Immunization with a Pseudomonas aeruginosa Immunotype 5 O Polysaccharide-Toxin A Conjugate Vaccine: Effect of a Booster Dose on Antibody Levels in Humans

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Received 17 November 1987/Accepted 14 March 1988

Healthy adult volunteers were vaccinated on days 0 and 28 and at 15 months with a Pseudomonas aeruginosa immunotype 5 O polysaccharide–toxin A conjugate vaccine. Immune response in mill, transient local reactions in less than 20% of the subjects. Maximal immunoglobulin G (IgG) antibody titers to both toxin A and lipopolysaccharide (LPS) as determined by enzyme-linked immunosorbent assay were seen at day 42, at which time 50% of the vaccinees showed a fourfold or greater rise in toxin A-neutralizing titers. By 15 months postvaccination, both antitoxin A and anti-LPS IgG antibodies had markedly declined. A booster dose of vaccine administered at 15 months evoked a vigorous anti-toxin A IgG antibody response with 100% of the volunteers showing a fourfold or greater rise in neutralizing antibody titer compared with preimmunization levels. In contrast, there was no significant elevation of anti-LPS IgG antibody levels. At 24 months postimmunization, only anti-toxin A antibody levels were significantly higher than preimmunization levels.

Human immunity to Pseudomonas aeruginosa appears to depend on humoral antibody directed against serospecific lipopolysaccharide (LPS) determinants and toxin A (1, 4, 12). We have previously described the synthesis of O polysaccharide (O-PS)–toxin A conjugate vaccines and their safety and immunogenicity in humans (5–8). Given the diverse patient populations at risk to P. aeruginosa infections (2), such vaccines have two potential uses. The first would be to actively vaccinate immunocompetent patients, such as those suffering from burn trauma, who are capable of mounting a protective immune response (1). Alternatively, plasma could be obtained from healthy immunized donors and be used to produce a hyperimmune globulin (5, 10). Such a hyperimmune globulin could be passively transferred to immunocompromised patients or patients at immediate high risk of acquiring an infection, such as those on the surgical intensive care unit. To evaluate the potential applicability of O-PS–toxin A conjugate vaccines for these purposes, we determined the duration of the immune response after primary vaccination with an immunotype 5 O-PS–toxin A conjugate and the capability of a booster dose administered at 15 months to further elevate antibody levels.

The synthesis and characteristics of the immunotype 5 O-PS–toxin A conjugate vaccine have been described elsewhere (5, 7). Twenty healthy adult volunteers received either 81.25 or 162.5 µg of conjugate (corresponding to 25 and 50 µg of O-PS, respectively, on the primary dose) subcutaneously on days 0 and 28. Fifteen months later, 10 subjects (4 of whom received the 162.5-µg dose and 6 of whom received the 81.25-µg dose) were available to be vaccinated. Each volunteer received 81.25 µg of conjugate subcutaneously. Serum samples were obtained on the day of immunization, 10 to 15 days later, and 8 months later. Anti-LPS immunoglobulin G (IgG) antibody and anti-toxin A IgG antibody were determined by enzyme-linked immunosorbent assay (7). Antibody levels are expressed as enzyme-linked immunosorbent assay units, which were calculated by choosing a serum dilution which fell in the linear portion of the serum titration curve and multiplying its reciprocal by the corresponding A415. Toxin A-neutralizing antibody was quantitated by a HEp-2 cell cytotoxicity assay (5, 7). Significance between antibody titers was determined by a one-way analysis of variance with a least-square-means t test.

Reactions to vaccination at 15 months post-primary immunization were infrequent and mild. Three volunteers had a slightly painful reaction at the site of injection which lasted less than 48 h. These reactions were similar in nature and frequency to those noted after primary immunization.

Anti-LPS IgG antibody engendered by primary immunization was long lived, being significantly (P < 0.05) higher than preimmunization levels after 15 months (Table 1). Anti-toxin A IgG antibody declined more rapidly over the same period. The booster dose of vaccine administered at 15 months evoked a rapid rise in anti-toxin A IgG antibody. Postboost antibody levels were approximately 10-fold above preimmunization titers (P < 0.01). A total of 50% of the subjects responded with a fourfold or greater rise after immunization, and 90% had titers fourfold or greater above preimmunization levels. In contrast, the booster dose of vaccine elicited only a slight increase in anti-LPS IgG antibody. Even so, 70% of volunteers possessed titers fourfold or greater above base-line levels. Nine months after boosting, IgG antibody levels to toxin A and LPS had declined considerably. Mean anti-toxin A titers were still significantly (P < 0.05) higher than preimmunization levels with five of eight subjects still having titers fourfold or greater above base-line levels. In contrast, anti-LPS antibody levels were not significantly elevated, with only three of the eight subjects having titers fourfold or greater above preimmunization levels.

To confirm that the anti-toxin A IgG engendered by boosting was functionally relevant, we tested serum samples for toxin A-neutralizing antibody (Table 1). Primary immunization evoked a detectable neutralizing antibody response in 80% of the subjects. Fifteen months later, 4 of these 10 subjects still had detectable levels of neutralizing antibody.

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TABLE 1. Immune response engendered after vaccination with O-PS–toxin A conjugate vaccine

<table>
<thead>
<tr>
<th>Time sample obtained</th>
<th>IgG ELISA(^a) units (mean with range)</th>
<th>Toxin A-antibody (\mu g) of toxin A neutralized/ml of serum (mean)</th>
<th>No. with detectable neutralizing antibody(^b)</th>
<th>No. with (\geq)fourfold rise in titer(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimunization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postimmunization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42 days(^d)</td>
<td>209 (28–704)*</td>
<td>113 (3–810)*</td>
<td>4.3</td>
<td>8/10</td>
</tr>
<tr>
<td>15 months</td>
<td>58.4 (16–200)*</td>
<td>12.3 (2.6–28)</td>
<td>0.56</td>
<td>4/10</td>
</tr>
<tr>
<td>16 months'</td>
<td>79 (17–272)*</td>
<td>53.3 (22–128)*</td>
<td>5.6</td>
<td>10/10</td>
</tr>
<tr>
<td>24 months</td>
<td>34 (4–140)</td>
<td>21 (4–40)*</td>
<td>1.1</td>
<td>5/8</td>
</tr>
</tbody>
</table>

\(^a\) ELISA. Enzyme-linked immunosorbent assay. \(^b\) \(\geq\)312 ng of toxin A neutralized per ml of serum. Number with detectable antibody/number tested. 
\(^c\) Compared with preimmunization levels. Number with rise in titer/number tested.
\(^d\) Volunteers were immunized on days 0 and 28 and at 15 months.

Immunization at this time evoked a vigorous neutralizing antibody response, with all 10 volunteers having at least a fourfold rise in neutralizing titer. At 24 months post-primary immunization, five of eight vaccinees had demonstrable toxin A-neutralizing antibody in serum.

The above results indicate that while primary immunization with an immunotype 5 O-PS–toxin A conjugate vaccine elicited good levels of antibody to both vaccine moieties, a booster dose given 15 months later succeeded in elevating only anti-toxin A antibody levels. In healthy adults, it would appear that low-molecular-weight (<50,000) polysaccharides behave as T-cell-independent antigens even when covalently coupled to a carrier protein capable of evoking an antibody response. Similar findings have been noted for a Haemophilus influenzae b polysaccharide-diphtheria toxoid conjugate vaccine as pertains to the antipolysaccharide response (9, 11). These results cannot simply be attributed to a carrier suppression effect (13), since most volunteers possessed very low anti-toxin A antibody levels before boosting. This would indicate that regulation of a humoral immune response in humans depends, at least in part, on the inherent chemical nature of the antigen.

The findings that immunization with the current O-PS–toxin A conjugate vaccine evokes a long-lasting IgG antibody response to both vaccine components and that antitoxin A levels can be increased by a single booster dose demonstrate the versatility of such vaccines. We are currently extending these studies to include toxin A conjugates produced with O-PS derived from additional serotypes of P. aeruginosa LPS.

LITERATURE CITED