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Improving the immunogenicity of a trivalent *Neisseria meningitidis* native outer membrane vesicle vaccine by genetic modification

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ABSTRACT

Trivalent native outer membrane vesicles (nOMVs) derived from three genetically modified *Neisseria meningitidis* serogroup B strains have been previously evaluated immunologically in mice and rabbits. This nOMV vaccine elicited serum bactericidal activity (SBA) against multiple *N. meningitidis* serogroup B strains as well as strains from serogroups C, Y, W, and X. In this study, we used trivalent nOMVs isolated from the same vaccine strains and evaluated their immunogenicity in an infant Rhesus macaque (IRM) model whose immune responses to the vaccine are likely to be more predictive of the responses in human infants. IRMs were immunized with trivalent nOMV vaccines and sera were evaluated for exogenous human serum complement-dependent SBA (hSBA). Antibody responses to selected hSBA generating antigens contained within the trivalent nOMVs were also measured and we found that antibody titers against factor H binding protein variant 2 (fHbpv2) were very low in the sera from animals immunized with these original nOMV vaccines. To increase the fHbp content in the nOMVs, the vaccine strains were further genetically altered by addition of another fHbp gene copy into the *porB* locus. Trivalent nOMVs from the three new vaccine strains had higher fHbp antigen levels and generated higher anti-fHbp antibody responses in immunized mice and IRMs. As expected, fHbp insertion into the *porB* locus resulted in no PorB expression. Interestingly, higher expression of PorA, an hSBA generating antigen, was observed for all three modified vaccine strains. Compared to the trivalent nOMVs from the original strains, higher PorA levels in the improved nOMVs resulted in higher anti-PorA antibody responses in mice and IRMs. In addition, hSBA titers against other strains with PorA as the only hSBA antigen in common with the vaccine strains also increased.

1. Introduction

Neisseria meningitidis is a Gram-negative diplococcus and an obligate human pathogen. Meningococcal strains can be divided into multiple serogroups based on chemically and antigenically distinct polysaccharide capsule expression. Six capsular polysaccharide serogroups of *N. meningitidis* (A, B, C, W, X, and Y) cause nearly all cases of invasive meningococcal disease [1]. These infections have different clinical consequences including meningitis and septicemia. For serogroups A, C, Y and W, capsular polysaccharide vaccines (such as Menomune® [2]) and capsular polysaccharide conjugate vaccines (such as Menactra® [3]) have been licensed, based on the induction of a functional antibody response only (serum bactericidal activity, SBA) [4]. A polysaccharide approach is not being pursued for serogroup B (MenB) due to poor immunogenicity and potential cross-reactivity between the MenB polysaccharide capsule and human tissue antigens [5–7]. Vaccine development efforts for MenB have therefore focused on non-capsular polysaccharide immunogens, particularly outer membrane proteins either in the form of purified recombinant proteins or outer membrane vesicles (OMVs) which are prepared from outer membrane "blebs" spontaneously produced by the bacteria. Recently, two recombinant protein containing MenB vaccines, Bexsero® and Trumenba®, have been approved [8,9].

The meningococcus has an outer membrane which contains numerous protein types. Some of these proteins are used for further classification of meningococci into serotypes and serosubtypes, based on antigenic differences in their major outer membrane proteins, PorB and PorA, respectively [10,11]. The

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Table 1
Summary of the strain designations, genetic modifications and antigens expressed with each of the vaccine strains.

Vaccine strain designation and antigens expressed by the parent strain	fHbp variant and NadA allele in the parent strain	Short name	Genetic modification and recombinant antigen expression						
			synX deletion	*lpxL1* deletion	*lgtA* deletion	Resultant LOS type	Insertion of 2nd *porA*	Expression of stabilized *opcA*	Increased expression of conserved proteins
44/76 HOPS-LD B:15:P1.7, 16:L3, 7	fHbp1.1	NB1	Yes	Yes	Yes	L8-3	Yes P1.7-1, 1	Yes	Yes NadA and fHbp v.2
8570 HOPS-LG1 B:4:P1.19, 15:L3-5, 7-5	fHbp1.1; NadA allele 1	NB2	Yes	Yes	Yes	L8-5	Yes P1.22, 14	Yes	Yes fHbp v.1 (2 copies)
B16B6 HPS-LG2 B:2a:P1.5, 2:L2	fHbp2.22; NadA allele 2	NB3	Yes	Yes	Yes	L8-2	Yes P1.22-1, 4	No	Yes fHbp v.2 (2 copies)

lipo-oligosaccharide (LOS), embedded in the outer membrane, determines 12 different immunotypes [10,12]. OMV-based vaccines have the advantage of including several outer-membrane proteins in properly folded conformations which might help to elicit a protective immunological response. OMVs may be prepared by known methods comprising a detergent extraction of the bacterial cells, which has the benefit of reducing LOS endotoxin and most of the capsular polysaccharide from the vaccine. However, detergent extraction also reduces or eliminates conserved protective lipoproteins such as factor H binding protein (fHbp) [13]. In children under two years of age, OMV vaccines produced from detergent extraction generate a predominately PorA-specific SBA response and will thus only protect against strains with the same PorA serosubtype [14]. Therefore, vaccines based on extracted OMVs from epidemic outbreak MenB strains were used with success to control local clonal outbreaks [15–18] but did not offer broad protection against heterologous strains with different PorA subtypes. This lack of broad protection restricts the ability of these OMVs to be used as a universal MenB vaccine.

Alternatively, native OMV (nOMV) have been purified from meningococci without detergent extraction [19–21]. These vesicles are not depleted of LOS or lipoproteins and the outer membrane proteins are likely to be present in their native conformation. Zollinger et al. designed a genetically engineered trivalent nOMV vaccine from three parent strains H44/76, 8570 and B16B6 [21] (Table 1). The vaccine was designed to include multiple outer membrane antigens such as PorA, fHbp, NadA, Opc and LOS, each with the capability to induce SBA. The trivalent nOMV vaccine was immunogenic in mice and rabbits, and elicited functional SBA antibody responses against strains from not only MenB, but also other serogroups, due to conserved cross-reactive antigens [21–23]. A small phase I study in healthy adults with a monovalent nOMV vaccine (from strain B2) was also conducted and the vaccine was shown to be safe and immunogenic [24]. However, the trivalent nOMV vaccine has not been tested in either humans or non-human primates.

To enhance safety of the trivalent nOMV vaccine, the *lpxL1* gene was deleted from the vaccine strains, resulting in mutant LOS with penta-acylated lipid A which has attenuated endotoxin activity [21]. Previous studies have shown that mouse dendritic cells were stimulated by the penta-acylated LOS, and the cytokine responses might result in a potent adjuvant effect [25], where human dendritic cells showed marked decreased responses to penta-acylated LOS [26]. The adjuvant effect of penta-acylated LOS in immunized non-human primates were much lower and likely to be similar to that in humans, suggesting that a non-human primate model might be more suitable to predict the antibody responses to these modified nOMV vaccines in humans [27]. Because human infants are the primary target for a MenB vaccine, we sought to evaluate the immunogenicity of this trivalent nOMVs vaccine in an infant non-human primate model. Infant Rhesus macaques (IRMs) were immunized with trivalent nOMV vaccines and sera were evaluated for hSBA and antigen-specific antibody responses. We found that antibody responses against fHbp variant 2 (fHbpv2) were very low, even though the vaccine strain B3 was genetically engineered to increase its expression level [21]. Therefore, we further genetically modified the vaccine strains by adding another fHbp gene copy into the *porB* locus of each vaccine strain to increase the fHbp content in all the three nOMVs. We showed here that genetic modification can further improve the immunogenicity and breadth of the nOMV MenB vaccines.

2. Material and methods

Details on material and methods can be found in Supplemental material section.