Safety and Immunogenicity of a Pseudomonas aeruginosa O-Polysaccharide Toxin A Conjugate Vaccine in Humans

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Abstract

Lipid A-free polysaccharide (PS) isolated from Pseudomonas aeruginosa immunotype 5 lipopolysaccharide (LPS) was covalently coupled to toxin A via reductive amination. The PS-toxin A conjugate was comprised of 29.8% PS and 70.2% toxin A, possessed a molecular weight of > 10^6, was nontoxic for animals and was nonpyrogenic for rabbits at a dose of 50 µg/kg body wt when administered intravenously. The conjugate evoked only mild, transient reactions upon subcutaneous administration to human volunteers. Vaccination engendered immunoglobulin G (IgG) antibody, which neutralized the cytotoxic effect of toxin A and promoted the uptake and killing of P. aeruginosa in the presence of human polymorphonuclear leukocytes. Passively transferred IgG isolated from the serum of immunized donors was far more effective at preventing fatal P. aeruginosa burn wound sepsis than paired preimmunization serum. These studies establish the potential usefulness of such a PS-toxin A conjugate as a vaccine against P. aeruginosa.

Introduction

Gram-negative aerobic bacilli are the leading cause of fatal nosocomial infections (1, 2). Preeminent among these pathogens is Pseudomonas aeruginosa, whose ability to cause life-threatening infections in compromised patients is well documented (3, 4). The case fatality rate for P. aeruginosa bacteremia reported in the literature from 1951 to 1981 has remained in the range of 25 to 50% (4, 5), indicating the limited impact of antibiotic therapy.

The use of immunological means as an adjunct to antimicrobial chemotherapy for the control of P. aeruginosa infections has recently gained much attention (4, 6). Human immunity to P. aeruginosa had been found to correlate with humoral antibody directed against serospecific epitopes expressed by LPS and against toxin A (7, 8). A reduction in fatalities due to P. aeruginosa among compromised patients has been obtained following vaccination with an LPS-based vaccine (9, 10). However, the high frequency of adverse reactions associated with vaccination has limited its use.

The above findings indicate that optimal protection against P. aeruginosa would be obtained by use of a vaccine capable of eliciting both anti-LPS and anti-toxin A antibody. We have previously demonstrated that serologically active nonimmunogenic O-polysaccharide (O-PS) derived from LPS could be covalently linked to toxin A, yielding a conjugate vaccine (11). This conjugate was found to be nontoxic for animals and capable of evoking both an anti-LPS and an anti-toxin A immune response. We now report on the safety and immunogenicity of an O-PS-toxin A conjugate vaccine in humans.

Methods

Purification of antigens. LPS was purified from P. aeruginosa strain W-18 (immunotype 5) as previously described (12). The final preparation of LPS contained < 1% (wt/wt) protein or nucleic acid (12).

PS was obtained from LPS by acid hydrolysis (11). Briefly, LPS (suspended in 1% vol/vol acetic acid) was heated for 90 min at 100°C. After cooling, the suspension was centrifuged at 5,000 g for 15 min. The lipid A-containing pellet was discarded and the supernatant extracted three times with chloroform/methanol (3:1). After concentration by rotary evaporation, the aqueous phase was passed over a 5 × 40-cm column of AcA34 (LBK-Produkter, Bromma, Sweden) equilibrated in distilled water. The PS fractions eluting with a relative molecular mass (Mr) ≤ 50,000 were pooled, concentrated, and lyophilized.

Toxin A was purified from the culture supernatants of P. aeruginosa PA103 as previously described (13). The purity of the toxin A preparations as measured by high pressure liquid chromatography (E. I. DuPont Co., Wilmington, DE, GF-250 column) (see below) was > 96%. The mean lethal dose when administered intraperitoneally to 18–20 g female NMRI mice was between 0.1 and 0.25 µg per mouse.

Synthesis of PS-toxin A conjugate. The PS-toxin A conjugate was produced essentially as described elsewhere (11). Briefly, PS was oxidized by treatment with NaOCl at 22°C, after which ethylene glycol was added to exhaust residual NaOCl. This procedure generated approximately seven equivalents of reducing activity. After extensive dialysis, the oxidized PS was lyophilized. Adipic acid dihydrazide (ADH) was introduced into the toxin A molecule by the use of carbodiimide (11, 14). Approximately 13 mol of ADH were incorporated into each mole of toxin A as determined by reaction with 2,4,6-trinitrobenzenesulfonic acid (TNBS) using ADH to generate a standard curve. Toxin A-ADH was covalently coupled to oxidized PS by mixing equal amounts at 22°C for 6 h. At this time, NaCNBH3 was added to reduce Schiff bases and the mixture stored at 22°C for 5 d. The mixture was then extensively dialyzed against phosphate-buffered saline (PBS) and filtered over an AcA34 column equilibrated in PBS. The conjugate contained no detectable reactive hydrazide groups as determined by reaction with TNBS. Collected fractions were analyzed by measuring the absorbance at 220 and 280 nm, respectively. The material that eluted in the void volume of the column (Mr > 350,000) was pooled and lyophilized.

Analytical methods. The protein content of the conjugate was measured by the method of Lowry et al. (15) using toxin A as a standard. The carbohydrate content was determined by the tryptophane reaction (16) using purified P. aeruginosa PS as a standard.

Abbreviations used in this paper: ADH, adipic acid dihydrazide; ADPR, adenosine diphosphatase; ELISA, enzyme linked immunosorbert assay; LPS, lipopolysaccharide; O-PS, O-polysaccharide from lipopolysaccharide; PBS-T; PBS containing 0.02% Tween-20.
Enzymatic activity assay. The adenosine diphosphate ribosyl (ADPR)-transference activity of toxin A was measured as previously described after activation in the presence of urea and diethiothreitol (17). All assays were performed in duplicate. The background value (all assay reagents except toxin A protein) was subtracted to yield specific counts. The sensitivity of this assay is < 1 ng toxin A. To determine if the conjugate possessed residual enzymatic activity, increasing amounts of toxin A protein as conjugate (100 ng/assay to 1 μg/assay) were tested. A negative result was defined as follows: specific counts < 5% above background value and no evidence of an increase in specific counts with an increasing amount of conjugate protein assayed. Enzyme neutralization assays were performed as follows. To 50 μg of toxin A in 10 μl of 50 mM Tris, pH 8, were added 10 μl of sera or IgG (see below). The mixture was placed at 37°C for 30 min, after which time the assay was completed as described above.

Cytotoxicity neutralization assay. The ability of sera to neutralize the cytotoxic effect of toxin A was tested as previously described except Chinese hamster ovary cells were used in place of HeP-2 cells (11).

Mouse lethality assay. The lethal effect of toxin A or conjugate vaccine for mice was evaluated by the intraperitoneal injection of graded doses of toxin A or conjugate. Mice (female, NMRI strain, weighing 18–20 g) each received 200 μg of conjugate protein, equal to 1,000 mean lethal doses of native toxin A. Mice were observed daily for 14 d postinjection.

Enzyme-linked immunosorbent assays (ELISA). ELISA for the quantitation of anti-toxin A and anti-LPS IgG were performed as previously described (11, 13). The conjugate used was peroxidase-labeled protein A produced by the method of Nakane and Kawai (18). Color was allowed to develop for 10 min after the addition of the substrate solution (11) and read at 405 nm on a Titertek Multiscan (Flow Laboratories, Hamden, CT).

A standard curve for the quantitation of IgG was produced as follows. Affinity purified goat anti-human IgG (Kirkegaard and Perry, Gaithersburg, MD) was diluted in PBS (10 μg/ml) and 200 μl added to each well of a flat-bottomed microtiter plate. The plates were incubated for 3 h at 37°C and then stored at 4°C until used. After three washes with PBS containing 0.02% Tween-20 (PBS-T), 200 μl of purified human IgG (Globuman, Swiss Serum and Vaccine Institute, Berne, Switzerland) from 0.009 μg/ml to 10 μg/ml in twofold serial dilutions were added per well. The plates were then processed in the same manner as described above. When the results were plotted as the absorbance at 405 nm versus the log of IgG concentration, the linear portion of the curve was found between 0.03 and 0.7 μg IgG/ml. Human IgG was excluded from control wells.

Specific IgG antibody to LPS or toxin A in human serum was derived as follows. A dilution of each test serum giving an absorbance within the linear region of the standard curve was used to calculate micrograms of IgG. This value was then multiplied by the dilution factor to yield micrograms IgG/ml of serum.

Opsonophagocytosis assay. Opsonophagocytic assays were performed as previously described (19).

Column chromatography. The conjugate vaccine was sized by gel filtration over Sephacryl S-500. The column (1 cm × 45 cm) was equilibrated in PBS and calibrated with Blue Dextran 2000 and 13Cdiodoacetate.

Vaccine. Conjugate-containing fractions that eluted in the void volume of an AcA34 column (see above) were pooled and the protein and carbohydrate content determined. The conjugate was diluted in an equal volume of 10% (wt/vol) lactose. This was further diluted in 5% (wt/vol) lactose in PBS to yield a final conjugate concentration of 100 μg carbohydrate and 225 μg protein/ml. This was passed through a 0.45-μm filter under aseptic conditions. The vaccine (1 ml) was placed in sterile glass vials and lyophilized under aseptic conditions. The vials were labeled, sealed, and stored at 4°C.

Tests for sterility and general safety of the vaccine were performed according to requirements set forth under articles V.2.1.1 and V.2.1.5 of the European Pharmacopoeia (20). Pyrogenicity testing was performed in New Zealand white rabbits as previously described (21).

Subjects and study design. Healthy volunteers of both sexes aged 16–59 participated in the trial. The vaccine was reconstituted in sterile distilled water immediately before use. Volunteers received either 0.5 ml (162.5 μg of conjugate equal to 50 μg of PS) or 0.25 ml (81.25 μg of conjugate equal to 25 μg of PS) by the subcutaneous route in the deltoid region on days 0 and 28. Adverse reactions including local pain, swelling, redness, induration, fever, chills, malaise, or headache were recorded on a control sheet for 5 d postimmunization.

Venous blood samples were obtained on days 0 (at the time of vaccination), 2–3, 14, 28, 30, and 42. Aliquots of serum were frozen at −20°C. Serum or heparinized whole blood from each timepoint was analyzed by the Clinical Laboratory Service at the Insel Hospital, Berne, Switzerland, for hemoglobin, hematocrit, erythrocyte, leukocyte, thrombocyte, and differential leukocyte counts, creatinine, serum glutamate-oxalacetate-transaminase and serum glutamate-pyruvate-transaminase.

Isolation of IgG. Two serum pools were made by combining 0.5 ml of serum from each volunteer collected on day 0 (preimmune pool) or on day 42 (postimmune pool). IgG was obtained from each pool by published techniques (22). The purified IgG was reconstituted to the original starting volume in saline, passed through a 0.45-μm filter and stored at −20°C. The concentration of IgG was determined by use of a Partigen test kit (Boehringer Mannheim, Mannheim, West Germany). Postimmune IgG was absorbed with the O-PS-toxin A conjugate as follows. To 2 ml of IgG was added 1.25 mg of conjugate. The mixture was incubated for 1 h at 37°C. The resulting precipitate was removed by centrifugation at 12,000 g for 10 min and the final supernatant passed through a 0.4-μm filter. This material was concentrated over a ym-10 filter (Amicon Corp., Danvers, MA) and applied to a Sepharose CL-4B column to separate free conjugate from IgG. The IgG fractions were collected and concentrated. This procedure was repeated once and the material filter sterilized before use.

Passive protection studies. Female NMRI mice weighing between 18 and 20 g were used. Each mouse received 40 μg of IgG via the tail vein at time 0. Approximately 24 h later, mice were burned and challenged with graded doses of P. aeruginosa strain W-18 (immunotype 5) or PA220 (immunotype 1) (12, 23). Groups of six mice were used. Mortality was recorded for 7 d post-challenge.

Statistical analysis. The mean lethal dose was calculated according to the method of Reed and Muench (24).

Results

Various characteristics of the O-PS-toxin A conjugate vaccine are shown in Table I. The conjugate was composed of toxin A and O-PS at a ratio of 2.3:1. The vaccine possessed a molecular weight of ~ 2 × 106 as determined by gel filtration and was devoid of ADPR-transferase activity. The conjugate was non- pyrogenic when tested at a dose of 50 μg/kg and nontoxic upon intraperitoneal administration to mice and guinea pigs. To document that the vaccine was not subject to toxic reversion when exposed to physiologic temperatures, the following experiments were performed. Reconstituted vaccine was incubated at 37°C for 28 d at which time 200 μg of conjugate protein (equivalent to 1,000 mean lethal doses for native toxin A protein) was injected intraperitoneally into mice. No overt signs of illness were observed and all mice survived. Similarly, there was no evidence of an increase in enzymatic activity after storage.

The above results, demonstrating the safety and stability of the conjugate vaccine, led us to evaluate its acceptability and immunogenicity in humans. Healthy adult volunteers received either 162.5 μg of vaccine (equal to 50 μg of O-PS and 112.5 μg of toxin A protein) or 81.25 μg of vaccine (equal to 25 μg of O-PS and 56.25 μg of toxin A protein) subcutaneously in the deltoid region. Reactions to vaccination are shown in Table II. Only a single vaccinee noted a local reaction after the first vaccination. One subject experienced malaise 24 h postimmunization that

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Table I. Characteristics of PS Toxin A Conjugate Vaccine

<table>
<thead>
<tr>
<th>Composition*</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>M*</th>
<th>ADPR-transferase activity</th>
<th>Pyrogenicity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>% %</td>
<td>µg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70.2</td>
<td>29.8</td>
<td>2 x 10⁶</td>
<td>ND‡</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* Values shown are on a weight basis.  
† Determined by filtration over Sephacryl S-500.  
‡ When administered intravenously to rabbits, 50 µg of vaccine per kg body weight evoked < 0.5° increase in temperature.  
ND = nondetected.

lasted for < 24 h. Reactions were more frequent following the booster dose of vaccine administered on day 28. A total of six subjects (30%) reported a local reaction, while two noted a systemic reaction (malaise and a slight headache). The majority of vaccinees who experienced a local reaction had received the higher vaccine dose. All reactions were mild, did not hinder normal functions and were spontaneously resolved within 48 h. No significant alterations in blood chemistry or serum liver enzyme profiles were noted that could be attributed to vaccination over the 6-wk observation period.

The ability of the O-PS-toxin A conjugate to elicit an IgG antibody response that recognized native LPS and toxin A is shown in Table III. The magnitude of the anti-LPS response was comparable at both immunizing doses with 70 to 90% of the vaccinees responding with a significant (fourfold or greater) rise in specific IgG. It should be noted that two of the three subjects, who received the 81.25-µg dose and did not respond, possessed the highest levels of anti-LPS IgG in their preimmune sera (12.1 and 13.9 µg/ml) seen in this study. The mean concentration of anti-LPS IgG in postimmune sera was comparable for both groups. In contrast, the immune response to the toxin A component was more vigorous in volunteers who received the higher immunizing dose. Whereas on day 42, 90% of the subjects who received the 162.5-µg dose of vaccine responded with a greater than or equal to fourfold rise in anti-toxin IgG, only 60% did so at the 81.25-µg dose. Similarly, the mean concentration of anti-toxin IgG in postimmunization sera was approximately threefold higher for the group that received the 162.5-µg dose. It is interesting to note that the second dose of vaccine administered on day 28 resulted in a substantially higher seroconversion rate to toxin A. This effect is most evident in the group which received the 162.5-µg dose of vaccine, where the percentage of subjects responding increased from 40% after primary immunization to 90% after the second dose of vaccine. A similar trend in the immune response to LPS was not observed. There was no detectable anti-ADH response after immunization, illustrated by the fact that postimmune sera did not bind to ADH-sensitized ELISA plates.

Next, functional activities of antibody elicited following immunization with the conjugate vaccine were evaluated. Antibody directed against the toxin A moiety of the conjugate were able to neutralize the cytotoxic potential of purified toxin A (Table IV). The mean toxin A-neutralizing capacity in postimmune sera and the meanfold rise in specific anti-toxin A IgG were comparable at both vaccine doses, as were the number of volunteers showing a more than fourfold rise in titer. Only 4 of 20 subjects (20%) possessed detectable levels of toxin A-neutralizing antibody in their preimmunization serum (0.4 to 0.8 µg of toxin A neutralized per ml of serum). All responded with a more than fourfold rise in neutralizing capacity after vaccination.

Vaccination with the conjugate did not evoke a significant rise in antibody capable of neutralizing the ADPR-transferase activity of toxin A. Pooled preimmune sera reduced the enzymatic activity of toxin A by an average of 15.4%, whereas the level of inhibition was increased to only 32% when pooled postimmunization (day 42) sera were tested.

Antibody directed against the O-PS moiety of the conjugate vaccine was capable of promoting the phagocytosis and killing of an IT-5 strain of P. aeruginosa by human polymorphonuclear leukocytes (Table V). Both immunizing doses were found to be equally effective at engendering an opsonic antibody response. The postimmune mean titer was comparable in both groups, as were the number of individuals responding with a significant (more than fourfold) rise in titer. All but one volunteer, who received the low vaccine dose, made a significant response.

The ability of antibody evoked following immunization with the conjugate vaccine to afford protection against fatal experimental P. aeruginosa immunotype 5 burn wound sepsis is shown in Table VI. Compared with mice that received human albumin, both preimmune and postimmune IgG were able to increase the LD₅₀. The level of protection was dependent upon the amount of specific antibody transferred. Therefore, while preimmune IgG raised the LD₅₀ value 14-fold over the human albumin-treated group, the administration of postimmune IgG with ~ 10-fold higher antibody content resulted in a further 100-fold increase. To confirm that protection was indeed me-

Table II. Reactions Noted after Immunization with O-PS Toxin A Conjugate Vaccine

<table>
<thead>
<tr>
<th>Immunizing dose*</th>
<th>Local reactions</th>
<th>Systemic reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>Pain</td>
<td>Swelling</td>
</tr>
<tr>
<td>81.25</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>162.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* Volunteers immunized with 81.25 µg of conjugate received 25 µg of O-PS and 56.25 µg of toxin A protein. Volunteers immunized with 162.5 µg of conjugate received 50 µg of O-PS and 112.5 µg of toxin A protein.  
† The first immunization was on day 0 and the second was on day 28.
Table III. IgG Response after Immunization with the O-PS Toxin A Conjugate Vaccine

<table>
<thead>
<tr>
<th>Immunizing dose*</th>
<th>Immune response to</th>
<th>Mean µg IgG/ml serum (range)</th>
<th>Nr. ≥ 4-fold rise/total^3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 14^1</td>
</tr>
<tr>
<td>81.25 µg</td>
<td>Toxin A</td>
<td>0.19 (0.07-0.54)</td>
<td>1.9 (0.07-40)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>5.8 (0.16-13.9)</td>
<td>49 (0.5-178)</td>
</tr>
<tr>
<td>162.5 µg</td>
<td>Toxin A</td>
<td>1.42 (0.09-12)</td>
<td>19.4 (0.1-80)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>1.57 (0.3-3.1)</td>
<td>30.1 (0.19-160)</td>
</tr>
</tbody>
</table>

* Volunteers were immunized on day 0 and 28. Subjects vaccinated with 81.25 µg of conjugate received 25 µg of O-PS and 56.25 µg of toxin A. Subjects vaccinated with 162.5 µg of conjugate received 50 µg of O-PS and 112.5 µg of toxin A.

^1 Relative to the time of immunization (day 0).

^3 Indicates the number of volunteers showing a fourfold or greater rise in specific IgG compared to preimmunization levels.

diated by anti-conjugate antibody, postimmune IgG was absorbed with the conjugate vaccine. This procedure, which reduced the levels of antitoxin A and anti-LPS antibody to slightly below those for preimmune IgG, resulted in a corresponding loss in protective capacity. It is interesting to note that submicromolar amounts of specific antibody could confer an extremely high degree of protection in this model system.

To determine the relative protective roles of antitoxin A versus anti-LPS antibody in this model system, the following experiment was performed. Groups of mice received either preimmune or postimmune IgG before burning and challenge with a toxin A-producing strain of a heterologous LPS immunotype (immunotype 1). The LD₅₀ value for both groups was identical (2 × 10⁴) indicating that the protection noted above was mediated primarily by anti-LPS antibody. These findings are in agreement with previously published studies showing that antitoxin A antibody is poorly protective in this model system against highly virulent challenge strains (13).

Discussion

The need for immunological agents to aid in the control of P. aeruginosa infections is documented by the fact that mortality rates for nosocomial bacteremic episodes have remained essentially unchanged for the past three decades (4, 5). Immunoprophylactic or immunotherapeutic intervention is complicated by the fact that various patient populations are at risk to acquiring a life-threatening P. aeruginosa infection (4, 6). Therefore, the mode in which a P. aeruginosa vaccine is employed would depend upon the immune status of the individual. Active vaccination is a feasible approach in certain high-risk groups, such as in burned or surgical patients who have been shown to mount a good humoral immune response to parenterally administered P. aeruginosa antigens (10, 25, 26). Immunosuppressed patients, on the other hand, would not be expected to benefit greatly from immunization due to their depressed capacity to mount an antibody response (9, 27, 28). In such cases, a hyperimmune gammaglobulin for intravenous use produced from the plasma of immunized donors would be the most logical approach.

Promising, but inconclusive results from clinical trials using a polyvalent LPS vaccine suggest that an immunological approach against P. aeruginosa infections is possible (10). Supporting this hypothesis is the finding that elevated levels of antibody to LPS and toxin A have been found to correlate with survival from P. aeruginosa bacteremia and may act in an independent and additive manner (7, 8). This would indicate that optimal protection against P. aeruginosa would be obtained by use of a vaccine capable of eliciting both anti-LPS and anti-toxin A antibody. We have, therefore, selected toxin A to serve as the carrier protein for nonimmunogenic serospecific O-PS in the construction of a conjugate to meet these requirements.

The O-PS-toxin A conjugate vaccine presently described was found to be safe when parenterally administered to healthy adult volunteers. No systemic reactions of consequence (fever, chills, etc.) were noted and local reactions were infrequent and mild in nature. No significant abnormalities in blood chemistry or

Table IV. Ability of the O-PS Toxin A Conjugate to Elicit Toxin A-Neutralizing Antibody

<table>
<thead>
<tr>
<th>Immunizing dose*</th>
<th>Mean µg of toxin A neutralized per ml of serum</th>
<th>Nr. ≥ 4-fold rise/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.25 µg</td>
<td>Preimmune^1 0.12 Postimmune^2 2.64</td>
<td>6/10</td>
</tr>
<tr>
<td>162.5 µg</td>
<td>Preimmune^1 0.16 Postimmune^2 2.9</td>
<td>6/10</td>
</tr>
</tbody>
</table>

* Volunteers were immunized on day 0 and 28. Subjects vaccinated with 81.25 µg of conjugate received 25 µg of O-PS and 56.25 µg of toxin A. Subjects vaccinated with 162.5 µg of conjugate received 50 µg of O-PS and 112.5 µg of toxin A.

^1 Preimmune = prior to vaccination (day 0).

^2 Postimmune = 2 wk after receiving the second immunization (day 42).

Table V. Opsonic Antibody Response after Vaccination with the O-PS Toxin A Conjugate Vaccine

<table>
<thead>
<tr>
<th>Immunizing dose*</th>
<th>Mean opsonic titer (range)</th>
<th>Nr. ≥ 4-fold rise/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.25 µg</td>
<td>Preimmune^1 40 (10-160) Postimmune^2 596 (40-2,560)</td>
<td>8/10</td>
</tr>
<tr>
<td>162.5 µg</td>
<td>Preimmune^1 58 (10-160) Postimmune^2 528 (160-1,260)</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Volunteers were immunized on days 0 and 28. Subjects vaccinated with 81.25 µg of conjugate received 25 µg of O-PS and 56.25 µg of toxin A. Subjects vaccinated with 162.5 µg of conjugate received 50 µg of O-PS and 112.5 µg of toxin A.

^1 Preimmune, prior to vaccination.

^2 Postimmune, 2 wk after receiving the second immunization as in Table IV (day 42).
Table VI. Capacity of Passively Transferred Human IgG Preparations to Prevent Fatal P. aeruginosa Burn Wound Sepsis

<table>
<thead>
<tr>
<th>Material transferred*</th>
<th>Toxin A</th>
<th>LPS</th>
<th>Mean lethal dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human albumin</td>
<td></td>
<td></td>
<td>1.4 × 10^3</td>
</tr>
<tr>
<td>Preimmune IgG^a</td>
<td>22.4</td>
<td>32</td>
<td>2 × 10^4</td>
</tr>
<tr>
<td>Postimmune IgG^b</td>
<td>250</td>
<td>376</td>
<td>2 × 10^4</td>
</tr>
<tr>
<td>Postimmune IgG absorbed^c</td>
<td>8</td>
<td>15</td>
<td>2 × 10^4</td>
</tr>
</tbody>
</table>

* Mice each received 0.2 ml of IgG preparation or 0.2 ml of human serum albumin (160 µg/mouse) intravenously 24 h before challenge with P. aeruginosa.
^a Indicated the amount (ng) of specific antitoxin IgG and anti-LPS IgG each mouse received.
^b Prepared from equal aliquots of serum taken from all volunteers before immunization.
^c Prepared from equal aliquots of serum taken from all volunteers 42 d postimmunization.
^d Postimmune IgG was twice absorbed with immunotype 5 O-PS toxin A conjugate prior to administration.

Infections (6, 32). Therefore, any vaccine using serospecific antigen would have to be multivalent. It is presently envisioned that such a polyvalent conjugate vaccine would be composed of the appropriate serotypes of O-PS coupled to toxin A. However, due to the considerable heterogeneity in the monosaccharide composition of O-PS between serotypes (34, 35), it remains to be seen if the conjugation strategy currently used can be applied with comparable results to other relevant O-PS antigens.

Acknowledgments

Excellent technical assistance was provided by J. Schup, S. Balmer, D. Pavlovic, and S. Futosky. We thank Dr. M. Maag for providing fermenter cultures, Dr. S. Varralay for safety, pyrogenicity, and sterility testing, and L. Cryz for preparation of the manuscript.

References


