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Human Antibody Responses to Meningococcal Outer Membrane Antigens after Three Doses of the Norwegian Group B Meningococcal Vaccine

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The antibody kinetics in sera from 27 adults after three doses of the Norwegian group B meningococcal outer membrane vesicle (OMV) vaccine was studied. The vaccinees received the third dose 4 to 5 years after the first two. Antibody responses against outer membrane proteins (OMPs) and lipopolysaccharides were studied by enzyme-linked immunosorbent assay and immunoblotting and with serum bactericidal assays (SBA) with three variants of the vaccine strain, 44/76. Six weeks after the second injection, the geometric mean (GM) of the levels of immunoglobulin G (IgG) against OMVs was about sevenfold higher than that of prevaccination levels, and 74% of the vaccinees developed a greater-than-twofold rise in SBA titer. After 6 months, the GM of IgG levels declined to about threefold higher, and after 4 to 5 years it declined to about twofold higher, than that before vaccination. The third dose induced a rapid increase in SBA titers in 96% of the vaccinees, and the GM of levels of IgG against OMVs rose to about 14-fold the prevaccination level. One year later, the IgG antibody levels had dropped to 4.6-fold the prevaccination level, but 88% of the vaccinees still showed bactericidal activity. The response after the two first doses was higher in individuals with prevaccination antibodies, but no such effect was found after three doses. The use of defined mutants in SBA and linear multiple regression analyses indicated that among the major OMPs, antibodies to the Opc and class 1 proteins made the most important single contributions to the bactericidal activity against the vaccine strain, but it also demonstrated the importance of antibodies against other antigens. After three doses, 68% of the vaccinees showed a significant SBA response against a strain lacking both the Opc and the class 1 proteins. Three doses converted almost all subjects to SBA responders and gave higher antibody levels and relatively less serosubtype-specific bactericidal activity than did two doses, probably indicating a broader cross-protection against heterologous strains.

The currently used polysaccharide vaccines against *Neisseria meningitidis* strains of serogroups A, C, Y, and W135 offer good protection in adults and in children over 2 years of age (13, 47), whereas the serogroup B capsular polysaccharide is poorly immunogenic (35, 77). Serogroup B meningococcal disease remains a significant health problem in many parts of the world. Therefore, alternative vaccine approaches, based on noncapsular surface antigens such as outer membrane proteins (OMPs), detoxified lipopolysaccharides (LPS), or LPS-derived oligosaccharides, have been suggested (13, 14, 41, 50, 51, 65, 68, 70, 78, 81, 82).

Norway has had a high incidence of serogroup B meningococcal disease since the mid-1970s. This motivated the development of an outer membrane vesicle (OMV) vaccine against group B meningococcal disease based on a representative, epidemic *N. meningitidis* strain. In a double-blind, placebo-controlled protection trial among Norwegian teenagers, two vaccine doses administered 6 weeks apart significantly protected against systemic serogroup B meningococcal disease (8). After 29 months, the point estimate of efficacy was 57% (95% confidence interval = 14 to 79%) (7). Almost 100% of the vaccinees developed significant immunoglobulin G (IgG) antibody responses to OMVs after two doses, although a relatively rapid decline in IgG levels after vaccination was observed (58), and 30% of the teenagers did not respond in serum bactericidal assays (SBA) (30). Most of the vaccine failures were observed during the later part of the observation period, indicating a decrease in protective efficacy with time (7, 57). Vaccine failures were found not to be due to complement deficiencies (28), low levels of mannan-binding protein in serum (20), or a predisposition through blood group ABH nonsecretor status (39), nor did genetic or antigenic analyses of the strains isolated from cases of vaccine failures and from subjects given a placebo indicate a selection pressure leading to a predominance of escape mutants (10, 18). Although the in vitro bactericidal activity of the sera was dependent upon epitopes on the class 1 OMP in the test organism (30, 60), the observed protection in humans did not appear to be serotype or serosubtype specific (18).

In order to increase the antibody levels and the proportion of responders in SBA, a third vaccine dose was administered 4 to 5 years later to a group of adult volunteers who had participated in previous phase II trials (6). In this paper, we describe the kinetics of the antibody responses against noncapsular antigens measured by enzyme-linked immunosorbent assays (ELISA), SBA, and immunoblotting. In addition, we have analyzed the relative contributions of antibodies against the various antigens in SBA with defined variants of the vaccine strain (44/76) and immunoblotting and by multiple regression analyses.

(A preliminary report of some of the data described here was presented at the Ninth International Pathogenic Neisseria Conference, Winchester, England, 26–30 September 1994 [31].)

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TABLE 1. Variants of meningococcal strain 44/76 and their reactivities with mouse MAbs

Variant strain	Colony blotting[a] with MAb[b]:			Bactericidal activity[c] with MAb[b]:		
	154,D-11 (Opc)	184,F-12 (P1.16)	188,C-1 (P15)	154,D-11 (Opc)	184,F-12 (P1.16)	188,C-1 (P15)
44/76-1	++	+++	+++	6.5	3.2	—[d]
44/76-SL	+/−	+++	+++	—	3.2	—
44/76-2	−	+++	+++	—	6.5	—
M14-2	−	−	+++	—	—	—

[a] +++, 100% of CFU positive; ++, 97 to 100% of CFU positive; +/−, 10 to 13% of CFU positive; −, 0 to 3% of CFU positive.
[b] The specificity of the MAb is indicated in parentheses.
[c] Concentration of IgG (nanograms per milliliter) needed to give >50% killing of the meningococcal variant in SBA.
[d] —, no killing with 25,000 ng of IgG per ml.

MATERIALS AND METHODS

Vaccine. The vaccine was deoxycholate (DOC)-extracted OMVs from meningococcal strain 44/76 (B:15:P1.7,16:P5.5:L3,7,9) adsorbed to aluminum hydroxide (17). One human dose contained 25 μg of protein, 2 μg of LPS, and 1.67 mg of $Al(OH)_3$ and was injected in 0.5-ml volumes into the deltoid muscle.

In addition to the major OMPs of classes 1 to 5 (67), the vaccine contained the Opc protein (2), small amounts of some less well characterized membrane proteins, and about 8% LPS (16, 17). The first two doses were with the same vaccine batches as were used in phase II trials and in the efficacy trial in Norway (8). The third dose was from a vaccine batch prepared in the same way but in another type of fermentor. Only minor quantitative differences between the batches were noted (16). The batch used for the third dose had been stored at 4°C for 2 years.

Vaccinees. Twenty-seven healthy adult volunteers (5 male and 22 female) participated in the trial (phase II-9). These subjects were medical students and laboratory personnel who previously had participated in other phase II trials (6). The first two injections were given about 6 weeks apart. Five individuals (from phase II-1), 17 individuals (from phase II-3), and five individuals (from phase II-4a) received their third doses 5, 4 1/2, and 4 years, respectively, after the first dose. Sera were drawn at week 0 (before the first dose), 6 weeks later (before the second dose), and then after 12 weeks, 26 weeks, and 4 to 5 years (before the third dose). After the third dose, sera were collected after 3 days, 1 week, 2 weeks, 6 weeks, and 1 year (see Tables 2 and 3).

The vaccination trials were approved by the Norwegian Medicines Control Authority and the Regional Ethical Committee for Medical Sciences, South East Norway.

MAbs. Monoclonal antibodies (MAbs) 279-5C (IgG1) (61) and 154,D-11 (IgG2a) were used to detect expression of the Opc protein in the strains used for SBA. These MAbs reacted identically with a panel of >100 different meningococcal strains in whole-cell ELISA (75) and bound identically in colony blots with the variants of meningococci used in SBA (Table 1). MAb 154,D-11 detected the Opc protein on immunoblots only in the presence of Empigen BB (Albright and Wilson, Cumbria, United Kingdom), while the reactivity of MAb 279-5C was detergent independent. MAb 154,D-11 was highly bactericidal against strain 44/76-1, but no activity against strains 44/76-SL and 44/76-2 was seen (Table 1). MAb 279-5C showed no bactericidal activity against any of the strains.

MAbs 3-1-P1.16 (83) and 184,F-12 (IgG3) were used as MAbs specific for the P1.16 epitope. These two MAbs reacted identically in whole-cell ELISA as well as against the denatured class 1 OMP from strain 44/76 on immunoblots. Both were highly bactericidal against strains 44/76-1, 44/76-2, and 44/76-SL but not against strain M14-2 (Table 1).

MAbs 2-2-P15 (83) and 188,C-1 (IgG3) (12) were serotype 15 specific and reacted identically in whole-cell ELISA and on immunoblots. Both recognized the class 3 protein only after renaturation with Empigen BB. With the concentrations applied in SBA, MAb 188,C-1 was not bactericidal against any of the variants of 44/76.

MAbs 154,D-11, 184,F-12, and 188,C-1 were prepared by immunizing BALB/c mice with OMVs from strain 44/76, as described previously (12). The MAbs were purified by affinity chromatography on protein A-Sepharose, and IgG concentrations were determined by measuring the optical density ($E^{1\%}_{1cm}$ = 14.0) at 280 nm.

MAbs 9-1-L3,7,9 (L3,7,9) and 2-1-L8 (L8) were provided by W. D. Zollinger (43), and MAb 4C4 (L11) was from M. Apicella (36); those MAbs were used to characterize LPS. MAb AE3, reacting with the class 4 OMP, was a gift from C. T. Sacchi, Adolfo Lutz Institute, Sao Paulo, Brazil.

Meningococcal strains. Three variants of the vaccine strain 44/76 (B:15:P1.7,16) were used as target strains in SBA. The variant 44/76-SL is the strain we use in our standard SBA, and it was also used in a multicenter trial recently performed in Iceland (48). When an inoculum of 44/76-SL was prepared as for SBA (30), repeatedly about 10 to 13% of the CFU expressed the Opc^{++} phenotype on colony blots (43). Two well-isolated single colonies, one Opc positive and one Opc negative, were subcultured from 44/76-SL and retested for Opc activity in a second colony blotting. Again one Opc^{++} colony and one Opc^{-} colony were picked, subcultured on agar plates, and batch frozen once in numerous tubes in Greaves' medium at −70°C. On further retesting, the Opc-positive variant (44/76-1) and the Opc-negative variant (44/76-2) proved to be stable. Only 1 to 3% of Opc-negative colonies were seen in 44/76-1, and conversely, Opc-positive colonies were less than 1% in 44/76-2 (Table 1).

The mutant strain M14, which is deficient in class 1 protein, was selected from single colonies after growth of 44/76 for 90 min at 37°C in tryptic soy broth containing 1.2% ethylmethane sulfonate (Eastman Kodak Co., Rochester, N.Y.). Two new variants, M14-1 (Opc^{++}) and M14-2 (Opc^{-}) were selected by colony blotting with MAb 154,D-11, as described above. All variants showed identical reactivity patterns with MAbs specific for the serotype 15 protein, LPS, and the serogroup B capsular polysaccharide.

The reference strains (15) M1080 (B:1:P1.1,7) and S3032 (B:NT:P1.12,16) were used as antigens in immunoblotting.

ELISA. IgG, IgM, and IgA antibodies to OMVs and LPS were analyzed by ELISA. The OMV ELISA was performed as described previously (27, 55). The antigen for sensitizing ELISA microtiter plates was OMVs from strain 44/76, prepared as for vaccine production (17).

LPS from strain 44/76 was obtained from a side fraction early in the vaccine production process as previously described (9). Briefly, the material precipitated by cold ethanol from the 0.5% DOC supernatant after ultracentrifugation of OMVs was extracted with hot aqueous phenol (76) and phenol-chloroform-hexane (19). LPS was further purified by gel filtration on a Sephadex G-75 column in the presence of 0.5% DOC in Tris-HCl-EDTA buffer (pH 8.6) followed by ethanol precipitation and ultracentrifugation. No protein contamination was detected by Coomassie blue B staining of sodium dodecyl sulfate-polyacrylamide gels loaded with 10 μg of LPS. The LPS preparation did not react with MAbs against the serotype 15 or subtype P1.16 proteins but did react with MAbs against L3,7,9, L8, and L11.

For LPS ELISA, microtiter plates (Immunoplate 1; Nunc, Roskilde, Denmark) were coated with 100 μl of 10-μg/ml LPS in 0.01 M phosphate-buffered saline (pH 7.2) (PBS)–0.1% DOC (PBS-DOC) per well. Alternatively, LPS was dissolved in PBS only. The plates were incubated at 22°C overnight and then stored at 4°C until used. When used, plates were blocked with 150 μl of PBS–0.5% bovine serum albumin (PBS-BSA) per well for 1 h and washed four times with PBS–0.05% Tween 20. Serum samples, diluted in PBS-BSA, were tested in triplicate (100 μl per well), and the plates were incubated for 3 h at 33°C. IgG, IgA, and IgM antibodies were detected as described previously (55). In addition to the vaccinee sera, a twofold dilution series of a positive reference serum (starting at a 1:200 dilution) and a negative reference serum (1:100 dilution) were tested on each plate.

All sera were initially tested at a 1:200 dilution for IgG and a 1:100 dilution for IgA and IgM. Samples with optical densities higher than 0.9 times the maximum optical density of the reference serum were retested at higher dilutions (1:400 to 1:6,400). The ELISA results were scaled relative to those for reference sera and transformed into units per milliliter by use of a sigmoidal standard curve (logit-log transformation) (53). The concentrations of specific antibodies in the reference sera for OMVs had previously been determined by a solid-phase radioimmunoassay (27, 55); in this assay 1 U corresponds approximately to 1 μg of Ig. The reference sera for LPS ELISA were arbitrarily defined as having 1,000 U/ml. All ELISA analyses were performed at least twice, and mean units per milliliter were calculated.

SBA. Bactericidal assays were performed in microtiter plates in the presence of 25% human complement (80) with a bacterial inoculum of 70 to 90 CFU per well, as described previously (30). With the complement alone, no reduction in CFU was detected after incubation of the bacteria for 30 min, and no reactivity was seen against OMVs from 44/76 on immunoblots. For each day's experiment, one frozen tube with bacteria was thawed and used as a starter culture. A twofold dilution series (starting at 1:2) for each serum was examined. The reciprocal of the highest serum dilution causing more than 50% killing of the target strain was recorded as the bactericidal titer (BT). Sera showing survival of more than 50% of the CFU in the first well were defined as having a $\log_2$ BT (LBT) of 0. Fourfold or greater rises in BT were considered significant responses. All sera from each vaccinee were tested in the same experiment.

MAb 154,D-11 was included as a control in all SBA, and gave consistently high titers with 44/76-1 but no bactericidal titer with 44/76-SL, 44/76-2, or M14-2 (Table 1).

Immunoblotting assay. IgG antibodies to the noncapsular surface antigens from strain 44/76 were analyzed by immunoblotting, as described previously (72–75). Each gel (7 by 8 cm) was loaded with 45 μg of the same OMV preparation as for the ELISA experiments. After electrotransfer at 100 V for 1 h and blocking, each blot was cut into strips with about 1.8 μg of protein per strip. The strips were incubated overnight at room temperature with 1:200 dilutions of sera collected at 0, 6, 12, and 26 weeks and 4 to 5 years after the first dose and 6 weeks and 1 year after the third dose. The sera were incubated in the presence and absence of 0.15% Empigen BB for partial renaturation of OMP epitopes (40, 73). All blots were incubated for 2 h with a 1:500 dilution of peroxidase-conju-

TABLE 2. Levels of OMV antibodies, determined by ELISA, in sera from 27 adults immunized with the Norwegian group B vaccine

Serum no.	Time[a] (vaccination no.)	Antibody level					
		IgG		IgM		IgA	
		U/ml[b]	Fold increase[c]	U/ml	Fold increase	U/ml	Fold increase
1	0 wk (1)	2.3 (1.0–5.4)		0.24 (0.16–0.35)		0.32 (0.20–0.51)	
5	6 wk (2)	7.7 (3.5–16.7)	3.3	0.29 (0.19–0.45)	1.2	0.45 (0.26–0.76)	1.4
7	12 wk	15.8 (6.3–39.5)	6.8	0.30 (0.17–0.52)	1.2	0.71 (0.33–1.53)	2.2
8	26 wk	7.4 (3.0–18.3)	3.2	0.27 (0.17–0.43)	1.1	0.52 (0.29–0.91)	1.6
11	4–5 yr (3)	4.5 (2.2–8.9)	1.9	0.26 (0.17–0.41)	1.1	0.36 (0.22–0.59)	1.1
12	+3 days	3.9 (1.8–8.4)	1.7	0.24 (0.16–0.38)	1.0	0.35 (0.22–0.56)	1.2
13	+1 wk	9.6 (5.2–17.5)	4.1	0.28 (0.19–0.43)	1.2	0.56 (0.34–0.93)	1.8
14	+2 wk	33.7 (15.8–71.9)	14.5	0.33 (0.21–0.50)	1.4	0.93 (0.47–1.85)	2.9
15	+6 wk	28.2 (12.4–64.1)	12.2	0.33 (0.19–0.56)	1.4	0.74 (0.40–1.38)	2.3
16	+1 yr	10.5 (5.2–20.9)	4.5	0.27 (0.17–0.44)	1.1	0.56 (0.28–1.12)	1.8

[a] Time after first vaccination. For sera 12 through 16, the time after the third vaccination is indicated.
[b] Expressed as GM with the confidence interval (1 standard deviation) given in parentheses. One unit of Ig to OMVs corresponds to approximately 1 μg of specific antibody.
[c] With respect to the level at 0 weeks.

gated rabbit anti-human IgG (Dakopatts a/s, Glostrup, Denmark) and stained for 10 min with 3-amino-9 ethylcarbazole and hydrogen peroxide. The intensity of IgG binding to the 70- and 80-kDa major high-molecular-mass proteins, to the class 1, 3, 4, and 5 OMPs (including Opc), and to LPS was scored visually on a scale from 0 to 4; all strips were evaluated at least twice. The binding strengths were then grouped as strong (score of 3 to 4), medium (2 to 2.5), or no or weak (0 to 1.5) responses. Control strips incubated with MAbs against the class 1 and 4 and Opc proteins were used to identify the major antigens.

Statistical analyses. The ELISA results (in units per milliliter) were transformed to logarithmic values for calculation of geometric means (GM). This transformation gave approximately normal distributions of the data. Differences between groups were tested for significance by Student's *t* test, the chi-square test, and/or the Mann-Whitney test. Linear multiple regression (LMR) analyses (37) of the immunoblotting and ELISA results were performed to assess the contributions of antibodies to the individual antigens and the antibody isotypes to the overall bactericidal activity. All statistical analyses were performed with SPSS/PC+ software (SPSS Inc., Chicago, Ill.).

RESULTS

Antibody responses to OMVs. The kinetics of the IgG, IgA, and IgM antibody responses are shown in Table 2. Six weeks after the first dose, 89% of the vaccinees responded significantly (>2-fold); the increase in the GM IgG level was 3.3-fold. Six weeks after the second dose, 96% of the vaccinees showed significant increases in the level of IgG antibodies, and the GM IgG level was 6.8-fold higher than it was before vaccination. Thereafter, the level of IgG antibodies decreased with time, and in sera collected 4 to 5 years after the first vaccination, the GM level was only 1.9-fold higher than it was before vaccination.

The third dose induced a strong IgG booster response. Two weeks after the third immunization, the GM IgG level was 14.5-fold higher than it was before vaccination and 1.7-fold higher than it was at week 12. Six weeks after the third injection, the level of IgG antibodies started to decrease, although it was still 4.5-fold higher than the prevaccination value 1 year later. The IgM and IgA responses to OMVs were generally weak compared with that of IgG, and no booster response was seen after the third dose.

Large individual variations in the kinetics of antibody development were observed. Some vaccinees showed a weak or moderate IgG response after the first two doses but a strong increase after the third dose. Others showed an IgG response after the third injection that was similar to or weaker than those after the first two injections. Those vaccinees who responded with levels of IgG antibodies to OMVs after the third dose (serum 14) that were greater than twofold higher than those after the second dose (serum 7) were defined as category I responders ($n = 15$); the remaining 12 vaccinees were defined as category II responders. The kinetics of the IgG responses for these two categories are shown in Fig. 1a. IgG and IgA levels in the prevaccination sera were significantly higher for category II responders than for category I responders ($P < 0.001$), and the response to the second dose was higher in category II responders. Six weeks after the third dose, the difference between the two categories was no longer signifi-

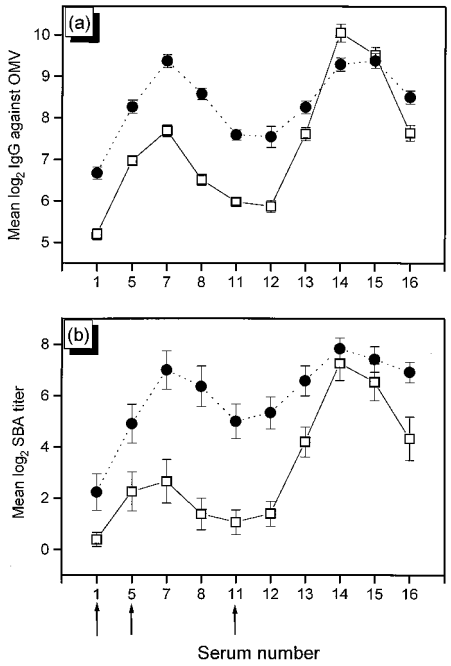

FIG. 1. Immune responses of 27 Norwegian adult vaccinees participating in the three-dose study with the Norwegian meningococcal group B vaccine. (a) Mean $\log_2$ IgG antibody levels against OMVs from strain 44/76 for category I and II responders; (b) mean LBTs of sera in SBA against strain 44/76-1. Squares, category I responders ($n = 15$); circles, category II responders ($n = 12$) (see text). Error bars indicate standard errors of the means. For serum numbers, see Tables 2 and 3. Arrows indicate individual vaccinations.

TABLE 3. Levels of LPS antibodies, determined by ELISA, in sera from 27 adults immunized with the Norwegian group B vaccine

Serum no.	Time[a] (vaccination no.)	Antibody level					
		IgG		IgM		IgA	
		U/ml[b]	Fold increase[c]	U/ml	Fold increase	U/ml	Fold increase
1	0 wk (1)	89 (46–171)		121 (56–260)		133 (98–182)	
5	6 wk (2)	234 (114–479)	2.6	224 (116–433)	1.9	200 (123–326)	1.5
7	12 wk	350 (140–876)	3.9	246 (107–546)	2.0	246 (113–536)	2.0
8	26 wk	238 (114–499)	2.7	202 (98–418)	1.7	207 (116–371)	1.7
11	4–5 yr (3)	91 (41–204)	1.0	103 (54–198)	0.9	115 (73–182)	0.9
12	+3 days	82 (20–147)	1.0	ND[d]		ND	
13	+1 wk	147 (71–308)	1.7	ND		ND	
14	+2 wk	240 (108–536)	2.7	ND		ND	
15	+6 wk	361 (149–873)	4.1	186 (78–445)	1.5	213 (102–445)	1.8
16	+1 yr	150 (63–355)	1.7	134 (65–279)	1.1	127 (70–233)	1.0

[a] Time after first vaccination. For sera 12 through 16, the time after the third vaccination is indicated.
[b] Expressed as GM with the confidence interval (1 standard deviation) given in parentheses. The units for antibodies to LPS are arbitrarily chosen. The relationship between units and micrograms of Ig has not been determined.
[c] With respect to the level at 0 weeks.
[d] ND, not determined.

cant, but 1 year later category II responders showed a lesser decrease in IgG antibody levels than did category I responders.

Antibody responses to LPS. A sensitive and reproducible assay was produced for coating plates with concentrations of 3 to 10 μg of LPS per ml when LPS was dissolved in PBS-DOC. If LPS was dissolved in PBS only, no maximal concentration for coating plates and large variations in the optical density readings were observed, even with LPS concentrations of up to 30 μg/ml. When the reference sera with anti-LPS antibodies were absorbed with LPS, binding to LPS afterwards was almost negligible, whereas the binding in OMV ELISA was reduced by only 10 to 15%.

The kinetics of antibody responses to LPS are shown in Table 3. After the first two doses, vaccinees responded with a GM increase in IgG levels of 3.9-fold. Somewhat weaker responses were observed for IgA and IgM. The anti-LPS antibody levels rapidly declined in most of the vaccinees, and 4 to 5 years later the GM of IgG antibody levels was not significantly different from that before vaccination. In contrast to the IgG response against OMVs, no booster response against LPS was seen after the third dose; an increase in the IgG level up to only about the same level as observed after the second dose was observed. The level of IgG antibodies against LPS peaked at 6 weeks after the third dose in most vaccinees, in contrast to the IgG response against OMVs, which peaked after two weeks. For antibody responses against LPS, large individual variations in the kinetics were also observed.

SBA. The kinetics of bactericidal antibodies against the three variants of strain 44/76 are shown in Table 4. Before vaccination, 37% of the vaccinees had SBA activity against strain 44/76-1 (Opc^{++}). Six weeks after first dose, 50% of the vaccinees had responded significantly and the GM titer (GMT) increased 4.5-fold. Six weeks after the second dose 74% of the vaccinees had responded, and 83% had bactericidal activity against this strain. After 6 months, 67% of the vaccinees retained SBA titers. Four to 5 years later (serum 11), the GMT was 2.8-fold higher than it was before vaccination; 61% of individuals had SBA activity against strain 44/76-1, and 35% had activity against M14-2.

The third dose induced a rapid SBA response. Thus, an

TABLE 4. Bactericidal antibody responses against variants of meningococcal strain 44/76 after immunization with the Norwegian serogroup B OMV vaccine.

Serum no.[a] (vaccination no.)	Activity against target strain:								
	44/76-1			44/76-SL			M14-2		
	MLBT[b] (SEM)	% 4× response[c]	% ≥1:2[d]	MLBT (SEM)	% 4× response	% ≥1:2	MLBT (SEM)	% 4× response	% ≥1:2
1 (1)	1.2 (0.4)		37	0.8 (0.3)		33	0.8 (0.3)		24
5 (2)	3.4 (0.4)	50	65	2.4 (0.5)	33	66	1.0 (0.3)	8	36
7	4.7 (0.7)	74	83	3.0 (0.5)	52	88	1.5 (0.4)	17	56
8	3.7 (0.7)	54	67	2.1 (0.5)	27	61	1.1 (0.3)	8	46
11 (3)	2.7 (0.6)	46	61	1.8 (0.4)	18	52	0.8 (0.3)	8	35
12	3.1 (0.5)	52	74	2.2 (0.5)	33	59	1.0 (0.3)	16	35
13	5.3 (0.5)	92	96	3.5 (0.4)	63	89	1.8 (0.3)	32	79
14	7.5 (0.4)	96	96	5.2 (0.4)	89	96	3.0 (0.3)	68	92
15	6.9 (0.5)	96	96	4.6 (0.3)	85	96	2.1 (0.3)	36	89
16	5.5 (0.6)	85	88	3.4 (0.4)	58	92	1.8 (0.3)	34	74

[a] The times when the sera were drawn are given in Tables 2 and 3.
[b] Arithmetic mean of LBT.
[c] Percentage of vaccinees with a fourfold or greater increase in BT.
[d] Percentage of vaccinees with bactericidal activity in a 1:2 serum dilution.

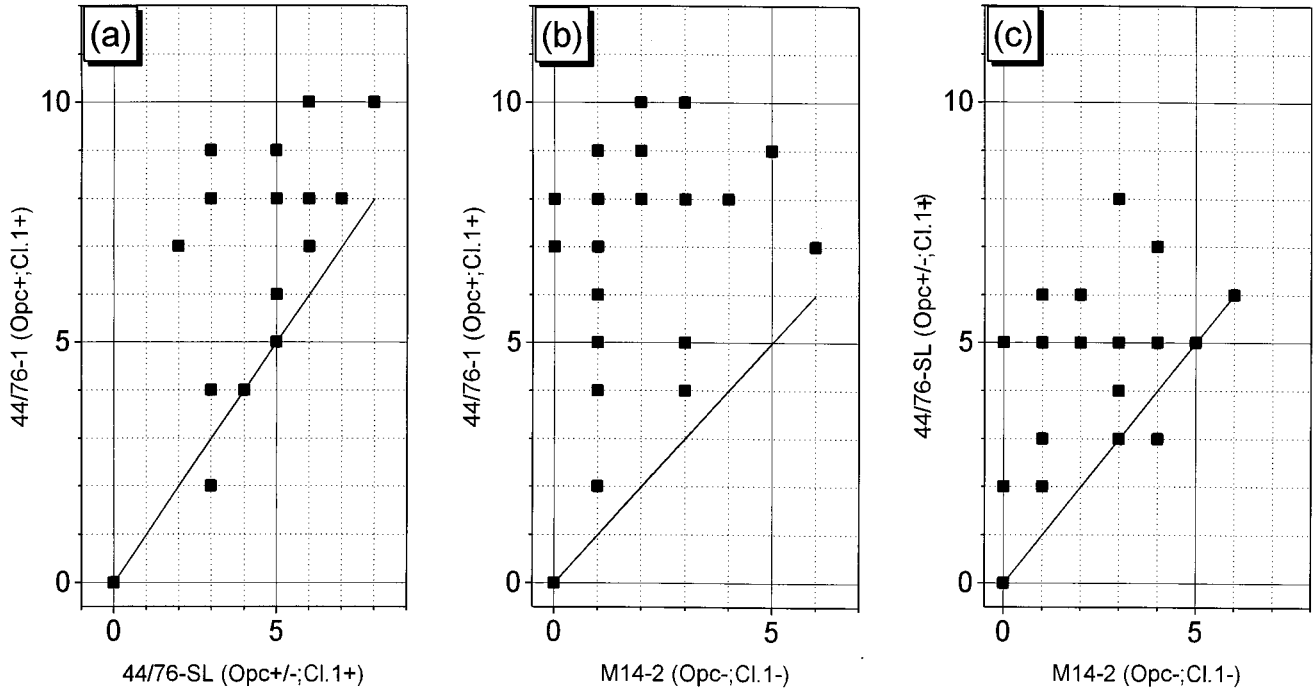

FIG. 2. Comparison of the LBTs of sera from 27 vacinees, collected 6 weeks after three vaccine doses, against different variants of strain 44/76. The relative contributions to SBA titers of Opc-specific antibodies (a), both Opc- and class 1 OMP-specific antibodies (b), and class 1 OMP-specific antibodies (c) are shown. The boxes indicate the paired data points, and the solid 45° lines denote no difference in LBT between strains.

eightfold increase in titer against strain 44/76-1 was observed in one vaccinee 3 days after vaccination (serum 12), and twofold increases were observed in eight individuals, but the GMTs in sera 11 and 12 were not significantly different. BTs reached a peak level 2 weeks after the third dose, with 96% of the vaccinees responding, and then started to decline. The decline was, however, less steep than that after the first two doses, and even 1 year later 74% of the vaccinees had detectable SBA activity against strain M14-2 (Table 4). None of the vaccinees showed significant decreases in titer after vaccination, but one individual did not show detectable SBA titers after any of the three injections. When the vaccinees were divided into category I and II responders, as defined above, differences similar to those seen with OMV ELISA were observed for the bactericidal antibody kinetics (Fig. 1b).

The relative importances of antibodies against the class 1 and Opc proteins in SBA were studied with the variants 44/76-1, 44/76-SL, and M14-2 as target strains. MAb 154,D-11 was bactericidal only to strains that expressed large amounts of Opc (44/76-1) (Table 1). Most sera showed reduced SBA activity with the variants 44/76-SL and M14-2 compared with 44/76-1, and bactericidal activity was completely abolished in some vaccinees (Fig. 2). In sera taken after three doses, a 70 to 80% reduction in GMT was observed with strain 44/76-SL. A similar effect was observed with strain M14-2 (Table 4). Notably, even with the double-negative variant (M14-2), 68% of the vaccinees responded after the third dose, and in five individuals no difference in titers with strains 44/76-SL and M14-2 was detected (Fig. 2c).

Immunoblotting experiments. The percentages of vaccinees showing strong or medium binding of IgG antibodies to the individual vaccine components, as a function of time after vaccination, are shown in Table 5. Four volunteers (15%) had high or medium levels of IgG antibodies against the class 1 OMP before vaccination. Six weeks after the second dose, this number had increased to 13 individuals (50%), and there was a reduction to 10 individuals (37%) 4 to 5 years later. Six weeks after the third injection, 23 individuals (85%) showed high or medium levels of antibodies against the class 1 OMP, with a reduction to 18 individuals (67%) 1 year later.

No vaccinee demonstrated distinct IgG antibodies to the class 3 protein before vaccination, and none responded after the first dose. After the second dose, antibodies against this protein were detected in six individuals (23%). The antibody levels dropped rapidly, and only two (8%) of the vaccinees retained a class 3 OMP band 6 months later. The third dose induced a distinct response against the class 3 protein in 23 (85%) of the vaccinees. Several immunoreactive bands were detected in the class 5 OMP region (25 to 30 kDa), but we did not distinguish between the Opc and Opa reactivities. The number of individuals with a strong binding to the 80-kDa protein increased from 1 before vaccination to 20 (74%) after

TABLE 5. Immunoblot responses against OMP and LPS antigens from meningococcal strain 44/76 after immunization of 27 adults with three vaccine doses

Serum no.[a] (vaccination no.)	% of vaccinees with strong or intermediate IgG binding to:						
	Class 1 OMP	Class 3 OMP	Class 4 OMP	Class 5 OMP[b]	80-kDa protein	70-kDa protein	LPS
1 (1)	15	0	15	12	4	19	4
5 (2)	33	0	15	19	11	26	15
7	50	23	23	35	42	31	46
8	38	8	16	38	27	27	23
11 (3)	37	4	8	26	8	19	11
15	85	85	22	78	74	26	78
16	67	63	30	74	52	30	59

[a] The times when the sera were drawn are given in Tables 2 and 3.
[b] Includes Opa and Opc proteins.

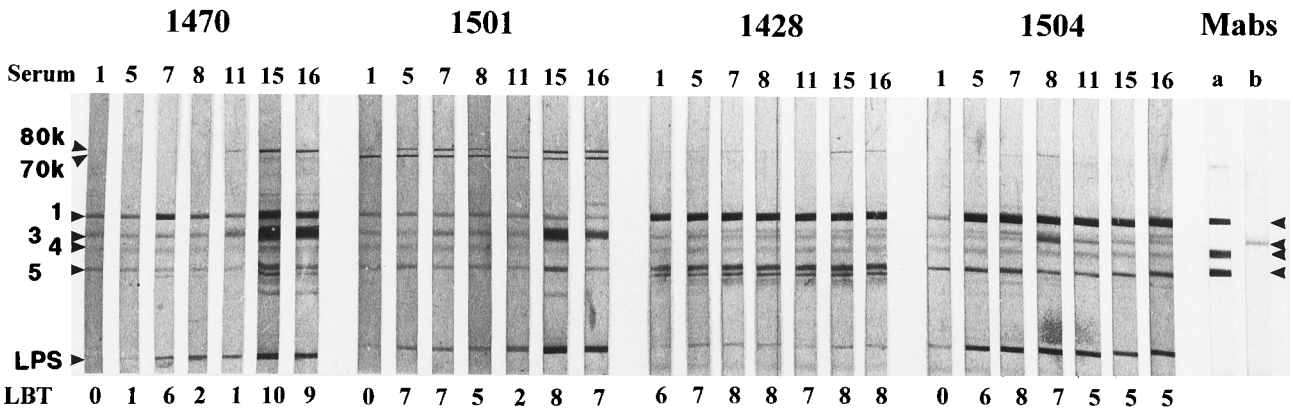

FIG. 3. Immunoblots of prevaccination (serum 1) and postvaccination (sera 5 to 16) sera from four vaccinees after immunization with the Norwegian group B meningococcal vaccine. The lanes show binding of IgG antibodies to outer membrane antigens from strain 44/76 in the absence of the detergent Empigen BB. The LBTs against strain 44/76-1 are shown below the lanes. Vaccinees 1470 and 1501 are category I responders, and vaccinees 1428 and 1504 are category II responders (see text). Lane a, reactions with MAbs specific for class 1, class 4, and Opc proteins; lane b, reaction with a MAb specific for class 3 protein. The last strip was incubated in the presence of 0.25% Empigen BB. 80k and 70k, 80- and 70-kDa proteins, respectively; 1, 3, 4, and 5, class 1, 3, 4, and 5 OMPs, respectively.

the third dose. The number of vaccinees with antibody binding to the class 4 and 70-kDa proteins varied less than that for the other antigens throughout the study period.

Most of the vaccinees responded with antibodies to LPS after three doses, and two immunoreactive bands could be detected in the LPS region. More vaccinees reacted more strongly with the lower- than with the higher-molecular-mass LPS band. The control L8 MAb (2-1-L8) reacted only with the lower-molecular-mass LPS band, and the L3,7,9 MAb reacted only with the upper band.

With most sera, binding of antibodies to the 70- and 80-kDa proteins and class 1, 3, and 4 OMPs did not depend on the presence of the detergent Empigen BB during the incubation. Empigen BB abolished the binding to LPS, whereas it increased the binding to the class 5 proteins.

The vaccinees showed individual reaction patterns on immunoblots, and blots of sequential sera from four selected vaccinees are shown in Fig. 3. The immunoblotting results gave a distinction between category I and II responders similar to that seen with ELISA. Category I responders showed distinct bands mainly after the third dose (vaccinees 1470 and 1501 in Fig. 3), while category II responders generally showed distinct bands after the first dose (vaccinees 1428 and 1504). Eighty-three percent of the category II responders showed distinct immunoreactive bands with their prevaccination sera, whereas 47% of the category I responders demonstrated such reactivity.

The immunoblotting method was also used to analyze the specificities of the anti-class 1 OMP antibodies against the two main variable regions VR1 and VR2 with the P1.7 and P1.16 epitopes, respectively. Sera showing strong binding to the class 1 protein (P1.7,16) of strain 44/76 were tested against strains M1080 and S3032, expressing either the P1.7 epitope (M1080, P1.1,7) or the P1.16 epitope (S3032, P1.12,16). Of the 15 vaccinees with strong binding to the P1.7,16 protein in one or more of their sera, 13 individuals (87%) had antibodies reacting with the class 1 protein in S3032 (P1.16 response), whereas only one (vaccinee 1504 in Fig. 3) reacted with M1080 (P1.7 response); one vaccinee reacted with the class 1 proteins from all three strains.

Statistical relationship between SBA and antibodies to OMVs. The Pearson correlation coefficients (r) between $\log_2$ levels of IgG (units per milliliter against OMVs) and LBTs were calculated at different time points. The r values depended on the strain used in SBA and the time elapsed after vaccination. With data from all time points, the r values for strains 44/76-1 and 44/76-SL were 0.81 and 0.67, respectively. The highest correlation between IgG levels and LBTs ($r = 0.87$) was found for strain 44/76-1 with sera collected after 4 to 5 years (Table 6), whereas the correlation between IgG levels and LBTs against strain M14-2 was low or not significant.

Some prevaccination sera had high levels of antibodies against LPS in ELISA but no bactericidal activity. The correlation between LBTs and levels of IgG against LPS was generally lower ($r = 0.3$) than that between LBTs and levels of IgG against OMVs.

When levels of IgG antibodies against both OMVs and LPS were used as independent variables in LMR analyses, only the level of IgG antibodies against OMVs was a significant positive predictive factor for LBT. When IgG, IgA, and IgM antibodies against OMVs were used as independent variables, only IgG consistently gave significant positive beta coefficients in the regression line, while the contribution from IgM in sera collected at weeks 6 and 12 was negative; for IgA the beta coefficient did not significantly differ from zero.

The relative contributions to bactericidal activity by antibodies to the individual outer membrane components were also studied by LMR analyses, using LBT as the dependent variable and scoring of the immunoreactive bands as predictors. The results depended upon the target strain used in SBA and on the time of serum sampling. Beta coefficients in best-fit regression lines with SBA data from strain 44/76-1 (Opc^{++}) are shown in Table 6. For the prevaccination sera both anti-class 1 and anti-80-kDa protein antibodies gave significant positive contributions to the LBT. In sera 7, 8, and 15, a significant positive contribution was observed with anti-class 5 protein antibodies for strain 44/76-1, whereas for 44/76-SL, anti-class 1 OMP antibodies were found to be the most important. With sera collected 4 to 5 years after the first vaccination (serum 11), the LMR analysis indicated that anti-class 1 protein, anti-80-kDa protein, and anti-LPS antibodies were all significantly positive predictive factors for SBA against strain 44/76-1. After the third dose, anti-class 3 protein antibodies also showed a significantly positive beta coefficient in the regression line. The combination of data from all time points yielded a regression line for which class 1 protein, class 5 protein, 80-kDa protein, and LPS antibodies all were significantly positive predictive factors (Table 6). No significantly negative beta coefficients for any of the single antigens were identified by these analyses.

TABLE 6. MLR analyses relating IgG binding to individual outer membrane antigens on immunoblots to BTs against meningococcal variant strain 44/76-1 and correlations between BTs and IgG anti-OMV level

Serum no.[a] (vaccination no.)	Beta coefficient (SE) in best-fit regression model							Correlation coefficient[b]	
	Class 1 OMP	Class 3 OMP	Class 4 OMP	Class 5 OMP[c]	80-kDa protein	70-kDa protein	LPS	r_1	r_2
1 (1)	0.09 (0.04)	NS[d]	NS	NS	0.20 (0.08)	NS	NS	0.59	0.78
5 (2)	NS	NS	NS	NS	NS	NS	NS	NS	0.64
7	NS	NS	NS	0.20 (0.07)	NS	NS	NS	0.51	0.73
8	NS	NS	NS	0.23 (0.06)	NS	NS	NS	0.60	0.84
11 (3)	0.12 (0.03)	NS	NS	NS	0.14 (0.05)	NS	0.15 (0.06)	0.77	0.87
15	NS	0.08 (0.03)	NS	0.06 (0.03)	NS	NS	NS	0.65	0.73
All sera	0.09 (0.02)	NS	NS	0.07 (0.02)	0.06 (0.02)	NS	0.06 (0.02)	0.72	0.81

[a] The times when the sera were drawn are given in Tables 2 and 3.
[b] r_1, correlation between LBT and best-fit regression line; r_2, correlation between LBT and $\log_2$ IgG anti-OMV level.
[c] Includes Opa and Opc proteins.
[d] NS, not significant ($P > 0.05$).

DISCUSSION

This paper reports the long-term kinetics of serum bactericidal activity and of antibodies to various meningococcal outer membrane components in a group of adults vaccinated three times with the Norwegian OMV vaccine. The study group includes only 27 vaccinees, and the individual variations in immune responses were relatively large. Nonetheless, the results confirm and extend our previous observations with larger groups of vaccinees (29, 58) and give new information about the antibody kinetics and the contributions of individual vaccine components to the bactericidal activity.

The antibody response to vaccination was primarily of the IgG isotype and directed against the OMPs (Tables 2 and 3). In agreement with previous findings (29, 30, 58), antibody levels decreased relatively rapidly after the first two injections. This fact may account for the possible waning in protection in the vaccine trial among teenagers in Norway (7). Six months after the first vaccination (serum 8), only 46 to 67% of the vaccinees demonstrated SBA activity against the three variants of strain 44/76 tested (Table 4). These percentages are in accord with the observed efficacy (57%) after 29 months in the Norwegian protection trial (8). When a third dose was administered 4 to 5 years later, strong booster responses were observed in most of the vaccinees, suggesting that the first two doses had induced immunological memory. A similar booster effect was observed when a third dose was administered about 1 year after the second dose in an immunogenicity study among teenagers in Iceland (48). After the third dose, the antibody levels also declined relatively rapidly; however, the decrease in SBA titers was less steep than that after the first two doses. The reduction was more pronounced for IgG antibodies than for BTs, suggesting that the efficiency of the IgG antibodies in inducing complement-mediated cell lysis increased with time (56). One year after the third dose, 74 to 92% of the vaccinees still had detectable bactericidal activity against all three variants of 44/76, and the GMT against strain 44/76-1 was 3.4-fold higher than it was 6 months after the second dose (Table 4 and Fig. 4). The Norwegian protection trial showed that during the first 10 months after vaccination, only one vaccine failure occurred, and the calculated efficacy after 10 months was 87% ($P = 0.035$) (7, 57). Therefore, if a third dose had been administered after about 10 months, the long-term efficacy of the vaccine would probably have been significantly increased.

On the basis of differences in antibody response patterns, the vaccinees were divided into two responder categories. Vaccinees in category II showed a good immune response after the first two doses and no further increase after the third dose, whereas those in category I reacted more weakly after the first doses but showed a strong booster response after the third dose (Fig. 1). Prevaccination sera from category I vaccinees contained significantly lower levels of IgG antibody to OMVs than those from category II vaccinees; 80% of the vaccinees without SBA activity in their prevaccination sera belonged to category I. This is in agreement with our previous findings (29) and with the results from a study of children in Brazil immunized with the Cuban BC meningococcal vaccine, in which significantly greater increases in BTs were observed in individuals with preexisting antibodies than in those without such antibodies (42). The immunoblotting experiments showed that the majority of the category II responders showed more distinct immunoreactive bands with their prevaccination sera than the category I responders, probably reflecting previous exposure to meningococcal or cross-reacting bacteria. Genetic factors (e.g., HLA class II haplotype) which may also be important for the differences in antibody kinetics have not been investigated.

In order to study the immune response to the individual

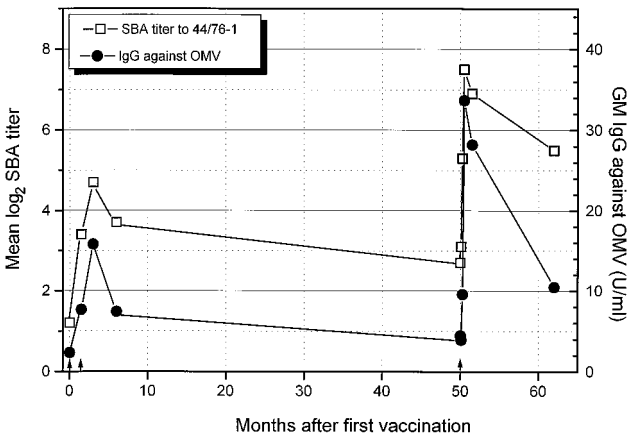

FIG. 4. GM levels of IgG against OMV, and serum LBTs against strain 44/76-1 in 27 vaccinees immunized with three doses of the Norwegian serogroup B OMV vaccine. One unit of IgG to OMV corresponds approximately to 1 μg. Arrows indicate individual vaccinations.

components of the vaccine, both OMVs and LPS were used in ELISA, and the sera were studied by immunoblotting with OMVs as the antigen. The antibodies detected in OMV ELISA were mainly directed against determinants on the OMPs. By detergent treatment, much of the LPS and lipids are stripped from the OMVs, revealing protein epitopes that normally are buried. After two doses, 96% of the vaccinees showed significant increases in the levels of IgG antibodies to OMVs, but only 74% had increases in SBA activity. This suggests that at least some of the antibodies raised were nonproductive in killing activity. We have analyzed the relevance of the antibodies detected in OMV ELISA by calculating correlation coefficients (r) between BTs and levels of the different antibody isotypes at different times after vaccination and further, by LMR analysis. High statistical correlations ($r = 0.7$ to 0.9) between levels of IgG antibody to OMVs and BTs against strain 44/76-1 were consistently observed, and significant positive beta coefficients were found by LMR analysis. Therefore, the levels of IgG antibodies in adults, detected by our OMV ELISA, give a good prediction of bactericidal activity against the vaccine strain, even if only a fraction of the antibodies are bactericidal. Other studies with sera from Norwegian vaccinees have shown similar results (1, 58). On the other hand, no significant correlation between anti-OMV IgG values and LBTs against strain M14-2 was observed, demonstrating the importance of the Opc and class 1 OMPs. A poor correlation ($r = 0.2$) between ELISA IgG values and BTs was also reported from a study of Brazilian children given the Cuban BC meningococcal vaccine (42); in this analysis, however, the correlation coefficient was calculated between fold increases in IgG levels and BTs.

Investigations on the bactericidal activity of serum IgA have led to various conclusions. Initial studies indicated that IgA inhibited complement-mediated bacteriolysis of meningococci (22–24). It was later shown that blocking IgA antibodies were directed against the capsule (34), whereas IgA against OMPs proved to be bactericidal (33). Our analysis did not indicate that high levels of IgA antibodies against OMPs had a blocking effect on bactericidal activity.

In contrast to the anti-OMV response, no booster response was observed in LPS ELISA after the third dose. This is in agreement with the assumption that LPS is a T-cell-independent type 1 antigen and does not induce immunological memory (50). However, on immunoblots, a higher percentage of the vaccinees showed strong IgG binding to LPS after the third dose than after the first two doses (Table 5 and vaccinees 1470 and 1501 in Fig. 3). A possible explanation for this difference may be changes in the specificity or affinity of the anti-LPS antibodies after the third dose, observed as increased binding to LPS on the blots.

Conflicting results have been obtained with respect to bactericidal activity of human antibodies against meningococcal LPS (23, 66). Analysis of the present data showed a poor correlation between bactericidal activity and levels of IgG antibodies against LPS. However, anti-LPS antibodies are opsonizing (71) and may also possibly neutralize the toxic effects mediated by LPS. In addition, LPS has an adjuvant effect and can induce and increase the expression of major histocompatibility complex antigens on antigen-presenting cells (46). LPS is also important for the conformation of porins (45) and the physical stability of vesicles and hence may be an important component in an OMV vaccine.

So far, human antibodies to only two defined OMPs in meningococci (Opc and class 1) have been shown to be bactericidal (59, 82). However, with an experimental B:15:P1.3 OMP vaccine in Chile, Zollinger and Moran (82) could not detect bactericidal activity by vaccine-induced anti-class 1 OMP antibodies; only class 1 OMP antibodies in convalescent sera from patients with meningococcal disease showed such activity. The vaccine used in Chile was rather different from ours; it was not in the form of vesicles, essentially free of LPS, and contained low levels of the class 5 protein (78).

We have tried to analyze the importance to SBA of antibodies against the class 1 and Opc proteins by using defined variants of the vaccine strain 44/76. Although we cannot completely rule out the possibility that the variants differed in properties other than class 1 and Opc proteins, our results indicated a significant contribution of antibodies against these two antigens for the bactericidal activity (Table 4 and Fig. 2) and agree with previous observations (30, 32, 61, 74). However, antigens other than the class 1 and Opc OMPs must also be important for SBA activity, since 68% of the vaccinees responded against the variant strain without these two antigens (M14-2) after the third dose. For some sera, we did not observe any difference in titers between strain 44/76-SL and M14-2 (Fig. 2c). These sera showed distinct immunoreactive bands for the 80-kDa protein, the class 3 and class 5 OMPs, and LPS antigens, as well as other weaker, undefined bands, but showed no distinct bands for the class 1 OMP (e.g., vaccinee 1501 [Fig. 3]). After two doses, only 8 to 17% of the vaccinees responded in SBA against strain M14-2. Therefore, after three doses, the relative importance of class 1 OMP-specific antibodies is probably reduced, and cross-reactivity against heterologous meningococcal strains should be expected to be higher than after two doses. Other experiments with sera from vaccinees who received three doses of the Norwegian vaccine in the vaccine trial in Iceland (48) showed that 50% of the subjects were SBA responders against a systemic serogroup A strain from Mali which lacked Opc and had a different class 1 protein (A:4/21:P1.9:L3,7) (59).

We used LMR analysis in an attempt to determine the relative importances of antibodies against the individual vaccine components for SBA activity, acknowledging that such analyses should be interpreted cautiously. Fifty percent of the vaccinees responded distinctly in immunoblots against the class 1 OMP after two doses, and 85% responded after three doses, demonstrating this to be a major immunogenic vaccine component. The regression analysis gave positive beta coefficients for class 1 protein antibodies and supported the conclusion from the SBA studies with the variants of strain 44/76. Blotting experiments with P1.7,1 and P1.12,16 class 1 proteins demonstrated that the specificity of the class 1 antibodies was directed mainly against the P1.16 epitope. This result agrees with previous observations (11, 32, 54, 69, 72) and indicates that the VR2 loop in the P1.7,16 class 1 OMP is more immunogenic than the VR1 loop.

None of the vaccinees showed a response to the class 3 protein in immunoblots after the first dose. Since the amounts of class 3 and class 1 proteins in the vaccine are similar (17), this indicates that the class 3 porin may be less immunogenic than the class 1 porin in this OMV preparation. However, after the third dose, the majority of the vaccinees responded distinctly against the class 3 protein. These IgG antibodies reacted with denatured class 3 protein and have been shown to be directed mainly against a linear epitope in the VR1 region of the serotype 15 molecule (12). In a study of convalescent-phase sera from patients surviving systemic meningococcal disease, using purified class 1 and 3 porins as antigens in ELISA, a higher-level IgG response against the class 3 protein than against the class 1 protein was observed (25). Possible explanations for this difference between vaccinees and patients may be the amount of or mode of presenting the antigen or that the

majority of the antibodies after one and two doses were directed against conformational epitopes which were not detectable in immunoblots. The biological significance of human anti-class 3 protein antibodies remains uncertain. Murine MAbs against class 3 protein are only occasionally bactericidal or are bactericidal only under certain conditions (43, 63, 64). Six weeks after three doses, 85% of the vaccinees developed high or intermediate levels of IgG antibodies to the class 3 OMP (Table 5), whereas only 36% responded significantly in SBA against M14-2 (lacking the class 1 protein and Opc), indicating that at least a fraction of the anti-class 3 OMP antibodies were not bactericidal. Guttormsen et al. (26) found that affinity-purified anti-class 3 OMP antibodies from patients were bactericidal and opsonic. The LMR analyses also indicated that such antibodies contributed significantly to the bactericidal activity in sera collected after the third injection (Table 6).

The Norwegian vaccine contains both the Opc and the P5.5 antigens (17, 61). Whereas the importance of Opc antibodies has been clearly demonstrated (61; this study), the significance of antibodies to P5.5 in humans remains less clear. Sacchi et al. (62) found the murine P5.5 MAb to be bactericidal but detected no difference in SBA titers of murine sera whether the antigen was present or not in a vaccine.

Most vaccinees also reacted with antibodies against the 80-kDa protein on blots, although this component was present in only small amounts in the vaccine (16). The LMR analysis (Table 6) indicated a positive contribution to the bactericidal activity from IgG antibodies against the 80-kDa protein. At present, the nature of the 80-kDa antigen is not clear; it does not react with antibodies against transferrin-binding protein 1 or 2 (4, 5) or with MAbs against any other defined proteins tried by us (16).

The 70-kDa protein, which is present in the vaccine in about the same amount as the 80-kDa protein, showed a low level of immunogenicity. Previous studies (16) showed that this protein reacted with a MAb specific for the iron-regulated protein FrpB (3), so it was concluded that this is either the same or a closely related protein. Although rabbit polyclonal antibodies (3) and some murine MAbs (3, 49) against FrpB were bactericidal, our study showed no correlation between the BTs and antibodies against the 70-kDa protein on immunoblots.

The class 4 protein shows considerable homology with the equivalent protein PIII in gonococci (38). Antibodies against PIII have the ability to block the bactericidal effect of antibodies directed against other surface antigens (52). A similar effect was observed with a murine MAb against the class 4 protein (44), and this protein has therefore been suspected to induce blocking antibodies in humans (21). Only a few vaccinees responded against the class 4 protein after vaccination with the Norwegian OMV vaccine. In those who responded, no decrease in SBA titers was observed after vaccination and no negative correlation between LBTs and anti-class 4 OMP antibodies was found. This is in agreement with the observation of Zollinger et al. (79) that the presence or absence of the class 4 OMP in a vaccine had no effect on the bactericidal antibodies induced.

In conclusion, since the vaccine contains many antigens and there is extensive heterogeneity in the human response, it seems likely that bactericidal antibodies may be induced by several antigens. This study has shown that a third dose of the Norwegian group B OMV vaccine is probably advantageous, since almost all subjects developed bactericidal antibodies and the SBA activity became relatively less serosubtype dependent.

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