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 (fH).
- 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 meningococcal strains.
- In our current study, we aim to develop a NspA OMV (outer membrane vesicle) vaccine with low-affinity for fH as previous studies from our group have shown that binding of fH 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 fH to NspA and PorB3.
- Currently licensed meningococcal vaccines containing NspA and PorB3.

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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 fH. We also extracted EOMVs expressing wild-type (WT) NspA or no NspA as controls. We immunized WT CD-1 mice and fH transgenic (Tg) mice with EOMVs expressing WT or mutant NspA. We measured anti-NspA IgG antibody titres of sera fromfH mice by whole bacterial cell ELISA and assessed the functional activity of anti-NspA antibodies by serum bactericidal assay using human complement.

Results

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

- Amino acid residues in external loop 3 of NspA are important for binding 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 fH Tg mice with fH binding to WT NspA, serum IgG antibody titres of mice immunized with WT NspA and L3-M2.1 NspA proteins were not significantly different. L3-M3 NspA IgG antibody titres were lower as observed with the WT mouse.
- Since loop 3 mutants have low affinity for fH, fH may not have interfered with the induction of antibody responses.

Conclusions and further work

- Amino acid residues in external loop 3 of NspA are important for binding 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 fH Tg mice with fH binding to WT NspA, serum IgG antibody titres of mice immunised with WT NspA and L3-M2.1 NspA proteins were not significantly different. L3-M3 NspA IgG antibody titres were lower as observed with the WT mouse.
- Since loop 3 mutants have low affinity for fH, fH may not have interfered with the induction of antibody responses.
- L3-M2.1 NspA appears to be a promising candidate for further investigation as the IgG antibody responses have increased in fH 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

This study was funded by NIH R01 AI1347868 to PTB

References