

# Vaccines against MenB disease: over-expression of factor H-binding protein (fHbp) in native outer membrane vesicles elicits broader strain-coverage than recombinant fHbp

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## Introduction

Recombinant factor H-binding protein (fHbp) from *Neisseria meningitidis* is included in vaccines for prevention of meningococcal serogroup B disease. Based on amino acid sequences, fHbp can be classified into two sub-families (A and B), or three variant groups (variant 1, 2 and 3).

While there is consensus that anti-fHbp bactericidal activity is sub-family (or variant group) specific and that low fHbp expression can render strains resistant, the extent of cross-protective bactericidal activity against strains with different fHbp sequence variants within a sub-family is controversial. This controversy is addressed in the following study.

Differences in the immune response against fHbp presented as a purified recombinant vaccine antigen or as an over-expressed protein in a native OMV vaccine formulation is also evaluated.

## Materials & Methods

**Bacterial isolates:** Of 50 MenB strains from a low-incidence situation in Norway (2005-06), 32 were fHbp family B. Based on fHbp family B diversity, 12 strains were selected for further study.

**Vaccines:** Recombinant fHbp; seven different sub-family B variants selected.<sup>1</sup> For comparison a native outer membrane vesicle (nOMV) formulation with over-expressed (OE ~12 times) fHbp ID 9 was used (OE-nOMV; KO-nOMV as control).<sup>2</sup> Note that the OE ID 9 was mutated, unable to bind fH (R41S). All vaccines were formulated with Al-hydroxide as adjuvant.

**Immunization and sera:** Four week old female CD-1 mice were immunized with 20µg per dose of the recombinant fHbp and with 2.5µg total protein of the nOMV vaccines; three times with three weeks interval and bled after three more weeks.

**Serum bactericidal activity (hSBA):** Target strains grown to early log-phase in Mueller-Hinton broth (MHB) %0.25% glucose and 0.02mM cytidine monophosphate N-acetylneuraminic acid. Adult serum, lacking bactericidal activity was the exogenous human complement source; depleted of IgG with a protein G column. Bactericidal titers were determined as the reciprocal serum dilution that yielded a 50% decrease in colony-forming units after 60min incubation (relative to that of control wells at 60min).

**Flow cytometry:** Bacteria grown to mid log-phase (OD ~0.6 at 600nm) in MHB%0.25% glucose. After washing, ~10<sup>7</sup> cells/mL incubated with specific sera for one hour at room temperature. After two washes with PBS-BSA the bacteria were incubated with anti-mouse IgG conjugated with Alexa Flour 488. Finally, the samples were inactivated with 0.5% formaldehyde in PBS and analyzed by a BD Fortessa flow cytometer.

**References:** <sup>1</sup>M. Konar *et al.* JID 2013; 208: 627-35. <sup>2</sup>R. Pajón *et al.* PLoS ONE 2013; 8: e66536.

## Results

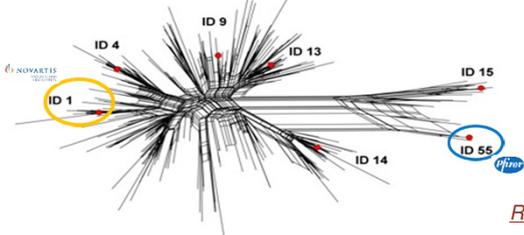
Against most of the strains susceptible to anti-fHbp bactericidal activity, the highest titers were elicited by antisera to the sequence variant that exactly matched the strain and lowest against the most distant (Fig. 2).

As seen in previous studies, when measuring human complement-mediated bactericidal activity, isolates with low fHbp expression are less susceptible to anti-fHbp bactericidal activity. In the present study, among strains with moderate to high fHbp expression, there was heterogeneity in the susceptibility to anti-fHbp bactericidal activity.

**Table 1.**  
 Characteristics for Men B strains presented in Figs. 3 and 4

Strain	Clonal Complex	PorA	PorB	fHbp
N10/05	cc32	7-2, 16	15	1,1
N33/05	cc32	5-13, 10-4	4,7	1,1
N28/06	cc60	5, 2	NT	1,13
N18/05	cc41/44	17, 16-3	14,19	1,13
N02/06	cc41/44	7-2, 4	4,7	1,4
N04/05	UA (ST30)	18-25, 25-7	4,7	1,4

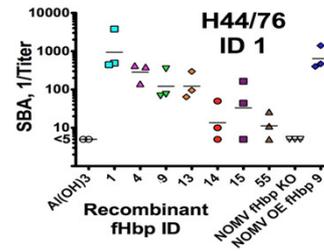
**Figure 1.**  
 "Split-Tree" phylogeny for fHbp sub-family B, with labels indicating the seven IDs selected for mouse immunization (adapted from M. Konar *et al.* JID 2013)



Results (continued)

## Results (continued)

**Figure 2.**  
 hSBA in various mouse serum pools with H44/76\* as target strain



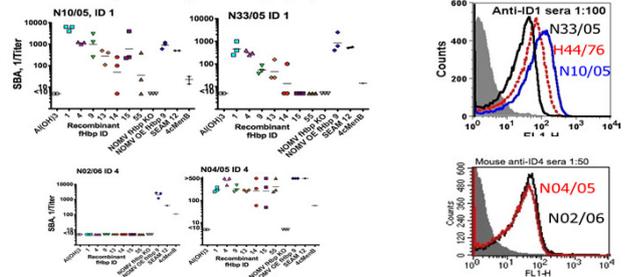
\*Strain H44/76 (ID 1), high fHbp expresser, used as reference.

Among the 12 Norwegian strains chosen to represent fHbp diversity within the sub-family B, three pairs of strains could be grouped as "discordant." Within each pair, the strains had an identical fHbp sequence variant and similar levels of fHbp expression, but different susceptibility to bactericidal activity elicited by anti-fHbp sera. For example, pairs of strains with identical, respective fHbp sequences ID 1, 4 or 14 and moderate to high expression of fHbp, one member of a pair was susceptible to bactericidal activity of antisera elicited by the recombinant fHbp vaccines, while the other was resistant or substantially less susceptible (Fig. 3; data for ID 14 strains not shown).

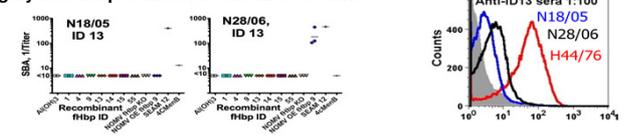
The respective pairs showed similar susceptibility to bactericidal activity of mouse monoclonal antibodies directed against the group B capsule or the matched PorA (not shown). Both members of each of the three pairs were killed by the anti-nOMV-fHbp serum, despite having heterologous PorA to the OE-nOMV-fHbp vaccine (P1.5.2). Estimated amount of fHbp in this formulation was substantially lower (i.e. 2.5µg total nOMV proteins versus 20µg purified recombinant protein). Thus, fHbp being a much stronger immunogen when presented in a native membrane, rather than as a purified recombinant protein.

The ID 13 strains tested were highly resistant to killing with anti-fHbp sera; one strain (N18/05) was even resistant to anti-OE-nOMV antibodies (Fig. 4). Note that N28/06 has identical PorA (P1.5.2) to the two nOMV vaccines.

**Figure 3.**  
 Anti-fHbp-hSBA against pairs of discordant target strains (left) and flow cytometry with the homologous fHbp-serum (right)



**Figure 4.**  
 Highly anti-fHbp-hSBA resistant ID 13 strains



## Conclusions

- Other, as yet unidentified factors, can render meningococcal strains resistant to anti-fHbp-bactericidal activity. Not only mismatched fHbp sequence and low protein expression.

- Strains resistant to anti-fHbp bactericidal activity appear rather common; measurements of antigen sequence and expression only, might overestimate strain-coverage.

- Antibodies elicited by the OE-nOMV-fHbp vaccine showed broader bactericidal reactivity than recombinant fHbp. The use of nOMV technology can in the future offer a more powerful approach to vaccine formulation than recombinant proteins.