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Immunogenicity of a meningococcal native outer membrane vesicle vaccine with attenuated endotoxin and over-expressed factor H binding protein in infant rhesus monkeys

Oliver Koeberling[1], **Anja Seubert**[1], **George Santos**[2], **Annalisa Colaprico**[1], **Mildred Ugozzoli**[2], **John Donnelly**[2], and **Dan M. Granoff**[3,*]

[1]Novartis Vaccines, Siena, Italy

[2]Novartis Vaccines, Cambridge Massachusetts, USA

[3]Center for Immunobiology and Vaccine Development, Children's Hospital Oakland Research Institute, Oakland, CA 94609

Abstract

We previously investigated immunogenicity of meningococcal native outer membrane vesicle (NOMV) vaccines prepared from recombinant strains with attenuated endotoxin (ΔLpxL1) and over-expressed factor H binding protein (fHbp) in a mouse model. The vaccines elicited broad serum bactericidal antibody responses. While human toll-like receptor 4 (TLR-4) is mainly stimulated by wildtype meningococcal endotoxin, mouse TLR-4 is stimulated by both the wildtype and mutant endotoxin. An adjuvant effect in mice of the mutant endotoxin would be expected to be much less in humans, and may have contributed to the broad mouse bactericidal responses. Here we show that as previously reported for humans, rhesus primate peripheral blood mononuclear cells incubated with a NOMV vaccine from ΔLpxL1 recombinant strains had lower proinflammatory cytokine responses than with a control wildtype NOMV vaccine. The cytokine responses to the mutant vaccine were similar to those elicited by a detergent-treated, wildtype outer membrane vesicle vaccine that had been safely administered to humans. Monkeys (N=4) were immunized beginning at ages 2 to 3 months with three doses of a NOMV vaccine prepared from ΔLpxL1 recombinant strains with over-expressed fHbp in the variant 1 and 2 groups. The mutant NOMV vaccine elicited serum bactericidal titers ≥1:4 against all 10 genetically diverse strains tested, including 9 with heterologous PorA to those in the vaccine. Negative-control animals had serum bactericidal titers <1:4. Thus, the mutant NOMV vaccine elicited broadly protective serum antibodies in a non-human infant primate model that is more relevant for predicting human antibody responses than mice.

Keywords

Neisseria meningitidis; fHbp; OMV; adjuvants

*Corresponding author, DMG, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland CA USA 94609. Telephone, (+1) 510 450 7640 (voice) and 510 450 7915 (fax). dgranoff@chori.org .

1. Introduction

Meningococcal outer membrane vesicle vaccines that have been extracted with detergents (dOMV) to decrease endotoxin activity are safe and effective for prevention of meningococcal disease caused by capsular group B strains (Reviewed in [1-3]. Their major limitation is induction of strain-specific serum bactericidal antibodies, particularly in infants [4], who are in the age group at highest risk of developing meningococcal disease [5, 6]. The strain-specificity is largely because the serum bactericidal antibody responses are directed primarily against the major porin protein PorA, which is antigenically variable. To broaden protective antibody responses, we prepared native OMV (NOMV) vaccines from genetically engineered strains that were not detergent treated [7, 8]. To decrease endotoxin activity, we deleted the *lpxL1* gene, which encodes an acyl-transferase that is involved in lipooligosaccharide (LOS) biosynthesis. In earlier studies, the resultant mutant LOS had been shown to have penta-acylated instead of hexa-acylated lipid A, and to have attenuated endotoxin activity [9-11]. Native outer membrane vesicle vaccines (NOMV) prepared from ΔLpxL1 recombinant strains also had decreased endotoxin activity as measured by decreased stimulation of human peripheral blood mononuclear cells (PBMC) to release proinflammatory cytokines [12-16]. To increase breadth of protective antibodies, the vaccine strains were engineered to over-express fHbp [12, 13]. Mice immunized with NOMV vaccines prepared from these genetically engineered strains developed broadly protective serum antibody responses against genetically diverse meningococcal strains with heterologous PorA proteins.

Meningococcal LOS has potent adjuvant activity from stimulation of Toll-like receptor 4 (TLR-4) [17], which activates cytokine release and maturation of dendritic cells that are required for robust immune responses [18, 19]. Studies of lipopolysaccharides from other Gram negative bacteria found human-specific TLR-4/MD-2 recognition of hexa-acylated lipid A whereas mouse TLR-4/MD-2 recognized tetra-, penta- and hexa-acylated forms of lipid A [17, 20, 21]. Similarly, Steeghs et al reported that bone marrow-derived dendritic cells from mice were activated by both wildtype meningococcal hexa-acylated and mutant penta-acylated LOS [9]. In contrast, dendritic cells from humans were activated primarily by the wildtype meningococcal hexa-acylated LOS. The attenuation in the human cells provided the rationale for development of NOMV vaccines from penta-acylated lipid A mutants as a way of avoiding the need of detergent treatment of NOMV vaccines to decrease endotoxin activity [22]. The broad protective antibody responses of mice immunized with NOMV vaccines prepared from mutant strains with penta-acylated LOS, however, may have resulted, in part, from a strong adjuvant effect of the LOS, which would be expected to be much lower in humans.

In this study we investigated the immunogenicity in an infant primate model of a NOMV vaccine prepared from strains engineered to express penta-acylated LOS and to over-express fHbp. Our hypothesis was that the adjuvant effects and resulting immunogenicity of vaccines containing penta-acylated LOS in infant primates would more closely mimic human responses than those in the mouse model.

2. Material and Methods

2.1. Vaccines

The vaccines used in this study are described in table 1. For immunization of the infant primates we prepared NOMV from two recombinant strains, which were constructed using methods previously described [12, 13]. One of the NOMV vaccines (designated NOMV 1) was a prepared from the same mutant of group B strain H44/76 used in our previous mouse studies [12, 13, 23]. To prepare this recombinant vaccine strain we had deleted the *lpxL1*

gene to attenuate endotoxin activity of the LOS [9, 10], and had engineered the strain to over-express fHbp variant 1 (ID 1) using a multicopy plasmid [7]. This recombinant strain was designated H44/76 ΔLpxL1ΔfHbp pFP12-fHbp v.1 (Table 1). The NOMV 1 vaccine derived from this mutant expressed approximately 10-fold higher amounts of fHbp than that from the parent H44/76 wildtype strain [23]. The second NOMV vaccine (designated NOMV 2) was prepared from a new mutant of group B strain NZ98/254. To prepare this recombinant strain, we deleted the *lpxL1* and *fHbp* genes and engineered the recombinant strain to over-express fHbp variant 2 (ID 77) using an expression vector, pComP1523, as previously described [12]. The resulting mutant was designated NZ98/254 ΔLpxL1ΔfHbp pComP1523-fHbp v.2 (Table 1). By Western blot (Figure 1), NOMV 2 contained approximately 5-fold higher amounts of fHbp v.2 (ID 77) than an NOMV vaccine (referred to as NOMV3con, Table 1) that had been used in a previous mouse immunogenicity study, and which also had low levels of endogenous fHbp variant 1 (ID 14) expression [12].

The two fHbp amino acid sequence variants, ID 1 (variant 1) and ID 77 (variant 2), were selected for over-expression based on previous data that mice immunized with NOMV vaccines that over-expressed these variants developed broadly protective antibody responses [8, 12, 24]. Further, a chimeric recombinant fHbp vaccine that contained the N-terminal portion of fHbp ID 1 and the C-terminal portion of fHbp ID 77 elicited bactericidal antibody responses against genetically diverse strains with fHbp variants 1, 2 or 3 [25].

The following additional control (con) vaccines were used in the present study to measure fHbp expression by Western blot, or for stimulation of proinflammatory cytokines by rhesus peripheral blood mononuclear cells (PBMC). NOMV 4con and dOMV 4con (detergent-extracted) vaccines were both prepared from the wildtype NZ98/254 strain, and NOMV 5con was prepared from a ΔLpxL1 mutant of the wildtype strain. dOMV 4con was from a clinical lot of a vaccine that had been licensed and used extensively in New Zealand [3].

2.2. Preparation of NOMV

The NOMV vaccines consisted of native outer membrane blebs that were spontaneously released into the culture supernatant during growth of bacteria. In brief, the bacteria were grown to early stationary phase (OD 1.0 to 1.2 after 6 hours of growth); phenol 0.5 % (w/v) was added and left overnight at 4°C to inactivate the bacteria, which is a procedure used previously during preparation of OMV vaccines [14, 26]. After centrifugation for 10 minutes at 7500 × g, the culture supernatant was filtered (pore size 0.45 μm) and concentrated by filtration through a membrane with 100 kDa cut off. NOMVs from the concentrated culture supernatant were collected by ultracentrifugation. The isolated blebs were washed twice with 3% sucrose, resuspended in 3% sucrose, and stored at − 20 °C. Protein concentration was measured with the BCA protein assay Kit (Thermo Scientific, Rockford, IL), and lipooligosaccharide (LOS) content was assessed by SDS PAGE and silver staining [13].

Vaccine formulation—Each 0.5 mL dose of vaccine contained 25 μg NOMV (12.5 μg NOMV 1 and NOMV 2) based on total protein. The amount of total LOS per dose was approximately 10 μg, based on SDS-PAGE and silver stain of LOS (data not shown). The two NOMVs were adsorbed separately to aluminum hydroxide (3 mg/mL final concentration) in the presence of histidine (10 mM) and NaCl (9 mg/mL) in sterile water for injection. To prepare the final formulation, equal volumes of each of the adsorbed NOMV vaccines were mixed. A fresh formulation was prepared one to two weeks prior to immunization and was stored at 4 °C until use.

2.3. Cytokine assays

Rhesus primate blood was obtained from healthy animals from the National Research Council, Institute of Neurobiology and Molecular Medicine, Rome, Italy. PBMCs were prepared and cytokine release was measured after 4 hour incubation with OMV preparations as described previously [12, 13]. Cytokines released were analyzed with the Milliplex non-human primate cytokine Kit (Millipore). Cytokines assayed were IL-1 beta, IL-6, IL-8 and TNF-alpha.

2.4. Immunogenicity

The immunization studies were performed at the California National Primate Research Centre, University of California, Davis under a protocol approved by the Institutional Animal Care and Use Committees. Animals were 2-3 months old at the time of the first immunization. The infants lived in an outdoor social housing with their dams and extended families for the duration of the study and were weaned naturally. The blood samples were taken from hand-restrained infants via femoral venipuncture. Immunizations were administered following the blood draws, and were given via intramuscular injection into the quadriceps muscle in the leg.

Four animals were immunized with the NOMV vaccine. Two doses were given intramuscularly (IM) separated by one month (day 0 and week 4) with a booster IM dose given at week 18. Six unimmunized animals of similar ages as the vaccinated animals served as negative controls. Blood was drawn on day 0, week 8 and week 20. Sera were separated and stored at −80 °C until use.

2.5. Serological analysis

Assays were performed on individual sera obtained 2 weeks after the booster dose (week 20). The sera were heat-inactivated at 56°C for 30 min to inactivate internal complement. IgG antibody responses to fHbp v.1, 2 and 3 were measured by ELISA, which was performed as described elsewhere [13]. The antigens on the plate consisted of 1 μg/mL of purified His-tagged recombinant fHbp ID 1 (variant 1), ID 77 (variant 2) or ID 28 (variant 3). Secondary antibody was alkaline phosphatase conjugated goat anti-monkey IgG, gamma chain (Rockland Immunochemicals, Gilbertsville, PA). Complement mediated bactericidal antibody activity was measured in serum using 2-fold serial dilutions starting at 1:4. The assay used had been standardized to measure bactericidal titers of sera from human clinical trials [27]. In brief, the bacteria were grown to mid-log phase in Mueller Hinton Broth supplemented with 0.25 % glucose, and diluted in Dulbecco's PBS buffer (Mediatech), which contained 0.1 g/L $CaCl_2$, 0.1 g/L $MgCl_2 \times 6\ H_2O$,1% BSA and 0.1% glucose. The final 40 μL reaction mixture contained 25% human complement, consisting of serum from healthy adults who had been screened for lack of intrinsic bactericidal activity. The bactericidal titer was defined as the reciprocal serum dilution resulting in 50% killing of bacteria after incubation for 60 min at 37°C.

2.6. Neisseria meningitidis test strains

Ten genetically diverse *Neisseria meningitidis* strains were used to measure serum bactericidal activity (Table 2). Strain NZ98/254 expressed a homologous PorA to one of the mutant strains used to prepare the NOMV vaccine. The other nine test strains expressed PorA proteins with heterologous VR types. The ten test strains expressed fHbp amino acid sequence variants belonging to antigenic variant 1 (N=3), 2 (N=4) or 3 (N=3). The PorA VR types, fHbp sequence IDs of and LOS immunotypes of each the strains are provided in Table 2.

2.7. LOS immunotype

The LOS immunotype of the *N. meningitidis* test strains was determined by whole cell ELISA using anti-LOS monoclonal antibodies (See legend to Table 2), which was performed as described previously [23].

3. Results

3.1. A NOMV vaccine from a ΔLpxL1 recombinant strain has decreased ability to stimulate cytokine release by monkey PBMCs

In previous studies, human PBMCs were less responsive in terms of cytokine release when stimulated with purified meningococcal endotoxin or NOMV vaccines prepared from mutants with penta-acylated LOS than with wildtype hexa-acylated LOS [9, 10, 12, 13, 16]. In contrast, mouse PBMCs were reported to show similar stimulation by the respective wild-type and mutant LOS preparations [22]. Stimulation of monkey PBMCs by NOMV from wild-type or a ΔLpxL1 recombinant strains was unknown. Therefore, we incubated PBMC from rhesus monkeys with a control NOMV vaccine from NZ98/254 wild-type (NOMV 4con) or the recombinant strain NZ98/254, ΔLpxL1, ΔfHbp, pComP1523 fHbp v.2 (NOMV 2) and measured concentration-dependent release of pro-inflammatory cytokines IL-1 beta, IL-6, IL-8 and TNF alpha. Fifty to more than 500-fold higher concentrations of the NOMV vaccine from the ΔLpxL1 recombinant strain were required to induce release of the four cytokines than of the NOMV from the wild-type strain (Figure 2). Further, the concentration of mutant NOMV required for cytokine stimulation was similar or lower than that required for stimulation by a control clinical lot of a detergent-extracted OMV vaccine (dOMV 4con) prepared from the corresponding wild-type strain. The respective results with the monkey PBMCs were similar to those we reported previously from cytokine stimulation experiments with human PBMCs. [12, 13].

3.2. The NOMV vaccine from recombinant strains elicits serum IgG antibody responses against fHbp v. 1, 2 or 3

The animals immunized with the NOMV vaccine prepared from the recombinant strains with fHbp variant 1 and 2 (NOMV 1 + NOMV 2) developed serum IgG anti-fHbp antibody responses (Figure 3). The reciprocal GMTs measured by ELISA were 29,000, 5700 and 5600, respectively, against fHbp variant 1, 2 or 3. In contrast, the unimmunized negative control animals had undetectable serum IgG anti-fHbp antibody responses (reciprocal GMT <50).

3.3. The NOMV vaccine from recombinant strains elicits broad serum bactericidal antibody responses

The serum bactericidal antibody responses against the ten test strains are summarized in Figure 4. One of the strains, NZ98/254, was the parent of one of the recombinant strains used to prepare the vaccine. The other nine test strains were unrelated to the vaccine strains and had heterologous PorA VR types. None of the test strains expressed fHbp variants with identical sequences to the fHbp variants in the mutant vaccine strains (ID 1 and 77). For nine of the strains, all four animals given the NOMV vaccine had serum bactericidal titers ≥1:4, which in humans predict protection against disease [2, 28, 29]. Against the tenth strain (M4287) three out of four animals developed titers ≥1:4. The reciprocal bactericidal GMTs were 67 to 121 for test strains expressing fHbp variant 1, 15 to 188 for the strains with fHbp variant 2, and 16 to 128 for strains with fHbp variant 3. There was no evidence of natural acquisition of serum bactericidal activity against the test strains by the six negative control animals (reciprocal titers <4),

4. Discussion

In previous studies, we found that mice immunized with meningococcal NOMV vaccines prepared from ΔLpxL1 recombinant strains with penta-acylated LOS and over-expressed fHbp developed broad serum bactericidal antibody responses [12, 13]. As described in the introduction, mouse bone marrow derived dendritic cells, however, were reported to be stimulated by the penta-acylated LOS in the NOMV [9], whereas human dendritic cells showed markedly decreased responses to penta-acylated LOS compared to the wild-type hexa-acylated LOS [22]. This species-specific activation of the mouse TLR-4/MD-2 complex by the penta-acylated LOS in the NOMV vaccine [22] limited interpretation of the immunogenicity results since the resulting cytokine responses in the mice likely exerted a potent adjuvant effect that would be much less in humans.

In the present study, we investigated the immunogenicity of an investigational NOMV vaccine in a non-human primate model whose immune responses to the vaccine are likely to be more predictive of antibody responses of humans. We first demonstrated that the cytokine responses of rhesus PBMCs stimulated by the mutant NOMV vaccine with penta-acylated LOS were much lower than those to the wild-type NOMV vaccine with hexa-acylated LOS. Further, the cytokine responses to the mutant vaccine were similar to those elicited by a wildtype dOMV vaccine that had been safely administered to humans. These results were similar to those obtained in previous studies with stimulation of human PBMC by these vaccines [12, 13]. Thus, the cytokine stimulation results with the non-human primate PBMCs indicated that in immunized monkeys the adjuvant effect of the penta-acylated LOS in the mutant NOMV vaccine was likely to be similar to that in humans.

This NOMV vaccine with increased expression of fHbp elicited broadly protective serum bactericidal antibody responses in the infant primates. These results were obtained with NOMV vaccines prepared from mutants containing antibiotic resistance genes and in one case a plasmid for over-expression of fHbp variant 1. The results provide the rationale for constructing additional recombinant strains devoid of antibiotic resistance genes and where fHbp expression is exclusively encoded by genes integrated into the chromosome, which are approaches more suitable for mutant strains intended for commercial production of an NOMV vaccine for humans.

The ten test strains used for measuring serum bactericidal antibody responses included fHbp variants in each of the three antigenic variant groups. Only one of the strains, NZ98/254, expressed the homologous PorA to one of the NOMVs (NOMV 2) included in the vaccine. We did not measure bactericidal activity against the second homologous vaccine strain H44/76 because of limited amounts of sera and because in previous studies this strain was highly susceptible to bactericidal activity of antibodies directed at either the homologous PorA and/or fHbp ID 1 [8, 12, 13].

One limitation of the present study was the small number (N=4) of animals immunized with the NOMV vaccine from the recombinant strains. However, with only a few exceptions, the respective antibody responses of the individual animals were similar when measured against the different strains, and there was no evidence of natural acquisition of antibody by the six negative control animals. Thus, despite the small sample size of animals immunized with the NOMV vaccine, the immunogenicity results provided proof of concept of the potential of a mutant NOMV vaccine with attenuated endotoxin and over-expressed fHbp to elicit broadly protective serum antibody responses.

Because of the large number of assays performed on individual sera from the infant primates, there were insufficient volumes of sera remaining to investigate directly the target of the bactericidal antibodies. Several lines of evidence from previous studies in immunized

mice indicated that serum bactericidal activity was mainly directed at fHbp [13, 23]. First, nearly all of the bactericidal activity of sera of mice immunized with NOMV vaccines with over-expressed fHbp was lost after depletion of anti-fHbp antibodies. Second, sera from mice immunized with control NOMV vaccines prepared from fHbp knock-out mutants strains had low or undetectable serum bactericidal antibody. Third, mixing sera from mice immunized with fHbp knock-out mutants with sera from mice immunized with a recombinant fHbp vaccine did not augment bactericidal activity of serum antibodies against the recombinant fHbp [23]. Therefore, there was no evidence of cooperative bactericidal activity between antibodies directed at fHbp and antibodies directed at other NOMV antigens such as LOS. Based on these mouse data, it is likely that the primary antigenic target of the serum bactericidal antibody responses of the monkeys immunized in the present study with the mutant NOMV vaccine was fHbp.

In a recent phase 1 study, the main serum bactericidal antigenic target of adults immunized with a different mutant NOMV vaccine was LOS [30]. The results in the humans were similar to those previously reported by the investigators from immunogenicity studies in mice [14]. The vaccine used in these studies was prepared from capsular deficient mutants with attenuated endotoxin activity (ΔLpxL1), enhanced expression of fHbp, stabilized expression of OpcA, and an LOS with a truncated oligosaccharide chain [14, 30]. One likely explanation for the different serum bactericidal antigenic targets in their studies and ours' is much greater over-expression of fHbp in the NOMV vaccine we prepared [23] and, possibly, the use of a mutant with truncated LOS to prepare their NOMV vaccine.

In our primate study it was not feasible to monitor tolerability and/or adverse effects related to vaccination since the animals were maintained with their mothers in an open space during the study. In the phase I clinical study in the adults immunized with an NOMV vaccine with attenuated endotoxin activity from deletion of *lpxL1*, the vaccine was well tolerated and febrile reactions were rare [30].

In conclusion, our data showed that a NOMV vaccine from recombinant strains with deleted *lpxL1* and over-expressed fHbps in the variant 1 and 2 groups elicited broadly protective serum bactericidal antibody responses in the infant primate model. The results are likely to be relevant for predicting immunogenicity of this vaccine in humans and support advancing this vaccine concept to humans.

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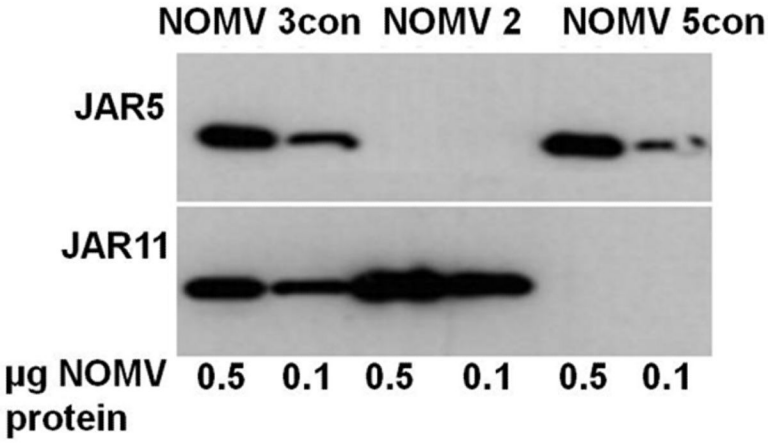

FIGURE 1.

Expression of heterologous fHbp variant 2 in recombinant NZ98/254 strains as measured by Western blot. NOMV 3con: control NOMV from a recombinant strain of NZ98/254 that expressed endogenous fHbp variant 1 (ID 14) and heterologous fHbp variant 2 (ID 77) (used in a previous mouse immunogenicity study)[12]. NOMV 2: NOMV from a recombinant strain of NZ98/254 with deleted endogenous fHbp variant 1 and expressing a heterologous fHbp variant 2 (ID 77). This vaccine was one component of the mutant NOMV vaccine used for immunization of the primates in the present study. NOMV 5con, control NOMV from a recombinant strain NZ98/254 expressing only its endogenous fHbp variant 1. JAR 5 and JAR 11 are murine anti-fHbp monoclonal antibodies specific for fHbp variant 1 and variant 2/3, respectively [36, 37].

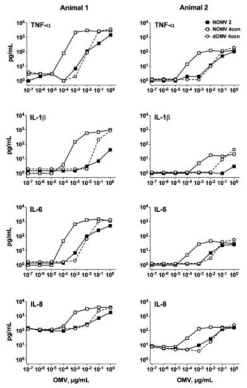

Figure 2.

Release of pro-inflammatory cytokines TNF-alpha, IL-1beta, IL-6 and IL-8 after incubation of rhesus primate PBMCs for four hrs with NOMV or detergent-extracted OMV from NZ98/254. PBMC were prepared from blood of two animals and assayed in parallel. black squares, NOMV 2 from recombinant strain NZ98/254, ΔLpxL1ΔfHbp, pComP1523 fHbp v. 2, which was one component of vaccine used for immunization of infant primates; white squares, NOMV 4con from NZ98/254 wild-type strain; Open circles, dashed line, dOMV 4 con, control clinical lot of a detergent-extracted OMV from NZ98/254 wild-type strain.

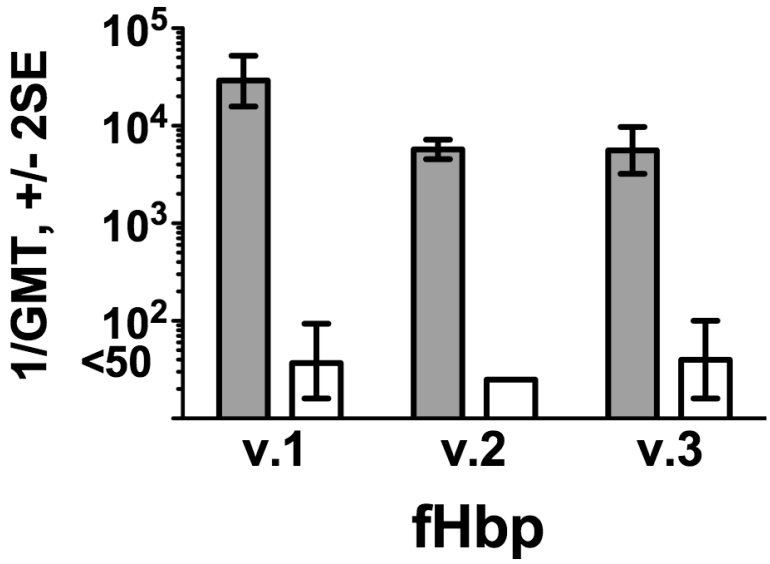

FIGURE 3.

Serum IgG anti fHbp antibody responses of infant primates as measured by ELISA. Gray bars: NOMV vaccine from recombinant strains; white bar: negative control animals. The antigen on the plate was recombinant fHbp variant 1 (ID 1), 2 (ID 77) or 3 (ID 28).

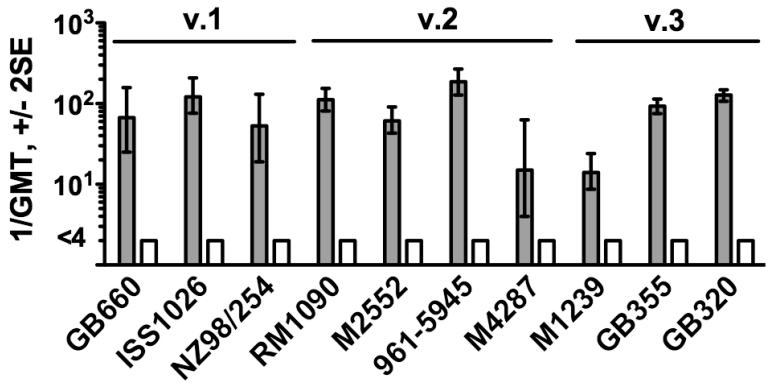

FIGURE 4.

Serum bactericidal antibody responses measured with human complement. Strain designations are shown on x-axis (See Table 2) and fHbp variant (v.) groups are shown above the bars. Gray bars: animals immunized with the NOMV vaccine from the recombinant strains. White bars: negative control animals

TABLE 1

Vaccines used for immunization or in vitro studies

Strain designation	Relevant Characteristics	Vaccine designation/description
H44/76, ΔLpxL1, ΔfHbp, pFP12-fHbp v.1	Derivative of H44/76, deleted *lpxL1;* deleted endogenous *fHbp*; over-expressing fHbp v.1 using a multicopy plasmid encoding fHbp v.1 (ID 1). LOS immunotype L3,7,9	NOMV 1, for immunization (prepared from a mutant used to prepare NOMV vaccines in previous studies [12, 13, 23])
NZ98/254, ΔLpxL1, ΔfHbp, pComP1523 fHbp v.2	Derivative of NZ98/254; deleted *lpxL1*; deleted endogenous *fHbp* v.1; over-expressing *fHbp* v.2 (ID 77) by heterologous integration of the *fHbp* v.2 gene into the chromosome under control of strong promoter from nmb1523. LOS immunotype L3,7,9	NOMV 2, for immunization (prepared from a new mutant)
NZ98/254, ΔLpxL1, pComP1523 fHbp v.2	Derivative of NZ98/254; deleted *lpxL1*; expressing endogenous fHbp v.1; fHbp v.2 (ID 77) integrated into the chromosome under control of strong promoter from nmb1523	NOMV 3con, for in vitro assays (used in a previous mouse immunogenicity study [12]).
NZ98/254	NZ98/254 wildtype	NOMV 4con and dOMV 4con, for in vitro assays
NZ98/254, ΔLpxL1	Derivative of NZ98/254; deleted *lpxL1*	NOMV 5con, for in vitro assays

NOMV, native outer .membrane vesicles (not treated with detergent). dOMV, clinical lot of detergenttreated OMV vaccine

Table 2

Neisseria meningitidis strains used to measure serum bactericidal activity

	fHbp					Genetic Lineage	
Strain[a]	**ID**[b]	**Variant**[c]	**Modular Group**[d]	**Capsule:Serotype:PorA VR type**	**LOS Immunotype**[e]	**ST**	**Clonal Complex**
NZ98/254	14	1	I	B:4:P1.7-2,4	L3,7,9	42	41/44
GB660	15	1	I	B:NT:P1.19,15	L1	1049	269
ISS1026	14	1	I	B:4:P1.13	L1,L3,7,9 and L8[f]	44	41/44
RM1090	22	2	III	C:2a:P1.5-1,2-2	L3,7,9	11	11
M2552	25	2	III	B:NT:P1.18-1,3	L1	103	103
961-5945	16	2	VI	B:2b:P1.21,16	L3,7,9	153	8
M4287	19	2	VI	B:NT:P1.7,1	L3,7,9 and L8[f]	44	41/44
M1239	28	3	II	B:14:P1.23,14	L3,7,9	437	41/44
GB355	31	3	V	B:1:P1.22,14	L3,7,9	213	213
GB320	45	3	V	B:1:P1.22,14	L1	213	213

[a] NZ98/254 has an homologous PorA to the NZ98/254 recombinant strains used to prepare the NOMV vaccine. The other nine strains had PorA proteins with heterologous VR types to both recombinant vaccine strains.

[b] fHbp identification number assigned in the fHbp database http://pubmlst.org/neisseria/fHbp/

[c] fHbp variant as described by Masignani et al [31].

[d] fHbp modular group as described by Beernink and Granoff [32] and Pajon et al [33].

[e] Determined by whole cell ELISA with anti LOS monoclonal antibodies 2-1-L8, 9-2-L379, 17-1-L1 and 9 14-1-L-10.

[f] Strong reactions with more than one anti-LOS mAb on four independent clones from each strain. lgt genes encoding glycosyltransferases involved in biosynthesis of LOS oligosaccharide chains are present at more than one chromosomal loci [34], and isolates with expression of more than one LOS immunotype have been described [35].