Neisseria meningitidis serogroup A capsular polysaccharide biosynthetic protein SacD / MynD is a potential transporter: structure-function characterization and molecular modelling

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Key words: *Neisseria meningitidis*, Capsular polysaccharides, innate immunity, cytokines, macrophages

Background: Capsular polysaccharides (CPS) is critical virulence factor for *N. meningitidis* invasive infections and forms the basis for vaccines. Meningococcal serogroup A CPS consists of O-acetylated α1→6 linked ManNAc 1-phosphate repeating units. The genetic biosynthetic cassette is a composed of specific genes sacA, sacB, sacC and sacD (known as mynA, mynB, mynC, mynD). The function of sacA is characterized as epimerase, sacB as polymerase of UDPManNAc and sacC as O-acetyltransferase responsible for transferring acetyl groups to the ManNAc residues. However, the function of sacD gene is not known. **Aim**: the aim of this study in to investigate the structure-function of sacD protein. **Methods**: Molecular dynamic (MD) modelling of sacD protein was used to predict the structure and function. Innate immune recognition of CPS purified from sacD mutant and isogenic WT NMA was investigated using HEK-TLR2/TLR6 transfected cell line, THP-1 and RAW264 macrophages. **Results**: sacD protein consists of 280 amino acids and 3D structure is generated using the Maestro12.3 module in Schrodinger software. Innate immune recognition experiments showed that CPS polymer purified from sacD-deficient mutant induced higher amount of IL-8 release when compared to CPS from isogenic WT or the vaccine grade polymer MAPS. Similarly, CPS from sacD-deficient mutant induced higher amounts of nitrite release from murine RAW264 macrophages in a dose-dependent manner. Moreover, whole cell formalin-fixed sacD-deficient mutant induced slightly higher TNFα release from THP-1 cells when compared to isogenic WT but similar to capsule negative NMA mutant. The data suggest that CPS from sacD-deficient mutant may possess higher immunostimulatory activity in vitro cell culture models. To determine CPS surface expression, serogroup A monoclonal antibody (14-1-A) did not recognize sacD-deficient mutant in whole cell ELISA similar to capsule-deficient NMA suggesting that CPS in sacD is not surface exposed. MD modelling of sacD protein predict that sacD could be involved in the transport of the CPS to the surface. In support of this transport function, MD modelling of sacD with CPS-A polymer docking showed that sacD can bind four to six repeating phospho-ManNAc units in its pocket. **Conclusion**: sacD-deficient CPS is more immunostimlatory than isogenic WT in an *in vitro* cell models and sacD protein may function as a transporter of CPS-A polymer to the surface.