(1) Submission ID#1537935

Neisserial surface protein A (NspA) residues that interact with human complement Factor H impact binding of monoclonal and polyclonal anti-NspA antibodies

---

Author(s)

Dhaarini Raghunathan, PhD

Associate Specialist

University of California San Francisco

Susie Lim, n/a

Staff Research Associate

UCSF

Gregory R. Moe, PhD

Chief Scientific officer

OMVax, Inc.

Peter T. Beernink, Ph.D.

Professor

University of California, San Francisco

Background

Neisserial surface protein A (NspA) is a highly conserved integral outer membrane protein of Neisseria spp. that has been tested as a vaccine candidate against meningococci in mice and humans. NspA vaccines elicited bactericidal antibodies in mice but not in humans, which might have been due to the different vaccine formulations. However, our colleagues subsequently showed that meningococcal NspA binds human Factor H (FH) and further that the protective antibody responses of human FH transgenic mice were lower than those of wild-type mice.

Aim/Methods

Our objective was to identify amino acid residues of NspA that are important for FH binding and thereby develop an improved mutant NspA vaccine antigen. Based on the crystal structure of meningococcal NspA, we targeted ten charged residues in the four surface exposed loops of NspA. Using E. coli cells with surface exposed NspA, we measured the ability of NspA mutants to bind human FH by whole bacterial cell ELISA. As controls, we tested the binding of two mouse monoclonal antibodies and mouse polyclonal antibodies to the NspA mutants by whole-cell ELISA and western blotting. We evaluated whether NspA mutants had decreased FH binding by whole cell ELISA, and purified them under denaturing conditions. We assessed the ability of the refolded NspA mutants to bind FH and anti-NspA antibodies by ELISA.

Results

We identified three NspA mutants, D77A in loop 2, and D113A and D118A in loop 3, that had significantly

decreased FH binding. Strikingly, we found that the two alanine substitutions in loop 3 also decreased binding of monoclonal antibodies AL-12 and 14C7, which are known to bind loops 2 and 3. The D77A mutation in loop 2 also decreased binding of polyclonal antibodies to NspA.

Conclusions

Our results indicate that the immunogenic regions of NspA overlap with the FH binding site. Therefore, engineering a meningococcal NspA antigen with increased immunogenicity in the presence of human FH will require decreasing FH binding while preserving immunogenic epitopes. In addition, gonococcal NspA, which naturally exhibits low FH binding but still functions as an FH receptor, might also be improved by further reductions in FH binding.

Uploaded File(s)

Supplemental Document Upload

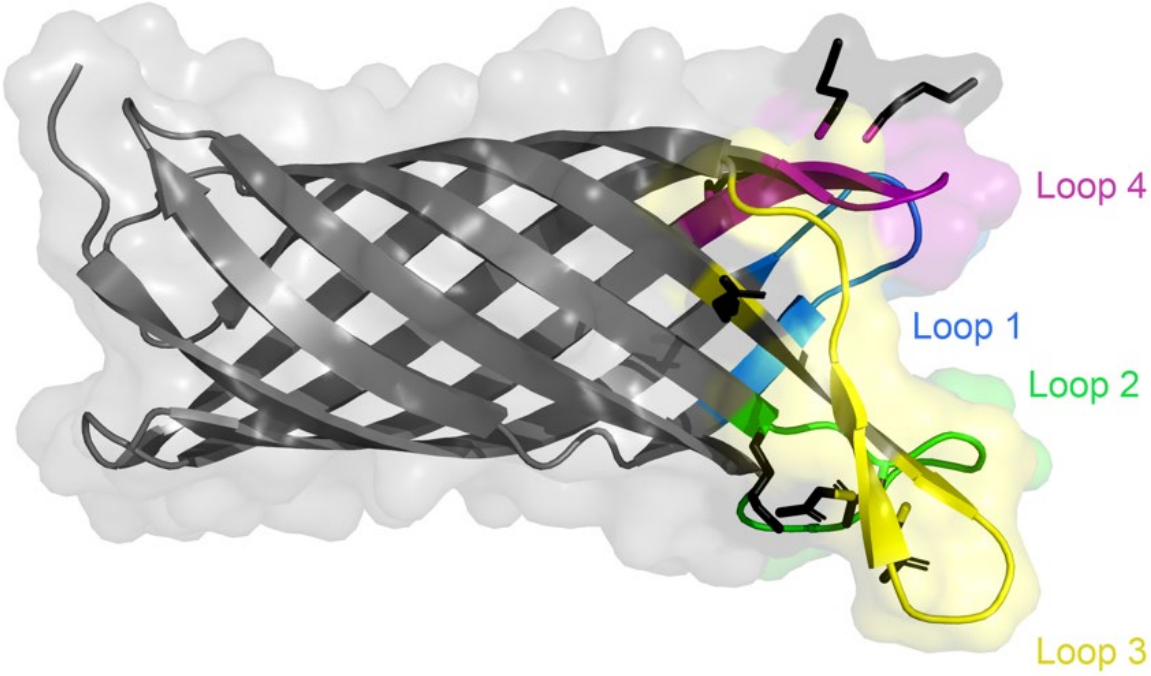

Figure. Crystal structure of NspA showing four extracellular loops. Loops 1 through 4 are shown in color and charged residues targeted for mutagenesis are in black. Loops 2 and 3 were identified experimentally to bind human complement Factor H.