Phase I study of detoxified \textit{Escherichia coli} J5 lipopolysaccharide (J5dLPS)/group B meningococcal outer membrane protein (OMP) complex vaccine in human subjects

Alan S. Cross\textsuperscript{a,\ast}, Steven M. Opal\textsuperscript{b}, John E. Palardy\textsuperscript{b}, Joseph J. Drabick\textsuperscript{c}, H. Shaw Warren\textsuperscript{d}, Christian Huber\textsuperscript{b}, Pamela Cook\textsuperscript{a}, Apurba K. Bhattacharjee\textsuperscript{c}

\textsuperscript{a} Department of Medicine, Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore Street, HSF 480, Baltimore, MD 21201, USA
\textsuperscript{b} Infectious Diseases Unit, Memorial Hospital of Rhode Island, Pawtucket, RI, USA
\textsuperscript{c} Department of Bacterial Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, MD, USA
\textsuperscript{d} Infectious Diseases Unit, Massachusetts General Hospital, Boston, MA, USA

Received 29 November 2001; received in revised form 8 April 2003; accepted 29 May 2003

Abstract

We previously observed that a detoxified \textit{Escherichia coli} O111, Rc chemotype J5 lipopolysaccharide (J5dLPS)/group B meningococcal outer membrane protein (OMP) vaccine protected animals from experimental lethal sepsis when immune antibodies were given passively as treatment at the onset of fever or when vaccine was given actively as prophylaxis. To test the safety and immunogenicity of this vaccine, we administered doses of 5, 10 and 25 \textmu g (based on dLPS) of vaccine at days 0, 28 and 56 to 24 human subjects (8 per group). Temperatures of 100.3, 99.5 and 99.4\degree F occurred in three subjects. At 24 h, pain at the injection site was moderate in 38%, mild in 44% and not present in 18%, while at 48 h, it was 1, 25 and 73%, respectively. No alterations in baseline renal, hepatic or hematologic functions occurred. There were two to three mean-fold increases in anti-J5dLPS IgG (range: 1.9–5.1) and IgM (range: 1.2–9.2) levels in subjects receiving the 10 and 25 \textmu g doses. At 12-month follow-up, three of the original responders had continued elevation of antibody levels. A 25 \textmu g booster dose of vaccine did not increase antibody levels among those responders and did not elicit antibodies among three subjects with no previous antibody response. The plasma from the six volunteers inhibited LPS-induced cytokine generation in human whole blood ex vivo. We conclude that this J5dLPS/OMP vaccine was safe and well-tolerated with transient, local pain at the injection site. Vaccine formulations with different adjuvants are currently under investigation.

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Keywords: Vaccine; Lipopolysaccharide; Phase I study; Sepsis; \textit{E. coli} J5

1. Introduction

Sepsis is a leading cause of death among patients in intensive care units and is an important cause of morbidity in patients undergoing complicated abdominal surgery and those suffering trauma or burns [1,2]. The mortality from this condition has changed little in the last few decades [1,3]. Consequently there has been a considerable effort to devise new therapies to complement the advances in supportive care and anti-microbial therapy. A clinical trial with antibodies directed against the core regions of the endotoxin or lipopolysaccharide (LPS) molecule of Gram-negative bacilli suggested a beneficial effect on sepsis [4]; however, additional studies with this preparation, other anti-core endotoxin antibodies as well as studies with anti-lipid A monoclonal antibodies were disappointing [5–8]. These efforts were followed by clinical studies that tested whether anti-inflammatory mediator therapy would be a more effective adjunct therapy of sepsis, but after over 20 studies of at least 6 different types of interventions in over 20,000 patients, immune-based therapy for sepsis remains elusive [9].

Most recently, the administration of activated protein C to patients with severe sepsis that included acute impairment to at least one organ, resulted in a 20% improvement in 28-day mortality compared to placebo-treated controls [10]. Because of the initial success of a clinical trial with anti-core LPS (J5), we decided to determine whether a
modification of this approach would be useful. We showed that the passive administration of polyclonal anti-J5 IgG protected neutropenic rats from lethal infection with both *Pseudomonas* and *Klebsiella* when given as therapy at the onset of fever [11]. The protection resided in antibodies specific for the core saccharides in the J5 LPS and not for the lipid A. Consequently we prepared a J5 LPS vaccine in which the J5 LPS was detoxified by alkaline-treatment to remove some of the ester-linked fatty acids. When complexed with the outer membrane protein (OMP) of the group B-meningococcus, the vaccine-induced IgG antibodies that were similarly protective as the IgG elicited with the lipid A. Consequently we prepared a J5 LPS vaccine in which the J5 LPS was detoxified by alkaline-treatment to remove some of the ester-linked fatty acids. When complexed with the outer membrane protein (OMP) of the group B-meningococcus, the vaccine-induced IgG antibodies that were similarly protective as the IgG elicited with whole-boiled bacterial vaccine [12]. This vaccine was also effective in protecting neutropenic rats from lethal infection when given as active immunization before the induction of neutropenia [13]. Based on these data, we prepared a detoxified J5 LPS (J5dLPS)–OMP complexed vaccine for human use. This study describes the results of a phase I clinical trial of this vaccine in 24 human volunteers.

2. Methods

2.1. Preparation of Escherichia coli J5 detoxified LPS (J5dLPS) non-covalent complex with Neisseria meningitidis group B outer membrane protein (GBOMP)

All processes for the production of the vaccine were performed at the Pilot Bioproduction Facility of the Walter Reed Army Institute of Research, Forest Glen, MD, which is approved for current good manufacturing practices (cGMP).

*E. coli* J5 cells (originally obtained from Dr. Elizabeth Ziegler, San Diego, CA and maintained at −70 °C) were grown in 3001 of Trypticase Soy Broth in a 4001 fermenter for 24 h at 37 °C. The cells were collected by centrifugation in a Sharples centrifuge and the cell paste was stored at −40 ± 5 °C.

Acetone-killed and dried cells were prepared by sequential washing of cells with water, ethanol, acetone and ether. The dry bacteria were extracted with phenol:chloroform:petroleum ether (2:5:8) by the method of Zollinger et al. [15,16]. The extract was evaporated to remove residual phenol and was then dried overnight in a vacuum dessicator. The dry powder was resuspended in water and stored frozen at −80 ± 10 °C.

2.2. Preparation of detoxified J5 LPS (J5dLPS)

The purified *E. coli* J5 LPS solution/suspension in water was adjusted to a concentration of 4 mg/ml and an equal volume of 0.2 M sodium hydroxide solution was added slowly with stirring. The mixture was placed into a screw-capped bottle and the bottle was placed in a 65 ± 2 °C water bath for 2 h. The mixture was shaken every 5 min for the first hour and every 10 min for the second hour. The cooled solution was neutralized with 1 M acetic acid and the detoxified lipopolysaccharide (J5dLPS) was precipitated by the addition of 4 volumes of ethanol. The J5dLPS was then dissolved in water, filtered through a 0.45 μm Millipak membrane and stored at −80 ± 10 °C.

2.3. Preparation of N. meningitidis group B outer membrane protein (GBOMP)

*N. meningitidis* group B strain 8532 cells were grown in 3001 modified Catlin’s medium in a 4001 fermenter for 20 h at 37 °C. Following growth, the cells were inactivated with 0.5% phenol for 60 min at 37 °C and the cells were harvested by centrifugation. The GBOMP was extracted from the cells by the method of Zollinger et al. [15,16]. The GBOMP preparation was dialyzed against TEEN 0.1% buffer (0.05 M Tris, 0.15 M NaCl, 0.1% Empigen BB), pH 8.0 and stored at −80 ± 10 °C.

2.4. Preparation of J5dLPS–GBOMP non-covalent complex vaccine

Filtered bulk J5dLPS (294 mg in 350 ml water) was mixed with purified bulk GBOMP (352 mg in 132 ml TEEN 0.1% buffer). Non-covalent complex formation was initiated by slow removal of detergent from the mixture by ultrafiltration/diafiltration against sterile 0.9% sodium chloride solution, using a 3000 molecular weight cut-off, hollow fiber cartridge (AG Technology Inc., Needham, MA). The filtration was continued until there was no detectable detergent (Empigen BB) in the filtrate. The final retentate was filter sterilized through a 0.45 μm membrane (Millipak). The LPS content was determined by the phenol–sulfuric acid method [17], using purified J5dLPS as standard.

The filtered bulk J5dLPS–GBOMP vaccine was diluted with sterile 0.9% sodium chloride to contain 100 ± 10 μg J5dLPS/ml. The diluted vaccine was filled into sterile 10 ml vials, each vial contained 4 ± 0.1 ml of the final vaccine formulation. The vials were closed with sterile Teflon septum and then sealed with sterile aluminum caps. The vials (designated lot number 0376, BPR-156-02) were stored at −80 ± 10 °C. The protein (GBOMP) content of this lot was 136 ± 10 μg/ml [15].

2.5. Study design

A phase I study was conducted with this J5dLPS vaccine to determine its safety and immunogenicity. This study was approved by the IRB of the Walter Reed Army Medical Center and the Surgeon General’s Human Use Review Board. It was conducted at the Clinical Trials Center of the Walter Reed Army Institute of Research in Washington, DC. Patients were excluded if they had either HIV infection or a known immunodeficiency disease. Twenty-four healthy
male and female volunteers between the ages of 18 and 50 years signed informed consents and were randomly assigned to one of three groups of differing LPS doses on the basis of computer-generated random numbers. The doses were selected on the basis of antibody responses to these vaccine doses in rabbits and rats.

Vaccine dilutions were prepared in sterile, endotoxin-free normal saline at the clinical site. The vaccine was administered in a volume of 0.5 ml in the deltoid muscle. Successful doses of vaccine were given in alternating arms. The first group received 5 g of vaccine (based on LPS content) at days 0, 28 and 56. Starting 1 week later a second group received 10 g of the vaccine at the same time intervals. One week after the second group began immunization a third group received a 25 g dose of vaccine again administered at the same intervals. Blood was drawn before and 14 days after each immunization. Blood was sent for electrolyte levels, renal (creatinine) and liver (AST, ALT, total bilirubin, and alkaline phosphatase) function tests. In addition, complete blood count and urinalysis were also obtained. Aliquots of the blood were saved for antibody analysis.

After each immunization volunteers were given a diary to record their symptoms and temperature for 7 days, and were examined at 24 and 48 h after immunization for both local and systemic reactions. A severe local reaction was one that was sufficiently painful that the volunteer was unable to sleep on it or had pain on movement (i.e. without palpation) (may have been accompanied by analgesic use). A mild reaction is one in which the pain was of lesser intensity and was elicited on palpation of the arm. The systemic reactions were graded by the amount of fever and whether they were accompanied by arthralgia, headache or fatigue.

2.6. Follow-up immunization

Twelve months after the initial immunization, 6 of the original 24 subjects were re-immunized: 3 whose ELISA antibody response was ≥3-fold of baseline ("high responders") and 3 whose ELISA antibody responses were ≥2-fold of baseline ("low responders"). The goal was to see whether a single booster immunization at 1 year could further improve the antibody response and whether a booster at 1 year would elicit an antibody response in those who perhaps were "primed" by the initial series of immunizations. After obtaining baseline laboratory studies (complete blood count, renal and liver function tests, urinalysis) and pre-immunization serum for antibody determination, subjects received a single 25 g dose of vaccine in the deltoid muscle. As before, they returned at 24 and 48 h for examination, and at 7 days for review of symptoms, submission of their temperature and adverse reaction diary, post-immunization laboratory safety tests and urinalysis and then underwent plasmapheresis. The plasma was used for antibody determination as well as for use in functional studies.

2.7. ELISA antibody assay

Anti-J5 antibody levels were measured by ELISA assay as previously described [11]. In 96-well flat-bottom polystyrene microtiter plates (Costar, Cambridge, MA), wells were coated with 50 µg/ml polyl-lysine type VIIIB (Sigma, St. Louis, MO) in 100 µl PBS (0.01 M sodium phosphate, 0.14 M NaCl, 0.02% NaN₃, pH 7.4) at 37 °C for 1 h. The wells were emptied and overlaid with J5 LPS at 10 µg/ml in PBS for 3 h at 37 °C. Excess binding sites were then blocked with 1% casein (Fisher Scientific, Columbia, MD) in PBS at 37 °C for 1 h. The wells were washed with PBS between steps to remove unbound material. The antigen-coated plates were incubated with serial two-fold dilutions of serum for 16 h at room temperature (25 °C). The second antibody was incubated for 20 h at room temperature. Disodium p-nitrophenyl-phosphate (Sigma) at a concentration of 1 mg/ml in 1 M diethanolamine buffer with 1 mM MgCl₂, pH 9.8 was used as substrate. Absorbance was read to 410 nm on a plate reader (Dynatech, Alexandria, VA). ELISA optical density (OD) units were calculated by multiplying the dilution of serum at A410 of approximately 0.5 with the OD reading near the midpoint of the linear part of the OD dilution curve in our assay.

ELISA using GBOMP as antigen was performed by coating the wells with GBOMP without the use of polyl-lysine. All other steps were the same as described above.

2.8. Functional assays

2.8.1. Whole blood assay

To test the potential anti-inflammatory capacity of immune plasma generated by the J5LPS/OMP vaccine, a human whole blood assay was performed as previously described [18]. A volume of 100 µl of immune plasma from each of six human subjects or pooled control plasma was tested with a single healthy human volunteer for whole blood samples. A highly purified, single source LPS reagent was used from a trichloroacetic acid and phenol-extracted LPS from E. coli O111:B4 (Sigma, St. Louis, MO). All the reagents in this whole blood assay were endotoxin-free (<10 pg/ml) and utilized endotoxin-free glassware and reagents.

E. coli LPS was added at increasing concentrations (10 pg/ml to 10 ng/ml) to whole blood samples that had 2.5 International Units/ml of endotoxin-free heparin along with RPMI medium 1640, and incubated for 24 h at 37 °C in a CO₂ incubator. The cell viability was tested at the end of the incubation using Trypan Blue dye exclusion. Cell supernatants were analyzed by ELISA in duplicate for tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-10 levels.
Twenty-three of the 24 volunteers enrolled completed the study. After the second dose of vaccine, one volunteer had recurrent pyuria secondary to a urinary tract infection which responded both times to anti-microbial therapy. We elected not to administer the final dose of vaccine. There were no serious adverse effects in any group from the 71 immunizations. All patients had use of their arm following immunization and no one either called or saw a physician because of adverse effects. There were four episodes of analgesic use. One volunteer took a acetaminophen because of a temperature elevation of 99.4 °F (she was not febrile, but recorded this temperature as part of her required monitoring and took the medication because of the temperature elevation). A second volunteer took ibuprofen before going to exercise in order to prevent the occurrence of pain and two volunteers required ibuprofen for pain at the injection site. In each case the pain subsided within 30 min and did not return.

3.3. Systemic reactions

Two subjects at the 5 μg dose and one at the 10 μg dose reported transient headaches and three subjects at the 5 μg dose experienced fatigue (Table 1). One volunteer at the 10 μg dose had an episode of light-headedness which was possibly related to the vaccine and another volunteer reported an anxiety reaction 24 h after the immunization. Three subjects had temperature elevations, all <100.4 °F.

3.4. Local reactions

Volunteers experienced moderate to mild pain at the site of immunization and this usually resolved within 24–48 h (Table 1). There were six instances of local induration among the 71 immunizations for the 3 groups and 6 instances of local erythema. Two of the six instances of erythema were probably caused by sensitivity to the adhesive tape that was placed over the injection site. Of interest, there were a number of instances of generalized induration of the deltoid muscle noticed only by the examiner. This subtle finding usually resolved within 24 h and was not dose-related.

3.5. Laboratory abnormalities

Fourteen days after each immunization urine and serum was submitted to the laboratory for analysis (Table 2). No increases in creatinine, liver transaminases or alkaline phosphatase were noted. Two volunteers had mildly elevated bilirubin levels before immunization and these levels remained stable. While hematuria and pyuria were present in some samples, it was difficult to determine the roles of either menstrual periods or exercise in these findings (many of the volunteers came to the clinic after physical training). After the second immunization, one volunteer had two separate episodes of asymptotic pyuria. She was sent on both occasions to her private physician who treated her with anti-microbials, but she was not given the third dose of vaccine. Two subjects had mild pre-existing anemia.
Table 1
Local and systemic reactions at 24 h (D1) and 48 h (D2) following each of three immunizations with different doses of J5d LPS/OMP complex vaccine

<table>
<thead>
<tr>
<th>Dose</th>
<th>5 µg dose*</th>
<th>10 µg dose*</th>
<th>25 µg dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Induration</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Swelling</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pain</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
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<td>0</td>
</tr>
</tbody>
</table>

* Volunteers were immunized at days 0, 28 and 56 with the indicated dose. There were eight subjects at each dosage level. The number of subjects having the indicated reaction at 24 h (D1) and 48 h (D2) is indicated.

3.6. Antibody response
Isotype-specific antibody levels against the J5dLPS vaccine antigen and GBOMP were performed (Table 3). Mean fold ± S.E.M. increases in antibody levels over baseline were 2.1 ± 0.3, 2.0 ± 0.3 and 2.9 ± 0.6 for the IgA at these same doses and 1.5 ± 0.1, 3.3 ± 1.0 and 2.9 ± 0.6 for the IgM (P = 0.0489 comparing 5 µg versus 10 µg dose groups). Pre-immunization levels of anti-J5 LPS IgM were higher than for the IgA and IgG isotypes. Maximal individual responses were a 5.1-fold increase for IgG (10 µg dose), 4.2-fold for IgA (25 µg dose) and 9.2-fold for IgM (10 µg dose). Anti-GBOMP IgG titers also rose two- to four-fold over baseline levels. The mean-fold increases over baseline levels (±S.E.M.) were 3.67 ± 0.84, 2.90 ± 0.64 and 4.11 ± 1.14 OD units for the 5, 10 and 25 µg dose groups, respectively (data not shown).

After the immunization of human subjects, rabbits were immunized with the same lot of vaccine to insure that the vaccine had not lost immunogenicity during the phase I study. Antibody levels in rabbits using the same immunization schedule were similar to what we previously reported [19], indicating that the vaccine remained immunogenic in rabbits.

3.7. Follow-up immunizations
At 12 months after the initial three dose series, we immunized six subjects with a 25 µg dose of vaccine. None of the six subjects who received the vaccine experienced any systemic adverse effect. Four of the subjects had mild to moderate local tenderness at the injection site at 24 h, but none experienced local tenderness to palpation at 48 h. All had full use of their arms and none used an analgesic. Two subjects had induration at the injection site only at 24 h, but no erythema. All six subjects had no change in their laboratory safety tests compared to baseline. Of interest, those three
Table 3
Anti-J5 LPS ELISA antibody titers of sera from volunteers in the phase I trial

<table>
<thead>
<tr>
<th>Subject</th>
<th>1 μg dose</th>
<th></th>
<th></th>
<th>10 μg dose</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>1</td>
<td>1.2</td>
<td>2.8</td>
<td>1.3</td>
<td>2.0</td>
<td>8.6</td>
<td>14.0</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>3.6</td>
<td>1.4</td>
<td>2.9</td>
<td>12.0</td>
<td>19.4</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
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<td>1.1</td>
<td>3.6</td>
<td>16.0</td>
<td>23.2</td>
</tr>
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<td>1.5</td>
<td>2.9</td>
<td>1.0</td>
<td>3.0</td>
<td>7.6</td>
<td>15.8</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>5.0</td>
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<td>2.2</td>
<td>12.4</td>
<td>15.2</td>
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<tr>
<td>7</td>
<td>1.1</td>
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<td>0.8</td>
<td>1.6</td>
<td>11.4</td>
<td>17.8</td>
</tr>
<tr>
<td>8</td>
<td>3.4</td>
<td>7.8</td>
<td>1.2</td>
<td>2.4</td>
<td>9.0</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>GMT ± S.E.M.</td>
<td></td>
<td></td>
<td>GMT ± S.E.M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.56 ± 1.14</td>
<td>3.16 ± 1.20</td>
<td>1.24 ± 1.11</td>
<td>2.45 ± 1.09</td>
<td>10.96 ± 1.07</td>
<td>16.21 ± 1.07</td>
</tr>
</tbody>
</table>

Eight volunteers in each group received J5dLPS/OMP vaccine at days 0, 28 and 56. Sera was obtained up to 3 months after initial dose. Data represent mean ± S.E.M. OD units, with the range for each group in the parentheses below. OD units are defined as the reciprocal titer of serum that gives an optical density value closest to 1.00 and is obtained by multiplying the dilution of serum by OD at an OD close to 0.50 (e.g. OD 0.400 at 1:100 dilution = 40 OD units). Post levels are from the peak antibody level measured on specimens obtained up to 3 months after immunization.

8 P = 0.0103, comparing GMT IgG levels after 5 and 10 μg doses of vaccine; P = 0.0142 comparing GMT IgG levels after 10 and 25 μg doses of vaccine; P = 0.0489, comparing GMT IgM levels after 5 and 10 μg doses of vaccine. All other comparisons not significantly different. Data analyzed by one-tailed Mann–Whitney test (GraphPad Prism).

3.8. Functional activity of plasma from immunized volunteers

3.8.1. Whole blood assay
We tested the plasma for the ability to decrease endotoxin-mediated responses. We pre-incubated different doses subjects who had a response to the initial series of immunization with the J5/OMP vaccine still had elevations at 12 months, before administration of the booster dose (Table 4, volunteers 4–6). None of these three subjects was able to increase the antibody level further in response to the vaccine. In contrast, none of the three subjects who did not have an antibody response initially (volunteers 1–3) was found to have a delayed increase in antibody response at the 12-month pre-immunization bleed and none was able to respond to the booster dose of vaccine.

Table 4
Anti-J5 LPS IgG antibody levels in six volunteers who received 25 μg vaccine boost at 12 months

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Study entry</th>
<th>Peak</th>
<th>Pre boost</th>
<th>Seven-day post boost</th>
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<tr>
<td>1</td>
<td>167</td>
<td>126</td>
<td>30</td>
<td>28</td>
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<td>2</td>
<td>25</td>
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<td>6</td>
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<td>238</td>
<td>231</td>
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</tr>
</tbody>
</table>

Six of the 24 subjects consented to receive a 25 μg booster dose of vaccine 12 months after the initial three dose immunization series. Three of the six had responses in excess of 200 OD units to the initial immunizations while three did not. After serum was obtained (“pre-boost”) each subject received the vaccine intramuscularly (i.m.) in the deltoid muscle. A serum sample was obtained 7 days post-booster immunization. Antibody levels are expressed in OD units. *Antibody levels in OD units. Peak refers to highest level of serum antibody attained after primary immunization series.
of LPS (1–1000 ng/ml) with a fixed volume of plasma from three low and three high responders (based on peak ELISA antibody levels post-immunization) who were plasmapheresed 1 week after the 12-month boost. A plasma pool was made from five healthy blood donors who were free of anti-J5 LPS antibody. Following the addition of LPS pre-incubated in whole blood from a healthy individual unexposed to the vaccine, IL-6 levels were measured in the supernatants. Pre-incubation of LPS with vaccinee plasma decreased the amount of IL-6 generated (Fig. 1A). This was true for each of the high and low responders. These data suggest that the ELISA antibody level alone might not accurately reflect vaccine potency. To investigate this further, we selected the plasma from one low and one high responder vaccinee, and mixed them at 1:1 and 1:1.6 dilutions in the normal, pooled human plasma with different doses of LPS (Fig. 1B). At each dilution, the low responding vaccinee was less able to reduce LPS-induced IL-6 generation compared to the high responder. Hence, while the plasma of all vaccinees tested reduced the ex vivo generation of cytokines by LPS, the greater level of anti-J5 LPS antibody had a greater LPS-inhibiting activity. When the plasma from one of the high responders was compared to that of controls, cytokine reducing activity also was shown for TNF-α and IL-10 generation (data not shown).

### 3.8.2. Clearance studies

We previously showed that IgG from rabbits immunized with three doses of the J5LPS/OMP vaccine promoted the clearance of bacteria from the circulation. Consequently, plasma from one volunteer (high responder) was also tested for its ability to promote the clearance of bacteria. A live bacterial challenge was given intravenously 30 min after the immune or control plasma with the following bacteria:
Fig. 2. Effect of post-immunization plasma from single high responder subject on quantitative blood culture and endotoxin levels following intravenous inoculation of 10^6 CFU of each microorganism (except 10^5 CFU for K. pneumoniae K2). Rats (six in the immune plasma-treated and six in the control group for each microbial challenge) were given 9 ml/kg i.v. of either control or immune plasma 30 min before administration of bacteria. Blood samples were obtained over 5 h and cultured quantitatively for bacteria and analyzed for circulating endotoxin levels. (A) P. aeruginosa 12.4.4; (B) E. coli O18.K1; (C) K. pneumoniae K2; (D) S. aureus Capsular Type 8.
$10^6$ CFU of either *E. coli* O18:K1, *P. aeruginosa* 12.4.4 immunotype 6, or *S. aureus* Capsular Type 8. A challenge dose of *K. pneumoniae* K2 was chosen at $10^5$ CFU based on previous experiments with this pathogen. Pretreatment with immune, but not non-immune plasma resulted in significant reductions in circulating bacteria and endotoxin following both the *E. coli* O18:K1 and *P. aeruginosa* 12.4.4 challenges (Fig. 2A and B). No similar reductions were observed after challenge with *K. pneumoniae* K2 (Fig. 2C). As expected, no decrements were observed in circulating levels of *S. aureus* Capsular Type 8 following administration of J5 plasma (Fig. 2D).
At 6 h after bacterial challenge, IL-6 levels were consistently attenuated by all of the Gram-negative bacterial pathogens tested. Median serum IL-6 levels (25–75% interquartile range) were 425 pg/ml (control plasma-treated) versus 63 pg/ml (immune plasma-treated) after E. coli challenge (P < 0.01); 778 pg/ml versus 1056 pg/ml, respectively, after E. coli K2 challenge (P < 0.01) and 1096 pg/ml versus 344 pg/ml, respectively, following K. pneumoniae K2 challenge (P < 0.05).

4. Discussion

In this study we found that a J5dLPS/OMP complex vaccine was well-tolerated by human subjects with mild, transient pain at the injection site, but weakly immunogenic (compared to our earlier studies in animals). There were no severe reactions to the vaccine at any dose and there was a dose-dependent increase in antibody levels to J5 LPS. Further there were no adverse effects of this vaccine on renal or liver function tests, nor any evidence of any endotoxemic reaction (falling blood pressure, falling white blood cell count, fever >100.4 °F).

There have been a number of studies in which volunteers were immunized with whole, boiled J5 bacterial vaccine, but in only one such study were the adverse effects enumerated [20]. On initial subcutaneous immunization, all 16 vaccinees had local reactions consisting of pain, tenderness, induration and/or erythema and 7 of the 16 had systemic reactions characterized by myalgias, low grade fever, chills, sweats, abdominal cramps, nausea, diarrhea and back pain, all of which subsided within 24 h. Nine of the 16 volunteers were available for re-immunization 30 days later. All nine re-vaccinees experienced mild local reactions and three had one or more of the systemic responses (one of the four re-vaccinees with a systemic reaction on the initial immunization had similar findings at re-immunization).

Ziegler et al. reported systemic reactions occurring in 25% of subjects immunized [4]. McCabe et al. also reported that administration of a heat-killed bacterial vaccine to human subjects resulted in an unacceptable rate of local discomfort, chills and low grade fever [21]; however, a modified version of this vaccine were administered at various doses and schedules to 122 subjects with less toxicity [22]. At doses of 4 × 10^10 CFU the systemic reactions occurred in 17%, with more severe reactions occurring at higher doses. At a dose of 2 × 10^10 to 3 × 10^11 CFU, 14 of 75 subjects experienced systemic reactions. Post-immunization serum from these volunteers protected mice from lethal challenge with both viable bacteria and LPS. The half-life of the protection afforded (20–24 weeks) was longer than the half-life of the measured antibody [22]. As in our study and that of Schwarzer et al. [20], no booster effect was evident with re-immunization. This behavior is consistent with a T cell-independent antigen.

The current detoxified J5 LPS/OMP vaccine caused no severe anaphylactic reactions after any of the three doses. In no instance did we observe an endotoxin-like reaction. Thus the vaccine appeared to be better tolerated than the whole cell bacterial killed vaccine. Two volunteers took single doses of a non-steroidal anti-inflammatory agent on three occasions. Interestingly, the local arm pain quickly responded (within 30 min) with complete resolution of the pain. Although local induration at the injection site was rare, volunteers experienced a subtle, generalized swelling of the deltoid muscle (non-tender) that typically resolved within 24 h. This was not dose-related and occurred more often in female volunteers.

The vaccine induced an antibody response to J5 LPS. In an earlier study in rabbits, this vaccine did not elicit antibodies to lipid A or to an unrelated LPS [2]. Thus in those studies the vaccine induced an antigen-specific and not a polyclonal antibody response. Interestingly, among the three subjects who responded to the initial immunization series, serum obtained at 12 months for antibody determination showed anti-J5 LPS levels still above the pre-immunization baseline. Administration of a booster dose at this time did not result in increased levels. Similarly, three subjects who failed to respond to the vaccine were still unresponsive when boosted at 12 months.

The vaccine previously elicited a greater than 20-fold increase in antibody levels above baseline in both rabbit and mice [11]. J5LPS-specific IgG prepared from post-immunization rabbit sera was protective in the treatment of sepsis in a neutrophil rat model of sepsis [11]. Active immunization of rats with this vaccine was also highly protective against lethal bacterial infection [13]. In contrast to these studies in animals, in human subjects the antibody response was lower; however, plasma from six vaccinees, both high and low responders, were able to attenuate an LPS-initiated cytokine response unlike the case with plasma from a pool of non-immunized donors lacking anti-J5 antibody. This raises a question whether ELISA antibody levels are the most appropriate measure of vaccine effectiveness compared to some functional assay. Similar discrepancies have been demonstrated between ELISA and functional assays in the evaluation of the response to pneumococcal vaccines [23,24]. We did note differences in attenuating capacity between the plasma with the high and low anti-J5 antibody levels. In addition, the plasma of one high responder volunteer promoted the clearance of E. coli and P. aeruginosa bacteria and endotoxin (but not that of Klebsiella or S. aureus) indicating that this anti-J5LPS antibody bound to both free LPS and to viable blood-borne Gram-negative bacteria. In order to identify a surrogate assay that best reflects the efficacy of the vaccine, we must correlate the protective efficacy of plasma from human volunteers with their ELISA antibody levels and functional capacity in these (e.g. ex vivo cytokine generation, bacterial clearance) and perhaps other (e.g. Limulus lysate neutralization, fluid phase binding) assays.

We previously showed that the protective effect of anti-J5 LPS IgG in an animal model of sepsis was dose-related [11] and that a high responding human subject may have enhanced functional activity (Fig. 1B). Consequently, we...
would predict that if we were able to elicit higher levels of antibody in humans with this well-tolerated vaccine, the antibody might provide anti-endotoxin activity in humans. Currently, we are assessing the ability of various adjuvants to increase the antibody response to the vaccine.

If the antibodies induced with this vaccine in humans have functional activity similar to what was observed in neutropenic rats, then the availability of a well tolerated, immunogenic vaccine may allow new therapeutic options. This vaccine could be used to elicit antibodies in normal volunteers for processing into a hyperimmune globulin which could be passively infused into patients stricken with sepsis. Alternatively, this vaccine could be used in the active mode in selected populations (e.g. firemen, police, soldiers or those undergoing elective surgery). Active immunization may induce a broader-based immune response than passive therapy and higher affinity antibody than what is achieved by screening out-dated blood.

Interestingly, patients who may benefit best from this vaccine may be those who may appear least able to respond; however, patients with burns or trauma have an initial Th2 response which favors the formation of antibody. Indeed, in a pilot study of experimental anti-<i>P. aeruginosa</i> and <i>Klebsiella</i> vaccines, all ten subjects immunized upon admission for trauma responded with levels of antibody observed following immunization of healthy volunteers [25]. If this were the case with this anti-endotoxin vaccine, then an active-passive approach in which patients actively immunized against sepsis have a break-through episode receive supplementation of antibody through passive infusion may provide optimal protection. The availability of a safe, immunogenic vaccine would allow such strategies to be tested.

Acknowledgements

This study was supported in part by a grant from the NIH (RO1AI42181-04), and also by a collaborative research and development agreement (CRADA) between the Walter Reed Army Institute of Research and Cheil America Inc. We are grateful for the excellent assistance provided by Patrick Grayson, CPT, MC and Jeremiah Stubbs, CPT, MC in this study. Veronica Greenidge gave invaluable assistance in the preparation of this manuscript.

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