Active Immunization with a Detoxified *Escherichia coli* J5 Lipopolysaccharide Group B Meningococcal Outer Membrane Protein Complex Vaccine Protects Animals from Experimental Sepsis

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The passive infusion of antibodies elicited in rabbits with a detoxified J5 lipopolysaccharide (LPS)/group B meningococcal outer membrane protein complex vaccine protected neutropenic rats from heterologous lethal gram-negative bacterial infection. In this study, active immunization was studied in neutropenic rats infected with *Pseudomonas aeruginosa*, in the presence or absence of ceftazidime therapy, and with *Klebsiella pneumoniae*. This vaccine elicited a >200-fold increase in anti–J5 LPS antibody, which remained elevated throughout the duration of cyclophosphamide-induced neutropenia and for ≈3 months. There was improved survival among immunized versus control animals: 48% (13/28) versus 7% (2/29) in *Pseudomonas*-challenged rats; 61% (11/18) versus 0% (0/10) in *Pseudomonas*- and ceftazidime-treated rats; and 64% (9/14) versus 13% (2/15) in *Klebsiella*-challenged rats (*P* < .01 for each comparison). Immunized animals had lower levels of bacteria in organs and lower levels of circulating endotoxin at the onset of fever. In conclusion, active immunization with an anti-endotoxin vaccine improved survival after infection with ≥2 heterologous, clinically relevant bacterial species in immunocompromised animals. Active immunization with this vaccine merits further investigation.

Gram-negative bacterial sepsis is a serious complication in patients residing in intensive care units (ICUs), undergoing abdominal surgery, or incurring trauma or burns and in patients who develop prolonged neutropenia. Although antibiotic therapy plays an important role in limiting the incidence of this complication, during the last 10 years there has been little change in the mortality of this condition once it develops [1]. Consequently, additional strategies have been examined in an attempt to decrease the mortality, which still approaches 50% in the presence of shock [2–5].

There has been considerable experience in both experimental and clinical studies with anti-endotoxin antibodies directed against widely conserved epitopes in the inner core region of the lipopolysaccharide (LPS, or endotoxin) of gram-negative bacteria. Therapy directed against bacterial components have the potential advantage over anti-inflammatory mediator therapy in that, unlike anti-cytokine-directed therapies (e.g., anti-tumor necrosis factor, IL-1Ra), anti-LPS therapy might not compromise host defenses and lead to uncontrolled infection. Antiserum prepared from a killed, mutant strain of *Escherichia coli* 0111 (J5 mutant; Rc chemotype) in which a core LPS epitope was exposed to the immune system provided a highly significant protective effect in patients with gram-negative bacteremia, particularly if they were in profound shock [5]. The investigators, however, were unable to show a correlation between patient survival and the antibody levels achieved in these passively immunized patients. We recently showed that the IgG fraction, purified from animals immunized with a similar whole-bacteria, killed *E. coli* J5 vaccine, conferred protection against lethal *Pseudomonas aeruginosa* infection in a neutropenic rat model of infection [6]. Antibody that was affinity purified on LPS extracted from *E. coli* J5 provided similar protection. On the basis of these results, we made a detoxified J5 LPS vaccine in which the detoxified LPS was complexed to group B meningococcal outer membrane protein (J5 dLPS/OMP). Anti-
body elicited by this vaccine similarly protected neutropenic rats from heterologous infection upon passive administration at the onset of fever [7]. This protection was dose dependent.

Since certain patient subpopulations are considered to be at particular risk of developing sepsis, it may be desirable to actively immunize such groups, in the expectation that a vaccine may either prevent or ameliorate the course of sepsis following infection with gram-negative bacteria. The availability of a detoxified J5 LPS/OMP complex vaccine that is immunogenic allowed us to test this hypothesis. In this study, rats immunized with this vaccine before being rendered neutropenic were protected from the development of lethal sepsis.

Materials and Methods

Bacterial challenge. Two bacterial pathogens were used in these experiments: *P. aeruginosa* 12.4.4. and *Klebsiella pneumoniae* K2. *P. aeruginosa* 12.4.4. (originally provided by A. McManus; US Army Institute of Surgical Research, San Antonio, TX) is a serum-resistant, human bloodstream isolate of *P. aeruginosa*. The organism, a Fisher-Devlin-Gnabasik immunotype 6 isolate, was stored in 10% glycerol at −70°C until ready for use. The day before the oral challenge, the isolate was incubated overnight in trypticase soy broth (TSB; Becton Dickinson) at 37°C. The following day bacteria were suspended in normal saline and adjusted spectrophotometrically to an inoculum size of 10^6 cfu/mL. This dose exceeds the LD_{50} for this experimental model in previous studies [6–8].

*K. pneumoniae* K2 (strain BS055) is a serum-resistant, rodent-virulent, encapsulated strain of *K. pneumoniae* (originally obtained from Drs. Frits Orskov and Ida Orskov, Statens Seruminstitut, Copenhagen). The organism was stored and prepared as described above, with exception that the challenge dose was 10^7 cfu/mL, since preliminary studies demonstrated that a higher inoculum was necessary to achieve a dose that exceeds the LD_{50} in this animal model.

Vaccines. The vaccine used in these experiments is a noncovalent complex vaccine consisting of detoxified *E. coli* J5 LPS and *Neisseria meningitidis* group B OMP [7]. When this de-O-acylated J5 LPS is complexed with the OMP, it remains highly immunogenic and is well-tolerated in experimental animals [7]. As a control, a similarly prepared LPS/OMP complex vaccine was made by extracting LPS from *Brucella abortus*, subjecting it to alkalization to remove ester-linked fatty acids (“detoxifying” the LPS) and mixing it with group B meningococcal OMP. In addition, PBS saline was administered in place of vaccine, to control for possible adverse reactions to either vaccine and to control for nonspecific protection, which may result from either component of the *Brucella* dLPS/OMP vaccine. The vaccines were stored at 4°C until ready for use. The vaccines were administered at a dose of 20 μg subcutaneously at intervals of 0 and 4 weeks (2-dose schedule) or 0, 2, and 4 weeks (3-dose schedule). The temperature of the animals was checked by infrared thermometer 24 h prior to and at 1, 2, 24, and 48 h after each immunization (see below). The weights were checked weekly.

Animal model. The basic design of the neutropenic rat model has been described in detail elsewhere [6–8]. Briefly, nonpregnant female, specific pathogen–free, albino Sprague-Dawley rats weighing between 125 and 150 g (Charles River Breeding Labs) were maintained in filtered, biological safety cages and allowed to eat and drink ad libitum. After a 7-day control period, the animals underwent baseline blood sampling and then were immunized with the J5 dLPS/OMP complex without the addition of an adjuvant. Two weeks after the last dose of the vaccine, repeat blood sampling was performed, to determine vaccine responsiveness. Four weeks after the last dose of vaccine, the animals were rendered neutropenic with an intraperitoneal dose (100 mg/kg) of cyclophosphamide (Bristol-Meyers) (time 0), followed by a second intraperitoneal dose of 50 mg/kg 72 h later.

Cefamandole (100 mg/kg; Eli Lilly) was given intramuscularly beginning 96 h before the first dose of cyclophosphamide, to facilitate colonization of the alimentary tract with the challenge strain of *P. aeruginosa*. Ampicillin (Sigma) was given at a dose of 25 mg/kg intramuscularly and orally on every other day basis, to disrupt colonization resistance against *K. pneumoniae*.

The activity of the vaccine was also tested in the presence of active antimicrobial therapy against the challenge strain of *P. aeruginosa*. At the onset of fever, ceftazidime (50 mg/kg; Glaxo Wellcome) was given intravenously (iv) in 1 experimental group, with (n = 18) and without (n = 10) the active vaccine. The treatment was given at a low iv dose (to promote antibiotic-induced endotoxin release [9]) every 12 h for 48 h after the onset of fever in these animals with sepsis.

A bacterial suspension was prepared to deliver 1 mL of 10^6 cfu *P. aeruginosa* 12.4.4 or 10^7 cfu *K. pneumoniae* K2 for each experimental group.

The bacterial challenge was given orally via an orogastric tube prepared from polyethylene tubing (Intramedic PE, 160; Clay Adams Division, Becton Dickenson). The bacterial challenge was given on day 0 (the first dose of cyclophosphamide) and again on days 2 and 4. PBS was given as a control for the intravenous injections and for the vaccine placebo groups.

All manipulations were done under light CO2 anesthesia to minimize any stress or trauma to the animals. Before onset of neutropenia, a patch of fur approximately 4 × 4 cm was shaved off the lateral thoracic region of the animal, to allow for accurate and repeated body temperature recordings. A Horiba noncontact digital infrared thermometer (Markson Science) was used to monitor the animal’s body temperature after each immunization and, after the onset of neutropenia, several times daily. Fever generally occurred in infected animals 4–5 days after the initial dose of cyclophosphamide; fever was defined as a body temperature measurement >38.0°C.

Blood determinations and necropsy studies. Blood samples were obtained from the retro-orbital plexus of each animal under CO2 anesthesia before immunization, 2 weeks after the 4-week immunization schedules, 2 days before the first dose of cyclophosphamide, at the onset of fever, and 24 h after the onset of fever. Each blood sample was tested for quantitative bacterial counts, serum endotoxin levels, and anti-J5 antibody levels. Quantitative bacteriology was performed using standard methods, with serial dilutions of whole blood performed in TSB. The limit of detection was 10 cfu/mL of blood. Blood and tissue specimens from animals challenged with *P. aeruginosa* 12.4.4 were plated on *Pseudomonas* isolation agar (Difco). Non–lactose-fermenting, oxidase-positive colonies were identified and immunotyped with polyvalent *P. aeruginosa* antisera (Difco). In *K. pneumoniae* K2 challenge experiments, cultures were plated on Simon’s citrate medium (Becton
Dickenson) and then were characterized by using standard microbiologic methods. The bacterial colony counts in cultures of tissue samples from the liver and spleen were measured separately for each animal, but, since the colony counts from the 2 sites were similar, the results were combined and were reported compositely as colony-forming units per gram of tissue.

Endotoxin levels were measured in serum samples that were heat-treated to 70°C after a 1:10 dilution in endotoxin-free water per manufacturer’s instructions. Endotoxin measurements were determined by turbidimetric quantitative limulus amebocyte lysate assay (Associates of Cape Cod). Anti-J5 antibody titers were measured by using an ELISA method described elsewhere [6, 7].

Each animal was examined daily throughout the experiment until 14 days after cyclophosphamide treatment. Previous experiments (6–8) have shown that the period of neutropenia (<50 granulocytes/mm³) induced by this dose regimen of cyclophosphamide begins 3 days after the first dose of cyclophosphamide and extends until days 10–12. Animals that remain alive for >14 days after the cyclophosphamide treatment were considered long-term survivors. All animals that died during the course of the experiment were subjected to necropsy examination, with quantitative cultures obtained from the cecum, liver, spleen, and lung tissue. Animals that survived the experiment were killed, and a necropsy examination with quantitative cultures of the same organ sample was done.

Data analysis and statistical methods. Survival functions were measured using Kaplan-Meier plots, and differences in survival time were measured using a nonparametric Kruskal-Wallis 1-way analysis of variance. Numeric data were compared using the Mann-Whitney U test. Numeric data are expressed as mean ± SE; P < .05 was considered significant.

Results

Vaccine response. Animals vaccinated with either 2- or 3-dose regimens of the J5 dLPS/OMP complex vaccine (n = 40) experienced no febrile reactions for up to 48 h after each immunization and had feeding and weekly weight gain patterns similar to those of saline-immunized (n = 31) or Brucella dLPS/OMP vaccine-immunized (n = 16) control groups. Both vaccine schedules resulted in anti-J5 antibody levels that exceeded the target antibody response of 800 ELISA units (horizontal dotted line, figure 1). This level of antibody response was predicted to be protective on the basis of previous experiments with passively administered, rabbit-derived antisera [6]. Since the 3-dose vaccine regimen resulted in significantly greater (P < .05) antibody titers (2440 ± 526 ELISA units; n = 40) than the 2-dose regimen (840 ± 175 units; n = 15), the 3-dose schedule was exclusively used in subsequent challenge experiments with P. aeruginosa and K. pneumoniae. No J5 LPS-specific antibody was detected after immunization with the Brucella dLPS/OMP vaccine.

Antibodies to the LPS extracted from the 2 heterologous bacterial challenge strains, P. aeruginosa, Fisher immunotype 6, and K. pneumoniae O1, as well as from an unrelated E. coli O18, were measured in serum samples from rats, obtained 4 weeks after the third dose of vaccine (just before the first dose of cyclophosphamide and of oral bacteria). In serum samples chosen from rats that had high (n = 6), intermediate (n = 2), or low (n = 2) levels of anti–J5 LPS antibody, there were barely detectable levels of antibody to these other LPS antigens (data not shown). This indicates that immunization with the J5 dLPS/OMP complex vaccine did not elicit a long-lived, nonspecific polyclonal anti-LPS response.

Vaccine effects on survival. A Kaplan-Meier survival plot of neutropenic animals showed that following P. aeruginosa 12.4.4 oral challenge, J5 dLPS–vaccinated animals had an overall survival rate of 48% (13/28), whereas saline-treated control animals had a survival rate of 7% (2/29) (P < .01; figure 2A). None of the 16 Brucella dLPS vaccine–immunized rats survived (data not shown).

Since antibiotic treatment may liberate endotoxin from the dying bacteria [9], we tested the ability of actively induced antibody to protect animals from lethal sepsis under conditions in which there may be an acute endotoxin load. A similar level of protection was observed in animals who received vaccine and ceftazidime at the onset of fever (figure 2B), as was observed in animals receiving vaccine alone (figure 2A). Ceftazidime was highly active in vitro against this strain of P. aeruginosa 12.4.4 (MIC, 0.25 µg/mL).

Ceftazidime-treated animals cleared the Pseudomonas bacteremia (0 cases of bacteremia/10 animals) after 24 h of therapy, yet this dose of ceftazidime, while prolonging survival compared to animals not receiving antibiotics, was unable ultimately to protect these neutropenic animals from lethality. In contrast, the J5 dLPS/OMP complex vaccine in combination with ceftazidime significantly lowered mortality (figure 2B); 11 (61%) of 18 animals receiving vaccine and ceftazidime survived,
Figure 2. Effect of J5 detoxified lipopolysaccharide/outer membrane protein (dLPS/OMP) vaccine immunization on survival after lethal heterologous sepsis. Survival was observed for 14 days, at which time the neutropenia resolved and Kaplan-Meier survival curves were plotted. A, Rats were challenged with *Pseudomonas aeruginosa* alone. B, Four doses of ceftazidime (CAZ), a potent liberator of endotoxin, were given with the *P. aeruginosa* challenge. Rats that received neither vaccine nor antibiotic all died of multisystem failure by days 6 and 10, respectively. There was an increase in survival among animals that were immunized with the vaccine and were given ceftazidime. C, Animals were given *Klebsiella pneumoniae* in place of *Pseudomonas*. Increased survival was observed among rats actively immunized with the J5 dLPS/OMP vaccine. Survival data at day 14 for each group are given in table 2.

compared with 0/10 receiving ceftazidime and saline and 0/4 receiving saline alone (P < .01).

Challenge with another heterologous gram-negative bacterial species, *K. pneumoniae*, was highly lethal in the saline control group (figure 2C). The Kaplan-Meier survival plots demonstrated that the J5 dLPS/OMP vaccine provided a highly significant survival protection in these neutropenic animals: 9 (64%) of 14 vaccine-treated animals survived, versus 2 (13%) of 15 saline-treated controls (P < .005). Thus, active immunization with the J5 dLPS/OMP vaccine provided a survival advantage for animals infected with both *Pseudomonas* and *Klebsiella* species.

**Antibody levels during infection.** After the third dose of vaccine, there were prompt (by day 35, 7 days following the last dose of vaccine) and sustained (>12 weeks) increases in anti-J5 LPS antibody levels, which were generally 100-fold in excess of prevaccine baseline levels at the time of the first dose of cyclophosphamide and of oral *Pseudomonas* (table 1). Antibody titers diminished slightly over the course of bacteremic infection in *P. aeruginosa*-challenged animals. Twenty-four hours after infection, anti-J5 LPS antibody levels decreased but then rapidly recovered to preinfection levels and remained elevated throughout the duration of the experiment (3 months). The saline- and *Brucella* vaccine–treated control animals had anti-J5 antibody levels that were at the lower limits of detection throughout the experimental period. As was observed in animals infected with *Pseudomonas*, there was a decrease in anti-J5 LPS antibody levels at 24 h after onset of fever in *Klebsiella*-infected animals, but here, too, the levels returned to prefebrile levels (data not shown).

**Endotoxin levels.** Circulating levels of bacterial endotoxin were undetectable or very low before the onset of infection in vaccinated and control animals challenged with *P. aeruginosa* in the absence of ceftazidime therapy (figure 3, baseline). Vaccinated animals had significantly lower levels of endotoxin at the onset of fever during the course of *P. aeruginosa* infection. However, endotoxin levels were elevated to a similar degree in vaccinated and control (both saline- and *Brucella* vaccine–treated) groups after 24 h of continued fever and overt illness in these neutropenic animals (figure 3 and table 2). In nonvaccinated animals treated with ceftazidime, endotoxin levels remained significantly elevated at the onset of fever (data not shown) and 24 h later (table 2), and these circulating endotoxin levels were not significantly different from those in the saline-immunized control group (table 2). Cefazidime-treated animals who also received the J5 dLPS/OMP vaccine, however, had the lowest endotoxin levels within the first 24 h after fever onset (table 2). Endotoxin levels in the circulation of animals infected with *K. pneumoniae* K2 were significantly reduced in the vaccine-treated group (table 2).

**Bacteria load.** Multisystem infection with either *P. aeruginosa* 12.4.4 or *K. pneumoniae* K2 occurred invariably in the control group, resulting in a >90% mortality (figure 2A–2C). In each experiment, vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group; nevertheless, quantitative bacterial counts of organ tissue cultures revealed that vaccinated animals had significantly lower tissue levels of the challenge organism when compared with the control groups, in both the *Pseudomonas*– and *Klebsiella*-challenged animals (table 2).
The primary finding of this study was that active immunization with the detoxified J5 LPS/OMP vaccine before bacterial encounter reduced the likelihood of lethal outcome following infection with 2 heterologous species of bacteria, *P. aeruginosa* and *K. pneumoniae*. Immunization with a similarly prepared LPS from *Brucella*, also noncovalently complexed to group B meningococcal OMP, did not provide such protection. Recipients of the J5 vaccine, but not the control vaccine, had a lower organ load of bacteria and decreased circulating levels of endotoxin.

During the last 4 decades, the potential use of antibodies to structures in the highly conserved core region of LPS as prophylaxis and/or treatment for sepsis has been highly controversial. Results of clinical trials using the passive infusion of anti-core LPS antibodies directed against lipid A or inner core regions of LPS have been inconsistent [4, 5, 10–15]. The lack of protection following passive therapy with anti-core LPS antibodies in these studies could be attributed to the infusion of antibodies directed against the wrong epitope, or to antibodies directed against the proper epitope that either were not given in adequate amounts initially or whose levels were not maintained during the course of therapy. Secondly, the vaccines, usually heat-killed bacterial cultures, have been poorly defined so that the nature of the effective immunogen (LPS, outer membrane protein or other component), its optimal presentation, dose and reproducibility have not been well-characterized [5,16–19]. Such vaccines have been poorly tolerated as well, with 40% of subjects having systemic reactions [19]. Thirdly, the role of antibodies, particularly in studies that use antiserum or plasma rather than purified immunoglobulin fractions, as well as the importance of specific immunoglobulin classes, has been debated [18, 20]. Importantly, in studies showing a beneficial effect of antibodies or antiserum to core LPS, no clear, reproducible mechanism for its action has been demonstrated. Consequently, no assay has been proposed to monitor the efficacy of immunotherapy with these products. Because of these difficulties, anti-endotoxin antibody therapy has received less attention by clinical investigators in recent years.

In an earlier study J5 LPS affinity-purified IgG was obtained from rabbit antisera induced with a killed bacterial vaccine prepared in an identical manner to that of Ziegler et al [6]. Passive administration of this material as treatment to neutropenic rats who became febrile and bacteremic with heterologous gram-negative bacteria demonstrated highly significant protection against lethal infection in a dose-dependent manner [6]. Thus we concluded that an epitope in J5 LPS may be the target

**Table 1.** *Escherichia coli* J5 antibody levels (in ELISA units) in neutropenic rats during experimental sepsis with *Pseudomonas aeruginosa* after immunization with J5 or *Brucella* vaccine or with normal saline.

<table>
<thead>
<tr>
<th>Treatment (n/a)</th>
<th>Prevaccine (day 0)</th>
<th>Postvaccine (day 35)</th>
<th>Onset of sepsis (day 66)</th>
<th>Postsepsis (24 h after onset of sepsis; day 67)</th>
<th>Recovery (day 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J5 vaccine (28)</td>
<td>6.4 ± 2.0</td>
<td>3.1 ± 2.3</td>
<td>3.0 ± 1.9</td>
<td>7.6 ± 2.8</td>
<td></td>
</tr>
<tr>
<td><em>Brucella</em> vaccine (16)</td>
<td>7.5 ± 1.9</td>
<td>3.1 ± 2.3</td>
<td>2.3 ± 5.7</td>
<td>627c 1827</td>
<td></td>
</tr>
<tr>
<td>Saline control (29)</td>
<td>7.5 ± 1.9</td>
<td>3.1 ± 2.3</td>
<td>2.3 ± 5.7</td>
<td>627c 1827</td>
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NOTE: J5 vaccine, J5 detoxified lipopolysaccharide/outer membrane protein (dLPS/OMP) vaccine.

* a Animals were immunized subcutaneously at days 0, 14, and 20 with 20 μg of the J5 dLPS/OMP or *Brucella* vaccine or with normal saline. IgG antibody levels of J5 LPS were measured 7 days after the third dose. Thirty-three days after the third dose (61 days after the first dose of vaccine), the rats were given the first dose of cyclophosphamide and *P. aeruginosa* (see Materials and Methods). Antibody levels were measured again at onset of sepsis (day 66), 24 h later (day 67), and, in surviving animals, at recovery (day 80).

b Data from the 2 long-term survivors in the saline control group and from 13 long-term survivors in the J5/OMP vaccine group; there were no survivors in the *Brucella*/OMP group.

c *P < .0001, compared with prevaccine levels.

d *P was nonsignificant for all postvaccine time points, compared with prevaccine levels. Data were analyzed by the Kruskal-Wallis test, and comparisons among groups were made with Dunn’s multiple comparison test.

**Figure 3.** Circulating serum endotoxin levels in rats immunized with J5 detoxified lipopoly saccharide/outer membrane protein (dLPS/OMP) vaccine or with normal saline (NS). Rats were immunized at time 0 and at 2 and 4 weeks. At 14 days after the last vaccine dose, animals were given the first doses of cyclophosphamide and *Pseudomonas*. The animals were then followed up every 12 h for the onset of fever. Serum samples drawn at the onset of fever (typically days 5–6 after the first dose of cyclophosphamide) and 24 h later were evaluated for endotoxin levels by a limulus amebocyte assay. At the onset of sepsis, J5 dLPS/OMP-immunized animals had 0.5 ± 0.3 ng/mL endotoxin, versus 1.3 ± 0.4 for NS-immunized controls (P = .02).
immunization studies. The vaccine was well-tolerated by the group B meningococcal OMP or by lipid A.

antibody induced by the whole bacterial vaccine. This protective degree of protection was observed with antisera obtained following immunization with this vaccine as was observed with antibody induced by the whole bacterial vaccine. This protection was not associated with antibodies elicited by either the group B meningococcal OMP or by lipid A.

Based on these data, we proceeded with the present active immunization studies. The vaccine was well-tolerated by the rats with no systemic signs usually associated with endotoxin administration (piloerection, diarrhea, huddling). The animals had levels of antibody directed to J5 LPS that was maintained throughout the duration of risk, up to 80 days after the initial immunization (table 1). Thus, unlike passive immunization, active immunization induced potentially protective antibody levels for extended periods of time. As was true in a previous study, immunization did not prevent either systemic infection or the onset of sepsis [12]. All immunized animals became infected and had a clinical appearance at the onset of fever similar to nonimmunized animals.

While the immunization induced both protection from lethal sepsis and highly significant increases in anti–J5 LPS antibody level, we cannot necessarily assume from these data that the observed protection was directly attributable to those antibody levels. The active immunization could have stimulated some other immune mechanism, such as an activation of the reticuloendothelial system (RES) or stimulation of an acute phase response. It is unlikely, however, that some ongoing acute phase response would account for the protection nearly 1 month after the last dose of vaccine.

It has been proposed that the protection from the whole cell vaccination to rough mutant bacteria may be due to the induction of a mitogenically induced polymicrobial anti–O chain antibody [22]. We do not believe that this was the case here because there were barely detectable circulating antibodies to the endotoxin prepared from the P. aeruginosa and K. pneumoniae challenge strains or to the LPS of an unrelated E. coli O18 at the time of initial bacterial challenge (i.e. 4 weeks after the last dose of vaccine). Furthermore the control Brucella LPS vaccine in the current study failed to protect.

The J5 dLPS–immunized animals exhibited decreased bacterial levels in liver and spleen and a significantly lower level of circulating endotoxin at the onset of fever (table 2), consistent with what others have shown with similar anti–core LPS preparations in animal models [23–25]. These data suggest that the antibody may have promoted the uptake and killing of Klebsiella and Pseudomonas challenge bacteria from the blood by reticuloendothelial tissue. The lower level of circulating endotoxin could be attributed either to the promotion of LPS clearance from the circulation, or to the reduction in bacteria load.

The availability of a well-tolerated anti–core LPS vaccine may permit new strategies for the prevention and treatment of sepsis in populations at risk of gram-negative bacterial sepsis.

If the protection were shown to be J5 LPS antibody-related, the potential advantages of active immunization would be (a) a more sustained level of circulating antibody so that adequate levels of antibody are more likely to be present at the time it is needed (i.e. at initial encounter with the pathogen) than is the case with passive prophylaxis, (b) the recruitment of other antibody isotypes, and (c) the possibility that this antibody may be subject to recall. Active immunization may potentially involve other arms of the immune system as well.
immunization with this vaccine may be a better strategy for preventing (as opposed to treating) sepsis than passive infusion.

An active immunization strategy may be limited by insufficient time for the antibody response to develop or by a poor antibody response among those patients most likely to require such a vaccine. Recent studies, however, have shown that (a) in the United States, most cases of sepsis occur in the ICU [26, 27], and (b) the development of septic shock occurs in recognizable, hierarchical stages, with a mean interval of 21 days between onset of SIRS and progression to sepsis [28]. Thus, if it were possible to induce anti-endotoxin antibodies within 7–14 days in patients with SIRS, it may be feasible and cost-effective to immunize patients upon entry to the ICU (and perhaps to supplement with passively infused J5 IgG at the onset of sepsis). Although it might be expected that such a population might not respond well to vaccination, in 1 study, active immunization of acutely traumatized individuals with polyvalent *P. aeruginosa* and *Klebsiella* vaccines resulted in levels of antibody similar to those observed in normal volunteers [29]. These observations suggest that active immunization of acutely traumatized individuals as well as populations at risk of sepsis (police, firemen and soldiers who might incur trauma or burns, and patients undergoing elective abdominal or complicated gynecotomic surgery) merits further investigation.

While immunization with this vaccine provided statistically significant protection against lethal sepsis, complete protection was not seen under any experimental conditions. Given the complexity of sepsis, however, we previously argued that combination therapy directed against multiple stages of the septic process (anti-bacterial, anti-endotoxin, and anti-inflammatory agents) may be required for optimal treatment [30].

Given the tremendous controversy surrounding the numerous previous studies relating to the use of anti-LPS passive immunization strategies, as well as the numerous conflicting reports of such protection, it seems prudent to be especially cautious with the development of new vaccines based on an anti-LPS strategy. The next steps will likely include further confirmation of its efficacy in numerous different models of gram-negative bacterial infection, further study and elucidation of its exact mechanism(s) of action, and development of an in vitro test that correlates (and predicts) protection.

References

23. Dunn DL, Ferguson RM. Immunotherapy of gram-negative bacterial sepsis:


