Quality and Immunogenicity of MenAfriVac, a Conjugate Vaccine Against Meningococcal Group A Disease

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Introduction

Bacterial meningitis causes significant morbidity and mortality worldwide. The highest burden of meningococcal disease affecting children under the age of 15, occurs within the meningitis belt in sub-Saharan African countries. Major epidemic outbreaks have occurred in Burkina Faso, Chad, Ethiopia, Niger and Nigeria which have been attributable mainly to *Neisseria meningitidis* serogroup A (Fig1).[1]

The Meningitis Vaccine Project (MVP), a partnership between WHO and PATH, aims to eliminate epidemic meningitis in Africa. Development of a monovalent MenA polysaccharide-tetanus toxoid (TT) conjugate vaccine, MenAfriVac, has shown promising results in phase I trials[2] and is currently undergoing Phase II. The object of this study was to analyze the quality and immunogenicity of MenAfriVac. The effects of adjuvant binding and the addition of an extra booster dose of polysaccharide/conjugate are also investigated in a mouse model.

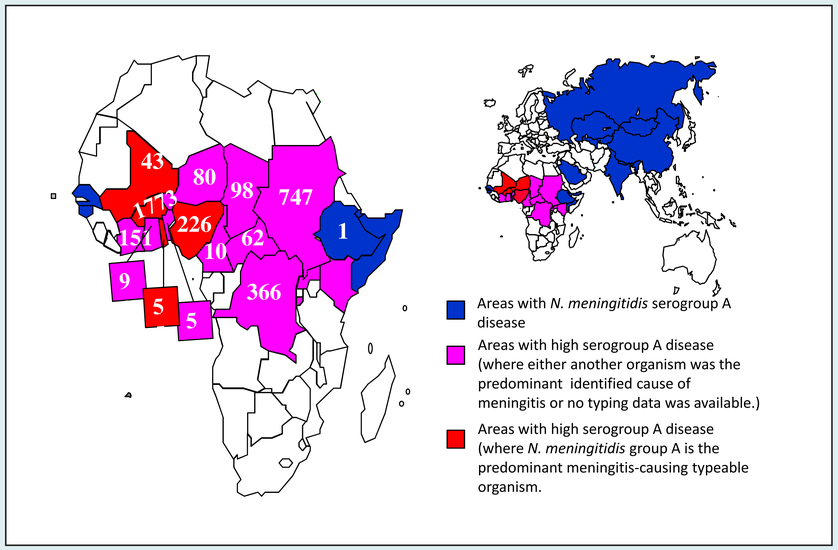

Fig 1 - Global distribution of N. meningitidis serogroup A: November 2006-June 2007 Epidemic Season

Numbers in white indicate the deaths due to meningitis reported in the year 2007[1].
There were 48,000 notified cases of meningitis in sub-Saharan countries under enhanced meningitis surveillance (www.meningivax.org/files/newsletter-2007-Q2-13-EN.pdf).

Methods

Vaccines:

Developmental MenA-TT bulk and MenA polysaccharide are labelled 'dev bulk' and 'native PS', respectively; MenA-TT clinical Phase I fills: Phase I-lot 'a', Phase I-lot'b' and Phase I-lot 'c' contained $AlPO_4$; clinical Phase II batch, 'Phase II', was tested with and without $AlPO_4$ adjuvant.

Molecular Sizing:

Vaccine molecular size was determined by HPLC Size-Exclusion Chromatography (SEC) with 18-angle laser light scattering (Wyatt Technology). Samples of 50 µg saccharide & TT or 69 µg conjugate (PS+prot) were isocratically eluted in PBS pH 7.4 on a TSK 5000PWXL analytical column. UV absorbance at 280 and 214 nm, refractive index and light scattering signals were collected and used to determine Mw.

Immunogenicity:

Female Balb/c mice (10/group) were immunised with a 1 µg/dose of vaccine following a 3-dose schedule, with injections at 0, 14, 28 days and serum samples taken 14 days after each vaccination. Total MenA-specific IgG response was quantified by ELISA and the mean geometric titre calculated. Vaccine protection induced in the mouse model was measured by Serum Bactericidal Assay (SBA), using baby rabbit complement and MenA strain F8238. The SBA titre was reported as the geometric mean of the reciprocal serum dilution yielding ≥50% bacterial killing as compared with the viable count control. Antibody persistence and effect of boosting were studied using a 3-dose schedule (0, 14, 28 days) with a 4th booster dose of conjugate or native polysaccharide at 26 or 36 weeks.

Adjuvant Binding:

Binding of the protein carrier to the adjuvant was tested by preparing 50-100 µg protein MenA-TT or TT samples in saline, Tris-saline or PBS with 1mg/ml $AlPO_4$ and measuring $UV_{280\ nm\text{-}baseline}$ after 2.5hr or ~18hr overnight incubation as indicated.

Results

Molecular Sizing

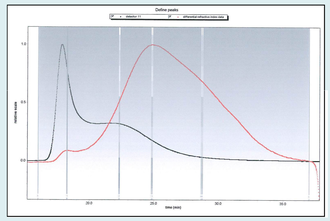

Fig 2 - Sizing Profile of MenA-TT from 90°Light Scattering (black) and RI (red) detectors.

Light scattering signals from 16 detectors at 14.5-163.3° and RI signal were used to determine the weight-average molecular weight of PS, carrier protein and conjugate samples, using Zimm formalism (Table 1).

Table 1 - Weight-Average Molecular Weight of MenA-TT and Components

Sample	dn/dc used	Mw (%error); g/mol Aggregate Peak	Mw (%error); g/mol Main Peak
MenA-TT	0.190 (Jumel)[3]	1.8 x 108 (2%)	2.0 x 106 (1%)
TT	0.231 (Jumel)[3]		182,000 (2%)
MenA PS	0.150 (assumed)		116,000 (10%)

Immune response to MenA-TT vaccine

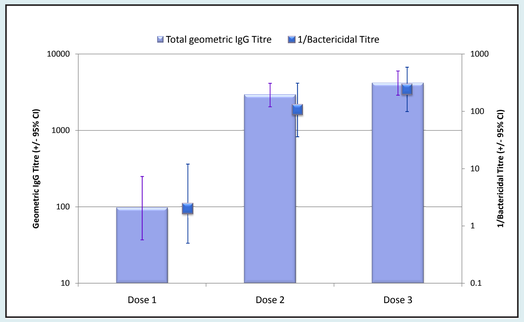

Fig 3 - Total IgG and Bactericidal Titres with 1, 2 and 3 Doses of MenA-TT Phase II/$AlPO_4$

Total IgG and bactericidal titres exhibited a similar correlation after receiving three separate doses of the Phase II MenA-TT vaccine with adjuvant (Fig 3).

Titres significantly increased ($p<0.05$) upon additional vaccination with a second dose. After the third dose the response appeared to increase slightly, but this did not reach statistical significance.

Batch-to-Batch Consistency

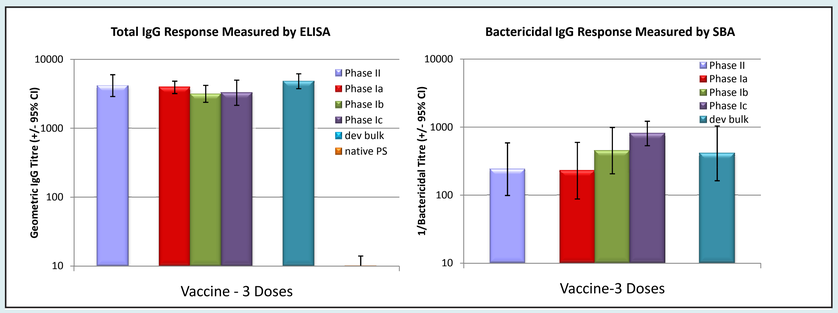

Fig 4 – Mouse Immune Response to MenA PS and MenA-TT Developmental/Clinical Batches

Total IgG and bactericidal responses were consistent among various MenA-TT vaccine formulations (Fig.4). Vaccination with native MenA PS alone elicited a significantly lower IgG response.

Adjuvant Effects

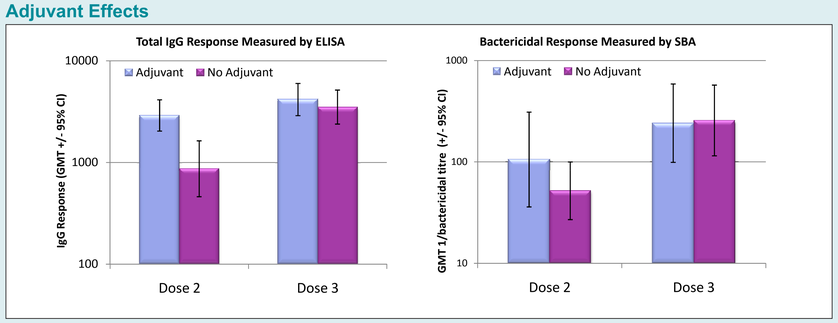

Fig 5- Effect of Adjuvant on MenA-TT Vaccine Response

The inclusion of adjuvant induced a higher MenA-specific total IgG and bactericidal response after two doses compared with the control vaccine lacking adjuvant (Fig 5).

A third dose boosted the control responses to reach equivalent titres to those receiving adjuvant.

Table 2 – Percentage Adsorption of MenA-TT Conjugates and Carrier Protein to $AlPO_4$

Sample	Buffer	pH	Percent Adsorption 2.5hr	Percent Adsorption overnight
MenA-TT	Saline	5.4 – 5.6	58%	87%
MenA-TT	Tris-Saline	6.6	54% 10 mM Tris	88% 25 mM Tris
MenA-TT	PBS	7.1	17%	
TT	Saline	5.4 – 5.6	76%	81%
TT	Tris-Saline	6.6	60% 10mM Tris	
TT	PBS	7.1	13%	

Binding of the conjugate to adjuvant was measured in Tris-saline (formulation), and PBS (buffer for immunogenicity studies.) Binding to $AlPO_4$ was higher in Tris than in PBS for both TT and conjugate.

Booster Dose

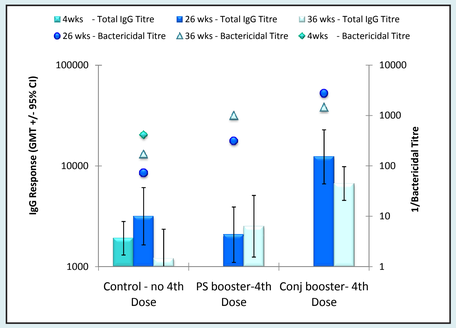

Fig 6 – Effect of a Delayed Booster Dose of Conjugate or Polysaccharide

A fourth dose of conjugate vaccine at 26 or 36 weeks, elicited a higher IgG and functional antibody response compared to polysaccharide with a significant increase above the pre-booster levels (Fig 6).

A polysaccharide booster at 36 weeks led to a further increase in functional antibody titre.

Conclusion

- The MenA-TT Phase II vaccine with adjuvant generated significantly elevated total IgG and bactericidal responses after two doses. A third dose did not significantly boost the response further.
- Immunogenicity data demonstrated consistency among clinical phase I and II conjugate vaccine formulations after three doses.
- MenA-TT & TT bind well to $AlPO_4$ adjuvant in saline and Tris-saline and to a lesser extent in PBS. Inclusion of adjuvant caused an increase in total IgG and functional antibody responses after two doses, however no differences between the adjuvant and control group lacking adjuvant were evident after a further dose.
- Persistence of antibody and the ability to boost the response with either polysaccharide or conjugate up to 8 months were demonstrated.

References

1. WHO, MDSC Meningitis Weekly Bulletin (Week 19, 2007) and ProMed up to April 2007
2. Kshirsagar N, Mur N, Thatte U, Gogtay N, Viviani S et al., Vaccine (2007), 25S,A101-A107.
3. Jumel K, Ho MM, Bolgiano B, Biotech App Biochem (2002) 36,219.