**Phase 1 and Phase 2 Studies of *Salmonella enterica* Serovar Paratyphi A O-Specific Polysaccharide-Tetanus Toxoid Conjugates in Adults, Teenagers, and 2- to 4-Year-Old Children in Vietnam**

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*Salmonella enterica* serovar Paratyphi A O-specific polysaccharide (O-SP) was activated with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) and bound to tetanus toxoid (TT) with adipic acid dihydrazide as a linker (SPA-TT1) or directly (SPA-TT2). In mice, these two conjugates elicited high levels of immunoglobulin G (IgG) anti-lipopolysaccharide (LPS) in serum with bactericidal activity (E. Konadu, J. Shiloach, D. A. Bryla, J. B. Robbins, and S. C. Szu, Infect. Immun. 64:2709–2715, 1996). The safety and immunogenicity of the two conjugates were then evaluated sequentially in Vietnamese adults, teenagers, and 2- to 4-year-old children. None of the vaccinees experienced significant side effects, and all had preexisting LPS antibodies. At 4 weeks after injection, there were significant increases of the geometric mean IgG and IgM anti-LPS levels in the adults and teenagers: both conjugates elicited a greater than fourfold rise in the IgG anti-LPS level in serum in ≥80% of the volunteers. SPA-TT2 elicited slightly higher, though not statistically significantly, levels of IgG anti-LPS than did SPA-TT1 in these age groups. Accordingly, only SPA-TT2 was evaluated in the 2- to 4-year-old children. On a random basis, one or two injections were administered 6 weeks apart to the children. No significant side effects were observed, and the levels of preexisting anti-LPS in serum were similar in children of all ages. A significant rise in the IgG anti-LPS titer was elicited by the first injection (P = 0.0001); a second injection did not elicit a booster response. Representative sera from all groups had bactericidal activity that could be adsorbed by *S. enterica* serovar Paratyphi A LPS.

Enteric fever, with its septicemia and complications, is caused by *Salmonella* serogroups A, B, C, and D. Although reported throughout the world several decades ago (2, 13), *Salmonella enterica* serovar Paratyphi A seems to be confined to Southeast Asia, where it is the second most common cause of enteric fever, accounting for about 10% of cases (1, 3, 19, 22, 25, 28, 29, 31, 36, 37, 39–43, 46–48, 55, 57, 62, 63, 66). Despite the frequency and severity of enteric fevers and efforts to control the diseases, there is no licensed vaccine for nontyphoidal salmonellae. TAB vaccine, composed of inactivated *Salmonella* serovar Paratyphi A (10, 15, 16, 50, 58, 64). Accordingly, we developed O-SP conjugates of *S. enterica* serovar Paratyphi A designed to elicit IgG LPS antibodies in serum (35).

The O-SP of *S. enterica* serovar Paratyphi A is composed of a trisaccharide backbone with a branch of d-paratose from the C-3 of α-mannose; C-3 of the adjacent α-1-rhamnose is partially O-acetylated (8, 9, 23, 27) (Fig. 1). *S. enterica* serovar Paratyphi A O-SP required O-acetyl groups to elicit serum LPS antibody with bactericidal activity in mice (33). O-SP was activated with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) at neutral pH and bound either directly or through an adipic acid hydrazide linker to the protein (33, 35, 38). In mice, *S. enterica* serovar paratyphi A conjugates induced LPS antibody with group-specific bactericidal activity against serogroup A.

We describe a clinical evaluation of *S. paratyphi* A O-SP, bound to tetanus toxoid (TT) directly or through an adipic acid hydrazide spacer, in adults, teenagers, and 2- to 4-year-old children.

**MATERIALS AND METHODS**

**Investigational vaccines.** The O-SP was prepared as described previously and had a molar ratio of 0.71 O-acetyl groups per repeating unit of polysaccharide (33). Pasteur-Mérieux Serum et Vaccins, Lyon, France, provided TT (lot GYA). Conjugates of the O-SP of *S. enterica* serovar Paratyphi A bound to TT through a linker, adipic acid dihydrazide (SPA-TT1; lot 64811), or directly (SPA-TT2, lot 64812) have been described previously (13, 35). SPA-TT1 contained 30.2 μg of protein per ml and 48.6 μg of polysaccharide per ml, and SPA-TT2 contained 40.7 μg of protein per ml and 51.3 μg of polysaccharide per ml; the dose for both
conjugates was 0.5 ml. Both lots passed the Food and Drug Administration requirements for sterility, pyrogenicity, and general safety.

**Study protocol.** The investigation was approved by the Ministry of Health of Vietnam; the Institutional Review Board of the National Institute of Child Health and Human Development, National Institutes of Health (protocol OH96 CH-N023); the Office of Protection from Research Risks; and the Food and Drug Administration (BB IND 6530). Informed consent was obtained from adult volunteers and from parents or guardians of vaccinated children younger than 18 years.

The site was Cao Lãnh District, Dong Thap Province, of the Mekong Delta region of southern Vietnam. Twenty healthy adults were randomly assigned to receive one 0.5-ml injection of either SPA-TT1 or SPA-TT2 in the deltoid muscle. The vaccinees were examined at 30 min and at 6, 24, and 48 h following immunization. Reactions at the immunization sites were inspected, and the temperatures of the volunteers were measured; these findings were recorded. Side effects were defined as a fever of >38.5°C, erythema of ≥2.5 cm, or induration of ≥2 cm within 48 h of the injection. Sera were obtained before and after each injection. Sera were collected before each injection and at 6, 24, and 48 h after each injection. Sera were collected before each injection and at 6, 10, and 26 weeks after the first injection. Sera were collected before each injection and at 6, 10, and 26 weeks after the first injection. 

**RESULTS**

**Clinical observations.** No significant side effects were reported for any of the three age groups.

**LPS antibody levels in serum.** (i) **Prevaccination.** All vaccinees had preexisting IgG and IgM antibodies in serum. The adults and 13- to 17-year-old teenagers had similar levels that were significantly higher than those in the 2- to 4-year-old children (1.46, 1.70, and 0.85 EU, respectively, for IgG \( P < 0.05 \); 17.5, 22.6, and 6.80, EU, respectively, for IgM \( P = 0.0001 \)).

(ii) **Adults.** At 6 weeks after injection, 75% of the adults responded with at least a fourfold rise in the IgG anti-LPS titer (Table 1). Recipients of SPA-TT2 had higher IgG anti-LPS levels than did recipients of SPA-TT1 (27.3 and 9.82 EU, respectively [not significant]) at 42 days following injection. These levels fell about threefold at 180 days but were still higher than the prevaccination levels (8.28 versus 1.69, and 3.73 versus 1.26 EU \( P < 0.005 \)). Recipients of SPA-TT1 had higher levels of both IgG and IgM than did recipients of SPA-TT2 (8.28 and 3.73 EU, respectively \( P = 0.03 \)).

Although significant, rises in the IgM anti-LPS levels induced by SPA-TT1 (35.2 versus 17.3 EU \( P = 0.0004 \)) and by SPA-TT2 (42.3 versus 17.7 EU \( P = 0.0044 \)) 6 weeks after injection were lower than that in the IgG level. IgM anti-LPS antibody levels elicited by SPA-TT1 were only slightly higher than those elicited by SPA-TT2 (42.3 and 35.2 EU, respectively \( P > 0.05 \)). IgM anti-LPS antibody levels at the 180-day interval declined to levels similar to those prior to the vaccination.

(iii) **Teenagers.** Conjugate-induced IgG anti-LPS levels in the 13- to 17-year-old volunteers were similar to but slightly lower than those in the adults (Table 1). At the 6-week interval, 85% responded with at least a fourfold rise in the IgG anti-LPS titer \( (P = 0.0001) \). Recipients of SPA-TT1 had a 7.6-fold rise to 12.8 EU, and recipients of SPA-TT2 elicited a 10.2-fold rise to 17.4 EU \( P > 0.01 \). At 180 days after vaccination, the IgG anti-LPS titers had declined about 50% compared to those at 42 days: the level elicited by SPA-TT1 was only slightly higher than that elicited by the SPA-TT2 (7.37 and 6.72 EU, respectively, \( P > 0.05 \)). These levels were higher than the prevaccination levels (6.72 versus 1.69, and 7.37 versus 1.70 EU \( P = 0.00011 \)).

As with IgG anti-LPS, the IgM responses in serum elicited by the two conjugates were similar to those observed in the adults. At the 42-day interval, the IgM anti-LPS titer had risen 1.4-fold \( (P = 0.0001) \) in the SPA-TT1 group and 1.5-fold \( (P = 0.0001) \) in the SPA-TT2 group. At 180 days, the IgM anti-LPS levels in the teenagers and adults were similar to each other and to the prevaccination levels.

Based on these results, only SPA-TT2 was evaluated in 2- to 4-year-old children.

(iv) **Children.** About 90% of the children had at least a fourfold rise in their IgG anti-LPS titer at the 42-day interval.

<table>
<thead>
<tr>
<th>Ig</th>
<th>Conjugate</th>
<th>Age (yr)</th>
<th>No.</th>
<th>GM LPS antibody titera (25th to 75th percentiles) (EU) on days after vaccination:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>IgG</td>
<td>SPA-TT1</td>
<td>18–44</td>
<td>10</td>
<td>1.26 (0.6–2.6)</td>
</tr>
<tr>
<td></td>
<td>SPA-TT2</td>
<td>18–44</td>
<td>10</td>
<td>1.69 (1.0–2.9)</td>
</tr>
<tr>
<td>IgM</td>
<td>SPA-TT1</td>
<td>13–17</td>
<td>56</td>
<td>1.69 (0.98–2.69)</td>
</tr>
<tr>
<td></td>
<td>SPA-TT2</td>
<td>13–17</td>
<td>52</td>
<td>1.70 (1.13–2.34)</td>
</tr>
</tbody>
</table>

* Serum standard is from a high-responder vaccinee and is assigned a value of 100 EU.
(19.3 versus 0.91, and 16.7 versus 0.77 EU \(P = 0.0001\))) (Table 2). Reinjection at 42 days did not elicit a booster response: the levels 2 weeks after the second injection (11.7 versus 11.9 EU [not significant] and 180 days after the first interval (4.08 versus 3.47 EU [not significant]) were similar in both groups. The IgG anti-LPS levels at 180 days were higher than at pre-vaccination (3.47 versus 0.91, and 4.08 versus 0.77 EU \(P = 0.0001\)).

IgM anti-LPS responses were slightly different from those of the teenagers and adults. The rises in IgM anti-LPS titers were slightly higher (ca. fourfold), although the levels at 42 days were similar to those in the teenagers and adults. As with IgG, there was no booster response to a second injection. At 180 days, IgM anti-LPS levels were higher in the children than in the teenagers and adults and were about twofold higher than the prevaccination levels (15.9 versus 6.65, and 17.6 versus 7.00 EU \(P < 0.0001\)).

Serum bactericidal levels. Representative sera (10) from the three age groups were assayed for their bactericidal titer (Table 4). Two pairs of sera from the children, prevaccination and after the first vaccination, were assayed, and their bactericidal titers were compared to the IgG and IgM anti-LPS titers by ELISA. All these sera had a bactericidal activity that required complement. The bactericidal activity was roughly related to the IgG anti-LPS titer. Adsorption of these sera with the LPS of \(S.\ enterica\) serovar Paratyphi A removed most of the bactericidal activity (data not shown).

**TABLE 2.** LPS antibodies in serum of children receiving one or two injections of SPA-TT<sub>2</sub>

<table>
<thead>
<tr>
<th>Ig</th>
<th>No. of injections</th>
<th>No. of children</th>
<th>GM LPS antibody titer&lt;sup&gt;a&lt;/sup&gt; (25th to 75th percentiles) (EU) on days after vaccination:</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1</td>
<td>63</td>
<td>0.91 (0.49–1.56) 19.3 (8.40–38.1) 11.7 (6.13–21.4) 3.47 (2.34–4.79)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47</td>
<td>0.77 (0.47–1.17) 16.7 (9.41–32.3) 11.9 (6.57–19.8) 4.08 (2.59–6.00)</td>
</tr>
<tr>
<td>IgM</td>
<td>1</td>
<td>63</td>
<td>6.65 (3.52–11.4) 28.8 (16.3–50.4) 22.0 (13.6–41.8) 15.9 (10.1–21.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47</td>
<td>7.00 (4.67–10.3) 32.3 (16.3–48.3) 24.7 (13.6–44.9) 17.6 (10.3–26.2)</td>
</tr>
</tbody>
</table>

<sup>a</sup> A high-responder serum was used as the standard and assigned a value of 100 EU.

**TABLE 3.** Age-related IgG levels of LPS antibody in serum before and 180 days after vaccination with SPA-TT conjugates

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Conjugate</th>
<th>No. of injections</th>
<th>No. of children</th>
<th>GM LPS antibody titer (EU) on days after vaccination:</th>
<th>Fold rise at 180 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18–44</td>
<td>SPA-TT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1</td>
<td>1.26</td>
<td>9.82</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SPA-TT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1</td>
<td>1.69</td>
<td>27.3</td>
<td>NA</td>
</tr>
<tr>
<td>13–17</td>
<td>SPA-TT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1</td>
<td>1.69</td>
<td>12.8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>SPA-TT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1</td>
<td>1.70</td>
<td>17.4</td>
<td>NA</td>
</tr>
<tr>
<td>2–4</td>
<td>SPA-TT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1</td>
<td>0.91</td>
<td>19.3</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>SPA-TT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2</td>
<td>0.77</td>
<td>16.7</td>
<td>11.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> NA, not applicable.

**TABLE 4.** Bactericidal levels elicited by SPA-TT conjugates in sera of vaccinees

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>IgG antibody titer (EU)</th>
<th>Bactericidal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1004 Adult</td>
<td>73.6  17.0</td>
<td>1,8000</td>
</tr>
<tr>
<td>P1012 Adult</td>
<td>15.5  31.5</td>
<td>1,2000</td>
</tr>
<tr>
<td>P2033 Teenager</td>
<td>10.1  36.8</td>
<td>1,2000</td>
</tr>
<tr>
<td>P2061 Teenager</td>
<td>133   73.6</td>
<td>1,8000</td>
</tr>
<tr>
<td>P3002 Child</td>
<td>78.8  32.4</td>
<td>1,1000</td>
</tr>
<tr>
<td>P3018 Child</td>
<td>1.76  11.4</td>
<td>1,500</td>
</tr>
<tr>
<td>P3094 Child</td>
<td>118  46.4</td>
<td>1,4000</td>
</tr>
<tr>
<td>P3094 Child</td>
<td>0.73  2.73</td>
<td>1,500</td>
</tr>
<tr>
<td>P3094 Child</td>
<td>58.5  10.8</td>
<td>1,8000</td>
</tr>
</tbody>
</table>

<sup>b</sup> Prevaccination sample.
We found a molar ratio of 0.71 Paratyphi A O-SP conjugate in infants, an age group that protein conjugates. We plan to evaluate the A, B, and D O-SPs of salmonellae (8–10, 17).

Earlier, we found that O-acetyl groups on the O-specific polysaccharide of S. enterica serovar Paratyphi A are essential for its immunogenicity and that conjugation with CDAP is useful for synthesis of S. enterica serovar Paratyphi A O-SP conjugates using only 1/10 of a human dose in saline elicited bactericidal antibodies specific for group A but not group B Salmonella (35). This is consistent with the immunodominant region of the group-specific antigen (factor 2), conferred by paratose, as predicted from the proposed structures of group A, B, and D O-SPs of salmonellae (8–10, 17).

We are grateful to Vee Gill and the staff of the Microbiology Branch, Clinical Center, NIH for their assistance. James C. Mond, Uniformed Services University of the Health Sciences, Bethesda, Md., and Andrew Lees, Virion Systems Inc., Rockville, Md., provided helpful advice about CDAP.

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