Identification of Neisseria surface protein A (NspA) mutants with low affinity for factor H as vaccine candidates against pathogenic Neisseriae

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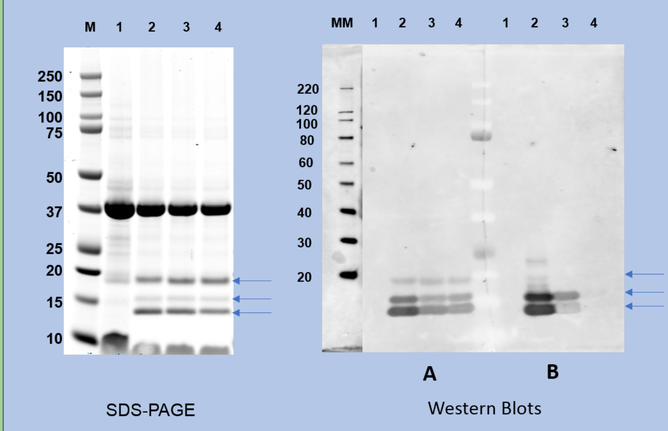
Introduction

- Neisserial surface protein A (NspA) is a small beta-barrel protein that binds to human complement Factor H (hFH).
- NspA is a highly conserved outer membrane protein of *Neisseriae*, present in both meningococcal and gonococcal strains.
- NspA elicits protective antibodies against *N. meningitidis* in mice. However, immunization trials in humans with a vaccine composed of an unfolded, recombinant NspA failed to induce protective serum bactericidal antibody responses against meningococci.
- In our current study, we aim to develop a NspA OMV (outer membrane vesicle) vaccine with low-affinity for hFH as previous studies from our group have shown that binding of hFH decreases NspA immunogenicity.
- Resistance of some *N. meningitidis* clinical isolates to bactericidal activity of anti-Factor H binding protein (FHbp) antibodies is attributed to the binding of hFH to NspA and PorB3.
- Currently licensed meningococcal vaccines containing FHbp have limited protection against strains with no or low expression of FHbp. Hence, inclusion of NspA could lead to better strain coverage and enhancement of protective antibody responses.

Immunization studies in mice

WT CD-1 mice and human FH transgenic mice were immunised with three doses of vaccine containing EOMVs expressing WT or mutant NspA proteins. The control group mice were either immunized with EOMVs not expressing NspA or the adjuvant, Alum. Sera were collected two weeks after the final dose by cardiac puncture.

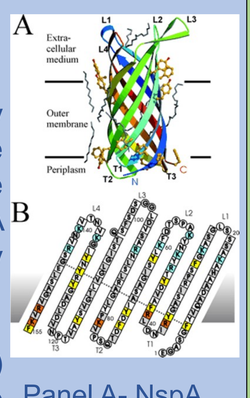


SDS-PAGE and western blot analysis of the extracted EOMVs.
M - Biorad Dual color Prestained protein standard, MM – MagicMark XP Western Protein Standard.
Lane 1 - Negative control OMV, 2-WTNspA OMV, 3- L3-Mt2.1 NspA OMV, 4 - L3-Mt3 NspA OMV. Blot A was detected with mouse polyclonal anti-NspA antibody. Blot B was detected with mouse monoclonal anti-NspA antibody, AL-12. Blue arrows correspond to NspA protein bands

Methods

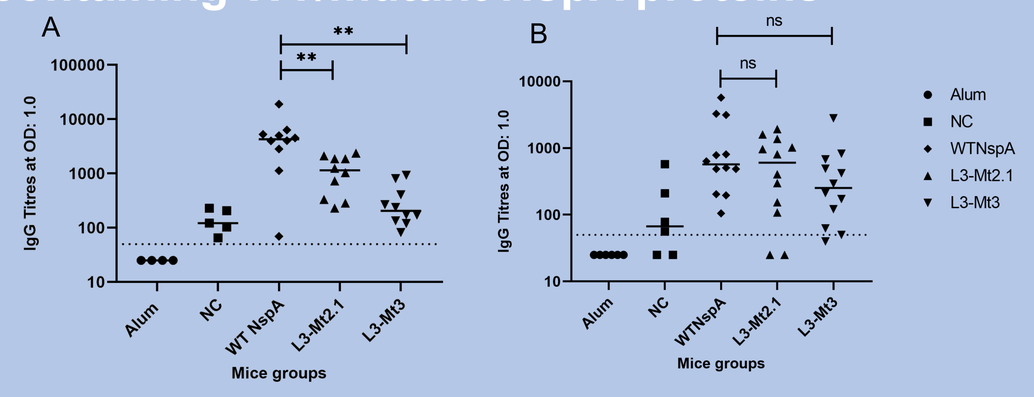
We generated point mutants of recombinant NspA in *Escherichia coli* by replacing residues in the external loop regions 1-4 (see Figure) by inverse PCR. We screened the point mutants for their ability to bind FH by whole bacterial cell ELISA. We also tested the ability of these recombinant NspA mutant strains to bind monoclonal antibodies, AL-12 and 14C7 by Western blot analysis and whole bacterial cell ELISA.

We purified outer membrane vesicles of *E. coli* BL21 (EOMVs) expressing recombinant NspA mutants with lower affinity for hFH. We also extracted EOMVs expressing wild-type (WT) NspA or no NspA as controls. We immunized WT CD-1 mice and hFH transgenic (Tg) mice with EOMVs expressing WT or mutant NspA. We measured anti-NspA IgG antibody titres of the sera from immunised mice by whole bacterial cell ELISA and assessed the functional activity of anti-NspA antibodies by serum bactericidal assay using human complement.



Panel A- NspA protein crystal structure; Panel B – NspA amino acid sequence (Vandeputte-Rutten *et al*., 2003)

IgG antibody titres of mice immunised with EOMVs containing WT/mutant NspA proteins

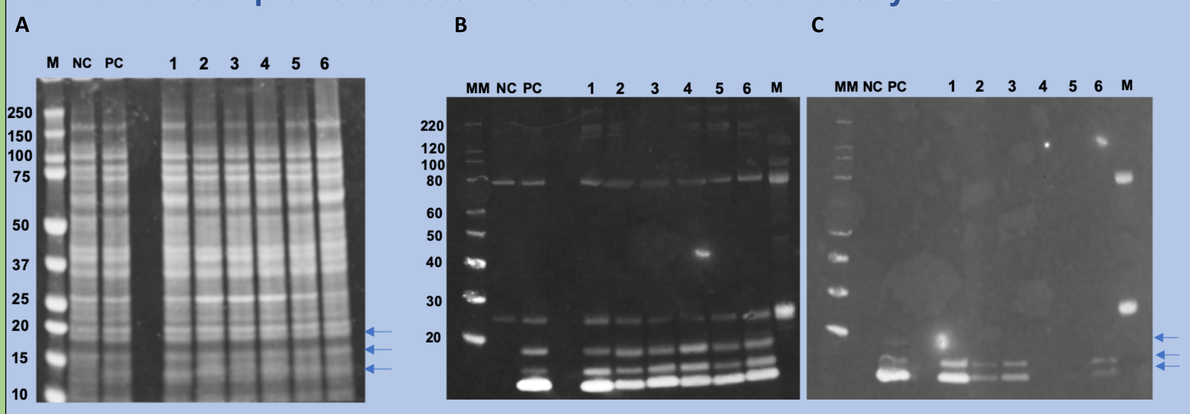


Panel A – IgG titres of sera from WT mice, B - IgG titres of sera from hFH Tg mice

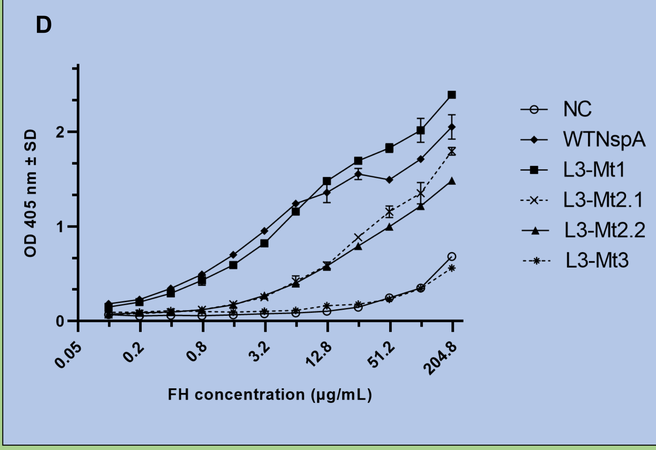
In WT and hFH Tg mice, anti-NspA IgG responses were higher in test groups than control mice immunised with aluminum hydroxide (alum) or negative control (NC) EOMV. In WT mice, IgG titres were highest in sera from mice immunised with WTNspA. Antibody titres are generally lower in hFH Tg mice due to the binding of FH to NspA. Interestingly, in hFH Tg mice, IgG titres against L3-Mt2.1 were similar to those to WTNspA. IgG titres against L3-Mt3 were lower in hFH Tg mice implying the importance of this residue in antibody generation against NspA.

Results

NspA mutants with modified residues in external loop 3 show decreased affinity for human complement Factor H and monoclonal antibody AL-12



Panel A – SDS-PAGE analysis of whole cell lysates of *E. coli* expressing WT and mutant NspA proteins; B and C – Western blots of whole cell lysates of *E. coli* expressing WT and mutant NspA proteins detected with polyclonal anti-NspA and monoclonal AL-12 Abs respectively; D – Whole cell ELISA to test binding of WT and mutant NspA proteins to hFH. M –Precision Plus Protein Standard, MM – MagicMark XP Western Protein Standard, NC – Negative control, PC – Positive control (WTNspA)
Lanes, 1 - L3-Mt1, 2 - L3-Mt2.1, 3 - L3-Mt2.2, 4 - L3-Mt3, 5 - L3-Mt1-2.1, 6 - L3-Mt1-2.2



L3-Mt1, L3-Mt2.1, L3-Mt2.2 are mutants with single point mutation in loop 3
L3-Mt1-2.1 and L3-Mt1-2.2 are mutants with two point mutations in loop 3

Conclusions and further work

- Amino acid residues in external loop 3 of NspA are important for binding hFH. L3-Mt2 and L3-Mt3 have low affinity for FH.
- The same loop 3 mutants showed weaker binding to anti-NspA monoclonal antibodies, AL-12 and 14C7.
- Both loop 3 mutants tested in immunization experiments induced significantly lower IgG titres in WT mice.
- In hFH Tg mice with hFH binding to WT NspA, serum IgG antibody titres of mice immunised with WT NspA and L3-Mt2.1 NspA proteins were not significantly different. L3-Mt3 NspA IgG titres were lower as observed with the WT mice. Since loop 3 mutants have low affinity for hFH, hFH may not have interfered with the induction of antibody responses.
- L3-Mt2.1NspA appears to be a promising candidate for further investigation as the IgG antibody responses have increased in hFH Tg mice and similar to anti-WT NspA IgG responses.
- We are currently evaluating the bactericidal activity of serum antibodies from immunized mice against *N. meningitidis,* which should provide more information about the pre-clinical efficacy of the modified NspA EOMV vaccines.
- A candidate mutant NspA will be tested in *N. meningitidis* OMV

Acknowledgements

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