

Active Immunization with a Detoxified Endotoxin Vaccine Protects against Lethal Polymicrobial Sepsis: Its Use with CpG Adjuvant and Potential Mechanisms

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Background. An experimental vaccine for sepsis, composed of detoxified *Escherichia coli* J5 lipopolysaccharide (LPS) complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B, induces anti-core glycolipid antibody and has been tested in pilot studies in human volunteers.

Methods. Mice were immunized with the LPS-J5/OMP vaccine with or without synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs as a vaccine adjuvant (CpG ODN). The efficacy of the vaccine-induced antibody response was tested in a cecal ligation and puncture model.

Results. Immunization resulted in a >20-fold increase in anti-core glycolipid antibody levels, which were further increased 5-fold by the addition of CpG ODN, compared with the levels in mice in the control group. The vaccine provided a survival advantage after a cecal ligation and puncture was performed ($P < .01$) and significantly decreased the levels of bacteria in organs. Immunoglobulin G (IgG) anti-core glycolipid antibodies were decreased in mice to a significantly greater extent than were levels of total circulating IgG or IgG to the OMP part of the vaccine complex, suggesting specific epitope binding and clearance.

Conclusions. These results indicate that the detoxified LPS-J5/OMP vaccine induces high levels of antibody against the core glycolipid of LPS and functions in vivo to promote clearance of gram-negative bacteria and improve the outcome of experimental polymicrobial intra-abdominal sepsis.

We have developed a vaccine to prevent and treat gram-negative bacterial sepsis [1]. IgG antibody that binds to conserved core regions within bacterial lipopolysaccharide (LPS) has been shown to cross-react with multiple heterologous LPS structures [1, 2]. The generation of such antibody is the therapeutic rationale for this vaccine strategy for immune protection against a wide variety of gram-negative bacterial pathogens. The LPS from an Rc chemotype mutant of *Escherichia coli* O111: B4 (*E. coli* J5) has been detoxified by alkaline treatment to cleave ester-linked fatty acids of the lipid A com-

ponent of LPS. This detoxified form of LPS (dLPS) was noncovalently complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B. This vaccine protects against lethal gram-negative bacterial sepsis when administered either actively (in the form of vaccine as a preventive strategy) or passively (in the form of immune plasma) in a neutropenic rat model of *Pseudomonas* sepsis [2–4]. A phase I clinical study of active immunization in humans has recently been performed [1].

Although immunization with the same vaccine in rabbits, mice, and rats demonstrated a >20-fold increase in levels of IgG antibody against the core glycolipid of LPS, human volunteers developed only a 2–3-fold increase above baseline antibody levels [1]. The antibody response was polyclonal, with both IgM and IgG antibodies generated that persisted for at least 12 months (the last time interval tested) [4]. Because previous passive protection studies indicated that protection against lethal sepsis was antibody level dependent, we inves-

Received 14 April 2005; accepted 1 July 2005; electronically published 10 November 2005.

Financial support: National Institutes of Health (grant AI-42181).

Potential conflicts of interest: none reported.

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The Journal of Infectious Diseases 2005;192:2074–80

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tigated the possibility of using synthetic oligodeoxynucleotides (ODNs) as immunoadjuvants to the dLPS-J5/OMP complex vaccine.

Bacterial DNA and synthetic ODNs that contain immunostimulatory unmethylated CpG motifs have been shown to be potent B cell activators and effective immunoadjuvants when combined with a wide variety of antigens, including peptide-based vaccines [5]. CpG motifs also promote a Th1-type immune response in human cells that may further promote a combined innate and adaptive immune response. Synthetic ODNs have been shown to have a potential benefit for patients with asthma, enhance innate host defenses against neoplasia [6, 7], and improve human vaccine responses [8]. To date, CpG ODNs have been used as vaccine adjuvants primarily with protein, protein-polysaccharide conjugate, and DNA vaccines [5–8].

In the present study, we investigated the possibility that CpG ODNs serve as immunoadjuvants to the dLPS-J5/OMP vaccine to increase antibody responses to the core glycolipid of bacterial LPS. The activities of antibodies raised against this vaccine were studied in the cecal ligation and puncture model of polymicrobial sepsis. This model generates a bacteremic infection by endogenous enteric bacteria and should serve as a clinically relevant test system. The mechanism of protection afforded by the vaccine was further investigated in this experimental model.

MATERIALS AND METHODS

Reagents

A rodent-specific CpG ODN adjuvant (no. 1826) was provided by Coley Pharmaceutical Group. The dLPS-J5/OMP vaccine was developed in a good manufacturing practices facility, as described elsewhere [1]. The meningococcal OMP was derived from LPS-free, membrane-free proteosomes and was noncovalently complexed with detoxified *E. coli* J5 LPS. Murine cytokine and chemokine levels were measured using the BioPlex 16 multiplex cytokine assay (Bio-Rad). LPS levels were measured using a quantitative turbidimetric Limulus amoebocyte lysate assay (Associates of Cape Cod). All other reagents and chemicals were provided by Sigma, unless otherwise stated.

Mouse Studies

Cecal ligation and puncture. Pathogen-free albino female BALB/c mice (Charles River Laboratories) were used in the experiments. The mice were 8–12 weeks old and were allowed to adapt to the laboratory for 7 days before the experiments were initiated. The mice were allowed to eat and drink freely. The University of Maryland Institutional Animal Care and Use Committee and veterinary staff approved all mouse studies before initiation of the experiments.

The experimental design was modeled after previously published investigations [9]. After an overnight fast, mice were

anesthetized with parenteral administration of 200 μ L of ketamine at 9 mg/mL (Abbott) and xylazine at 1 mg/mL (Phoenix Pharmaceuticals). Under sterile conditions, a midline abdominal incision was made, and the cecum was identified and brought outside of the abdomen. The cecum was then ligated with a 4-0 monofilament suture, and the antemesenteric side of the cecum was punctured twice with a 23-gauge needle. To ensure patency, a scant amount of luminal contents was expressed through each puncture site. The cecum was then returned to the abdomen, and the fascia and skin were closed in 2 layers.

Lidocaine (1% without epinephrine) and topical antibiotic (bacitracin) were applied to the surgical site. A single dose of trovafloxacin (20 mg/kg; Pfizer) was administered intramuscularly (im) with 1.0 mL of normal saline given subcutaneously. The mice were warmed externally until they were able to regain normal mobility. Mortality was monitored for 7 days. Moribund mice that were unable to right themselves and were hypothermic (temperature $<33^{\circ}\text{C}$ by digital infrared thermometry) were considered to be lethally infected and were euthanized. Each mouse underwent a necropsy, during which liver and spleen tissues were removed for quantitative cultures on MacConkey media and *Enterococcus*-specific media (BBL). A 1-mL sample of peritoneal fluid was obtained by lavage of the peritoneum with 5 mL of normal saline during the necropsy.

Vaccine schedule. The dJ5LPS/OMP vaccine was administered im in doses of 10 μ g or 20 μ g (based on dLPS content) at 0, 2, and 4 weeks. After a 1-month rest period, the cecal ligation and puncture was performed. Blood samples were obtained at baseline, 1 month after the final immunization (before the cecal ligation and puncture was performed), and 48 h after the cecal ligation and puncture was performed. The CpG ODN immunoadjuvant (25 μ g/mouse) was administered admixed with the vaccine in the same syringe. The control group received CpG ODN with saline at the same dosing and on the same schedule used for the vaccine. In a single experiment, CpG ODN with saline was administered to a separate control group of mice ($n = 5$) at 25 μ g/mouse 6 days before the cecal ligation and puncture was performed. A previous study [10] indicated that CpG alone may have significant immunoprophylactic effects when administered shortly before major systemic insults. Levels of antibodies against the core glycolipid of LPS were measured using a standard ELISA method described elsewhere [1].

Statistical analyses. Numeric data were analyzed by a nonparametric Kruskal-Wallis 1-way analysis of variance with Dunn's multiple comparisons test for multiple groups or the Mann-Whitney *U* test for 2 groups. A Kaplan-Meier survival plot was used to analyze outcome in each treatment group, and differences in survival time were measured by the log-rank test. A paired Student's *t* test was used to measure antibody levels and

Table 1. IgG response in mice to vaccines with and without adjuvants.

Treatment	IgG level, mean \pm SE (range), ng/mL
dLPS-J5/OMP vaccine	11,259 \pm 3253 (3036–23,880)
dLPS-J5/OMP vaccine with CpG ODN	72,052 \pm 29,889 ^a (26,333–219,650)
dLPS-J5/OMP vaccine with alum	25,472 \pm 9306 (5522–54,355)
dLPS-J5/OMP vaccine with CpG ODN and alum	6197 \pm 828 ^b (4380–8976)
CpG ODN with alum	85 \pm 15 (51–151)
CpG ODN with saline	60 \pm 13 (0–87)

NOTE. The vaccine consisted of detoxified lipopolysaccharide (LPS) from *Escherichia coli* J5 noncovalently complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B (dLPS-J5/OMP) with or without synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs as a vaccine adjuvant (CpG ODN). Mice in the experimental groups ($n = 6$ mice/group) were actively immunized with vaccine (10 or 20 μ g on the basis of LPS content) with or without CpG ODN (25 μ g) or alum (10 μ g) as indicated on days 0, 14, and 28. Mice in the control groups received CpG ODN with either alum ($n = 6$ mice) or saline ($n = 5$ mice). Serum was obtained on day 35, and serum samples from individual mice were examined in an anti-J5 LPS ELISA.

^a $P < .01$, vs. the dLPS-J5/OMP vaccine alone.

^b $P < .01$, vs. the dLPS-J5/OMP vaccine with CpG.

ratios of antibody response. $P < .05$ was considered to be statistically significant.

RESULTS

Immunogenicity of the dLPS-J5/OMP vaccine with or without adjuvants.

The ability of the vaccine constructs to induce antibody responses is summarized in table 1. Antibody responses were tested after mice were administered 3 doses of 10 or 20 μ g of the dLPS-J5/OMP vaccine 14 days apart. The antibody response to the dLPS-J5/OMP vaccine alone, with CpG ODN, with alum, or with a combination of CpG ODN and alum was also investigated. As previously reported [4], the dLPS-J5/OMP vaccine alone was highly immunogenic ($P < .005$, vs. control groups) and well tolerated. The geometric mean antibody level in response to the dLPS-J5/OMP vaccine alone was increased 5-fold ($P < .01$) by the addition of CpG ODN. Mice receiving alum had a slight increase in the antibody response, compared with that in mice receiving the dLPS-J5/OMP vaccine alone, but had a significantly decreased antibody response, compared with that in mice receiving the dLPS-J5/OMP vaccine with CpG ODN ($P < .01$). Thus, alum may interfere with epitope processing of the vaccine construct when given with CpG ODN. Consequently, alum was not included in subsequent experiments.

Active protection conferred by the dLPS-J5/OMP vaccine with or without CpG ODN. Mice ($n = 15$ /group) were actively immunized with the dLPS-J5/OMP vaccine with or without CpG ODN. One control group ($n = 5$) received CpG ODN with saline on the same schedule used for the vaccine. The results of the cecal ligation and puncture experiment are presented in figure 1. Another control group received CpG ODN alone 6 days before the cecal ligation and puncture was performed ($n = 5$). The dLPS-J5/OMP vaccine with or without CpG ODN provided significant protection against lethal sepsis after the cecal ligation and puncture ($P < .01$). Because CpG

ODN alone demonstrated protection in a similar murine model of intra-abdominal polymicrobial sepsis [10], we endeavored to determine whether the survival advantage observed for the dLPS-J5/OMP vaccine with CpG ODN was, in fact, due to the vaccine response rather than the immunomodulatory properties of CpG ODN alone. When CpG ODN alone was given 6 days before the cecal ligation and puncture was performed, it provided some protection against lethal sepsis (4/5 mice survived), but when it was given 1 month before the cecal ligation and puncture was performed, no mice survived (figure 1).

Levels of IgG against the core glycolipid of LPS were measured 28 days after the final immunization (before the cecal ligation and puncture was performed) and 48 h after the cecal ligation and puncture was performed (figure 2). Although the dLPS-J5/OMP vaccine alone was highly immunogenic (geometric mean IgG anti-core glycolipid antibody level, 151 μ g/mL), administration of the dLPS-J5/OMP vaccine with CpG ODN increased the geometric mean IgG anti-core glycolipid antibody titer ~3–5-fold to 552 μ g/mL ($P < .005$, vs. geometric mean IgG anti-core glycolipid antibody level induced by the dLPS-J5/OMP vaccine alone). Mice receiving CpG ODN alone had a flat response, with geometric mean IgG anti-core glycolipid antibody levels remaining at baseline values of 0.12 μ g/mL. Mice receiving CpG ODN with saline all died (figure 1). Mice receiving the dLPS-J5/OMP vaccine with or without CpG ODN were highly protected against lethal sepsis ($P < .01$).

Plasma LPS levels were significantly lower and peritoneal LPS levels were lower in the mice receiving the dLPS-J5/OMP vaccine with or without CpG ODN than in mice in the control group (table 2). Levels of bacteria in organs were decreased in the vaccine groups, compared with those in the control group. Peritoneal, but not plasma, tumor necrosis factor (TNF)- α levels were significantly lower in the vaccine groups than in the control group ($P < .01$). Peritoneal gram-negative bacteria levels and TNF- α and LPS levels were lowest in the group receiving

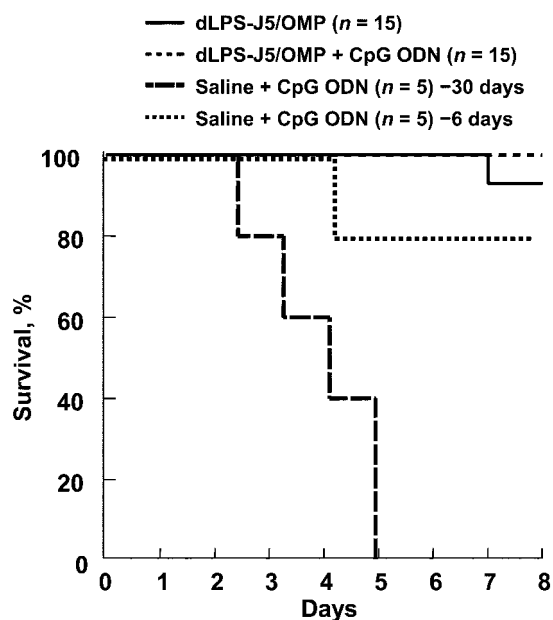


Figure 1. Kaplan-Meier survival plot of BALB/c mice. The vaccine consisted of detoxified lipopolysaccharide (LPS) from *Escherichia coli* J5 non-covalently complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B (dLPS-J5/OMP) with or without synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs as a vaccine adjuvant (CpG ODN). Mice in the experimental groups ($n = 6$ mice/group) were actively immunized with the dLPS-J5/OMP vaccine (10 or 20 μg on the basis of LPS content) with or without CpG ODN (25 μg). Mice in the control groups received CpG ODN (25 μg) with saline on day -6 ($n = 5$ mice) or -30 ($n = 6$ mice). On day 0, mice underwent cecal ligation and puncture. Mice in both vaccine groups had a significantly greater survival rate than did mice receiving CpG ODN with saline on day -30 ($P < .01$ for both vaccine groups vs. the relevant control group).

the dLPS-J5/OMP vaccine with CpG ODN but they were not statistically significantly different from those in the group receiving the dLPS-J5/OMP vaccine alone (table 2).

If a mechanism of action of anti-core glycolipid antibody is to bind and promote the clearance of bacteria by the reticuloendothelial system, then a decrease in levels of serum antibody against the core glycolipid of LPS, and not a decrease in levels of serum anti-OMP antibody, would be expected. This decrease in anti-core glycolipid antibody could be due to a generalized decrease in IgG (e.g., a hypermetabolic state during sepsis) or to a decrease in specific antibody (as may occur with the binding of antigen and subsequent clearance of the complex). Although a brisk antibody response to the OMP component of the vaccine complex was observed after immunization, the OMP-specific IgG levels measured 48 h after the cecal ligation and puncture were only slightly lower (1–2-fold) than those measured before the cecal ligation and puncture was performed. The decrease in OMP-specific IgG levels was comparable to the ratio of total IgG levels before and after the cecal ligation and puncture was performed (figure 2).

In contrast, serum anti-core glycolipid IgG levels were decreased 3–4-fold 48 h after the onset of intra-abdominal sepsis. We therefore compared the ratio of levels of total IgG and IgG specific for J5 LPS with the level of OMP-specific IgG before and after the cecal ligation and puncture was performed (figure 2). The decrease in antibody levels was specific for the target epitopes found within the core glycolipid portion of the vaccine formulation. As we expected, the administration of CpG ODN alone on the same schedule used for the vaccine induced minimal IgG antibody responses to both OMP and the core glycolipid of LPS.

DISCUSSION

The present study demonstrated that active immunization with the dLPS-J5/OMP vaccine elicited protection against death in mice with polymicrobial sepsis secondary to cecal ligation and puncture. The circulating endotoxin levels and the quantity of gram-negative bacteria in cultures of liver and spleen tissue were significantly decreased in mice receiving the vaccine. These results are compatible with the hypothesis that anti-core glycolipid antibody binds to microbial antigens and is cleared in vivo at a greater rate than are other circulating immunoglobulins in mice with polymicrobial gram-negative sepsis. There also was a decrease in the levels of peritoneal inflammatory cytokines in immunized mice, compared with those in mice in the control group. Peritoneal LPS levels were decreased, although not significantly, after active immunization with the dLPS-J5/OMP vaccine. Specifically, anti-core glycolipid antibody levels were decreased after the cecal ligation and puncture was performed. The lack of a comparable reduction in the level of circulating OMP-specific IgG argues against a generalized, nonspecific decrease in antibody levels from increased catabolism, altered tissue distribution, or decreased synthesis of immunoglobulins. These results in the cecal ligation and puncture model support our previous findings of vaccine protection in the neutropenic rat model of sepsis due to either *P. aeruginosa* or *Klebsiella pneumoniae* [2, 4].

We have previously reported that a whole bacterial vaccine prepared from boiled *E. coli* J5 (Rc chemotype) O111:B4 elicited antibody in rabbits that protected neutropenic rats from lethal gram-negative infection [11]. Because affinity-purified IgG prepared from this antiserum was protective, we developed a vaccine candidate with detoxified LPS from *E. coli* J5 [1]. The detoxified *E. coli* J5 LPS was noncovalently complexed to group B meningococcal OMP to maintain a critical conformational epitope present in the core glycolipid of LPS [2]. This vaccine, like the heat-killed whole bacterial vaccine, has been shown to be protective in both active and passive models in the neutropenic rat model [3, 4]. This protection is associated with decreased levels of both circulating cytokines and bacterial endotoxin and decreased levels of bacteria in target organs. These

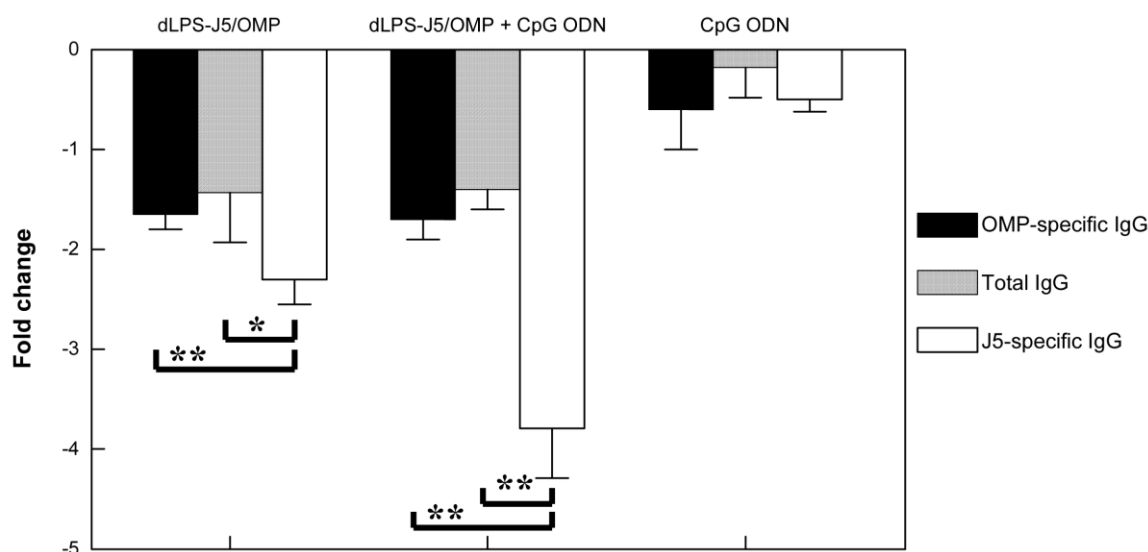


Figure 2. Decrease in geometric mean antibody level as a ratio of specific IgG antibody level before and 48 h after cecal ligation and puncture. The vaccine consisted of detoxified lipopolysaccharide (LPS) from *Escherichia coli* J5 noncovalently complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B (dLPS-J5/OMP) with or without synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs as a vaccine adjuvant (CpG ODN). Mice in the experimental groups ($n = 6$ mice/group) were actively immunized with the dLPS-J5/OMP vaccine (10 or 20 μg on the basis of LPS content) with or without CpG ODN (25 μg). Mice in the control group ($n = 6$) received CpG ODN alone. * $P < .05$; ** $P < .005$.

studies form the basis for further clinical development of the vaccine.

Phase I clinical testing of the dLPS-J5/OMP vaccine in healthy human volunteers has been completed [1]. The vaccine was given in doses of 5–25 μg (based on its LPS content) to 24 volunteers on days 0, 28, and 56. The vaccine was well tolerated, and no significant systemic toxicity and no abnormal laboratory values were attributed to the vaccine [1, 11]. Approximately two-thirds of the volunteers had some mild to

moderate pain at the injection site, which usually resolved in 24–48 h. Although preclinical studies consistently demonstrated >20-fold increases in antibody levels in other mammals, human volunteers had only a 3-fold increase over preimmune baseline levels. Despite the rather small increase in antibody levels, plasma from immune volunteers reduced cytokine generation in a whole-blood assay [11]. Because studies in the neutropenic rat model indicate the need for high levels of anti-core glycolipid antibody to offer protection against lethal sepsis [3], an

Table 2. Immune responses and bacterial levels in mice.

Parameter	CpG ODN with saline (control group) ($n = 5$)	dLPS-J5/OMP vaccine ($n = 15$)	dLPS-J5/OMP vaccine with CpG ODN ($n = 15$)	P^a
Plasma endotoxin level, ng/mL	7.7 ± 5.5	0.2 ± 0.12	1.8 ± 1.6	<.001
Plasma TNF- α level, pg/mL	32.0 ± 10	11.6 ± 7.9	18.1 ± 5.8	NS
Bacteria in organ samples, cfu/mg	350 ± 774	40 ± 97	0 ± 31	<.05
Peritoneal TNF- α level, pg/mL	52.6 ± 3.0	23.3 ± 6	15.0 ± 4.5	<.01
Peritoneal endotoxin level, ng/mL	92.4 ± 44	10.6 ± 32	3.1 ± 9	.1

NOTE. The vaccine consisted of detoxified lipopolysaccharide (LPS) from *Escherichia coli* J5 noncovalently complexed with the outer membrane protein (OMP) of *Neisseria meningitidis* group B (dLPS-J5/OMP) with or without synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs as a vaccine adjuvant (CpG ODN). Mice in the control group ($n = 5$) received CpG ODN (25 μg) with saline 6 days before the cecal ligation and puncture was performed; mice in the experimental groups ($n = 6$ mice/group) received the dLPS-J5/OMP vaccine (10 or 20 μg on the basis of LPS content) with or without CpG ODN 30 days before the cecal ligation and puncture was performed. Other cytokines and chemokines measured showed no differences between groups. NS, not significant; TNF, tumor necrosis factor.

^a P values represent differences (mean \pm SE) between the control group and either vaccine group. No significant differences between vaccine groups were found.

effort was undertaken to determine if immunoadjuvants would increase the vaccine-induced antibody response.

Microbial deoxynucleotides or synthetic ODNs with unmethylated CpG motifs are potent toll-like receptor 9 agonists [12, 13] and promote Th1 proinflammatory cytokine responses and B cell clonal expansion [5]. Through these mechanisms, CpG ODN simultaneously activates the innate and adaptive immune response essential to resist microbial invasion and promote antibacterial defense mechanisms [10]. CpG ODN is now being developed for a variety of purposes, including generation of antineoplastic immunity, promotion of antiviral activity, and treatment for Th2-associated diseases, such as asthma [5–7]. CpG ODN has also been used as an immunoadjuvant to various types of vaccines, including those for hepatitis B [8, 14] and influenza [15] in humans. Although most previous studies have focused on the use of CpG ODN as an adjuvant to protein-based vaccines, the present study focused on the ability of CpG ODN to increase antibody response to an LPS-based vaccine. Recent data suggest that CpG ODN may also stimulate polysaccharide antibody responses to *Haemophilus influenzae* type b conjugate vaccine [16] and a pneumococcal vaccine [17].

The addition of CpG ODN to the dLPS-J5/OMP vaccine led to a marked increase in anti-J5 LPS antibody levels (table 1). There was a 5-fold increase in the IgG antibody level when CpG ODN was administered with the dLPS-J5/OMP vaccine. Mice receiving the dLPS-J5/OMP vaccine with CpG ODN had higher IgG levels than did mice receiving the vaccine alone. Although alum and CpG ODN have shown synergy in various preclinical vaccines, as well as in a hepatitis B vaccine in clinical testing [8, 18, 19], the addition of alum to the dLPS-J5/OMP vaccine significantly decreased antibody responses to it (table 1). Alum, when administered with CpG ODN, may block the alignment and/or exposure of a critical conformational epitope in this vaccine that is recognized by the host immune system.

The adjuvant effect of CpG ODN demonstrated in our preliminary experiments was also evident in mice that underwent active immunization before the cecal ligation and puncture procedure. As was the case in previous experiments, mice had a significant increase in geometric mean anti-J5 dLPS IgG levels after receiving a 3-dose series of immunizations, and the dLPS-J5/OMP vaccine provided a high level of protection against lethal sepsis. Because the protection observed with the dLPS-J5/OMP vaccine alone was >90%, it was difficult to show that the addition of CpG ODN increased survival.

The administration to mice of a single dose of CpG ODN 6 days before the cecal ligation and puncture was performed offered protection against lethal sepsis, as was reported elsewhere [10]. This protection has been attributed to enhanced phagocytic function and immune clearance induced by CpG ODN. However, the administration of CpG ODN alone 30 days

before the cecal ligation and puncture were performed did not increase survival. The CpG ODN we used in our experiments appears to function as an adjuvant to the vaccine, with enhancement of adaptive immune responses, and not as an independent nonspecific stimulant of innate host defenses [20, 21].

We were unable to demonstrate that the addition of CpG ODN offered increased protection against lethal sepsis, compared with that conferred by the dLPS-J5/OMP vaccine alone, in the cecal ligation and puncture model (figure 1). This may be because the antibody response induced by the vaccine alone, even with its partial depletion during sepsis, produced antibody levels far in excess (geometric mean anti-core glycolipid antibody level, 151 $\mu\text{g/mL}$) of the level required for protection. Mice receiving vaccine alone and mice receiving vaccine with CpG ODN had lower peritoneal levels of local cytokines and lower levels of bacteria in organs than did mice in the control group after the cecal ligation and puncture was performed. Even though the dLPS-J5/OMP vaccine administered with or without CpG ODN elicited high levels of anti-core glycolipid antibody, the lowest TNF- α and LPS levels were found in mice receiving the dLPS-J5/OMP vaccine with CpG ODN. This suggests that the administration of CpG ODN with the vaccine may provide better protection than that conferred by the vaccine alone, particularly in more-severe models of sepsis and septic shock.

The mechanism of protection afforded by the dLPS-J5/OMP vaccine has not been fully elucidated. Because mice in the vaccine groups had a decreased level of aerobic organisms in organs, compared with that in the control group, one mechanism of protection of the vaccine may be the uptake and killing of bacteria by tissue phagocytes. Our previous studies involving passive administration of anti-core glycolipid antibody have shown that postimmunization serum samples promote the clearance of both LPS and bacteria from the blood [4]. It is critically important to identify whether this is a primary mechanism of protection, so that surrogate markers for vaccine efficacy can be used in the development of a human vaccine. It is possible that vaccine-induced antibody might promote clearance of *Bacteroides fragilis* or other anaerobic gram-negative enteric microflora as well.

In the case of pneumococcal or hepatitis B vaccines [19, 22], it has been shown that functional assays, and not simple binding assays, correlate with vaccine-elicited protection. Unlike the situation with these microbial pathogens, in which only a single activity (opsonization or neutralization) appears to be of primary importance, the host response to bacterial LPS is considerably more complicated. A wide range of potential, clinically relevant activities is initiated by this microbial mediator, and it is difficult to predict which function is the most appropriate target for inhibition by antibody induced by the vaccine. We

are currently undertaking a systematic survey of the effect of vaccine-induced antibody on LPS-induced activities.

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