Introduction

The Meningitis Vaccine Initiative (MVI) has been created in 2001 by a grant from the Bill and Melinda Gates Foundation as a partnership between WHO and PATH to develop and introduce affordable meningococcal conjugate vaccines for elimination of meningococcal epidemics in Sub-Saharan Africa [1]. A serogroup A meningococcal conjugate vaccine using tetanus toxoid as a carrier protein (PSA-TT) has been developed at the Serum Institute of India Ltd., using a new licensed conjugation technique from Biologics Evaluation and Research/Food and Drug Administration.

Immunogenicity and antibody persistence and safety in healthy volunteers aged 18-35 yrs was demonstrated in a double-blind randomised controlled phase I study [2]. Kshirsager et al. [2] reported immunogenicity as determined by SBA using baby rabbit complement and standardised ELISA for capsular antigen total IgG.

The aim of the study was to analyse sera by additional immunological assays, in order to investigate the relationship between different serogroup A immunogenic assays.

Methods

Study group

Healthy adult volunteers (18-35 yrs) were recruited from three sites within India [1] and randomised to receive intramuscularly in the deltoid region one dose of

- PSA-TT conjugate vaccine (Serum Institute of India, Pune, India)
- Meningococcal polysaccharide Vaccine A&CTM (Sanofi Pasteur, Lyon, France)
- Tetanus Toxoid AdsorbedTM (SII, Pune, India)

Blood samples were taken for immunogenicity studies on the day of immunisation and 4, 24 and 48 weeks later.

The immune response to PSA-TT and PsA/C was compared in 6 immunological assays. Data from the Tetanus Toxoid vaccine group is not included in this analysis

Immunological assays

- Serum bactericidal antibody (SBA) assay

  SBA assays were performed against the serogroup A target strain, CDC no FB282 (phenotype A-4;2;1-P1.20,20.9) [3]

- Complement source used was either serum from 2-4 week old rabbits (Pel-Freez Biologicals, WI) [Performed at HPA, Manchester, UK]

- Pooled human complement (from individual sera with no intrinsic killing of FB282, SBA titres ≤ 8 and total IgGAM < 30 μg/mL and showed no intrinsic complement activity against FB282 and showed preservation of complement activity as determined by CH50 titres (performed at DFA, Bethesda, MD, USA)

SBA titres were expressed as the reciprocal serum dilutions yielding ≥ 50% killing after 60 min for SBA and 90 min for hSBA.

- Titres <4 were assigned an arbitrary value of 2 for data analysis

- Serogroup A-specific IgG

  Serogroup A-specific IgG levels were determined using:

  - standardised ELISA using CDC 1992 and mouse monoclonal-PAN anti-human IgG Fc labelled with horseradish peroxidase [4] (performed at CDC, Atlanta, GA, USA)

  - Multiplex bead assay [5] (performed at HPA Manchester).

  Briefly, meningococcal serogroup A polysaccharide was attached to poly-L-lysine and then covalently attached to carboxylated microspheres using a two-step carbodiimide reaction. Serum and beads were incubated for 20 min at room temperature of a plate shaker followed by washing and incubation with R-phycocerythrin-conjugated anti-human IgG. The plate was then read on a Bio-plex reader and data analysed using Bio-plex Manager. Data for unknown test sera was generated from a 5-parameter logistic standard curve of CDC 1992 and converted to pg/mL.

- Oposphagocytic assay

- The oposphagocytic activity (performed at the CDC) was measured by incubating serogroup A polysaccharide conjugated to fluorescent beads, with serum dilutions followed by the addition of rabbit complement and H60 cells which had been chemically induced into monocytes [6].

Results

4 weeks post-vaccination there was a significant difference between the SBA GMTs of the 2 vaccine groups (p<0.005) which was not seen with the hSBA GMTs (p>0.05) which was not seen with the SBA GMTs (p<0.005) which was not seen with the hSBA GMTs (p<0.005) (data not available at 1 year for hSBA).

Table 1. SBA GMTs, IgG GMCs, and NIPH OPA GMT. At 48 weeks post-immunisation, the proportions achieving a titre of 4 fold rises were similar.

<table>
<thead>
<tr>
<th>Vaccine Group</th>
<th>SBA (n=47)</th>
<th>hSBA (n=45)</th>
<th>PsA/C (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMT (n=100)</td>
<td>44</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>IgG GMC (n=96)</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>NIPH GMT (n=96)</td>
<td>0.53</td>
<td>0.38</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table 2. Serogroup A-Specific IgG GMTs

<table>
<thead>
<tr>
<th>Vaccine Group</th>
<th>SBA GMTs (n=47)</th>
<th>hSBA GMTs (n=45)</th>
<th>PsA/C GMTs (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMT (n=100)</td>
<td>22</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>
| 4 weeks post-vaccination | 6 (48 weeks post-vaccination (p<0.005) (data not available at 1 year for hSBA).

Table 3. OPA GMTs

<table>
<thead>
<tr>
<th>Vaccine Group</th>
<th>SBA GMTs (n=47)</th>
<th>hSBA GMTs (n=45)</th>
<th>PsA/C GMTs (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMT (n=100)</td>
<td>21</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>4 weeks post-vaccination</td>
<td>11</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

Conclusions

- The proportion of subjects determined to be protectively by hSBA (titres ≥ 4) and SBA (titres ≥ 8) and also ≥ 4 fold rises were similar.

- Four weeks post-vaccination the vaccine groups differed by hSBA four-fold rise, achieving a titre of ≥ 2 of ≥ 8 GMT, IgG GMC, and NPH OPA GMT. At 48 weeks post-immunisation, the vaccine groups differed by ≥ 8 GMT, IgG GMC, and NPH OPA GMT.

- Four weeks post-vaccination a difference between the vaccine groups was seen by hSBA and serogroup A-specific IgG concentration (by either assay).

- Serogroup A-specific IgG concentrations determined by ELISA and the multiplex bead assay were reported comparably.

- OPA GMTs shows a rapid decline in antibody in the year post-vaccination whereas both the SBA titres and serogroup A-specific IgG concentrations show antibody persistence.

Both ELISA and multiplex bead assay showed a good correlation with hSBA.

References


Figure 1. Percentage of subjects before and 4 weeks following vaccination with either PSA-TT in serum bactericidal titre cutoffs of ≥ 4, 8, 16, ≥ 256 and ≥ 128.

Figure 2. Correlation of serogroup A-specific IgG determined by ELISA and multiplex bead assay (both vaccine groups and all vaccines combined).

Figure 3. Comparison of subjects with serogroup A-specific IgG ≥ 2 μg/mL ELISA and multiplex bead assay.

Pre-vaccination the proportion of subjects with serogroup A-specific IgG ≥ 2 μg/mL differed but at all other time points the proportions were similar.