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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

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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 2–3 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 $\geq$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.

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 (PBMCs) 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

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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 detergent-treated OMV vaccine.

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 for 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. Materials 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 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 ΔLpxL1 ΔfHbp 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 ΔLpxL1 ΔfHbp pComP1523-fHbp v.2 (Table 1). By Western blot (Fig. 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 (PBMCs). 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

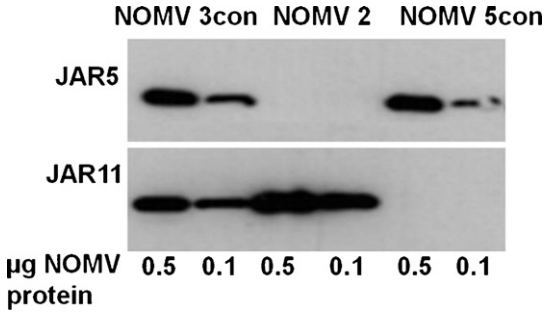

Fig. 1. Expression of heterologous fHbp variant 2 in recombinant NZ98/254 strains as measured by Western blot. NOMV 3con: control NOMV from a recombinant strain of NZ98/254 that expressed endogenous fHbp variant 1 (ID 14) and heterologous fHbp variant 2 (ID 77) (used in a previous mouse immunogenicity 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].