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Nanobody Based Nanosystems to Contend *Neisseria meningitidis* and West Nile Virus

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Extended Abstract

Neisseria meningitides (NM), commensal bacteria of the nasopharyngeal region and West Nile Virus (WNV), a mosquito-borne flavivirus are having the propensity to cause neuroinvasive meningitis and/or encephalitis. Several mechanisms of traversal through blood-brain barrier are suggested for both the pathogens, however, interaction with human brain microvascular endothelial cells (hBMECs) is pivotal. As an attempt to contend their pathogenesis in the brain, we aimed to generate nanobodies also known as VHH (variable heavy chain antibodies) against NM and WNV using phage display. These nanobodies are intended to specifically recognize NM and WNV at hBMECs and block the ligand-receptor interactions. Additionally, traversal of nanobodies through *in vitro* BBB after their conjugation with drug-loaded nanocarriers (e.g. polymers and dendrimer) will be assessed.

Although NM possesses several adhesins, NadA (Neisseria adhesin A) is a potent immunogen expressed in most hypervirulent strains and forms a component of the licensed vaccine. Likewise, the envelope (E) protein of WNV mediates receptor binding and host-membrane fusion. Domain DIII of E protein is reported to produce neutralizing antibodies. Therefore, we target proteins NadA and DIII as the antigens of NM and WNV to produce nanobodies.

Instead of producing nanobodies against entire epitopes of antigens, the receptor binding pockets (domains) of NadA and DIII interacting with hBMEC proteins were identified by limited proteolysis and mass spectrometry. The domains were recognized as NadA-gd$^{A33\text{-}K69}$ and NadA-cc$^{L121\text{-}K158}$ and DIII-915$^{G299\text{-}K307}$ and DIII-2003$^{V371\text{-}R388}$. Thereafter, synthetic analogs of NadA-gd$^{A33\text{-}K69}$, NadA-cc$^{L121\text{-}K158}$, DIII-915$^{G299\text{-}\ K307}$ and DIII-2003$^{V371\text{-}R388}$ were used in phage display to generate nanobodies against the domains of NadA (VHH_{F3} and VHH_{G9}) and WNV ($VHH_{A1,}$ $VHH_{A6,}$ VHH_{A9} and VHH_{A10}). Functional assays confirmed that the developed nanobodies block the interaction of recombinant NadA / DIII with hBMEC in western blot and immunocytochemistry or on-cell ELISA. Moreover, pre-incubation of live NM with nanobodies - VHH_{F3} and VHH_{G9} abrogated bacterial traversal through *in vitro* BBB. On the other hand, nanobodies targeting DIII showed neutralization ability on WNV like particles (pseudo virus) *in vitro*.

Alongside, polycaprolactone based nanoparticles were conjugated with aforementioned nanobodies. Subsequently, functional assays on polycaprolactone conjugated nanobodies to confirm their ligand/pathogen binding and traversal through *in vitro* BBB models are being performed. Further attempts to produce efficient nanosystems and interfere with the infections of NM and WNV will be discussed in our presentation.

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