Structure-immunogenicity studies of MenC-TT conjugate vaccines show advantage of larger conjugates

Kay Lockyer[1,2], Robert Francis[1], Fang Gao[1], David Eastwood[1], Sarah Davis[1], Richard Stebbings[1], Jeremy Derrick[2], Barbara Bolgiano[1]

1) National Institute for Biological Standards and Control, MHRA, Potters Bar, U.K. 2) Faculty of Life Sciences, The University of Manchester, Manchester, U.K.

INTRODUCTION

Since the Meningococcal serogroup C (MenC) conjugate vaccine was introduced into the UK vaccination schedule in 1999, mean disease incidence has decreased substantially in England from more than 800 cases per year (1999/2000 epidemiological year) to fewer than 40 cases per year (2016/2017 epidemiological year) (PHE, 2019). Conjugate vaccines are biologically diverse in terms of both the saccharide unit and the carrier protein, with potential for large variations in both size and structure. To determine whether there is a link between conjugate size and immunogenic responses a defined panel of MenC-TT conjugates were designed with control for all physical parameters excluding size.

MATERIALS AND METHODS

- Four MenC-TT conjugate vaccines were synthesized from monomeric TT and sized MenC PSs using carbodiimide coupling chemistry with sizes from 2,348,000 g/mol (MenC-TT 1) to 191,500 g/mol (MenC-TT 4).
- They were characterised as being physically similar to manufactured vaccines in terms of free saccharide and free protein content.
- Through SEC-MALS-viscometry profiling larger conjugates (MenC-TT 1 and 2) were found to be elliptical in shape, the smaller conjugates (MenC-TT 3 and 4) more spherical.
- Immunological profiling was carried out to determine if differences in size or shape contributed to different humoral or innate responses.

RESULTS

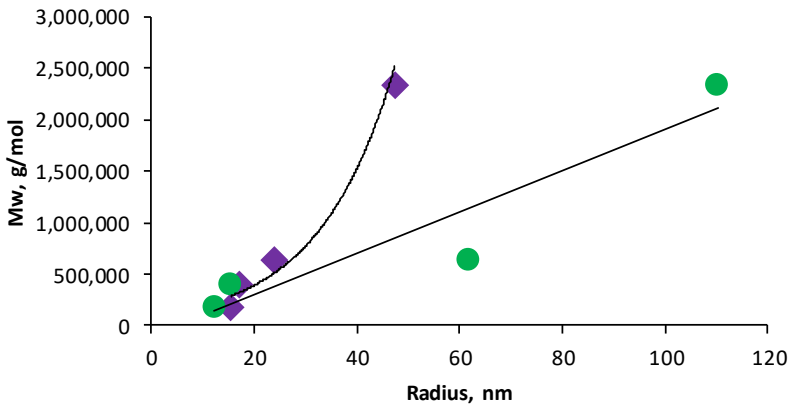

Figure 1: MALS/RI/Viscometry structural profiling showing panel conjugate configuration plots for conjugates MenC-TT 1 to 4: Conjugate panel radius plotted against molecular mass showing hydrodynamic radius (Rh) (purple diamond) and RMS radius moments (Rn) (green circles).

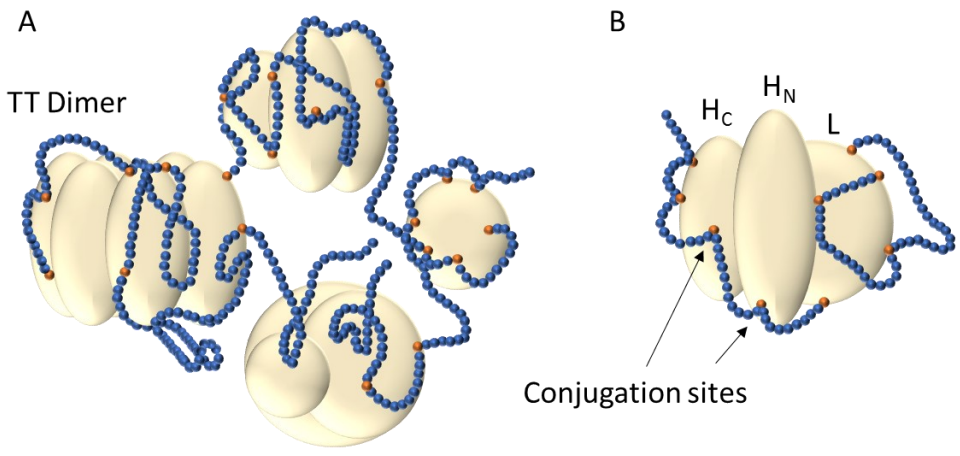

Figure 2: MenC-TT Conjugate configuration model for A) MenC-TT 1 and B) MenC-TT 3 molecules. MenC-TT 1 model with 3 PS chains connecting 3 monomeric proteins and 1 dimeric protein and Group 3 model with 2 PS chains connecting to 1 TT protein according to experimentally determined stoichiometries. Model A shows asymmetry of the larger conjugate and B shows a more symmetrical shape for the smaller panel conjugate.

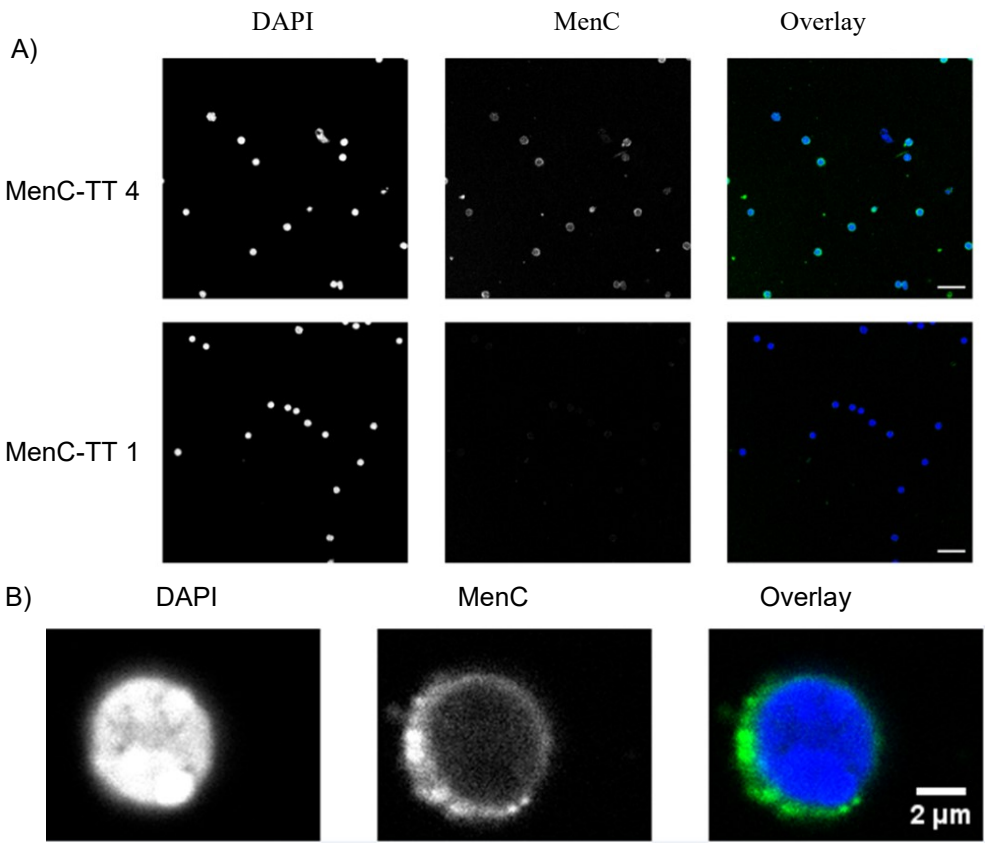

Figure 3: Confocal imaging showing A) Mouse splenocytes from MenC-TT 1 and 4 immunizations and B) Single splenocyte from MenC-TT 4. DAPI binds to nucleic acids enabling cell visualisation. Surface MenC Ag can be visualised and compared using the Image Overlay.
Scale bar is 10 µm for Figure A and 2 µm for Figure B.

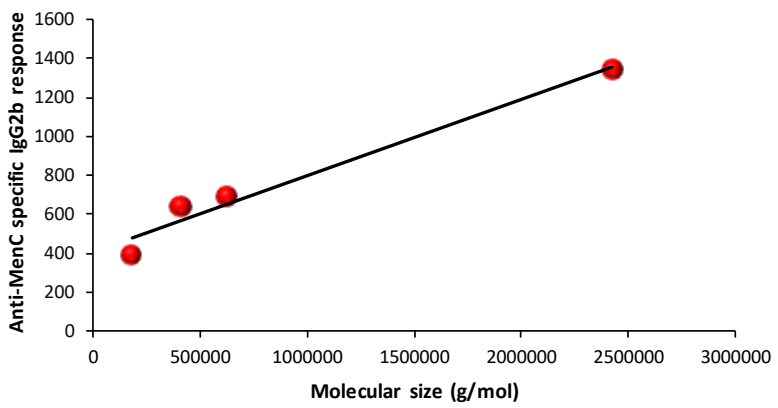

Figure 4: Relationship between molecular size (g/mol) and anti MenC specific IgG2b (r = 0.9857)

CONCLUSIONS

- Immune response generated by MenC-TT conjugates increases as a function of molar mass and chain length for the conjugate sizes used in this study (generally smaller than manufactured vaccines).
- A correlation between anti-MenC IgG2b quantities and molecular mass was determined.
- Smaller conjugates had a symmetrical spherical shape. As size increased the conjugates became more elliptical.
- Confocal imaging provided a useful analytical tool to visualize surface binding of MenC Ag on splenocytes, likely occurring through binding to TLR2/TLR4 or other MHC II receptors (Lai and Schreiber, 2009).
- Longer chain or networked saccharides could resist acidic depolymerization in the endosome (Sun *et al*, 2019).

REFERENCES

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- Sun, X., Stefanetti, G., Berti, F., and Kasper, D., Polysaccharide structure dictates mechanism of adaptive immune response to glycoconjugate vaccines (2019) PNAS, 116, pp 193—198
- Lai and Schreiber, Antigen processing of glycoconjugate vaccines; the polysaccharide portion of the pneumococcal CRM197 conjugate vaccine co-localizes with MHC II on the antigen processing cell surface, Vaccine, 27 (2009) pp 3137—3144