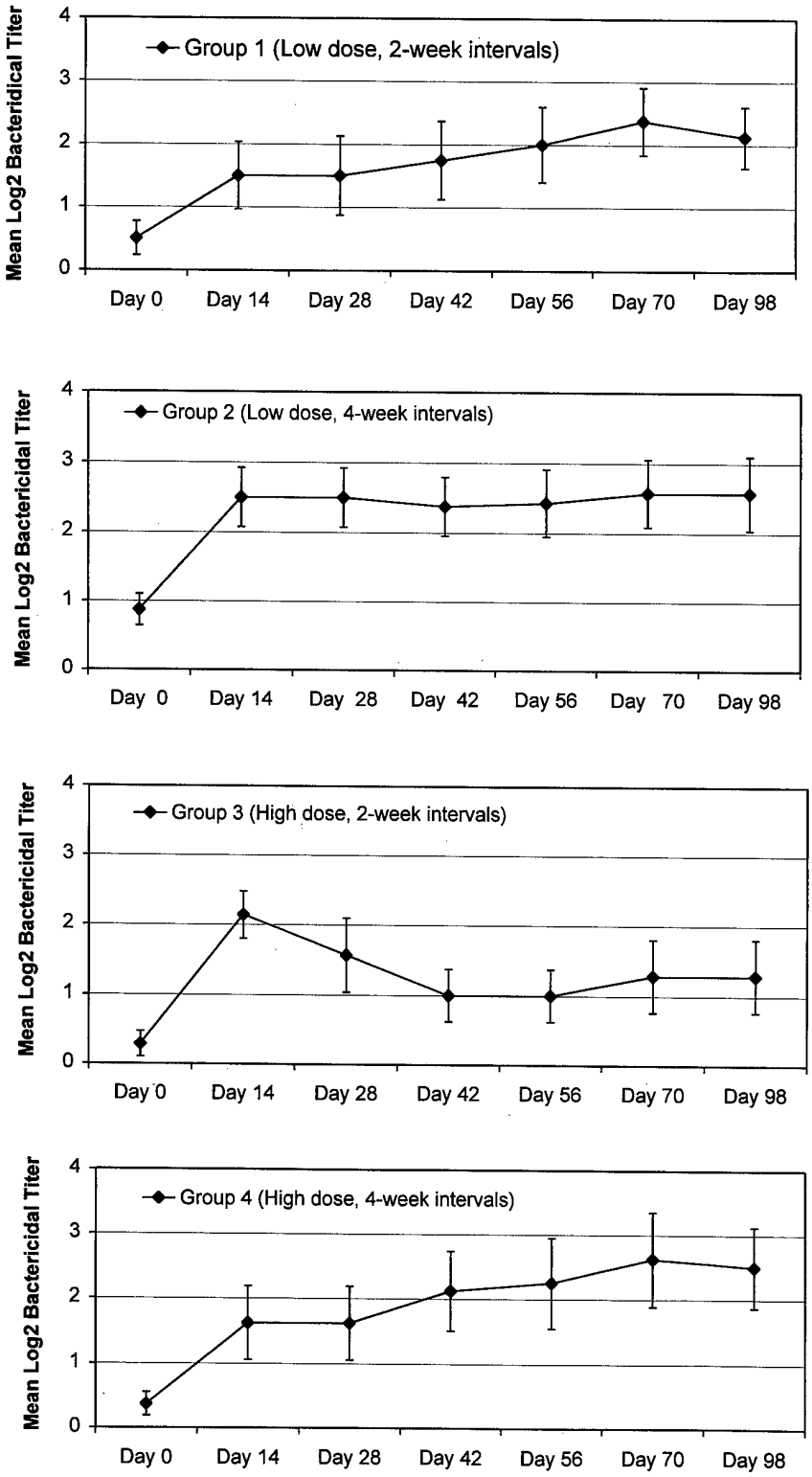


Fig. 4. Geometric mean bactericidal antibody responses versus strain 9162 of human subjects vaccinated intranasally with meningococcal NOMV vaccine.

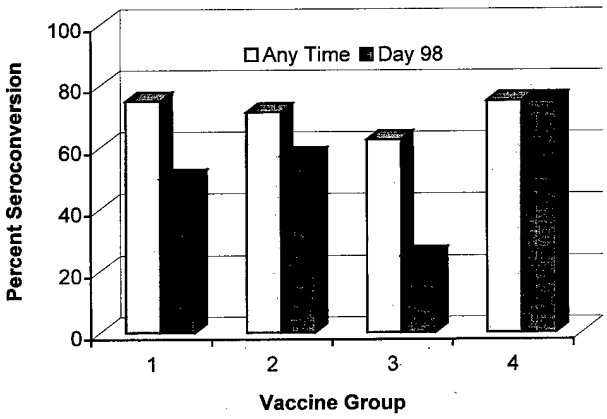


Fig. 5. Percentage seroconversion of volunteers vaccinated intranasally with the NOMV vaccine. Seroconversion is defined as a four-fold or greater increase in bactericidal titer either at any point after the initial vaccination or at day 98 (the end of the study).

ized IgG or IgA antibody levels in saliva were detected (data not shown) using NOMV as antigen.

3.3. Serum bactericidal activity

Serum bactericidal activity was measured against the encapsulated parent of the vaccine strain before vaccination and at 2, 4, 6, 8, 10 and 14 weeks after the initial vaccination. The geometric mean titers for each of the four vaccine groups are plotted as a function of time in Fig. 4. All four groups had geometric mean 3 to 4-fold increases in serum bactericidal antibodies at one or more time points following intranasal vaccination. Except for group 3, the antibodies appeared to be stable, persisting through the end of the study. There was no clear dose response that could be demonstrated with the small number of volunteers in the study. The greatest increase in bactericidal titer was observed following the initial dose of vaccine. Seroconversion, defined as a 4-fold or greater increase in bactericidal antibody titer, is shown in Fig. 5. Seroconversion was determined both for the highest point following initial vaccination and also at the end of the study (day 98). Group 4 had 75% seroconversion for both determinations.

3.4. Specificity of bactericidal antibodies

To obtain information about the specificity of the bactericidal antibodies, the sera were tested against 4 additional strains (Fig. 6). Two of the strains were similar to the vaccine strain (same serotype and serosubtype). These were isogenic variants of strain 8532 that expressed or did not express PorA. These were included to determine whether PorA was an important inducer of bactericidal antibodies in this study. The two other strains were representative of epidemics in Norway and Cuba. The Norwegian strain, 44/76, has the same serotype, but a different subtype, than the vaccine strain 9162. The Cuban strain differs both in serotype and subtype from the vaccine strain. All of the strains express L3,7,9 LOS, but not necessarily exclusively. The results of these bactericidal assays,

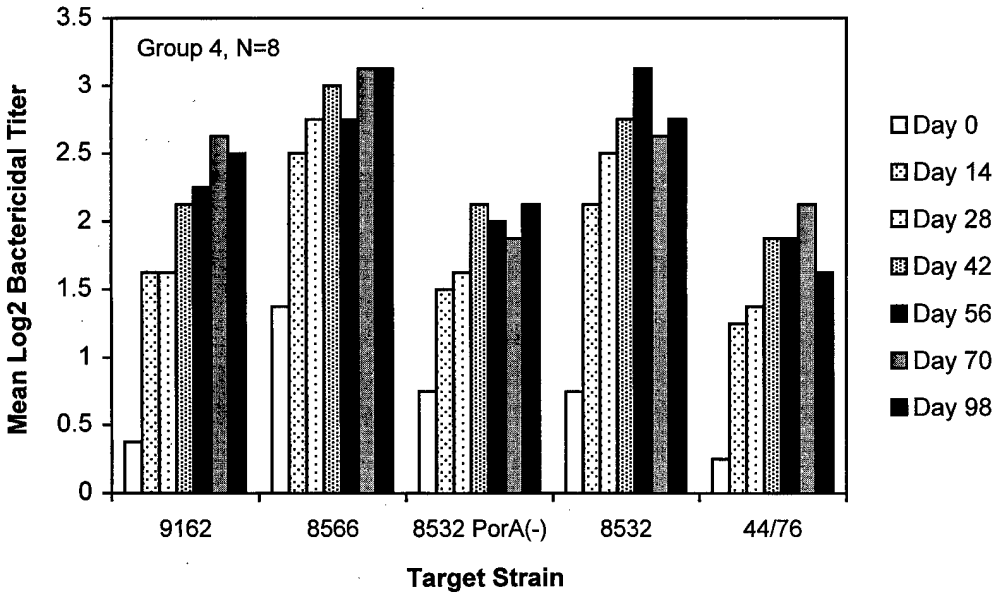


Fig. 6. Bactericidal activity of sera from group 4 volunteers against five different test strains. Data are expressed as mean $\log_2$ titers. The antigenic characteristics of the strains used are as follows (group:serotype:serosubtype: class 5 protein type: LOS immunotype): 9162(B:15:P1.7^h,3:P5.10,11:L3,7,9), 8566(B:4:P1.19,15:P5.5,11:L3,7,9), 8532(B:15:P1.–:P5.5,10:L3,7,8,9), 8532(B:15:P1.7^h,3:P5.5,10:L3,7,8,9), 44/76(B:15:P1.7,16:P5.5,c:L3,7,9).

Table 2
Seroconversion[a] against different test strains

Test strain	Vaccine group	*N*	Any time No.	At day 98 No.	Any time (%)	At day 98 (%)
9162	1	8	6	4	75	50
	2	7	5	4	71	57
	3	7	5	2	71	29
	4	8	6	6	75	75
8566	1	8	4	3	50	38
	2	7	3	1	43	14
	3	7	5	1	71	14
	4	8	4	3	50	38
8532C1-1	8	1	0	13	0	
	2	7	1	0	14	0
	3	7	4	3	57	43
	4	8	5	4	63	50
8532	1	8	3	0	38	0
	2	7	3	1	43	14
	3	7	4	2	57	29
	4	8	4	4	50	50
44/76	1	8	3	0	38	0
	2	7	1	1	14	14
	3	7	3	1	43	14
	4	8	5	3	63	38

[a] Four-fold or greater increase in bactericidal antibody titer.

expressed as geometric mean titers, are given in Fig. 6 and as seroconversion percentages in Table 2.

The specificity of the bactericidal antibodies was further investigated by selective removal of antibodies using a solid phase mini-adsorption technique. Eight selected sera were incubated in wells coated with serial dilutions of NOMV or purified LOS prior to testing for bactericidal activity. The results for two representative sera are shown in Fig. 7. Homologous NOMV was able to remove essentially 100% of the bactericidal antibody. NOMV that shared only the outer membrane proteins, but not the LOS, with the test strain was able to remove only about 40% of the antibody. Purified homologous (L3,7,9) LOS was able to remove 50 to 70% of the bactericidal activity from these sera. Purified L1,8 LOS had no effect (data not shown). All of the eight post-vaccination sera analyzed appeared to contain some bactericidal antibody specific for LOS.

4. Discussion

Meningococcal infection begins with colonization of the nasopharynx followed by invasion into the vascular system leading, to bacteremia and seeding of the meninges [13]. Protection against invasive meningococcal infection is mediated by systemic bactericidal antibodies [13]. Secretory antibody against meningococci may play an additional protective role at the mucosal surface [23], but that is as yet undetermined. An appropriate animal model or *in vitro* correlate of protection by mucosal antibodies needs to be developed to better ascertain the role of secretory antibodies in protection against meningococcal disease. Parenteral vaccines are generally not effective inducers of local antibody responses at mucosal surfaces [24]. This has led to attempts to induce both local and systemic immunity by immunization at the mucosal surface itself [25,26]. The pathogenesis of meningococcal disease suggests that such an approach may be advantageous compared to parenteral immunization. This thinking led to the production of this vaccine and phase I clinical trial.

Intranasal immunization with the NOMV in this study, using the metered inhalers, was an easily performed, non-invasive procedure and was very accurate in terms of delivered dose. The vaccine proved to be extremely well-tolerated, despite the fact that there was 25% LOS by weight (Table 1). This study demonstrates that immunization with vaccines containing relatively large amounts of bacterial endotoxin can be safely administered by the intranasal route. The pyrogenicity constraints that are placed on parenteral vaccines do not appear to apply when the vaccine is administered intranasally.

The vaccine appeared to be poorly immunogenic in terms of the total serum antibody response as measured by ELISA. Volunteers in group 4 who received the high dose at 4 week intervals seemed to have the best response. However, less than 30% of this group had a 2-fold or greater increase in serum IgA or IgG antibodies to NOMV by ELISA. Overall there was a significantly higher serum IgA response in this group at 42 and 98 days post-vaccination (Fig. 1).

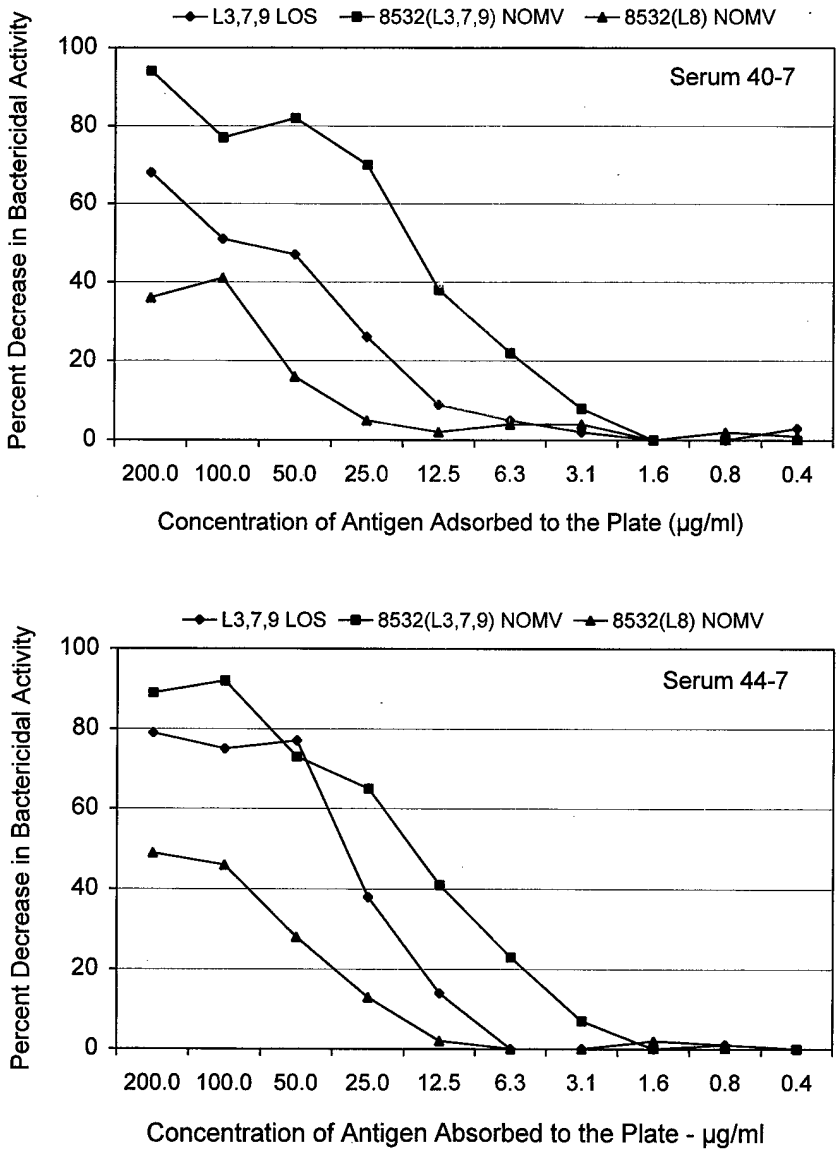


Fig. 7. Effect of solid phase adsorption with serial dilutions of different antigens on the bactericidal activity of two post vaccination sera.

There was a similar trend with antibodies directed against the LOS component of the vaccine (Fig. 2). The specific mucosal antibody response in nasal wash fluid is summarized in Fig. 3. The mean increase in specific secretory antibody was significant for all groups combined. Groups 2 and 4 (high dose groups) demonstrated the best responses. Little, if any, change in the level of antibodies in saliva was detected. Of note, the antibody levels in the nasal wash and saliva may be underestimated because protease inhibitors were not added to neutralize residual protease activity. These data nonetheless suggest that the NOMV vaccine could induce sustained amounts of secretory antibodies at the mucosal site.

Despite the poor quantitative response to the vaccine as measured by ELISA, the functional bactericidal antibody response was similar to that obtained by some parenteral OMP-based vaccines [11,27]. All 4 groups had a 3 to 4 fold increase in bactericidal antibodies following intranasal vaccination (Fig. 4). Group 4 seemed to have the best response in terms of bactericidal antibody production, but there was no clear dose-response that could be demonstrated given the small number of volunteers in the study. The greatest increase in bactericidal titer in most individuals occurred after the initial vaccination. Interestingly, the titers remained stable with little evidence of drop off in all groups, except group 3, out to the end of the study at 96 days. This suggests that intranasal vaccination may be more effective than parenteral vaccination for inducing long-lasting systemic bactericidal antibodies. This possibility requires further investigation in ad-

ditional studies with longer follow up times. The rates of seroconversion (4-fold increase in titer compared to baseline) for several different test strains are summarized in Table 2. Group 4 had the best rates of seroconversion with 75% experiencing seroconversion by the end of the study. Of the conditions tested in this small study, it appears that intranasal immunization with the high dose at 4 week intervals gave the best response in terms of total antibody, seroconversion rates, duration of the antibodies and bactericidal titers. Larger numbers of volunteers are needed, however, reach a firm conclusion on optimal vaccine presentation. Despite the relatively poor overall response in terms of total antibody as measured by ELISA, the bactericidal response was surprisingly good. This suggests that small amounts of systemic antibody are induced by intranasal vaccination with NOMV, but these antibodies are qualitatively superior since they are bactericidal. Bactericidal antibodies correlate with protective immunity in meningococcal disease [11,28,29].

The serum bactericidal antibodies induced by the NOMV intranasal vaccine appeared to include both anti-PorA antibodies and anti-LOS antibodies specific for the L3,7,9 LOS immunotype. Based on the results of the solid phase absorption experiments it appeared that up to 50% of the bactericidal antibody may be directed against the LOS. This antibody may be partially responsible for the cross-reactive bactericidal activity that was observed. The degree to which sialylation affects the binding of this antibody and its ability to cause complement mediated lysis of meningococci carrying the L3,7,9 LOS is being investigated. The test strain (wild type 9162) used in these experiments was approximately 25% sialylated as judged by a silver stained polyacrylamide gel of the LOS expressed by the organism when grown under exactly the same conditions as for the bactericidal assay. The safety of antibodies to the L3,7,9 LOS has been questioned due to the presence of the lacto-*N*-neotetraose oligosaccharide group present on the LOS [30]. However, large numbers of people have been safely vaccinated with deoxycholate extracted vesicle vaccines containing significant amounts of L3,7,9 LOS [10, 31]. We have observed carriage-induced bactericidal antibodies with the same L3,7,9 LOS specificity in the sera of healthy children (Zollinger et al., manuscript in preparation). The precise epitope on the L3,7,9 LOS to which these antibodies bind is unknown. The L3,7,9 LOS is expressed on a relatively high percentage of group B case isolates, especially those associated with recent epidemic outbreaks [32, 33]. If antibodies to L3,7,9 can be safely induced by intranasal vaccination and are effective against group B strains *in vivo*, they may be able to provide relatively broad protection against group B disease.

The results obtained in this study are similar to the results of an intranasal vaccine study recently reported by Haneberg et al. [19]. The vaccine they used, which consisted of meningococcal outer membrane vesicles extracted with deoxycholate, had been previously tested in a phase III efficacy trial as a parenteral vaccine given intramuscularly. Extraction with deoxycholate results in removal of most of the LOS and phospholipids from the membrane vesicles. Our vaccine, on the other hand, was prepared without exposure to detergent and therefore contains all the LOS and phospholipid present in the intact outer membrane. Additional differences in the two vaccines and the study designs make it difficult to directly compare the results of the two studies. Our vaccine did not contain the Opc protein which apparently induced significant bactericidal antibody in the Haneberg study. We believe that the Opc protein may be an important vaccine component for an intranasal vaccine, but were unable to obtain consistent expression of this protein by our vaccine strain at the time we initiated these studies. The two vaccines were prepared from strains expressing different subtype proteins and different Opa proteins. The intrinsic immunogenicity of the different subtype proteins appears to differ [27]. Also, the organisms from which our vaccine was prepared were grown on limiting iron and therefore expressed the iron uptake proteins in the outer membrane. In addition, the number and schedule of doses differed between the two studies. In spite of these differences a number of striking similarities are evident in the results of the two studies. In both studies, only small increases in total serum antibody as measured by ELISA were observed, but increases in serum bactericidal antibodies were quite good and similar to increases seen following intramuscular vaccination. The bactericidal antibody levels persisted in most cases through the end of the study.

This phase I clinical study demonstrates that intranasal vaccination with NOMV against group B meningococcal infection is feasible. The immunizations are easy to perform, accurate in terms of delivered dose and extremely well-tolerated despite containing 25% bacterial endotoxin by weight. For intranasal immunization, it would appear that efforts to remove endotoxin and potentially denature important protein epitopes in the process, are not necessary. Immunization with NOMV appears to mimic natural colonization with viable meningococci by inducing long-lasting serum bactericidal antibodies in the majority of volunteers despite relatively low total antibody titers. Some of these functional antibodies are cross-reactive with other virulent Group B meningococci. The vaccine also induces secretory IgA antibodies in nasal secretions, but with the particular doses and schedules used induced little if any antibody response in the saliva. We would anticipate that se-

cretory IgA at the nasal mucosal surface may play a role in protection against initial colonization with virulent meningococci. Further studies will be required to define the best vaccination strategy for intranasal vaccination with NOMV, but the safety and immunogenicity results of this trial suggest that further studies, possibly including an efficacy study are warranted.

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