# Mapping of binding sites on NadA protein of *Neisseria meningitidis* and blocking its interaction with human brain microvascular endothelial cells

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*Nisseria meningitidis* casing cererospinal meningitis utilizes several of its membrane proteins to interact with human Brain Microvascular Endothelial Cells (BMECs). *Nisseria* adhesin A -NadA an outer membrane protein has been recognized to mediate adhesion of bacteria and evokes strong bactericidal antibodies when used as vaccine candidate. In the present study, binding pockets of NadA (domains) interacting with the receptors of BMECs were mapped using mass spectrometry. In brief, binding of BMECs proteins and recombinant NadA (rNadA) expressed in *E. coli* M15 cells was determined by western blotting. On membrane tryptic digestion (partial) was performed and the non-interacting rNadA fragments were washed to retain BMEC protein-rNadA peptide complex. Further, mild striping was performed on the complex to isolate the domains of rNadA from BMEC proteins. MALDI-TOF/MS was employed to identify the amino acid sequences of the domains. Next, synthetically produced NadA peptides (37 mer peptides) corresponding to the interacting domains of rNadA were used to capture single domain antibodies (VHH) raised against rNadA through M13K07ΔpIII hyper phage system. At least 96 VHH clones were found to have an affinity for rNadA peptide 1 and 2. Among them 20 clones were tested for blocking the interaction of rNadA with BMEC proteins. However, only two clones of VHH binding to globular domain of rNadA and one clone of VHH binding to coiled coil region were able to block the interaction of rNadA with hBMEC proteins.

## Biography

Amod Kulkarni has completed his PhD in Biosciences from Nord University, Bodo, Norway and has been a Postdoctoral Researcher at the Laboratory of Biomedical Microbiology and Immunology, UVLF, Kosice, Slovakia since April 2018. He has published his research in 15 papers in reputed journals and has been an active Researcher in the field of host-pathogen interactions.

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